Development of Flash Détente Applications for Impacting Red Wine Style and for Production of Colour Stable Red Wines and Concentrate

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THESIS SUMMARY

The basic process flow for making red wines has barely changed over several millennia. Recent advances in technology have led to the development of new or modified processes for red wine production, for example, flash détente (FD). FD processing consists of heating grape must to ~85 °C followed by application of vacuum to boil or 'flash off' water in grape tissues, resulting in cellular damage that aids extraction of key grape skin compounds into the juice. This technology offers the opportunity to reconfigure the red winemaking process by separating grape colour and phenolic extraction from fermentation. Potential financial benefits include less capital expenditure by using inexpensive white wine fermenters for red wine production, and decoupling fermentation from harvest, through production of red juice concentrate. However, since FD is relatively new, very little research has been undertaken to evaluate the application of this technology to the production of either red grape concentrate or red wine. The research outlined within this thesis therefore aimed to develop an understanding of the impact of FD treatment on red colour extraction, quality, and stability during red juice concentrate production, as well as to assess the impact of fermenting FD treated red juice or must (under different fermentation conditions) on the style, composition and sensory properties of red wine. This work also investigated treatments for stabilising the colour of FD-derived red grape concentrate and red wine.

The use of FD in the production of red juice concentrate as a food and beverage colouring has not been well studied. Colour concentration, quality, stability, and filterability were compared and contrasted for Rubired concentrates obtained with traditional conventional must heating (CMH) or FD extraction. FD concentrate had similar levels of red colour and concentrations of C_6 ('green') aroma compounds, a lower ratio of brown colour, and higher concentrations of caftaric acid and

catechin, compared to CMH concentrate. FD processing also generated a significantly higher concentration of grape suspended solids and the juice had four times lower flux compared to CMH juice. Red colour stability was greater for CMH concentrate under normal concentrate storage conditions.The colour stability of Rubired concentrate produced via FD and CMH was compared under accelerated ageing conditions, i.e. by heating at 50, 60 and 70 °C, with or without different chemical treatments (i.e. acid addition to lower pH, acetaldehyde or commercial seed tannin addition, applied either individually or in combination) to assess their impact on colour stability. Compositional analyses were performed after 0, 3, 6, and 9 days, to gain insight into colour stability, 5-hydroxymethylfurfual (5-HMF) formation and browning. CMH concentrate had significantly greater 5-HMF formation and red colour stability compared to FD concentrate, as shown by a half-life and activation energy of 233.9 hours and 65.2 kJ/mol versus 203.3 hours and 59.2 kJ/mol respectively, after heating at 50 °C. Red colour, malvidin-3,5-*O*-diglucoside and malvidin-3-*O*-glucoside loss followed first order reaction kinetics.

Acetaldehyde, low pH, and their combination increased red colour stability, as well as violet and brown colour with heating at 50 and 60 °C whereas seed tannin had no significant effects under all treatment conditions. At 70 °C, only acetaldehyde treatment increased red, brown and violet colour, while all treatments involving low pH decreased red, brown and violet colour units, due to acid hydrolysis at the higher temperature.

Making red wines from FD derived grape juice or possibly even reconstituted concentrate requires understanding the impact of ‘off-skins’ fermentation conditions, including fermentation temperature and suspended solids content, on wine sensory profile. FD-derived Cabernet Sauvignon juice was fermented ‘off-skins’ with or without suspended grape solids at three

different temperatures, i.e. 16, 24 and 32°C. Low fermentation temperature and low suspended solids content increased the concentration of most esters, while high fermentation temperature and high suspended solids content increased the concentration of fusel alcohols, polysaccharides and glycerol. High temperatures also increased linalool concentrations, which were unaffected by solids content. Classic maceration ferment (i.e. the control) gave the highest concentrations of fusel alcohols and 1-hexanol. Descriptive analysis results showed that removing grape solids from FD-derived Cabernet Sauvignon juice prior to fermentation led to wines with increased red fruit (raspberry and strawberry) and confectionery (candied fruit) attributes, while fermenting on 3.5% grape solids increased dark fruit (blueberry and plum jam) notes. Traditional maceration fermentations had significantly higher green and savoury notes compared to all FD treatments.

The impact on wine composition and style of fermenting FD treated Merlot must with different levels of skin contact was also investigated. Skin contact ranged from fermentations of juice with no solids contact or zero contact time, to 'on-skins' fermentations for which draining and pressing occurred at 17 °Brix, 7 °Brix and 0 °Brix, representing 1, 2 and 5 days of skin contact respectively. On-skin fermentations of FD treated must produced wines with significantly higher intensity ratings for dark fruit, body and astringency, compared to fermentations without skin contact (i.e. 'off-skin' fermentations). FD wines received significantly lower green, savoury and dusty ratings compared to control wine from unheated 'on-skins' ferment.

One of the key challenges with 'off-skins' red wine production is colour loss during fermentation. For 'on-skins' fermentations, colour loss is mitigated by continuous anthocyanin extraction from skins. Pre-fermentation addition of oenological tannins or toasted oak chips were investigated as methods for stabilising the colour of red wines made from FD-derived juice. Off-skins fermentations, with or without the addition of 0.4 g/L of oenological tannin, produced wines with

significantly higher red fruit and confectionery ratings, while fermentations with 4 g/L of medium toasted oak chips gave wines which exhibited increased dark fruit, vanilla, toasty and allspice characters. However, pre-fermentation addition of oenological tannin or toasted oak chips did not improve wine colour stability.

This research demonstrated that FD can be used to produce higher quality Rubired concentrate with a lower ratio of brown colour and 5-HMF formation compared to CMH. However, FD had the downside of producing concentrate with poorer filterability and less stable red colour, which could potentially increase production cost and present challenges in some food applications where high colour stability is a key requirement. FD followed by 'off-skins' or 'on-skins' fermentations under different fermentation temperature, suspended solids concentration, and skin contact time (accompanied by different alcohol strengths) conditions was demonstrated to create differentiated wine styles that can potentially be used as stand-alone wines or blending legs for wine style attainment. Other findings from this research offer insights into the capacity of chemical treatments (pH adjustment, seed tannin and acetaldehyde) and the use of winemaking additives (oak wood and seed tannin) to stabilise the colour of red grape concentrate and FD derived 'off-skins' wines respectively. The knowledge gained from this research will help guide winemakers' use of FD in red winemaking, including 'off-skins' fermentations which offer greater logistical flexibility, to deliver differentiated wine styles.

DECLARATION

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CHAPTER 1

The bulk of this literature review was prepared in the first 6 months of candidature, i.e. from July 2016 to January 2017. It therefore mainly covers literature up until early 2017. The relevant literature beyond this review is included in the introduction sections of papers presented in Chapters 2 to 5.

Chapter 1.

Literature Review and Research Aims

1.1 Introductory background

Flash Détente (FD) was developed and commercialised by the French National Institute of Agricultural Research (INRA) in 1993. FD involves red must being heated to a temperature of ~88 °C and pumped into a vacuum chamber to explode grape skin cell membranes, rendering phenolics, aroma compounds, and other cellular components more easily extractable. Cellular damage due to heating results in very rapid extraction compared to the extraction that occurs during the traditional red winemaking process. The pressure drop from vacuum application causes some water to evaporate and therefore, must temperature to instantaneously drop to ~30 °C due to the latent heat of vaporisation. FD-derived must can then either be fermented 'on skins', or pressed and fermented 'off skins', in a similar approach used for white winemaking.

In traditional red winemaking, fermentation and extraction occur simultaneously. However, FD enables separation of extraction from fermentation. The traditional process employed in red fermentations requires complex and expensive fermenters that are capable of discharging and conveying compacted skins and seeds (i.e., the 'cap') to presses at the end of fermentation. These fermenters typically also need to be capable of irrigating or 'punching down' the cap (several times per day), to aid extraction. This complexity makes red fermenters more expensive to install and operate compared to the liquid phase fermenters traditionally used for white wine production.

The application of FD to red wine making, without the need for skin contact during fermentation, could yield significant financial benefits, by circumventing the need for costly red fermenters. However, there are technical challenges, chief among them anthocyanin degradation post FD, that needs to be overcome before adoption of red fermentations without skin contact/extraction can

occur. Another potential application of FD that could yield significant financial benefits is in the production of red grape concentrate from FD-derived juice.

1.2 Classic Maceration

Red wines are traditionally made by classic maceration or fermentation ‘on skins’, a process whereby extraction of phenolic compounds (proanthocyanidins, flavan-3-ol monomers, anthocyanins, flavonols, hydroxycinnamic acids and other minor phenols) (Figure 1) and aroma compounds (free and bound monoterpenoids, C_{13}-norisoprenoids, C_6 compounds, volatile sulphur compounds, alcohols esters, and other carbonyls) takes place over several days, at relatively low temperatures ranging from ~24–32 °C. Factors that drive the extraction of phenolic and aroma compounds during classic maceration are well documented [1-3]. These factors, which include fermentation temperature, maceration time, the use of pectolytic enzymes, cap management, must or grape freezing, saignée and yeast selection, are important for determining wine quality.

Classic maceration takes place ‘on skins’ and therefore does not readily present options for experimenting with different levels of grape solids to influence wine style. As a result, most of the research undertaken to investigate ways to influence the sensory profile of wine from liquid-only fermentations has involved white winemaking. Only a few studies have investigated the fermentation of heat-extracted red juice [1, 4, 5]. Considering heating is employed to aid extraction during classic maceration, there is not much room to significantly lower fermentation temperatures as a means of impacting wine style.

Classical red fermentations are heterogeneous, with respect to having both solid (i.e., the ‘cap’) and liquid phases (juice), and temperature gradients [6, 7]. This makes their automation for real-time analytical measurements and temperature control challenging.

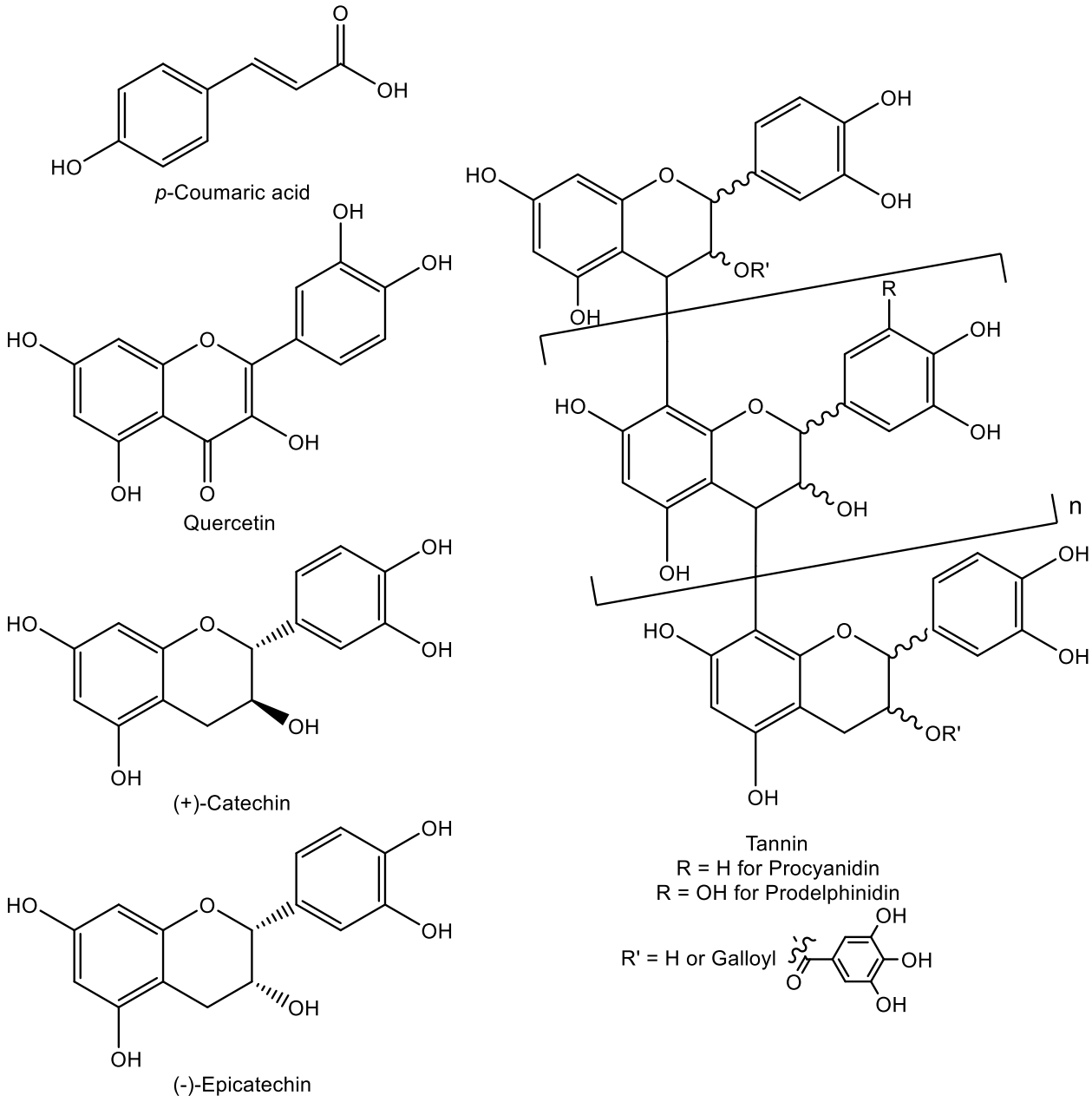

Figure 1. Chemical structures of p-coumaric acid (a hydroxycinnamic acid), quercetin (a flavonol) and a tannin molecule (where n represents the number of repeating units) comprising flavan-3-ol monomers, including (+)-catechin and (-)-epicatechin, in different configurations.

In addition, classic maceration is not an efficient extraction process, but rather a slow process involving extraction over several days. At the end of fermentation, less than 40% of grape tannins

will have been extracted, whereas FD has been demonstrated to boost extraction to 60% [8]. The quest to achieve faster and greater extraction has led to the development of heat-based extraction methods, such as microwave, thermo-vinification and Flash Détente. Other technologies such as Pulsed Electrical Field and ultra-sonication have also been investigated as a means for improving the efficiency and/or extent of extraction [9, 10]. This research will focus on developing applications for Flash Détente extraction.

1.3 Thermovinification

Thermovinification was a predecessor to FD technology. It is a winemaking technique that employs heat to damage cells and vacuole membranes to quickly release phenolic and aroma compounds [2]. Destemmed must or skins are heated to 60–70 °C and held at that temperature for a period of time ranging from ~0.5–2 hours. The hot must or skins will then be either cooled and fermented together, or pressed, cooled and fermented in liquid phase.

With thermovinification, the concentration of anthocyanins and other phenolic compounds extracted into wine is strongly influenced by fermentation ‘on’ or ‘off’ skins [11-13]. Inconsistent phenolic and colour extraction results have been reported for comparisons between thermovinification followed by liquid phase fermentation vs. classic maceration [11, 12]. Higher phenolic extraction was reported for wines made using classic maceration, compared to thermovinification followed by liquid phase fermentation, for some grape varieties. For example, [11] reported higher phenolic extraction in Cabernet Sauvignon and Noble wines that were made from classic maceration compared to wines made from heated must that was subsequently pressed and fermented off skins.

However, other varieties have showed opposing trends. Higher phenolic extraction was observed

in Chambourcin wines made using thermovinification followed by liquid phase fermentation, compared to wines made from classic maceration. Results for 'on skin' Chambourcin fermentations showed a decreasing trend in total phenol extraction as skin contact time increased, suggesting that analytical errors may be the reason for the two conflicting trends. In addition to higher phenolic extraction [12], higher anthocyanin extraction was observed in liquid phase fermentations compared to classic maceration. However, the heated must was cooled over a 24 hour period before inoculation and extended skin contact at elevated temperature might explain the higher concentrations of colour and phenolic compounds detected in the liquid phase fermentations.

Colour loss during and after fermentation is a major challenge associated with liquid phase fermentations [8, 13]. These researchers [13] noted that the anthocyanin concentration of heated juice was at a maximum concentration, being three times that of classic maceration, at the start of fermentation. The anthocyanin concentration of heated juice then decreased during fermentation at a higher rate compared to that of classic maceration. Classic maceration fermentations achieved peak anthocyanin concentration at day 3 or 4, followed by a slow decrease, in comparison to thermovinification. Thermovinified wines showed a similar increase in polymeric pigment concentration over time compared to classic maceration.

Some researchers [5] compared the aroma composition of wines made by heating red must prior to fermentation (on or off skins) with that of wines made via classic maceration. Their results appear to suggest loss of grape-derived aroma compounds in heated musts, and an increase in ethyl esters in heated musts that were subsequently subjected to liquid phase fermentation. However, these trends were not consistent across different varieties and vintages, so further investigation is needed to fully understand the chemistry involved.

Previous research [14] investigated the effect of fermentation temperature on the development of yeast derived aroma, using model wines. These researchers [14] reported higher concentrations of fresh fruity aroma compounds when fermentations were conducted at 15 °C, and higher concentrations of compounds associated with floral notes for fermentations at 28 °C. Others [1] compared fermentation of heat extracted Pinot Noir juice at 15 °C with classic maceration at 20 and 30 °C. They reported more intense fruity aromas and a total ester concentration four times higher in heat treated juice compared to the classic fermentations. However, this study did not separate the effect of thermovinification from that of fermentation temperature. It is therefore not clear if heat treatment, fermentation temperature, or both explain the higher concentrations of fruity compounds that were reported, and the effects of temperature are yet to be determined by fermenting juice at different temperatures, post-flash treatment.

Fine grape solids derived from berry skin and pulp breakdown during grape destemming, must movement and other processing operations that generate shearing forces, have been reported to contain important nutrients, such as lipids, that are metabolised by yeast during fermentation. Lipids, especially fatty acids and sterols, are important nutrients for fermenting yeasts. Since liquid phase red fermentations are relatively new, the effects of solids on red wine fermentation kinetics, wine style and quality have not been studied [15]. Most of the studies to date have involved white wine ferments. As such, the proposed research will investigate the effect of solids concentration on post-flash juice fermentation kinetics, wine aroma, phenolic composition and sensory profiles. In addition, interactions between solids content and fermentation temperature will be studied.

1.4 Flash Détente

Flash détente (FD) (Figure 2) is considered an improvement on the traditional thermovinification process. Extraction is enhanced by a combination of the higher temperature and flash evaporation, which causes greater cellular damage compared to thermovinification. Unlike in traditional red winemaking, where fermentation and extraction occur simultaneously, FD enables separation of extraction from fermentation. This means FD-derived must can be pressed, cooled and fermented in liquid phase.

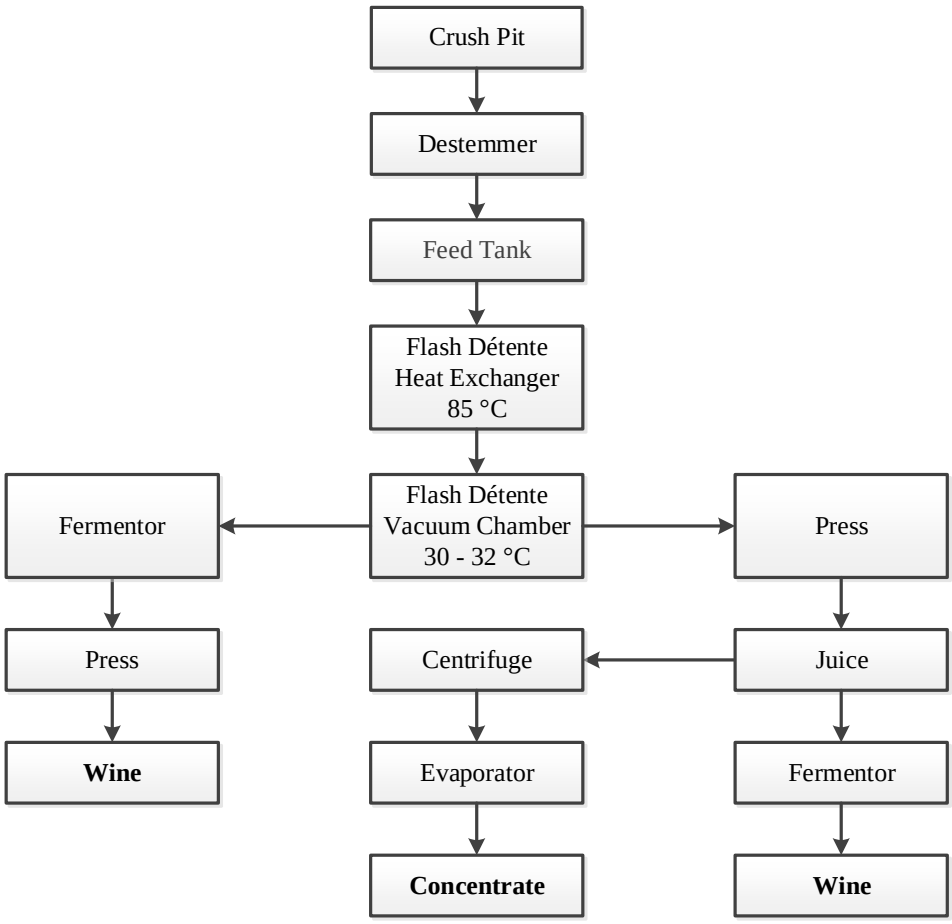

Figure 2. Flash Détente process for wine and concentrate production.

Because FD is a relatively new process, indeed there are only ~100 units in operation worldwide, very little research has been undertaken to investigate the technology [8, 16, 17]. The potential for FD to influence wine style has not been fully explored. Earlier research [16] demonstrated that there was potential for FD to be used to influence red wine mouthfeel properties through polysaccharide extraction. These researchers showed that flash extraction followed immediately by pressing of skins prior to fermentation, produced wines with lower concentrations of polysaccharides compared to classic maceration, whereas FD followed by 'on skin' fermentation resulted in wines with higher concentrations of polysaccharides.

Using FD for production of red wines without skin contact during fermentation offers significant financial benefits by overcoming the need for costly red fermenters. However, there are technical challenges, chief among them being anthocyanin degradation post-flashing [8]; challenges which need to be addressed before full adoption of liquid phase red fermentations can occur. A ground breaking study [8] showed that wines from FD followed by liquid phase fermentation had much lower concentrations of all major phenolic compounds compared to both classic fermentations and FD 'on skin' fermentations. The only exception was for sulphite bleaching-resistant pigments, whose concentration was higher in liquid phase fermentations. The anthocyanin concentration of wine from liquid phase fermentations was only ~67% that of wines from 'on skin' fermentations. For 'on skin' fermentations, extending the 95 °C temperature hold time from 6 to 15 minutes gave wines with ~25% less anthocyanins, even though more colour was initially extracted into juice. Holding must at higher temperatures for longer periods of time resulted in a higher rate of anthocyanin degradation during fermentation, irrespective of skin contact.

These researchers [8] demonstrated that there is a very high degree of anthocyanin degradation, or conversion into derived pigments, during both classic maceration and FD fermentations. For

classic fermentations ~25% of anthocyanins in grapes were recovered in wine and pomace, whereas for FD, ~40% was recovered. Recovery after a 15 minute temperature hold time was lower than for a 6 minute hold time, but still higher than for classic maceration.

Their findings [8] revealed that heat exposure was not the only variable driving anthocyanin reactivity. Their results showed that increasing heat exposure favoured conversion of anthocyanins to yellow/brown pigments but did not change the amount of sulphite bleaching-resistant pigments. Formation of anthocyanin tannin adducts, which are resistant to bleaching, appears to be in competition with the formation of bleaching resistant pigments such as pyranoanthocyanins. They argued that the tannin to anthocyanin ratio played a role in determining what reaction products were formed. Liquid phase fermentations which had a lower tannin to anthocyanin ratio favoured reactions that did not involve tannins. They postulated that products from such reactions are more orange/brown and sulphite resistant than anthocyanin tannin adducts. The proposed research will therefore investigate the addition of skin and seed extracted tannins to liquid phase fermentations, to increase the tannin to anthocyanin ratio. This may stabilise colour by converting anthocyanins to anthocyanin-tannin adducts.

1.5 Grape Juice and Concentrate for Food Colouring and Red Wine Production

Grape derived red concentrate for use as a food and beverage coloring is traditionally made from Rubired, a teinturier grape with color in the skins and pulp, which is a cross between Alicante Ganzin and Tinta Cao, using a conventional must heating extraction process. The high color content of Rubired makes it an attractive feed source for the production of red food colouring for the food and beverages industries. Rubired colour is comprised of anthocyanin mono and diglucosides with the later being the predominant type. The anthocyanins that have been identified

in Rubired include malvidin 3,5-diglucoside, peonidin 3,5-diglucoside, malvidin 3-glucoside, peonidin 3-glucoside, delphinidin 3-glucoside, petunidin 3-glucoside, petunidin 3,5-diglucoside, malvidin 3,5-diglucoside acylated with p-coumaric acid, malvidin 3-glucoside acylated with p-coumaric acid, peonidin 3-glucoside acylated with p-coumaric acid, and delphinidin 3,5-glucoside.

High temperature (~60 °C) extraction is traditionally used for production of red grape juice and concentrate for direct to consumer sale, or for use as colouring and sweetening ingredients in processed foods and beverages. The absence of off-flavors and presence of stable red colour with no apparent browning, are key quality expectations in either application. Producers of red juice and concentrate are therefore constantly looking for processing methods that stabilise red colour and/or minimise browning and off odours.

High juice extraction temperature has been reported to increase the extraction of anthocyanins and phenolic compounds [18]. Others [4] demonstrated that must treatments involving heating at higher temperatures but for shorter periods of time yielded juices with greater colour stability than treatments involving heating at lower temperatures for longer periods of time.

Extraction temperature determines whether or not polyphenol oxidase (PPO) is inactivated during juice extraction. PPO accelerates the rate of brown colour formation during the juice extraction process. Any PPO activity remaining in juice is degraded during juice pasteurisation or high temperature evaporation to produce concentrate. Post-pasteurisation or high temperature evaporation juice browning is therefore the result of non-enzymatic browning from oxidation of phenols and the Maillard reaction [19].

Previous research [18] has found that juice extracted at higher temperatures (i.e. 85 and 99 °C) was of superior quality, and had higher total anthocyanin concentrations and less browning than

juice extracted at 60 °C. However, it was observed that juice extracted at higher temperatures experienced a more rapid rate of browning during the first month of storage compared to juice extracted at lower temperatures. These results highlight the need for other processing technologies to be explored, for example FD, which incorporate a rapid cooling step after heat extraction, that may decrease the extent of browning (whether due to oxidation, thermal degradation of anthocyanins, or Maillard reactions).

1.6 Red Wine and Concentrate Colour Stability

Red wine colour arises from a complex mixture of pigments and is dependent on the concentrations of free anthocyanins, pyranoanthocyanins and polymeric anthocyanins (Figure 3), as well as on co-pigmentation [20]. Anthocyanin degradation during storage has been well documented [21]. This research [21] reported increased anthocyanin losses when either storage temperature or the duration of storage were increased.

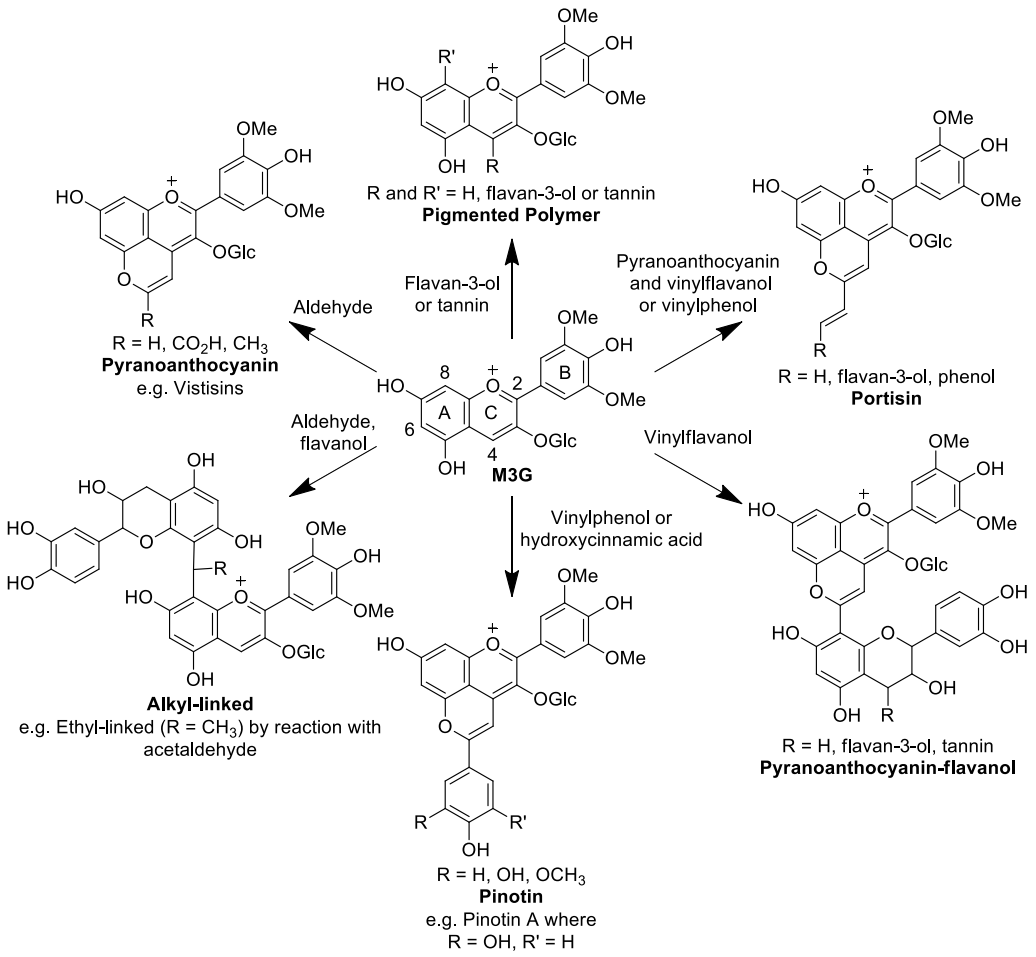

Figure 3. Red wine pigments derived from the grape anthocyanin malvidin-3-glucoside (M3G)[22]. Reproduced with permission from Oxford Publishing Limited.

As wine ages, anthocyanins react with phenolic compounds, and other wine constituents, to form derived pigments such as pyranoanthocyanins and polymeric anthocyanins. Pyranoanthocyanins are formed from reactions between anthocyanins and carbonyl compounds present in red wines, such as acetaldehyde and pyruvic acid [23]. They absorb light at 490–510 nm to give a red–orange colour [24]. Polymeric anthocyanins are formed via direct or indirect addition reactions between anthocyanins and tannins, yielding tannin-anthocyanin adducts. Acetaldehyde and glyoxylic acid are the key cross-linking agents for indirect addition reactions in wine [25].

Colour stability in red wine and processed red juice or concentrate is influenced by several factors including pH, the presence of antioxidants, flavanols or transition metal ions, and storage temperature. Of these factors, only antioxidant addition and cold temperature storage are regularly used to control colour degradation during processing and ageing. The most common antioxidant used in juice and wine processing is sulphur dioxide (SO_2), although ascorbic acid is occasionally used. Some recent studies have shown that ascorbic acid may act as a pro-oxidant under certain juice or wine conditions [26]. This is particularly true where ascorbic acid is not used in combination with SO_2. Ascorbic acid reacts with oxygen to form hydrogen peroxide, leading to more oxidation and browning if there is no free SO_2 to quench the hydrogen peroxide. In grape juice concentrate, SO_2 is not an effective antioxidant because it binds with sugars, which are present at a very high concentration. As such, there is a need to explore other ways of stabilising colour. For grape juice concentrate used for food and beverage colouring applications, both anthocyanin loss and brown colour formation during processing and ageing are major concerns.

The mechanism of colour loss and browning in juice and wine is not well understood [26]. Anthocyanin loss during winemaking can be due to degradation caused by ring opening of the heterocyclic ring to form colourless by-products, such as syringic acid [8]. Another explanation might be reaction of anthocyanins with flavanols and other molecules to form derived pigments. The transition metals iron and copper may play a central role in initiating autoxidation of phenolic compounds [26, 27], which leads to browning and precipitation of pigments.

Very few studies have investigated shelf life stability of grape juice concentrate [18]. These researchers found that juice extracted at a higher temperature had greater browning and loss of anthocyanins during ageing. As expected, colour loss in juice stored at a higher temperature (35 °C) was much more rapid compared to that of juice stored at a lower temperature (24 °C).

To date no comprehensive study has been undertaken to investigate ways of stabilising colour in FD-derived red wine or grape concentrate. Knowledge gained from this study is therefore expected to enhance the wine industry's ability to make FD-derived colour stable red wines from concentrate, to deliver significant economic benefit to wineries.

1.7 Research Question and Aims

In summary, the proposed research will investigate the effect of FD on the chemical composition and sensory properties of red wines made from must or juice. The project aims to investigate the impact of fermentation temperature, skin and seed tannin supplementation, and grape solids concentration during 'off skins' fermentation of FD-derived red juice on the phenolic and aroma composition, and sensory profiles, of resulting wines. Additional experiments will be conducted to investigate the impact of flashed skin contact time on phenolics and aroma extraction, and wine sensory profiles. It is hypothesised that flashed must or juice fermentation conditions can be manipulated to influence wine sensory profiles. There is currently no research available in the literature reporting the impact of fermentation conditions on the sensory profiles of red wine made from FD-derived must, fermented either 'on' or 'off' skins. Understanding the fermentation conditions that drive phenolic composition and aroma development in FD-derived red juice or must is expected to enable the creation of wines with distinctive sensory profiles, which can be used as blending components to help with attainment of targeted wine styles.

The potential for flash détente to produce red grape concentrate of enhanced colour concentration, quality, and stability, compared with traditional extraction processes, has not previously been investigated. This project aims to increase red colour extraction, quality and stability during production and storage of Rubired concentrate. In particular, the research will evaluate the use of

flash détente as a novel approach to the preparation of red juice concentrate, as well as effective ways for stabilising concentrate, through addition of grape seed tannin and/or food grade chemical treatments.

1.8 Objectives of the Project

Project aims will be addressed through four studies with the following objectives:

1. To investigate if FD can be used to produce a red grape concentrate with increased red colour extraction, higher colour quality, higher colour stability, and improved filterability without diminishing sensory quality compared to traditional thermovinification extraction.

2. To compare and contrast red colour stability and degradation kinetics of flash détente derived Rubired concentrate with that of traditional thermovinification derived concentrate, as well as investigate the effect of low pH, acetaldehyde, and seed tannin treatments on red colour stability and brown and violet colour formation, under accelerated ageing conditions.

3. To determine the impact of fermentation temperature and grape suspended solids content, as well as their potential interactions on the composition of phenolic compounds, aroma volatiles, polysaccharides, colour and the sensory profiles of red wine made from flash détente treated must fermented off-skins.

4. To investigate the impact of time on skins during fermentation on the chemical composition and sensory profiles of flash détente derived red wine. This study also aimed to investigate whether or not pre-fermentation addition of oenological tannin or toasted oak chips can be used for long term colour stabilisation of bottled red wines made via off-skin fermentation of flash détente derived juice.

Objective 1:

The amount, quality and stability of colour extracted into red grape juice concentrate can be impacted by extraction conditions including temperature and duration of skin contact time, among other factors. This study compared the effect of thermovinification and flash détente extraction methods on Rubired concentrate colour, quality and stability, as well as filterability. The two extraction methods had different extraction temperatures, skin contact times, as well as different levels of skin disintegration. Rubired grapes were harvested at commercial maturity and treated using commercial scale thermovinification and flash détente processing equipment. The resulting juices and concentrates were subjected to various compositional and spectrophotometric colour analyses, as well as filterability assessment. Compositional analyses included determinations of phenolics, protein, suspended solids, and aroma compounds. The two concentrates were also subjected to colour stability testing under normal cold storage conditions for a period of 12 months, with samples drawn for chemical and colour analysis at 3 monthly intervals. This study is reported in Chapter 2.

Objective 2:

Colour stability is an important quality attribute for red grape concentrate destined for use as a food colourant. Experiments were conducted to investigate the kinetics of red colour and anthocyanin degradation, as well as brown colour formation during accelerated ageing at different temperatures for flash détente and traditionally extracted Rubired concentrate. The rate of colour degradation for the two concentrates was assessed at 50, 60 and 70 °C over 9 days with samples taken for analysis at 3 day intervals, starting at time zero. Colour concentration was plotted against storage time to determine the rate constant K and half-life values. The temperature dependency of

colour degradation was estimated using the Arrhenius equation. The study also investigated treatments for stabilising Rubired colour, involving lowering concentrate pH and the addition of acetaldehyde or seed tannin, under accelerated ageing. This study is reported in Chapter 3.

Objective 3:

This study investigated the impact of fermentation temperature and suspended grape solids content on the colour, phenolics, aroma composition and sensory profiles of flash détente treated Cabernet Sauvignon wines, fermented off-skins. Flash détente derived juice was fermented with or without grape solids at three different temperatures, being 16, 24 and 32 °C, while classic maceration control fermentations were conducted at 30 °C. All fermentations were carried out in 40 L pilot scale fermenters, in duplicate. Finished wines were chemically analysed to determine colour, phenolic composition and wine aroma profiles; descriptive analysis was also performed to evaluate wine sensory profiles. This study is reported in Chapter 4.

Objective 4:

This study investigated the impact of fermenting flash détente treated Merlot must with different levels of bulk and suspended grape solids contact on wine style. Fermentations were carried out using 40 L fermenters with skin contact times ranging from no skin contact juice fermentations to draining and pressing on-skins fermentations at 17, 7 and 0 °Brix. The study explored pre-fermentation addition of oenological tannin and toasted oak chips to stabilise the colour of red wines made from flash détente derived juice. Finished wines were chemically analysed to determine colour, phenolic composition and wine aroma profiles; descriptive analysis was also performed to evaluate wine sensory profiles. Wine colour was measured immediately after

fermentation and again after 12 months of bottle ageing, to assess colour stability. This study is reported in Chapter 5.

1.9 Significance/Contribution to the Discipline

Flash détente is a relatively new technology that to date has only been adopted by a small number of wineries around the world. As such, the potential opportunities and challenges that can arise from the application of flash détente to juice and concentrate production, and winemaking, have not been fully explored.

FD technology presents a potential opportunity to produce red wines from red juice concentrate. Making red wine from fresh juice or reconstituted juice from concentrate has the prospect of offering significant economic benefit to wineries. In addition to potentially creating differentiated wines that stand out in the market, making red wine from juice would allow red wine to be produced using relatively inexpensive white fermenters, thereby reducing capital expenditure on more complex red fermenters. Making red wine from juice reconstituted from concentrate would also make it possible to decouple grape harvesting from fermentation. Grapes could potentially be flash treated, pressed, and juice concentrated and shipped anywhere in the world to be fermented close to where the wines will be sold. This would significantly reduce shipping costs and importation taxes as concentrate often incurs lower duties compared to finished wine.

There are however technical challenges associated with the use of FD, such as colour loss during concentrate and wine production, storage or ageing, that would need to be overcome to make this approach feasible. This research therefore explores different approaches to stabilising red concentrate and wine colour as well as to provide guidance on how fermentation conditions can be leveraged to attain differentiated wine styles from flash détente derived must or juice.

Chapter 2.

Color Extraction and Stability of Rubired Juice Concentrate Produced via Conventional Must Heating or Flash Détente Processing

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Contribution to the Paper	Designed experiments, planned and executed production and laboratory scale trials, performed statistical analyses on data sets, interpreted the data, and drafted manuscript.		
Overall percentage (%)	85%		
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.		
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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.

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Color Extraction and Stability of Rubired Juice Concentrate Produced via Conventional Must Heating or Flash Détente Processing

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ABSTRACT: This study compared the effects of conventional must heating (CMH) and flash détente (FD) extraction methods on color extraction, quality, stability, and juice filterability during the production of Rubired grape concentrate. FD concentrate had similar red color intensity and concentrations of C_6 volatiles, a lower brown color index, and higher concentrations of caftaric acid and catechin in comparison to CMH concentrate. The longer duration of heating employed with CMH led to enhanced formation of brown color (+10%) and pigmented polymers (+23%), as well as extraction of gallic acid (+67%) and proanthocyanidins (+50%). FD processing generated significantly higher concentrations of grape suspended solids compared with CMH, but following centrifugation CMH and FD juices had similar suspended solids. FD juice nevertheless had a 4-fold lower flux during filtration, which likely reflected an increased extraction of polysaccharides and/or other wine macromolecules as a result of the extraction method. CMH concentrate had greater long-term red color stability, and after 12 months of cold storage (2 °C) significantly higher red color was observed for CMH concentrate, compared to FD concentrate.

KEYWORDS: *flash vacuum expansion, anthocyanins, evaporation, filterability, phenolics, thermovinification, colorant*

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INTRODUCTION

Color is intrinsically linked to the appearance and perceived quality of foods and beverages, and a range of natural and synthetic coloring options exist.[1] Color extracts derived from fruits, including grapes, can be used as natural food and beverage colorants[1] and could replace synthetic food dyes, which have no nutritional value and may have adverse effects on human health,[2] for example, hyperactivity in children.[3] In contrast, the anthocyanin pigments in red grape varieties such as Rubired[4] have been reported to possess various beneficial properties.[5] Widespread adoption of natural colorants in food and beverage applications is limited, however, by higher production costs, less vivid colors, and/or lower color stability.[1] Development of benign production methods, for example, physical extraction, that increase color yield, quality, and stability of concentrates is therefore key to increasing the use and application of fruit-derived colorants in more foods and beverages.

Rubired, a teinturier grape cultivar with dark skin and red pulp, is a cross between Alicante Ganzin and Tinta Cao developed at the University of California, Davis, primarily for red concentrate production due to its depth of color.[6] For red colorants, the expression of red color is associated with high quality, whereas brown tones arising from enzymatic browning, chemical oxidation and/or Maillard reactions,[7] indicate low quality.[8] Enhancing red color stability during processing and storage and preventing browning from occurring are therefore key control points for increasing the quality and attractiveness of red concentrate as a food and beverage colorant. Following extraction, the stability of red color in processed juice or concentrate is influenced by factors including pH, the presence of antioxidants, flavan-3-ols or transition metal ions, and storage temperature.[9] Optimizing the extraction of Rubired grapes and stabilizing the color of the resulting concentrate would increase color yield and therefore economic value, leading to greater uptake of red concentrate as a natural food and beverage colorant.

Rubired grapes are typically extracted using conventional must heating (CMH), an extraction process that employs heat to degrade grape cell and vacuole membranes to rapidly release phenolics, pigments, and aroma compounds.[10] CMH (or "hot pressing") involves heating grape must (juice and solids) to 55−70 °C and holding it at that temperature for ∼0.5−2 h.[11] The heated must will then be pressed, giving juice that undergoes clarification (by centrifugation and filtration) before evaporation, to yield a juice concentrate.

A number of factors including grape variety, maturity at harvest, extraction temperature, and duration of skin contact influence color extraction into juice, while the temperature applied during evaporation could potentially also influence the color of concentrate. With CMH extraction, must and juice are typically held at elevated temperature for several hours (throughout draining and pressing, clarification, and evaporation processing steps), thereby increasing the risk of color

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degradation. In contrast, flash détente (FD), also known as flash vacuum expansion or flash release, is a relatively new continuous extraction method that has been used for processing grapes and has been demonstrated to have potential to be used for processing other fruits such as avocados, persimmons, passionfruit, and other tropical fruit.[12] When applied for grape processing, the technology involves heating must to ~85 °C before pumping it into a vacuum chamber to rupture grape skin cells,[13] rendering phenolic and aroma compounds and other cellular components more readily extractable. Extraction of red juice at high temperature (i.e., at 85 and 99 °C) has been reported to facilitate the extraction of anthocyanins and other phenolic compounds and to diminish browning, compared with extraction at 60 °C.[14] The pressure change from vacuum application causes water to "flash off", such that the must temperature instantaneously drops to ~32 °C due to latent heat of vaporization. Greater cellular degradation and more rapid extraction would be expected from the combined heating and flash extraction process compared with CMH.[13] Postextraction processing (i.e., draining and pressing, clarification, and evaporation)[15] then takes place at ambient temperature, potentially enhancing color yield by limiting color degradation[7] and mitigating brown color and off-flavor formation.

In addition to improved color yield and stability, factors that might impact the financial viability and attractiveness of employing FD for concentrate production need to be considered. These include suspended solids generation, which impacts clarification cost, along with juice yield and filterability. To enable use in food industry applications without further clarification, most of the commercial red concentrate produced in the U.S.A. undergoes solids removal using technologies such as dissolved gas flotation, centrifugation, cross-flow filtration, or dead-end filtration with diatomaceous earth (or its substitutes).[11] Juices that filter poorly will decrease the available filtration capacity, thereby increasing processing costs and possibly necessitating investment in infrastructure.

Limited research has been undertaken to investigate applications of FD technology in either winemaking or the production of concentrate from grapes and other fruit.[16] To date, there appeared to be no published research comparing color extraction, juice filterability, and color stability during cold temperature storage of FD and CMH derived concentrates. Addressing these gaps, this study aimed to investigate the potential for FD to produce red juice concentrate with increased color extraction, quality and stability, and improved filterability without diminishing other sensory qualities relative to traditional concentrate production using CMH. The knowledge gained from this study will increase the understanding of the impact of FD on juice chemistry and help juice processors make informed decisions regarding the adoption of FD for red concentrate production.

MATERIALS AND METHODS

Chemicals. Analytical grade chemicals were purchased from Sigma-Aldrich (St Louis, MO). Concentrated hydrochloric acid (37% w/v) was sourced from EMD (Burlington, MA). Unlabeled standards were sourced from Sigma-Aldrich and Indofine Chemicals (Hillsborough, NJ) and deuterated internal standards were sourced from CDN Isotopes Inc. (Pointe-Claire, QC, Canada). HPLC grade solvents were sourced from BDH (Radnor, PA).

Grapes. Rubired (*Vitis* hybrid (no authority), Vitaceae) grapes were sourced from a vineyard located in the Central Valley region of California (36.57° N, 119.61° W). Fruit (360 t in 2016 and 180 t in 2017) was harvested by machine when total soluble solids (TSS) had reached 23−24 °Brix, (i.e., commercial maturity) with fruit destined for commercial scale CMH and FD treatments harvested from alternate rows to ensure homogeneity. Fruit was sampled at the winery for compositional analysis as follows: 4 × 10 kg samples were taken from each of four trucks (per treatment, that is, $n = 4$) using a zone sampler (Yuba City Steel, Yuba City, CA). Samples were pooled and a portion was lightly pressed with 500 mL of the resulting juice being analyzed by Fourier transform infrared spectroscopy (FTIR) using a WineScan FT120 interferometer (Foss Electric, Hillerød, Denmark) for determination of basic grape chemical parameters (Table S1 of the Supporting Information). The remaining sample was used to determine material other than grapes (MOG). In 2017, an additional parcel of Rubired grapes was harvested from a vineyard in the Central Valley region of California (36.30° N, 119.14° W). Fruit (16 t) was machine harvested on each of two consecutive days for pilot scale FD treatment, to determine the impact of the duration of high-temperature extraction on juice and concentrate color. Fruit was sampled at the winery and basic grape chemistry parameters were determined as specified above (without replication, Table S1).

Preparation of Rubired Juice Concentrate. Rubired juice concentrate was produced via commercial scale CMH and FD processes. Commercial scale trials were replicated over two growing seasons because tank space constraints prevented replication during one harvest.

For CMH extraction, grapes (180 t in 2016 and 90 t in 2017) were destemmed and crushed and 50 mg/L of sulfur dioxide (as an 8% solution of potassium metabisulfite) and pectinase (Rohavin MX, AB Enzymes, Darmstadt, Germany, 28 mL/metric ton) were added before the must was heated to 57 °C at 127 t/hour using a shell and tube steam heated heat exchanger (Wiegmann and Rose, Oakland, CA). Following CMH, the heated must was held in a crush tank for 2 h at 57 °C before being pressed with a Diemme screw press (Diemme Enologia, Lugo, Italy). Solids were removed from the resulting juice using a Westfalia decanter centrifuge (Westfalia, Northvale, NJ) running at 230 L/min prior to evaporation with a high-temperature (HT) APV plate and frame evaporator (SPX Flow, Crawley, U.K.) to give a 55−56 °Brix concentrate. A portion (~1300 L) of centrifuged juice was evaporated (also to 55−56 °Brix) using a Reda low-temperature (LT) high vacuum evaporator (Reda, Isola Vicentina, Italy) to evaluate the potential for low evaporation temperatures to be used to enhance color yield. The maximum juice temperature reached in the APV evaporator was 80 °C, compared with 25 °C for the Reda evaporator.

For FD extraction, grapes (180 t in 2016 and 90 t in 2017) were destemmed and crushed, and the resulting must was heated to 85 °C (for 5−10 min) using a Della Toffola flash détente unit (Della Tofolla, Trevignano, Italy) operating at 27 t/h. The vacuum pressure in the flash chamber was maintained at −0.94 bar. Following FD, 50 mg/L of sulfur dioxide and pectinase (28 mL/metric ton) were added before pressing the must with the Diemme screw press; additions were made after extraction in this case to avoid losses due to vacuum flashing and inactivation, respectively, during FD processing. The resulting juice was chilled to 4 °C (to prevent fermentation), and solids were removed using the Westfalia decanter centrifuge, prior to evaporation with APV evaporator, to give 55−56 °Brix concentrate (as for CMH). A portion (~1300 L) of juice was also evaporated (to 55−56 °Brix) using the Reda LT evaporator.

In 2017, Rubired juice concentrate was also prepared using a pilot scale FD unit (TMCI Padovan, Vittorio, Italy), to investigate the influence of different durations of heat treatment prior to vacuum flashing, on color extraction. Grapes (2 × 16 t parcels, harvested and processed on consecutive days) were destemmed and crushed, and the resulting must divided into three lots and heated to 85 °C for 15, 30, or 45 min prior to flash treatment at −0.94 bar. Following FD, samples were treated with sulfur dioxide and pectinase (as above), pressed, centrifuged, and concentrated using the Reda LT evaporator to generate a 55−56 °Brix concentrate.

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Table 1. Composition of Juice and Concentrate Derived from CMH and FD Treatments[a]

parameter	juice		concentrate		*P*-value
	CMH	FD	CMH	FD	
Color					
red color units	2490 a	2640 a	2091 b	2213 b	*0.0001*
brown color units	992 a	914 b	920 b	832 c	*0.0001*
violet color units	632 a	610 ab	618 ab	564 b	*0.011*
brown index	0.40 b	0.35 d	0.44 a	0.38 c	*0.0001*
violet index	0.25 b	0.23 c	0.30 a	0.26 b	*0.0001*
Phenolic Compounds					
caftaric acid (mg/L)	36 c	96 a	22 d	80 b	*0.0001*
malvidin-3,5-*O*-diglucoside (mg/L)	5786 a	5436 b	4740 c	4659 c	*0.0001*
malvidin-3-*O*-glucoside (mg/L)	357 b	521 a	274 c	410 b	*0.0001*
pigmented polymers (mg/L)	156 a	110 b	114 b	78 c	*0.0001*
proanthocyanidin (mg/L)	1383 a	855 c	1194 b	799 c	*0.0001*
gallic acid (mg/L)	29 a	21 c	25 b	15 d	*0.0001*
catechin (mg/L)	101 d	199 a	107 c	181 b	*0.0001*
quercetin glycosides (mg/L)	214 ab	222 a	194 c	205 bc	*0.003*
grape reaction product (mg/L)	59 a	56 ab	46 b	46 b	*0.013*
total phenolics (g/L)	17	18	17	17	*0.050*
Basic Chemistry					
pH	3.89	3.90	3.95	4.03	*0.050*
potassium (mg/L)	9052 a	8890 a	6629 b	6127 b	*0.002*

[a]Values are means of two vintage replicates (i.e., 2016 and 2017, $n = 2$). Means followed by different letters (within rows) are statistically different ($\alpha = 0.05$, Tukey pairwise comparisons). Data were normalized to 68° Brix, except pH and color indices, which were measured at juice or concentrate TSS.

Juice samples (375 mL, in triplicate) were collected from centrifuged juice tanks throughout processing (from both commercial and pilot scale treatments) for compositional analysis, together with samples from the final concentrate product tanks (375 mL, in triplicate).

Color Stability of Rubired Juice Concentrate. CMH and FD concentrates (from 2016, each at ∼55 °Brix) were stored (in triplicate) at 2 °C and color monitored over a one year period with samples collected for compositional analysis at ∼2 monthly intervals. Concentrates were kept in glycol jacketed 190 000 L tanks during the first 6 months. Thereafter, concentrates were transferred into 200 L drums and stored in a cold room at 2 °C.

Compositional Analysis of Rubired Juice and Juice Concentrate. Juice and concentrate samples were centrifuged (4000×*g* for 15 min) using a temperature-controlled centrifuge (Beckman Coulter, Brea, CA) prior to analysis.

Color Analysis. Color measurements were performed as previously described,[15] using a USDA spectrophotometric method for red juice concentrate[17] that specifies measuring absorbance after adjusting pH to 3.2 with McIlvaine buffer. An HP 8453 UV−vis spectrophotometer (Agilent Technologies, Palo Alto, CA) with sipper and flow through cuvette, and using Chemstation v. B.02.01 control software. Centrifuged juice or concentrate samples were diluted with McIlvaine buffer (pH 3.2) and filtered through a 1 μm glass fiber filter (Pall, Port Washington, NY), as previously described.[21] Red, brown and violet color were then measured (in 1 cm cuvettes) via absorbance at 520, 430, and 580 nm, respectively. Color units (CU) normalized to 68 °Brix, brown index and violet index were calculated as previously reported.[15]

Phenolic Analysis. The total phenolics concentration of juices and concentrate samples (as mg/L of gallic acid equivalents) were measured using the Folin−Ciocalteu method.[18] Phenolic profiling of Rubired juice and concentrate samples was performed (in duplicate) by HPLC. Samples (25 mL) were diluted with 0.01 M hydrochloric acid to a total volume of 200 mL and analyzed by reversed-phase chromatography using an Agilent 1200 HPLC (Agilent Technologies, Santa Clara, CA) equipped with a quaternary pump, diode array detector (DAD) and PLRP-S Column (250 × 4.6 mm, 5 μm particle size, Varian Inc., Palo Alto, CA) fitted with a PLRP-S guard cartridge. A binary solvent gradient was used consisting of water with 0.5% (v/v) orthophosphoric acid (85% w/v, mobile phase A), and acetonitrile with 0.5% (v/v) orthophosphoric acid (85% w/v, mobile phase B). The gradient conditions were as follows: 5% mobile phase B at time 0; 0−10 min, 5−19% mobile phase B; 10−12.5 min, 33% mobile phase B; 12.5−13.5 min, 33−95% mobile phase B; and 13.5−14.5 min, 95% mobile phase B. The column thermostat was set at 50 °C and the injection volume was 20 μL.

Individual phenolic compounds and groups of related compounds were measured at the following wavelengths: proanthocyanidins at 230 nm; epicatechin, gallic acid, and catechin at 280 nm; grape reaction product (GRP), caftaric acid, and caffeic acid at 320 nm; quercetin glycosides (total of quercetin glucoside and quercetin glucuronide) at 360 nm; and malvidin-3-*O*-glucoside, malvidin-3,5-*O*-diglucoside, and pigmented polymers at 520 nm. Standard solutions of these compounds were used for identification and quantification. Because of unavailability of pure standards, pigmented polymers were reported as malvidin-3-*O*-glucoside equivalents and proanthocyanidins were reported as catechin equivalents.

Analysis of Total Organic Nitrogen (as Protein). Protein analysis was performed according to the Kjeldahl method[19] using Buchi K449 digestion and K375 distillation units (Buchi, Flawil, Switzerland). Total Kjeldahl nitrogen (TKN) was converted to percentage protein using a conversion factor of 6.25

$$\%\text{Protein} = \frac{\text{TKN (mg/L)} \times (\text{protein factor})}{10000}$$

Suspended Solids Measurement. Must handling and CMH and FD treatments generated suspended grape solids in juices. Samples of mixed free run and pressed juice (10 replicates per treatment) were collected from the centrifuge inlet and outlet at 20 min intervals during juice centrifugation (described above). Samples were immediately transferred into 15 mL Nalgene polycarbonate conical-bottom/open-top graduated centrifuge tubes before settling of solids. The suspended solids content of juice (% v/v) was measured by centrifuging juices (4000×*g* for 15 min) using a swinging bucket centrifuge (Beckman Coulter, Brea, CA).

Volatile Analysis. The concentration hexanal, *trans*-2-hexen-1-al, 1-hexanol, *trans*-3-hexen-1-ol, *cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol, 1-

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Table 2. Color and Volatile Composition of Juice and Concentrate Derived from CMH and FD Treatments with High (HT) and Low (LT) Temperature Evaporation used to Transform Juice into Concentrate[a]

			CMH			FD		
				concentrate			concentrate	
parameter	descriptor	thresholds (μg/L)	juice	HT	LT	juice	HT	LT
Color								
red			2490	2091	2242	2640	2213	2341
brown			992	920	871	914	832	844
violet			632	618	598	610	564	586
Volatiles (μg/L)								
hexanal	grassy, green	4.5[33]	**19.4**	**5**	**8.1**	**5.1**	**3**	**3.3**
1-hexanol	cut grass	8000[47]	2175.1	3.6	171.9	983.4	3	72.1
trans-2-hexen-1-al	green, fresh, fruity	17[33]	16.4	3.4	5.8	18.4	3.2	3.8
trans-3-hexen-1-ol	green	70	16.4	0.3	0.9	5.6	0.2	1.4
cis-3-hexen-1-ol	green	400[47]	39.2	2.3	6.4	19.9	4.8	13.1
trans-2-hexen-1-ol	green	400[48]	209.6	1.1	4.3	0.9	0.7	0.5
total C_6 compounds			2440	15	197	1012	11	94
1-octen-3-ol	mushroom	1.0[49]	**1.39**	**0.15**	**0.22**	**0.91**	**0.14**	**0.2**
geraniol	flowery	30[48]	0.97	0.26	1.06	2.47	0.18	0.57
linalool	lavender, citrus	25[50]	2.39	2.22	0.42	2.38	2.26	1.03
β-damascenone	stewed apples	0.05[47]	**2.74**	**2.07**	**5.37**	**1.71**	**1.8**	**1.17**
IBMP	bell pepper		nd	nd	nd	nd	nd	nd
Basic Chemistry								
TSS (°Brix)			22.5	25.0	25.1	24.0	24.8	25.1

[a]Bold denotes volatiles with an OAV > 1. Values are means of two vintage replicates (i.e., 2016 and 2017, $n = 2$). Analyses were performed at 22.5−25.1 °Brix., except color data, which were normalized to 68° Brix) nd = not detected.

octen-3-ol, 3-isobutyl-2-methoxypyrazine (IBMP), linalool, β-damascenone, and geraniol was determined. Juice samples were analyzed directly, whereas concentrate samples were diluted with water to 25 °Brix prior to analysis. Volatile compounds were extracted using solid-phase microextraction (SPME) with a DVB/CAR/PDMS fiber (1 cm, 50/30 μm, Supelco, Bellefonte, PA) and analyzed using an Agilent 7890B series gas chromatograph (GC) coupled to an Agilent 5973 mass selective detector and equipped with a Gerstel MPS2 multipurpose sampler, Gerstel CIS 4 inlet system. Separation was conducted with an Agilent J&W DB-WAX Ultra Inert capillary GC column and mass spectra were recorded at 70 eV in selected ion monitoring (SIM) mode. The oven temperature program was as follows: 35 °C for 1.5 min, increased by 6 °C/min to 42 °C, 11 °C/min to 75 °C, 6 °C/min to 135 °C, 11 °C/min to 195 °C, and then 120 °C/min to a final temperature of 240 °C and held at this temperature for 2 min. Helium was used as carrier gas at 1 mL/min. External calibration standards of the individual volatile compounds together with internal standards (d_{12}-hexanal, 2-heptanol, d_{13}-hexanol, 2-octanol, d_3-IBMP, d_3-linalool, and methyl decanoate) were used for identification and quantification using EnviroQuant software (Agilent Technologies, Palo Alto, CA).

Filterability of Rubired Juice Concentrate. Rubired concentrates (~400 mL) were centrifuged (4000×g for 15 min) to reduce suspended solids to <0.5% v/v before reconstitution with water to obtain ~800 mL of diluted juice with TSS of 25 °Brix. Filterability was then measured using a bench scale perpendicular flow filter (Millipore Corporation, Burlington, MA) with Celite Standard G diatomaceous earth filtering medium (Imerys, Paris, France). Flux measurements for each reconstituted juice were performed (in triplicate) at 10 °C using a juice pressure of 24−34 kPa. Time taken to filter successive 100 mL increments was recorded and used to calculate juice flux in L/hour.

Statistical Analysis. Data were analyzed by one-way analysis of variance (ANOVA) and repeated measures ANOVA with Tukey's posthoc test at $\alpha < 0.05$ using Minitab statistical software (State College, PA).

RESULTS AND DISCUSSION

A key aim of this study was to investigate whether FD can be used to increase red color extraction from Rubired grapes compared to that achieved using traditional CMH extraction. Analysis of the basic chemical parameters (TSS, pH, titratable acid, and potassium) confirmed that the chemical composition of Rubired grapes used in commercial and pilot scale experiments was comparable (Table S1 of the Supporting Information). A detailed study considering the molecular changes and assessing a range of quality parameters was undertaken as described in the following sections.

Influence of CMH vs FD Treatment on Juice and Concentrate Color. The extraction treatments did not significantly differ in terms of red color when comparing either juice or concentrate, whereas CMH enhanced the brown color of juice and concentrate compared with FD, resulting in a significantly higher brown index (BI) (Table 1). Irrespective of the extraction method, evaporation of juice into concentrate gave significantly lower red and brown color units, by approximately 16% and 8%, respectively. The decrease in red color during evaporation, due to degradation of red pigments or their conversion into nonred pigments, was greater than that for brown and violet color and effectively increased the concentrate BI and VI values (Table 1). It is hypothesized that brown pigments precipitated once their solubility limit was reached as a consequence of the juice being concentrated. The loss of brown color during evaporation of red grape juice was consistent with results reported for pomegranate juice concentrate.[20]

FD derived concentrate had 10% lower brown color compared to CMH concentrate (Table 1), which might reflect an effect of extraction temperature on polyphenol oxidase (PPO) activity. This enzyme catalyzes the oxidation of *o*-diphenols to *o*-quinones, which can polymerize and form

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brown pigments.[21] In the FD treatment, must was heated to a higher temperature (85 °C) even though over a shorter time (5−10 min) than for CMH, which could have more effectively inactivated PPO. The higher level of oxidation for CMH is evidenced by the 3.6-fold higher caftaric acid concentration in concentrate derived from FD treatment (Table 1). Previous research reported a low level of PPO inactivation (∼12%) after 20 min of heating at 57 °C and a pH of 7.[22] The increased oxidation and browning observed in the current study can likely be attributed to a combination of the lower extraction temperature and juice being held at ∼45 °C for an extended period of time due to the absence of a cooling step prior to evaporation; holding juice at this temperature could result in nonenzymatic browning. Use of a low temperature (LT) evaporator decreased red color loss by ∼30%, compared to the use of a high temperature (HT) evaporator (Table 2).

Influence of CMH versus FD Treatment on Phenolic Composition of Juice and Concentrate. The CMH process extracted significantly more malvidin-3,5-*O*-diglucoside, the most abundant anthocyanin present in Rubired grapes,[4] compared with FD treatment (Table 1). However, a greater proportional decrease of malvidin-3,5-*O*-diglucoside was observed during postextraction processing of juice from CMH, such that there was no significant difference between the two concentrates (Table 1). Malvidin-3-*O*-glucoside showed an opposing trend with FD juice and concentrate having significantly higher concentrations.

The extraction of malvidin-3-*O*-glucoside was consistent with previous studies[14,23] that reported an increase in anthocyanin extraction from grapes at increased temperatures. Other researchers similarly reported higher malvidin-3-*O*-glucoside concentrations in juice extracted at higher temperatures, although there were no differences in the anthocyanin concentrations of grape skins postextraction.[24] This led to the postulation that differences in juice anthocyanin concentrations were due to postextraction losses.[24] Holding must or juice at an elevated temperature for a prolonged period of time (i.e., several hours), as occurs under CMH extraction and evaporation conditions, appeared to cause greater degradation of malvidin-3-*O*-glucoside than of malvidin-3,5-*O*-diglucoside (Table 1) with the latter being more stable due to increased glycoconjugation.[25]

The loss of malvidin-3,5-*O*-diglucoside during evaporation seemed to parallel that of overall red color loss at 14−18%, whereas malvidin-3-*O*-glucoside and pigmented polymer loss (which were at least an order of magnitude higher compared to the diglucoside) ranged from 21−23% and 27−29%, respectively (Table 1). Polymeric pigments are generally observed to be more stable than monomeric forms during juice or wine storage, and thus it was surprising to see that polymeric forms decreased more rapidly than monomeric anthocyanins during concentration. The higher proportional loss of pigmented polymers might have been attributable to either their precipitation or conversion to compounds with different absorbance maxima (e.g., hypsochromic shift to wavelengths associated with orange/yellow color) during evaporation. Because of the very high concentrations of malvidin-3,5-*O*-diglucoside and other anthocyanin diglucosides in Rubired, loss of these anthocyanins contributed most to the observed loss of red color. In addition to glycosylation, other factors that influence anthocyanin stability, such as acylation of the glucose moiety and the presence of copigments, metal ions, enzymes, oxygen, and antioxidants,[26−29] may also have played a role in determining the final concentrate color.

CMH extracted significantly more proanthocyanidin and gallic acid into juice than FD, which instead yielded twice as much catechin (Table 1). The result for gallic acid appears to suggest that the longer duration of extraction for CMH favored extraction of phenolics from seeds, which takes place at a relatively slower rate compared to extraction of skins,[30] whereas enzymatic and/or nonenzymatic oxidation arising respectively from a lower extraction temperature and holding juice at a higher temperature for longer, might account for the loss in catechin. Normalized concentrations of almost all phenolic measures (i.e., catechin, quercetin glycosides, caftaric acid, and GRP) decreased during evaporation of juice to concentrate, most likely due to thermal degradation, acid hydrolysis, and/or precipitation as concentrations reached solubility limits. The proanthocyanidin loss observed during evaporation of CMH juice occurred to a lesser extent for FD juice (Table 1), although CMH still had a higher proanthocyanidin concentration after evaporation. It is hypothesized that higher polysaccharide extraction in FD might have favored formation of soluble complexes which mitigated proanthocyanidin precipitation.[31] The relatively short extraction duration of FD under aqueous conditions might have also favored extraction of lower molecular weight proanthocyanidins that were less prone to precipitation.

Influence of CMH versus FD Treatment on Volatile Composition of Juice and Concentrate. The volatile composition of Rubired juice and concentrate was determined after extraction, including volatiles associated with green aromas,[32,33] such as 3-isobutyl-2-methoxypyrazine (IBMP) and C_6 compounds (*cis*-3-hexenol, hexanal, 1-hexanol, *trans*-2-hexenal, *trans*-2-hexenol, and *trans*-3-hexenol). FD processing lowered the total concentration of C_6 compounds in juice by ∼65% compared to CMH (Table 2). Although heating must has been reported to lower the concentration of C_6 compounds in juice (due to inactivation of lipoxygenase enzyme systems[34]), the decrease in concentration observed in FD-derived juice in the current study was likely due to their volatilization into the condensed vapor stream during the flash cooling process.[35] 1-Hexanol was the most abundant C_6 compound observed in juice and concentrate samples, while IBMP remained below analytical detection levels, that is, ∼1−2 ng/L.[32]

The evaporation process used to convert juice into concentrate removed C_6 compounds more effectively than FD treatment with the evaporation temperature found to have a greater effect on volatile composition of the concentrate than the juice extraction method (Table 2). Concentrating juice to 55 °Brix using the APV plate and frame evaporator resulted in low concentrations of C_6 compounds (≤5 μg/L) in both FD and CMH concentrate. In contrast, the low-temperature, high-vacuum Reda evaporator was less effective at eliminating C_6 compounds, such that total C_6 compounds were 5−10 times higher than for APV concentrates (Table 2). A similar trend was observed for 1-octen-3-ol and geraniol; concentrates generated from HT evaporation had considerably lower concentrations of these compounds compared to the LT evaporation, irrespective of the juice extraction method. The decrease in 1-octen-3-ol, which was present in juice at concentrations close to sensory detection threshold levels (Table 2), would be expected to improve the organoleptic quality of concentrate, given the mushroom/metallic attribute

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imparted by 1-octen-3-ol is often perceived to be an undesirable food taint.[36] The trend toward lower volatile concentrations at the higher evaporation temperature was not seen for linalool, and β-damascenone concentrations did not show any consistent pattern (Table 2). When aroma detection thresholds are taken into account, only hexanal, 1-octen-3-ol, and β-damascenone were detected at concentrations that gave odor activity values (OAVs) > 1 (Table 2), that is, concentrations that would likely impact the sensory profiles of juice and concentrate. These compounds were observed at higher concentrations in juice and concentrate from CMH than from FD. Given that most Rubired concentrate is sold to food processors for use at relatively low addition rates, only β-damascenone, which had an OAV of ∼20−100, could be expected to meaningfully impact food and beverage flavor profiles. However, sensory evaluation of concentrate and/or color-enhanced food and beverage products would be needed to establish any aroma/flavor impact of CMH and FD concentrates.

Influence of CMH versus FD Treatment on Suspended Grape Solids of Juice and Concentrate. Knowing that suspended solids content of juice can negatively affect centrifuge throughput and juice yield after solids removal, the solids content of CMH and FD juice was monitored. FD-derived juice contained a significantly higher proportion of suspended solids (i.e., grape tissue fragments, expressed as % v/v) than CMH-derived juice, being about double at ∼7.4% compared with ∼3.6% (Table 3). Electrical impedance measurement of grape skin after FD treatment has shown that considerable cellular damage occurs during this process.[13] Greater skin and pulp disintegration would be expected to increase the level of grape solids in the resulting juice. Findings from the current study were contrary to results reported previously,[37] where the dry suspended solids content (expressed as % w/w) of must from CMH was higher than for FD. Differences in grape variety, the conditions employed during extraction, and the methods used for must pressing might explain the conflicting outcomes from these studies. In the current study, as may be expected, there was no significant difference in the suspended solids content of centrifuged CMH and FD juices (Table 3).

Table 3. Suspended Solids Content of Juice derived from CMH and FD Treatments[a]

extraction method	decanter inlet solids (% v/v)	decanter outlet solids (% v/v)
FD	7.4	1.3
CMH	3.6	1.2
P-value	*0.0001*	*0.461*

[a]Values are means (n = 10); α = 0.05, Tukey's pairwise comparisons.

Influence of CMH versus FD Treatment on Juice Filterability. The influence of extraction method on juice filterability was also investigated. FD juice was found to have 4-fold lower flux through a dead-end diatomaceous earth filter compared with CMH juice (Figure 1). The average flux during filtration was 2.4 L/h for FD juice compared with 9.3 L/h for CMH juice. Macromolecules such as proteins, proanthocyanidins, and polysaccharides have been reported to cause poor filterability in juice and wine.[38,39] In the current study, CMH juice had a higher proanthocyanidin concentration than FD juice, while total organic nitrogen concentrations (measured as protein) were similar at 1.33 and 1.35%, respectively (data not shown). Since the Kjeldahl method measures total organic nitrogen (i.e., proteins and amino acids), the potential for protein to impact filterability could not be established. The other likely cause of poor filterability was increased polysaccharide extraction from FD treatment, as reported previously.[40]

Influence of the Duration of Heat Treatment Prior to Flash Cooling on Juice and Concentrate Composition. Results from the pilot scale FD extraction trials showed that increasing the duration of FD extraction (at 85 °C) from 15 to 45 min did not significantly impact red, brown, or violet color in either juice or concentrate (Table 4), in agreement with previous research.[23]

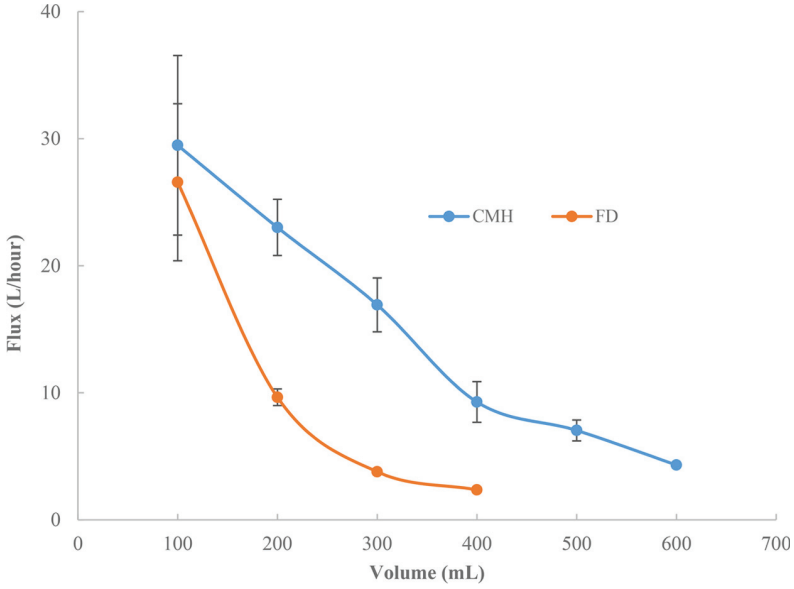

Figure 1. Effect of CMH versus FD on filterability of juice through diatomaceous earth. Values are means of three replicates from 2016 (n = 3) ± standard deviation.

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Table 4. Effect of Different Durations of Heat Treatment on Composition of Juice and Concentrate Derived from Pilot-Scale FD Treatment[a]

	juice extraction time			concentrate extraction time				
parameter	15 min	30 min	45 min	15 min	30 min	45 min	*P*-value	model adjusted R^2
Color								
red color units	1790 a	1743 ab	1745 ab	1644 bc	1585 c	1578 c	*0.0001*	69.5%
brown color units	649	649	649	592	593	586	*0.06*	26.0%
violet color units	458 ab	461 ab	463 a	413 b	421 ab	415 ab	*0.005*	46.8%
brown index	0.36	0.37	0.37	0.36	0.37	0.37	*0.78*	0.0%
violet index	0.26 ab	0.27 a	0.27 a	0.25 b	0.27 a	0.26 ab	*0.023*	34.9%
Phenolic Compounds								
malvidin-3,5-*O*-diglucoside (mg/L)	4422 a	4252 ab	4206 b	3830 c	3691 cd	3601 d	*0.0001*	93.8%
malvidin-3-*O*-glucoside (mg/L)	352 a	317 b	317 b	303 b	275 c	273 c	*0.0001*	86.2%
pigmented polymers (mg/L)	106 a	109 a	110 a	88 b	89 b	88 b	*0.0001*	82.6%
proanthocyanidin (mg/L)	1210	1299	1303	1193	1282	1259	*0.38*	30.4%
catechin (mg/L)	402 b	471 a	469 a	335 c	405 b	390 b	*0.0001*	97.7%
epicatechin (mg/L)	55 c	90 a	93 a	42 d	67 b	69 b	*0.0001*	98.2%
gallic acid (mg/L)	36 c	44 a	43 a	31 d	39 b	38 bc	*0.0001*	92.7%
grape reaction product (mg/L)	51 a	51 a	51 a	43 b	42 b	41 b	*0.0001*	73.5%
quercetin glycosides (mg/L)	200	195	194	176	175	174	*0.335*	4.8%
caftaric acid (mg/L)	107 a	111 a	107 a	93 b	95 b	91 b	*0.0001*	80.8%
total phenolics (g/L)	16 b	17 a	17 a	16 b	16 b	16 b	*0.0001*	65.9%
Basic Chemistry								
pH	3.89	3.89	3.89				*0.991*	0.0%
potassium (mg/L)	6469	6285	6206				*0.732*	0.0%

[a]Values are means of two replicates from 2017 (n = 2). Means followed by different letters (within rows) are significantly different (α = 0.05, Tukey's pairwise comparisons). Data were normalized to 68 °Brix, except pH and color indices, which were measured at juice or concentrate TSS.

Table 5. Percentage Change Relative to Initial Values in Composition of Concentrate Derived from CMH and FD Treatments, During Cold Storage (2 °C) for 12 Months, and *P*-Values from Repeated Measures Analysis of Variance[a]

			P-values			
parameter	CMH	FD	production method	time	concentrate type × time	model adjusted R^2
Color						
red color	95.3%	93.4%	*0.006*	*0.0005*	*0.583*	87.6%
brown color	98.4%	100.3%	*0.194*	*0.202*	*0.990*	
violet color	110.5%	113.2%	*0.266*	*0.0005*	*0.949*	74.4%
brown index	0.45	0.39	*0.0005*	*0.008*	*0.946*	86.5%
violet index	0.34	0.30	*0.0005*	*0.0005*	*0.995*	80.4%
Phenolic Compounds						
caftaric acid	213.2%	120.7%	*0.0005*	*0.0005*	*0.0005*	99.1%
malvidin-3,5-*O*-diglucoside	85.9%	83.4%	*0.006*	*0.0005*	*0.903*	96.0%
malvidin-3-*O*-glucoside	73.3%	75.2%	*0.479*	*0.0005*	*0.613*	92.0%
pigmented polymers	142.4%	168.9%	*0.011*	*0.0005*	*0.712*	78.0%
epicatechin	17.7%	17.5%	*0.819*	*0.0005*	*0.278*	99.5%
gallic acid	100.8%	126.5%	*0.029*	*0.0005*	*0.055*	77.5%
proanthocyanidin	108.2%	106.4%	*0.583*	*0.0005*	*0.531*	82.1%

[a]Values are means of three replicates from 2016 (n = 3); α = 0.05. Data were normalized to 68 °Brix, except color indices, which were measured at concentrate TSS. (Color indices are calculated ratios after 12 months of cold storage, and not percentage change).

Anthocyanin extraction achieved equilibrium within a holding time of 15 min, such that extending the time did not significantly increase the concentrations of malvidin-3,5-*O*-diglucoside or malvidin-3-*O*-glucoside. A hold time of 45 min actually decreased the malvidin-3,5-*O*-diglucoside concentration, whereas a decrease in malvidin-3-*O*-glucoside was seen after 30 min. The small decrease in anthocyanin concentrations (~5%) did not seem to affect red color and did not appear to be reflected in the concentrations of pigmented polymers. These results suggest that increased heating is unlikely to improve color extraction. In contrast, increasing the duration of extraction resulted in higher gallic acid, catechin, and epicatechin concentrations in the juice, with no significant effect on caftaric acid, proanthocyanidin, total phenolics, pigmented polymers and quercetin glycosides (Table 4). The observed extraction trends suggest that increasing the duration of extraction appears to aid extraction of relatively more hydrophobic compounds such as catechin and epicatechin but not extraction of more polar compounds such as potassium salts, anthocyanins, caftaric acid or quercetin glycosides. Proanthocyanidins did not increase with extraction duration as would be expected based on the above trend possibly due to precipitation. Increased heating did not increase pigmented polymer or grape reaction product formation, possibly due to

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concurrent thermal degradation and inactivation of oxidative enzymes, respectively.

Evaporation was found to have a greater effect on anthocyanin concentrations than hold time, with approximately 14% less malvidin-3,5-*O*-diglucoside observed after evaporation. The concentrations of anthocyanins, caftaric acid, grape reaction product, pigmented polymers, gallic acid, epicatechin, and catechin decreased by ∼14−26% upon evaporation of the juice, whereas total phenolics decreased by only ∼7%, and quercetin glycosides and proanthocyanidin concentrations remained unchanged. Heating grape juice has previously been shown to accelerate reactions between anthocyanins, monomeric flavan-3-ols and proanthocyanidins, thereby decreasing the concentrations of anthocyanin and flavan-3-ol remaining in the concentrate.[41,42] The apparent disconnect in the concentrations of anthocyanins/flavan-3-ol (decreasing) and proanthocyanidins (stable) in the pilot scale trial was consistent with the trend observed during commercial scale evaporation of FD juice. Proanthocyanidin concentration did not increase, likely due to precipitation and/or acid catalyzed cleavage. The decreased concentrations of quercetin glycosides and GRP after evaporation might reflect acid hydrolysis and thermal degradation, respectively.

Influence of CMH versus FD Treatment on Color Stability of Rubired Juice Concentrate. The color stability of CMH and FD-derived juice concentrate was compared via a 12 month storage study. Concentrates were kept at low temperature (2 °C) to prevent fermentation and to reduce the rate of color loss and browning. The juice production method was found to affect concentrate color stability under normal cold storage conditions. The red color of CMH concentrate was more stable than FD concentrate, such that after 12 months of storage at 2 °C, FD concentrate had lost a significantly higher proportion of its initial color (Table 5), making the technology less attractive for producers who store concentrate for prolonged periods prior to use.

Irrespective of the juice extraction method, concentrate brown color did not change significantly during storage (Table 5), which was a positive outcome from a color quality standpoint. This finding was consistent with results reported for peach concentrate stored at 2 °C for 4 months.[43] The observed increase in caftaric acid concentration during storage (Table 5), suggests the absence of enzymatic oxidation which would otherwise lead to browning.[7] Little or no enzymatic activity was expected, given the heat treatments that concentrates were subjected to during both extraction and evaporation. The brown index gradually increased, however, from 0.44 to 0.48 and from 0.36 to 0.42 for CMH and FD concentrates, respectively, due to red color loss during storage (Figure S3c of the Supporting Information). This was in agreement with previous research that showed an increase in the ratio of brown to red color in CMH-derived Concord grape juice stored at 24 or 35 °C for 18 months.[14] That study also reported an increase in brown color, which differed from the outcomes of the current study. This might be attributed to a decrease in the rate of Maillard reaction[7] due to the lower storage temperature used in the present work, resulting in less browning. Importantly, FD concentrate was consistently lower in brown and violet color ratios than CMH concentrate throughout the 12 month storage period (Table 5, Figure S3c of the Supporting Information).

Violet color increased by a similar margin (∼10%) during cold storage for both CMH and FD concentrate (Table 5) but increased markedly between 9 and 12 months (Figure S4). Notably, after 12 months of storage, both CMH and FD concentrate contained ∼2% alcohol by volume due to slow fermentation. Thus, the observed increase in violet color might reflect copigmentation reactions between anthocyanins and other phenolics compounds (e.g., flavonols and phenolic acids) that cause a bathochromic shift in λ_{max},[26] as well as reactions with pyruvic acid and acetaldehyde produced by fermenting yeast, to form pyranoanthocyanins such as vitisins and portisins.[44]

In contrast to the postextraction changes noted above (Table 1), CMH concentrate retained a significantly higher proportion of its malvidin-3,5-*O*-diglucoside during storage compared to FD concentrate, whereas there was no significant difference in malvidin-3-*O*-glucoside retention (Table 5). The decrease in malvidin-3,5-*O*-diglucoside in FD concentrate might be explained by the significantly higher conversion to pigmented polymers (Table 5). The comparatively lower formation of pigmented polymers in CMH concentrate with a higher initial concentration may point to equilibrium effects limiting anthocyanin conversion to more stable pigments. With the exception of caftaric acid which increased during storage at a much higher rate in CMH concentrate, there were no significant concentrate type × time interactions (Table 5), suggesting that changes in the color and phenolic composition of CMH and FD concentrates exhibited similar trends over time.

Overall, the concentration of malvidin-3,5-*O*-diglucoside decreased by 30−32% in both CMH and FD concentrate following storage (Figure S5 of the Supporting Information). In contrast, malvidin-3-*O*-glucoside concentrations decreased by 60−70%. This was consistent with previous studies,[45] as well as results presented above. The decrease in anthocyanin concentration after 12 months of cold storage was much greater than the 8−10% red color loss observed, suggesting that a greater portion of anthocyanins may have been converted into other forms of red pigments (e.g., anthocyanin-flavanol adducts, pyranoanthocyanins, or pigmented polymers), than were lost through adsorption to tartrates precipitating during cold storage[46] or converted to colorless forms or nonred pigments, especially when considering that the concentration of pigmented polymers increased significantly during storage (Table 5). Formation of anthocyanin-flavanol adducts, pyranoanthocyanins, and polymeric pigments would be expected to increase color stability.[9]

In conclusion, although FD did not significantly increase red color extraction relative to CMH nor did increasing the duration of FD extraction, the technology produced concentrate which was less oxidized and of higher color quality. FD technology could therefore be more suitable for use in minimally processed ready to consume food and beverage coloring applications. The lower color stability of FD-derived concentrate may present challenges in some product applications, especially those that involve further heating in particular, which might exacerbate color loss. Color stability might also limit the use of FD-derived concentrate as a coloring for products that have an extended shelf life, but this requires further investigation.

Similar aroma profiles of concentrates derived from CMH and FD were expected despite differences in β-damascenone, 1-octen-3-ol, and hexanal concentrations in the corresponding juices due to the mitigating effect of juice evaporation to generate the concentrates. The impacts of FD processing on

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the sensory properties of juice and concentrate were not evaluated in the current study, however, and warrant further investigation. The FD process generated a greater amount of suspended grape solids than CMH, which has potential implications for the efficiency of subsequent solids removal processes and juice yield. The lower flux for FD juice would similarly impact throughput during filtration and may require additional capital investment to increase filtration capacity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsfoodscitech.1c00004.

Flowchart of experimental treatments and analyses; basic chemistry of Rubired grapes used in commercial and pilot scale extraction trials; percentage red color loss during cold storage (2 °C) of concentrate derived from CMH versus FD for 12 months; changes in red, brown and violet color during cold storage (2 °C) of concentrate derived from CMH versus FD for 12 months; change in brown and violet indices during cold storage (2 °C) of concentrate derived from CMH versus FD for 12 months; percentage malvidin-3,5-*O*-diglucoside and malvidin-3-*O*-glucoside loss during cold storage (2 °C) of concentrate derived from CMH versus FD for 12 months (PDF)

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Color Extraction and Stability of Rubired Juice Concentrate Produced via Conventional Must Heating or Flash Détente Processing

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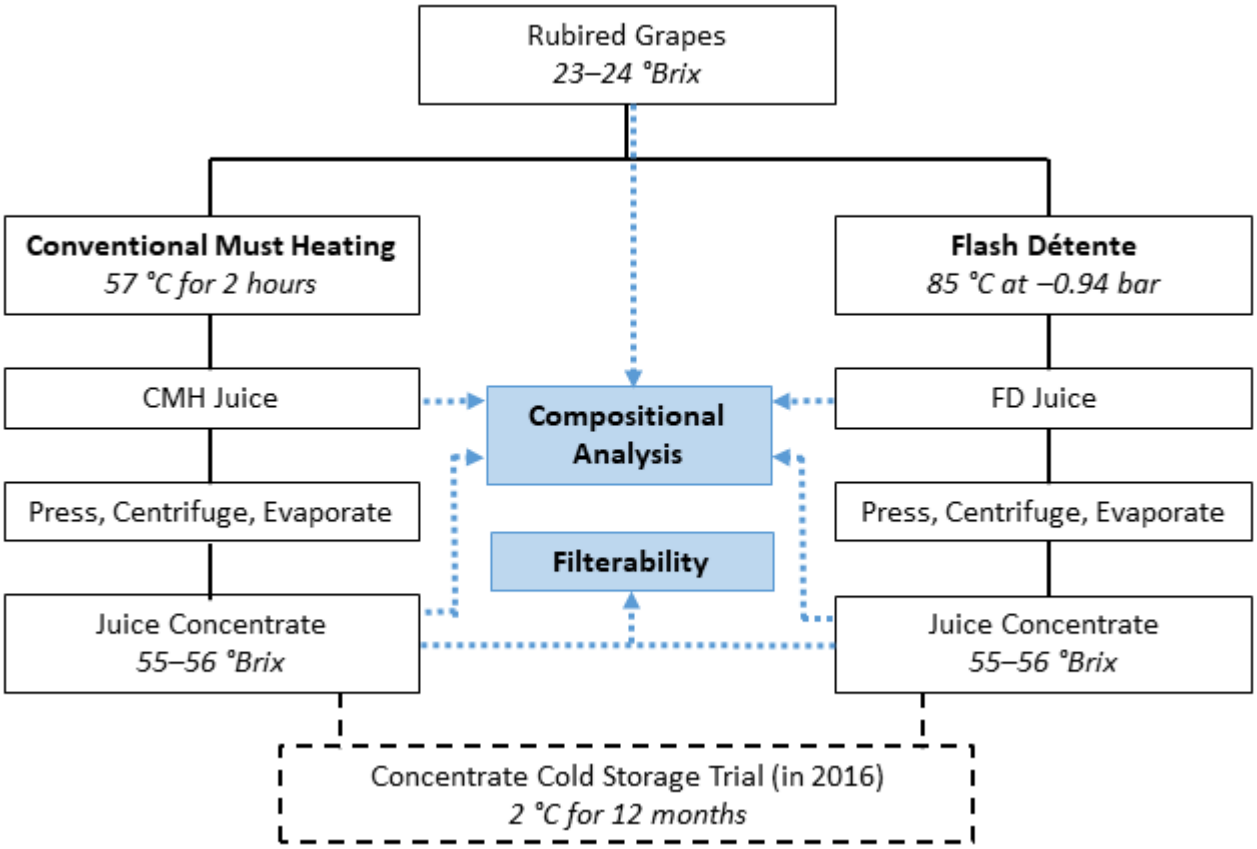
Supplementary Table S1. Basic Chemistry of Rubired Grapes used in Commercial and Pilot Scale Extraction Trials.

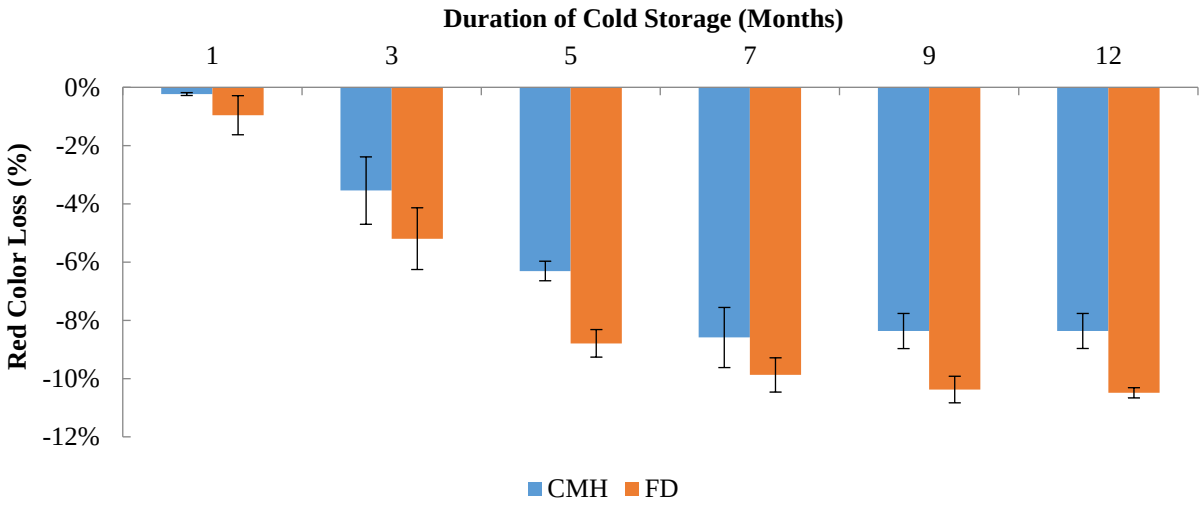
vintage	scale	extraction method	TSS (°Brix)	pH	TA (g/L)	potassium (mg/L)
2016	commercial	CMH	23.0	4.0	5.9 ab	2197
		FD	23.8	4.0	6.1 a	2186
2017	commercial	CMH	24.0	3.9	5.5 bc	2274
		FD	23.5	3.9	5.2 c	2146
2017	pilot	FD	25.6	3.8	4.3	1766
		FD	26.3	3.8	4.7	1971

For commercial scale trials, values are means (n = 4). Means followed by different letters (within a column for commercial scale) are significantly different ($\alpha = 0.05$, Tukey's pairwise comparisons). For pilot scale trials, measurements were not replicated (n = 1).

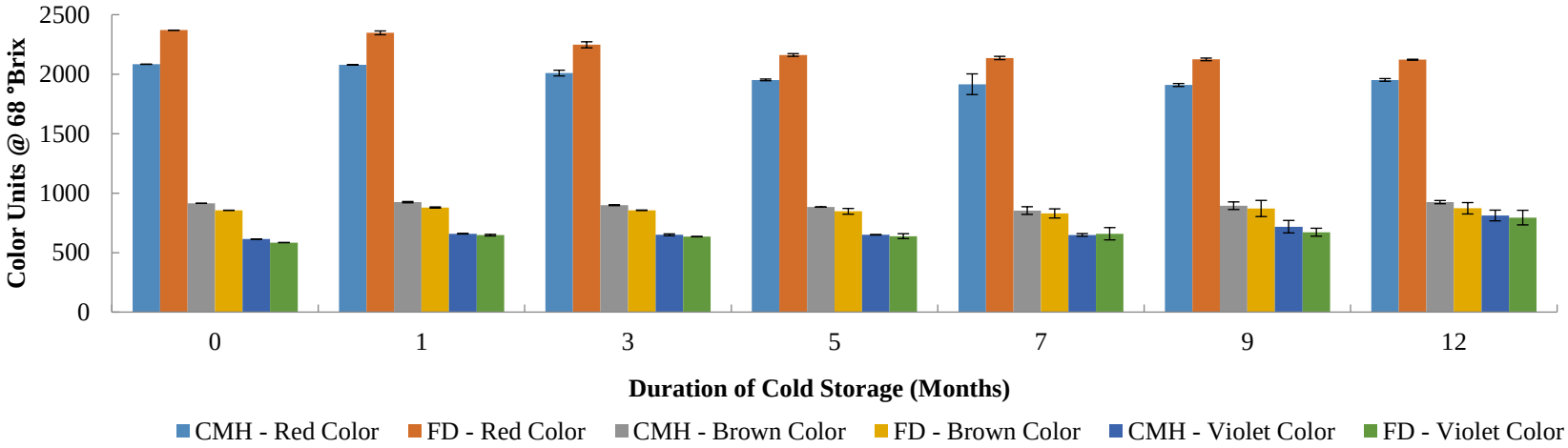
Supplementary Figure S1. Flowchart of experimental treatments and analyses.



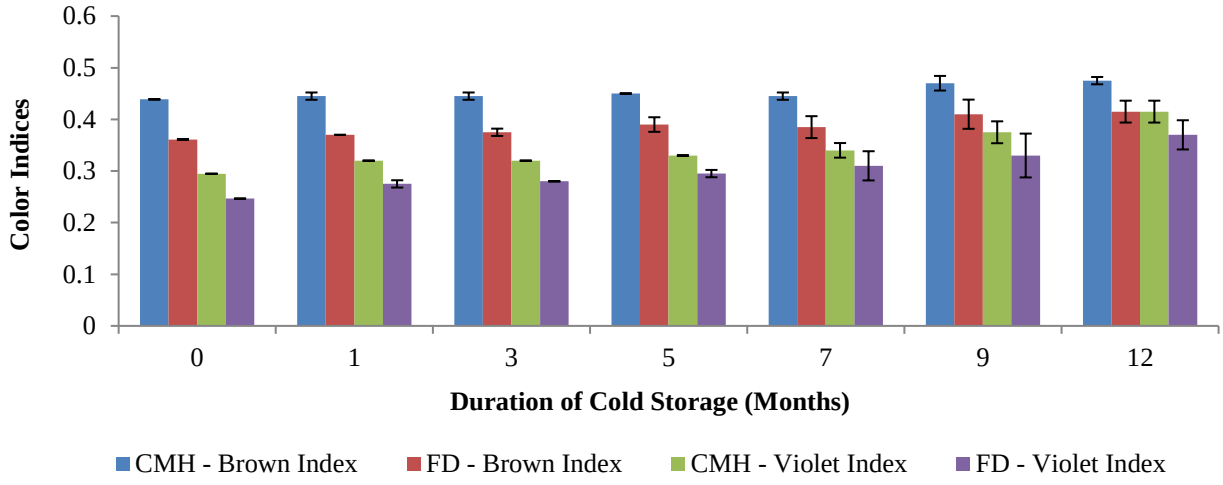
Supplementary Figure S2. Percentage red color loss during cold storage (2 °C) of concentrate derived from conventional must heating (CMH) vs. flash détente (FD) for 12 months. Values are means of three replicates from 2016 (n = 3) ± standard deviation. Data normalized to 68 °Brix.



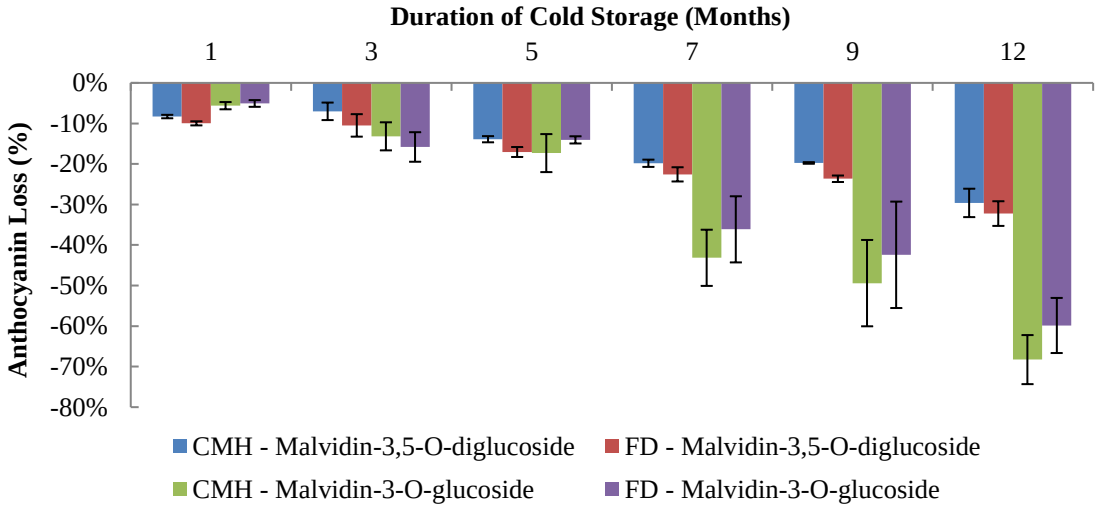
Supplementary Figure S3. Changes in red, brown and violet color during cold storage (2 °C) of concentrate derived from conventional must heating (CMH) vs. flash détente (FD) for 12 months. Values are means of three replicates from 2016 (n = 3) ± standard deviation. Data normalized to 68 °Brix.



Supplementary Figure S4. Change in brown and violet indices during cold storage (2 °C) of concentrate derived from conventional must heating (CMH) vs. flash détente (FD) for 12 months. Values are means of three replicates from 2016 (n = 3) ± standard deviation.



Supplementary Figure S5. Percentage malvidin-3,5-*O*-diglucoside and malvidin-3-*O*-glucoside loss during cold storage (2 °C) of concentrate derived from conventional must heating (CMH) vs. flash détente (FD) for 12 months. Values are means of three replicates from 2016 (n=3) ± standard deviation.



Chapter 3.

Impact of Juice Extraction Method (Flash Détente vs. Conventional Must Heating) and Chemical Treatments on Color Stability of Rubired Juice Concentrates under Accelerated Aging Conditions.

Ntuli, R. G., Ponangi, R., Jeffery D. W. and Wilkinson, K. L. (2020). Impact of Juice Extraction Method (Flash Détente vs. Conventional Must Heating) and Chemical Treatments on Color Stability of Rubired Juice Concentrates under Accelerated Aging Conditions. Foods, 9, 1270.
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Name of Principal Author (Candidate)	Richard G. Ntuli		
Contribution to the Paper	Designed experiments, planned and executed production and laboratory scale trials, performed statistical analyses on data sets, interpreted the data, drafted the manuscript and then revised the manuscript to address reviewer comments.		
Overall percentage (%)	85%		
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	25/07/2020

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.

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Contribution to the Paper	Contributed to the research idea and experimental design, assisted with data interpretation and edited the manuscript.		
Signature		Date	29/07/2020

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Impact of Juice Extraction Method (Flash Détente vs. Conventional Must Heating) and Chemical Treatments on Color Stability of Rubired Juice Concentrates under Accelerated Aging Conditions

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Abstract: Low color stability of Rubired food and beverage coloring negatively impacts color yield during production and storage while also limiting the use of this type of food colorant in applications where color stability is a key requirement. This study investigated the impact on color stability of using flash détente (FD) for Rubired color extraction in comparison to a conventional must heating (CMH) extraction process, in conjunction with the use of commercial seed tannin, acetaldehyde, or acid to lower the pH. Rubired concentrate color was evaluated under accelerated aging conditions at 50, 60, and 70 °C, over zero, three, six, and nine days for the different treatments. FD concentrate had lower color stability, with a half-life of 203.3 h and activation energy of 59.2 kJ/mol at 50 °C compared to the CMH concentrate with 233.9 h and 65.2 kJ/mol. FD concentrate generated less 5-hydroxymethylfurfural (5-HMF) during accelerated aging regardless of treatment. Acetaldehyde, low pH, and the combination of these two treatments increased red color stability as well as violet and brown color, whereas seed tannin had no effect. Low pH treatments increased 5-HMF formation and browning, which was detrimental to concentrate quality. Although promising in terms of color stabilization, implementation of these treatments will require development of solutions to mitigate the production of 5-HMF.

Keywords: grape color extraction; grape food coloring; color units; color degradation kinetics; grape concentrate

1. Introduction

Color stability is an important quality attribute for Rubired grape concentrate, considering its main application as a beverage or food colorant. This is especially important in view of the increased consumer interest in the use of natural food colorants due to their health benefits [1], in place of synthetic counterparts that have been reported to adversely affect human health [2]. The biggest challenge in replacing synthetic dyes with natural ones is matching the vivid colors and high color stability of the former. Enhanced red color stability during processing and storage of Rubired concentrate could potentially increase the range of food and beverage applications for the concentrate and increase its attractiveness as a replacement for synthetic food dyes.

Red color stability in processed red grape juice or concentrate is influenced by grape variables, as well as production and storage conditions. Production conditions that reportedly influence red grape concentrate color stability include extraction temperature and duration [3]. These factors impact the

stability of anthocyanins by affecting enzymatic activity of polyphenol oxidase, peroxidases, and other oxidative enzymes, as well as β-glucosidase enzymes that can accelerate anthocyanin degradation [4]. Increasing juice extraction temperature from 60 to 80 °C has been reported to increase acidity, as well as the concentrations of total anthocyanins and phenolic compounds, whereas at higher temperatures, i.e., around 90 °C, acidity, anthocyanins, and phenolic compounds decreased [5]. Increased acidity and extraction of phenolic compounds would be expected to increase color stability, as anthocyanins are more stable at lower pH values (i.e., at pH < 3) [6], and various phenolic compounds are known to act as color stabilizing co-pigmentation cofactors, whereas others react with anthocyanins to form more stable polymeric pigments [7].

In terms of extraction technologies, flash détente (FD) employs a high extraction temperature of ~85 °C, compared to ~60 °C for conventional must heating (CMH) extraction [8]. The former involves a relatively short duration of heating followed by rapid vacuum cooling of must. In contrast, the CMH extraction process typically has longer extraction times, ranging from 30 to 120 min, and then very slow, natural cooling of must and juice, prior to evaporation into concentrate. Based on these different heating conditions, it could be expected that concentrates produced via the two processes would have different color stabilities.

Degradation of juice or concentrate color during storage is impacted by several factors, including extraction temperature [3], contact time, grape variety, maturity at harvest [3], pH, light, the presence of antioxidants, pro-oxidants, oxidative enzymes [4,6,9,10], sodium chloride and transition metal ions [11–13], flavanols and other grape derived compounds, and storage temperature [3,14,15]. Of the factors that have been shown to influence red color stability, only antioxidant addition and cold temperature storage are regularly used to control color degradation during processing and storage. The most common antioxidant used in juice and wine processing is sulfur dioxide, although ascorbic acid is occasionally used. However, these two antioxidants are not effective in juice concentrate due to their tendency to bind with sugars or act as a pro-oxidant, respectively [13]. The only effective, widely used color preservation method is therefore cold storage of juice, which is a costly option, but one still practiced by commercial producers as a practical way to achieve stability. The need to develop more effective ways to stabilize red juice or concentrate color is therefore an obvious one.

Approaches that show promise for Rubired concentrate color stabilization, based on findings in other fruit juices, concentrates and wines, include the use of co-pigmentation cofactors [16–21], pH adjustment, and reaction of phenolic and non-phenolic compounds with anthocyanins to form more stable pigments, such as polymeric pigments and pyranoanthocyanins [22,23]. Because of consumer demand for minimally processed foods and food additives derived from natural food materials [24], priority should be given to treatments utilizing grape or fermentation-derived cofactors, and phenolic and non-phenolic compounds that can stabilize anthocyanins. However, more research is needed to screen these grape and wine constituents to determine which are the most effective, and to develop practical methods for stabilizing red concentrate color.

The goal of this work was to compare and contrast the red color stability and degradation kinetics of Rubired concentrate derived from FD with that from CMH. To date, no study has been documented that compares the red color stability, brown and violet color change, and formation of 5-hydroxymethylfurfural (5-HMF) in FD-derived red grape concentrate to that of traditionally extracted concentrate. The authors acknowledge that there are variations of the FD process in commercial application and this study was an evaluation of concentrate generated by a generic FD process rather than an evaluation of FD equipment itself. The study also aimed to investigate the effect of low pH, acetaldehyde (an alcoholic fermentation-derived additive), and grape-derived seed tannin addition on red color stability and brown and violet color evolution (using color measurements employed by commercial producers and end-users), as well as their effect on compounds that are thought to influence color expression under accelerated aging conditions.

2. Materials and Methods

2.1. Chemicals

Chemicals (analytical grade) were purchased from Sigma Aldrich (St Louis, MO, USA). Standards were sourced from Sigma Aldrich and Indofine chemicals (Hillsborough, NJ, USA). Solvents (HPLC grade) were sourced from BDH (Radnor, PA, USA). Deuterated internal standards were sourced from C/D/N Isotopes Inc. (Pointe-Claire, QC, Canada).

2.2. Preparation of Rubired Juice Concentrate

The Rubired juice concentrate used for this study was produced via commercial scale CMH and FD processes (Figure S1), with grapes (approximately 400 metric ton) harvested at commercial maturity (23–24 °Brix) from a vineyard located in the Central Valley region of California (36.57° N, 119.61° W), during the 2016 vintage. For CMH extraction, 180 metric tons of grapes were destemmed and crushed, and 50 mg/L sulfur dioxide (as an 8% solution of potassium metabisulfite) and pectinase (Rohavin MX, AB Enzymes, Darmstadt, Germany, 28 mL/metric ton) were added. Processing at 127 metric tons/hour, the must was heated to 57 °C using a steam-heated shell and tube heat exchanger (Wiegmann and Rose, Oakland, CA, USA). Following CMH, hot must was held for 2 h before pressing with a Diemme screw press (Diemme Enologia, Lugo, Italy). Solids were removed from the resulting juice using a Westfalia decanter centrifuge (Westfalia, Northvale, NJ, USA), prior to evaporation with a high temperature (HT) APV plate and frame evaporator (SPX Flow, Crawley, UK) to give a 55–56 °Brix concentrate. For FD extraction, 180 metric tons of grapes were destemmed and crushed, and the resulting must heated to 85 °C (for 5–10 min) using a Della Toffola flash détente unit (Della Tofolla, Trevignano, Italy) at 27 metric tons/h. Vacuum pressure in the flash chamber was maintained at -0.94 bar. Following FD, 50 mg/L of sulfur dioxide and pectinase (28 mL/metric ton) were added to the must before pressing; additions were made post-FD to avoid their loss due to vacuum flashing and inactivation, respectively, during FD processing. The resulting juice was chilled (to 4 °C to prevent fermentation) and solids were removed prior to evaporation to give 55–56 °Brix concentrate (as for CMH). FD and CMH concentrates were stored at 2–4 °C to prevent fermentation and minimize red color loss and browning.

2.3. Accelerated Color Stability Testing under Different Treatment Conditions

A laboratory scale accelerated aging experiment was conducted to compare the color stability of CMH and FD processes, and to investigate the impact of various chemical treatments on the color stability of Rubired concentrates produced by the two thermal processes. Treatments (performed in duplicate) comprised a control (i.e., no additions) and additions of seed tannin, acetaldehyde, acid, and combinations of these additions, as outlined below, to both CMH and FD concentrate prior to heat treatment.

2.3.1. Commercial Grape Seed Tannin

A commercial grape seed tannin powder (10 g) was dissolved in 60% aqueous ethanol solution (15 mL) and added to concentrate to give 1000 mg/L gallic acid equivalents (GAE) of tannin, as measured by the Folin-Ciocalteu method [22]. The concentrate was thoroughly mixed after tannin addition.

2.3.2. Acetaldehyde

Acetaldehyde in ethanol solution (50% *w*/*w*, Penta International Corp., Livingston, NJ, USA) was added to concentrate (at 4 °C, to prevent acetaldehyde from flashing off) to give a concentration of 300 mg/L. The concentrate was then thoroughly mixed.

2.3.3. Acid

Concentrated hydrochloric acid (37% *w*/*v*, EMD, Burlington, MA, USA) was added to concentrate to adjust the pH to 2.8, the lowest pH permitted by Alcohol and Tobacco, Tax and Trade Bureau (TTB) regulations [25], although hydrochloric acid was used for experimental purposes only. The concentrate was thoroughly mixed during pH adjustment.

2.3.4. Acetaldehyde and Acid

Concentrated hydrochloric acid was added to concentrate to adjust the pH to 2.8 before acetaldehyde was added to achieve a concentration of 300 mg/L.

2.3.5. Seed Tannin, Acetaldehyde and Acid

Concentrated hydrochloric acid was added to concentrate to adjust the pH to 2.8 before acetaldehyde and seed tannin were added (as above) to give an acetaldehyde concentration of 300 mg/L and a seed tannin concentration of 1000 mg/L GAE of tannin.

2.3.6. Heat Treatments

Control and treated FD- and CMH-derived concentrates (1.3 L, minus two 50 mL aliquots, as the time zero samples) were transferred into 6 × 200 mL amber colored glass bottles with black plastic lids. The bottles were filled with concentrate to leave minimal headspace and sealed to prevent moisture loss during heat treatment. Two bottles were heated in each of three ovens held at 50, 60, and 70 °C to cause accelerated aging. After three, six, and nine days of heating, samples were thoroughly mixed before aliquots (50 mL from each bottle) were collected for determination of red, brown, and violet color, total tannin, anthocyanins, monomeric flavan-3-ols, polymeric flavan-3-ols (proanthocyanidins), hydroxycinnamic acids, flavonols, and 5-HMF.

2.3.7. Color Degradation Kinetics

Changes in color and phenolic data were used to determine the kinetics of red color loss, brown color evolution, and phenolic transformation in FD and CMH concentrates under accelerated aging conditions (i.e., at elevated temperatures of 50, 60, and 70 °C over nine days). The concentration, natural logarithmic concentration, inverse of concentration, and inverse of squared concentration of red, brown, and violet color and other phenolic compounds were plotted against storage time to determine reaction orders, rate constants (k), half-life, and Q_{10} values to estimate temperature dependency. Linear regression was used to determine reaction orders and Arrhenius plots (log k vs. 1/T K) were used to determine activation energies (E_a) for the two concentrate types.

2.4. Compositional Analysis of Rubired Juice Concentrate

Concentrate samples were centrifuged (4000× *g* for 15 min; Beckman Coulter, Brea, CA, USA) prior to color and compositional analysis.

2.4.1. Color Analysis

Color measurements were performed using a United States Department of Agriculture (USDA) spectrophotometric method for red juice concentrate [26] that specifies measuring absorbance after adjusting pH to 3.2 with McIlvaine buffer. An 8453 UV-Vis spectrophotometer (Agilent Technologies, Palo Alto, CA, USA) with sipper and 1 cm flow through cuvette were used with ChemStation control software (version B.02.01). Centrifuged juice or concentrate samples (~1 g) were accurately weighed and diluted with McIlvaine buffer (pH 3.2) to a total volume of 100 mL. The pH-adjusted samples were thoroughly mixed before being filtered through 1 µm glass fiber filters (Pall, New York, NY, USA). This sample weight was chosen to obtain an absorbance at 520 nm in the range of0.3–0.7 absorbance units. Absorbances were measured at 520, 430, and 580 nm for red, brown, and violet color measurement, respectively.

Color units (CU) were calculated as follows:

$$CU_{(wavelength)} = (Absorbance \times 2000)/[(sample\ weight\ (g)) \times (dilution\ factor)] \quad (1)$$

Color units were normalized to 68 °Brix to compare color and compositional data in concentrates of different °Brix values.
Brown and violet indices were calculated as follows:

$$Brown\ index = CU_{430nm}/CU_{520nm};\ Violet\ index = CU_{580nm}/CU_{520nm} \quad (2)$$

Because the color unit scale was based on measuring the absorbance of 2 g of sample made up to 100 mL with buffer [26] (rather than 1 g in the present case), the absorbance values were multiplied by 2 and divided by sample weight to normalize absorbance readings to 2 g of sample. The normalized absorbance was then multiplied by 1000 to convert from a decimal absorbance reading to a color units scale with whole numbers, which are easier to evaluate.

2.4.2. Phenolic Analysis

The total phenolics in concentrate samples and seed tannin additive were measured using the Folin-Ciocalteu method [27], with results reported as mg/L GAE.

Phenolics profiling of Rubired concentrate samples was undertaken (in duplicate) via HPLC analysis. Samples (25 mL) were diluted with distilled water that had been acidified to a pH of 2.0 with 1 M hydrochloric acid, to a total volume of 200 mL. Samples were then analyzed by reversed-phase chromatography using an Agilent 1200 HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump, a diode array detector (DAD), and a Varian PLRP-S column (250 × 4.6 mm, 5 µm particle size, Varian Inc., Palo Alto, CA, USA) fitted with a PLRP-S guard cartridge. A binary solvent gradient was used consisting of water with 0.5% (*v*/*v*) orthophosphoric acid (85% *w*/*v*, mobile phase A), and acetonitrile with 0.5% (*v*/*v*) orthophosphoric acid (85% *w*/*v*, mobile phase B). The column thermostat was set at 50 °C and the injection volume was 20 µL.

Individual phenolic compounds were measured at the following wavelengths: proanthocyanidins at 230 nm; gallic acid, catechin, and epicatechin at 280 nm; grape reaction product (GRP), caftaric acid, and caffeic acid at 320 nm; quercetin glycosides (total of quercetin glucoside and quercetin glucuronide) and quercetin at 360 nm; and malvidin-3-*O*-glucoside, malvidin-3,5-*O*-diglucoside, and pigmented polymers at 520 nm. Standard solutions of these compounds were used for identification and quantification. Due to unavailability of pure standards for pigmented polymers and proanthocyanidins, malvidin-3-*O*-glucoside and catechin standards, respectively, were used. Pigmented polymers were reported as malvidin-3-*O*-glucoside equivalents, whereas proanthocyanidins were reported as catechin equivalents.

2.4.3. 5-Hydroxymethylfurfural Analysis

Concentrate samples were diluted, extracted, purified, and analyzed (in duplicate) using a previously described method [28]. The 5-HMF concentrations of the resulting extracts were determined using an Agilent 6890 gas chromatograph coupled to an Agilent 5973 mass spectrometer (Agilent Technologies, Santa Clara, CA, USA).

2.5. Sediment Quantification and Testing

Heating the CMH and FD concentrates resulted in sediment formation. To measure the amount of sediment, duplicate concentrates heated at 70 °C for 12 days were thoroughly mixed and then centrifuged (in pre-weighed centrifuge tubes) at 4000 × *g* for 15 min using a swinging bucket centrifuge

(Beckman Coulter, CA, USA). The resulting supernatant was decanted, and centrifuge tubes were re-weighed to determine the weight of solids. The solubility of sediments in water and 30% and 50% ethanol was also determined. All of the sediment dissolved in 30% ethanol and was subsequently analyzed for phenolic compounds (as above).

2.6. Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) and repeated measures ANOVA using Minitab (State College, PA, USA). Tukey-HSD was used for mean comparisons of the treatments. The level of significance was set at $\alpha = 0.05$.

3. Results and Discussion

3.1. Red Color Stability

All color and phenolic compound composition data (with the exception of color ratios and sediment data) from the accelerated color stability trials were normalized to 68 °Brix to account for differences in the initial °Brix values of CMH and FD concentrates. Color stability testing showed that the CMH concentrate had greater red color stability compared to the FD concentrate (Table 1). After nine days at elevated temperature, FD concentrate had lost 3.0% to 4.8% more of its original color, depending on temperature.

Table 1. Percentage change in red, violet, and brown color of concentrate derived from commercial must heating (CMH) vs. flash détente (FD) after nine days of accelerated aging (at 50, 60, and 70 °C).

Concentrate	Accelerated Aging Temperature								
	50 °C			60 °C			70 °C		
	Red	Violet	Brown	Red	Violet	Brown	Red	Violet	Brown
CMH	−47.3	−11.0	−17.0	−64.3	−21.9	−3.0	−69.4	−41.2	77.4
FD	−52.1	−10.7	−22.9	−68.8	−24.2	6.9	−72.4	−39.4	74.6

Values are means from two replicates.

Color and phenolic compound composition data (with the exception of color ratios) from the accelerated color stability trials were normalized by calculating the impact of three, six, and nine days of heat exposure as the percentage change from their initial concentrations, to account for differences in the initial composition of CMH and FD concentrates. Repeated measures ANOVA of color data normalized to 68 °Brix and relative to the initial concentrate composition confirmed that CMH concentrate retained significantly more red color after nine days of accelerated aging at 50 °C (Table 2). The CMH concentrate had a 20% higher concentration of the more stable pigmented polymers (114 vs. 95 mg/L at 68 °Brix). Treating FD concentrate with acetaldehyde, lowering the pH, and combining these two treatments increased red color stability at 50 and 60 °C, but not at 70 °C; whereas the addition of seed tannin, either alone or in combination with other treatments, had no significant effect on color stability, regardless of temperature (Figure 1, Table 3). The ineffective red color stabilization role observed for seed tannin in concentrate was consistent with previous studies on wine [29,30], but contrary to another [31] that suggested a beneficial effect of enological tannin addition on color stability. Similar trends were seen for CMH concentrate under the same treatment conditions and temperatures, as demonstrated by the lack of significant interactions between concentrate type and treatment (Table 2).

Lowering the pH or adding acetaldehyde stabilized the red color of concentrates stored at 50 or 60 °C, and combining these treatments gave even greater stabilization due to the increased formation of pigmented polymers (Table 4) and pyranoanthocyanins such as vitisins [32,33]. After nine days at 60 °C, concentrate treated with acid or acetaldehyde had on average 35% to 38% more red color, whereas concentrate from the combined treatment had on average 62% more red color compared to untreated concentrate (Table 3).

Table 2. Change in chemical composition relative to initial values of concentrate derived from commercial must heating (CMH) vs. flash détente (FD) after nine days of accelerated aging (at 50 °C) and *p* values from repeated measures ANOVA.

	Means		***p* Values**							**Model Adjusted R^2**
	CMH	**FD**	**Concentrate Type**	**Treatment**	**Time (days)**	**Concentrate Type × Treatment**	**Concentrate Type × Time**	**Treatment × Time**	**Concentrate Type × Treatment × Time**	
Red color (%)	82.0	79.7	0.0005	0.0005	0.0005	0.201	0.007	0.0005	0.894	98.80%
Brown color (%)	98.5	99.0	0.242	0.0005	0.0005	0.0005	0.140	0.0005	0.0005	97.21%
Violet color (%)	110.1	114.1	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	98.79%
Brown index	0.56	0.49	0.0005	0.0005	0.0005	0.005	0.050	0.0005	0.417	98.00%
Violet index	0.46	0.43	0.0005	0.0005	0.0005	0.001	0.034	0.0005	0.500	98.72%
5-Hydroxymethylfurfural (%)	9300	1900	0.0005	0.0005	0.0005	0.0005	0.0005	0.001	0.062	92.09%
Malvidin-3,5-*O*-diglucoside (%)	68.2	69.6	0.043	0.0005	0.0005	0.940	0.123	0.001	0.806	98.39%
Malvidin-3-*O*-glucoside (%)	52.3	53.0	0.398	0.0005	0.0005	0.961	0.308	0.0005	–	99.04%
Pigmented polymers (%)	131.2	158.2	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	99.36%
Caftaric acid (%)	116.0	109.8	0.0005	0.0005	0.0005	0.018	0.0005	0.007	0.726	93.26%
Epicatechin (%)	38.9	39.2	0.870	0.245	0.0005	0.219	–	–	–	98.48%
Gallic acid (%)	132.9	150.5	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	97.43%
Grape reaction product (%)	88.3	89.6	0.002	0.001	0.0005	0.350	0.132	0.058	0.998	96.92%
Proanthocyanidins (%)	126.8	128.1	0.086	0.0005	0.0005	0.0005	0.0005	0.0005	0.002	98.30%
Quercetin glycosides (%)	87.0	89.2	0.0005	0.0005	0.0005	0.527	0.005	0.0005	0.855	97.14%

Values are means from control and treated samples for each concentrate type. All data normalized to 68 °Brix to enable calculation of percentage change; $\alpha = 0.05$; $n = 2$.

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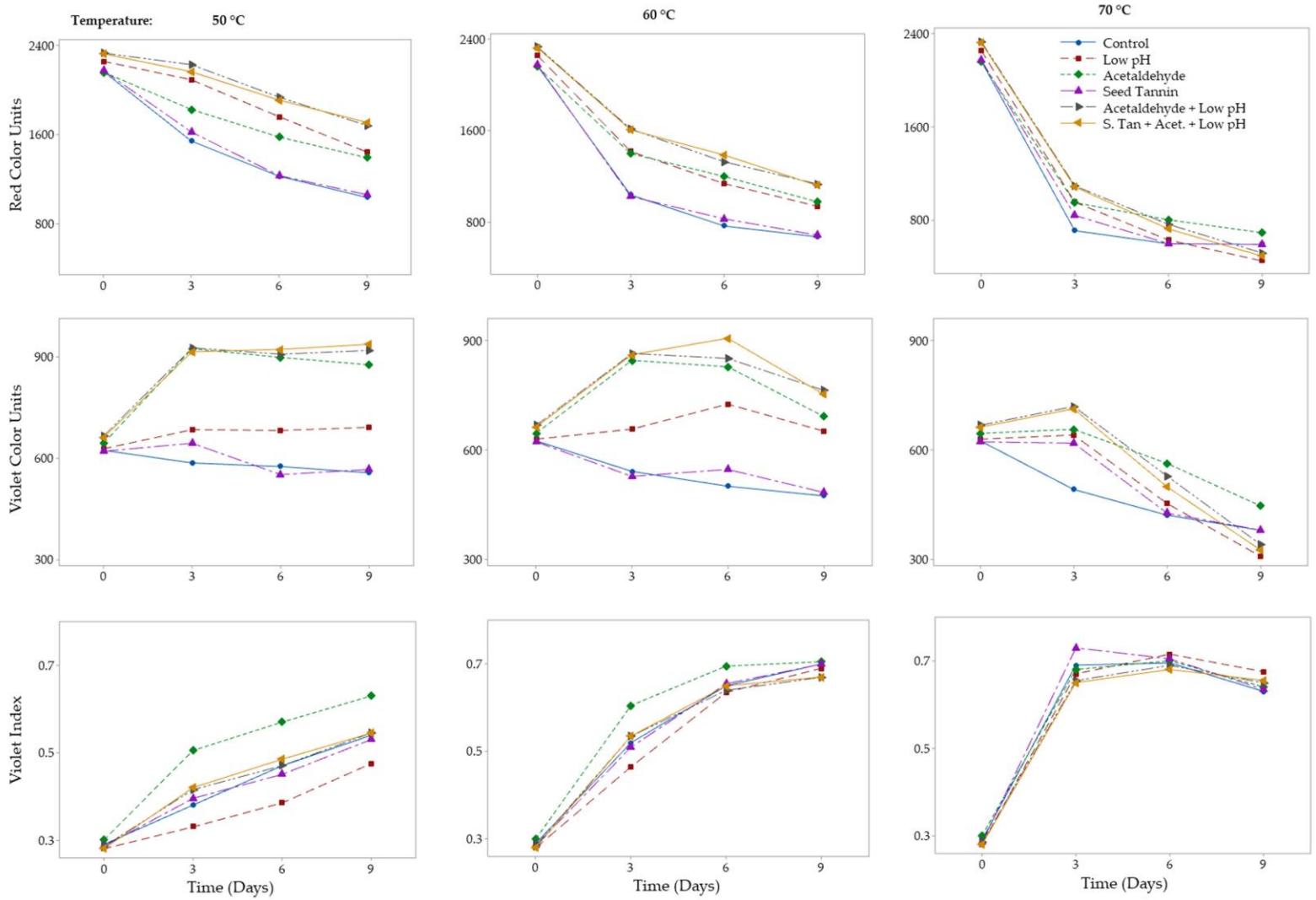

Figure 1. Effect of seed tannin, low pH, and acetaldehyde, individually or in combinations, on red and violet color stability (top and middle rows) and violet color index (bottom row) of Rubired concentrate derived from flash détente, heated at 50 ˚C (left column), 60 ˚C (middle column), and 70 ˚C (right column).

Table 3. Comparison of red, violet, and brown color (normalized to 68 °Brix), and brown index of concentrate derived from commercial must heating (CMH) and flash détente (FD) after treatment with different additives and nine days of accelerated aging at different temperatures.

Treatment	Temperature	CMH				FD			
		Red Color	Violet Color	Brown Color	Brown Index	Red Color	Violet Color	Brown Color	Brown Index
Acetaldehyde + low pH	50 °C	1534 a	865 a	981 a	0.64 c	1685 a	920 a	959 ab	0.57 c
Seed tannin + acetaldehyde + low pH		1531 a	877 a	988 a	0.65 bc	1711 a	937 a	1007 a	0.59 bc
Low pH		1352 b	711 c	860 bc	0.64 c	1445 b	692 b	794 c	0.55 c
Acetaldehyde		1255 c	784 b	911 ab	0.73 ab	1394 b	877 a	934 b	0.67 a
Control		1017 d	569 d	735 d	0.73 ab	1039 c	558 c	655 d	0.63 ab
Seed tannin		1017 d	567 d	773 cd	0.79 a	1063 c	568 c	703 d	0.66 a
Acetaldehyde + low pH	60 °C	1068 a	749 a	1092 a	1.03 d	1136 a	764 a	1053 a	0.93 c
Seed tannin + acetaldehyde + low pH		1026 b	711 b	1051 a	1.03 d	1123 a	752 a	1029 ab	0.92 c
Low pH		895 c	644 c	989 b	1.10 c	942 b	651 b	946 b	1.01 b
Acetaldehyde		905 c	657 c	1046 ab	1.16 b	981 b	692 ab	982 ab	1.00 b
Control		688 d	499 d	913 c	1.33 a	677 c	474 c	790 c	1.17 a
Seed tannin		697 d	502 d	928 c	1.33 a	689 c	483 c	793 c	1.15 a
Acetaldehyde + low pH	70 °C	658 a	421 a	1569 a	2.38 b	698 a	446 a	1497 a	2.15 b
Seed tannin + acetaldehyde + low pH		591 b	376 b	1572 a	2.66 a	599 b	379 b	1482 ab	2.47 a
Low pH		577 b	368 b	1510 b	2.60 a	595 b	379 b	1428 b	2.40 a
Acetaldehyde		452 c	304 c	947 c	2.10 c	523 c	340 c	1007 c	1.93 c
Control		418 cd	277 c	893 d	2.14 c	498 cd	325 c	953 c	1.92 c
Seed tannin		404 d	276 c	919 cd	2.28 b	457 d	308 c	970 c	2.12 b

Values are means from two replicates. Means followed by different letters (within columns, at each temperature) are significantly different; $\alpha = 0.05$; $n = 2$; Tukey's pairwise comparisons.

Table 4. Comparison of phenolic compounds (mg/L, normalized to 68 °Brix) in concentrate derived from flash détente (FD) after nine days of accelerated aging at different temperatures.

Treatment	Temperature	Malvidin-3,5-*O*-diglucoside	Pigmented Polymers	Malvidin-3-*O*-glucoside	Proanthocyanidins	Gallic Acid	Quercetin Glycosides	*trans*-Caftaric Acid	GRP
Control	50 °C	2098 ab	104 b	79 c	1293 b	37 a	154 abc	106 a	44 a
Seed tannin		2142 a	109 b	90 bc	1418 b	41 a	157 ab	108 a	45 a
Acetaldehyde		1801 cd	243 a	29 d	2072 a	36 a	138 d	108 a	43 a
Low pH		1839 bc	139 b	241 a	1541 b	22 b	160 a	100 b	46 a
Acetaldehyde + low pH		1539 de	246 a	135 b	1970 a	21 b	150 bc	100 b	46 a
Seed Tannin + acetaldehyde + low pH		1436 e	253 a	119 bc	2197 a	27 b	145 cd	99 b	45 a
Control	60 °C	431 a	99 d	42 c	1883 e	43 b	83 abc	97 a	29 a
Seed tannin		439 a	101 d	43 c	2030 e	49 a	84 ab	96 a	29 a
Acetaldehyde		377 b	169 b	18 d	2479 d	41 b	73 d	95 a	29 a
Low pH		157 c	149 c	139 a	2965 c	31 cd	88 a	81 b	30 a
Acetaldehyde + low pH		127 c	211 a	72 b	3250 b	28 d	81 bc	81 b	30 a
Seed tannin + acetaldehyde + low pH		124 c	220 a	67 b	3589 a	34 c	79 c	80 b	30 a
Control	70 °C	51 a	148 c	nd	4605 b	36 a	16 a	79 b	28 a
Seed tannin		48 ab	139 d	nd	4637 b	42 a	15 a	82 a	28 a
Acetaldehyde		45 b	183 a	nd	5092 a	36 a	15 a	83 a	28 a
Low pH		30 c	137 d	nd	4462 b	37 a	18 a	58 c	22 b
Acetaldehyde + low pH		29 c	177 a	nd	4910 a	41 a	17 a	59 c	24 ab
Seed Tannin + acetaldehyde + low pH		29 c	164 b	nd	4525 b	44 a	17 a	59 c	22 b

Values are means from two replicates. Means followed by different letters (within columns, at each temperature) are significantly different; $\alpha = 0.05$; $n = 2$; Tukey's pairwise comparisons. All data normalized to 68 °Brix. nd, not detected.

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At 70 °C, only acetaldehyde was effective at stabilizing red color. In contrast, all pH-adjusted concentrates had significantly lower red color units than their corresponding control, regardless of other treatments. The result at 70 °C under low pH conditions was in contrast to the outcomes at 50 or 60 °C and most likely due to acid hydrolysis of red pigments, which is favored at higher temperature and longer treatment duration [34]. This seemingly reversed the beneficial effect of low pH on color stability that was initially seen through increased pigmented polymer formation up to day six (data not shown). As such, anthocyanins were likely to have hydrolyzed into their less stable anthocyanidins [35], which degraded at a faster rate and resulted in less red color compared to the control.

3.2. Violet Color Stability

Violet color units decreased in control FD and CMH concentrates after nine days of accelerated aging, with the extent of color loss increasing with increased temperature (Table 1). Repeated measures ANOVA showed that the overall violet color units for all treatments combined (i.e., low pH, and acetaldehyde and seed tannin additions) and control significantly increased with accelerated aging (i.e., relative to time zero) to a greater extent in FD concentrate compared to CMH concentrate (Table 2). Seed tannin had no effect on violet color formation or stability, regardless of temperature (Table 3). Tannin-only treatments and controls exhibited violet color loss at all temperatures, whereas low pH, acetaldehyde, and combinations of these treatments showed violet color increases at 50 and 60 °C, and only exhibited color loss at 70 °C (Table 3). Unlike at 50 and 60 °C, where violet color markedly increased during the first three days of aging for combined low pH and acetaldehyde treatments, less of an increase was seen at 70 °C, likely due to the rapid loss of anthocyanins at the higher temperature (Figure 1).

In a similar pattern to red color, low pH, acetaldehyde addition, and a combination of these treatments gave 24–38%, 32–57% and 50–66% higher violet color units, respectively, compared to the control after nine days of accelerated aging at 50 or 60 °C (Table 3). Likewise, violet color units decreased 10–27% compared to the control for all three low pH treatments but increased 12–18% in acetaldehyde-only treatments after nine days of heating at 70 °C for both CMH and FD concentrates (Table 3). Acetaldehyde reacts with anthocyanins to form the more stable pyranoanthocyanin vitisin B [36,37], which has been reported to have greater stability [38] and contributes 11–14 times more color compared to unmodified anthocyanins due to vitisins having higher extinction coefficients [32]. Pyranoanthocyanin formation arising from reaction with acetaldehyde likely resulted in greater violet and red color expression due to a bathochromic shift in λ_{max}, and the relatively high concentrate pH of ~4.0 would have favored pyranoathocyanin color expression over that of anthocyanins.

CMH concentrates had significantly higher violet to red color ratio (violet index) compared to FD concentrates after accelerated aging (Table 2). The violet index increased for all treatments involving acetaldehyde and/or low pH due to the combination of red color loss and violet color formation (Figure 1). For control and seed tannin concentrates, the rate of violet color loss was slower than for red color loss, thereby increasing the violet index. At 70 °C, the violet index plateaued for all treatments after three days of heating (Figure 1). Thereafter, the violet index remained fairly constant, potentially indicating a similar rate of violet and red color degradation. After three days of heating at 70 °C, most of the anthocyanins present in the concentrate had either been converted into non-red compounds or more stable pigments such as pigmented polymers (Table 4) and pyranoanthocyanins. This slowed red color degradation to that of violet color loss, resulting in a fairly constant violet index from three to nine days of aging (Figure 1). Red color loss from untreated concentrate was much higher than violet color loss after nine days of accelerated aging (Table 1). As such, using low pH, the addition of acetaldehyde, or a combination of these treatments to promote conversion of anthocyanins to more stable pigmented polymers and pyranoanthocyanins may provide a strategy for improving the color stability of Rubired concentrate.

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3.3. Anthocyanins and Pigmented Polymers

Loss of malvidin-3,5-*O*-diglucoside, the most abundant anthocyanin in Rubired juice and concentrate [39], was significantly lower in FD concentrate compared to CMH concentrate (Table 2). The concentrate type by treatment interaction was not significant, suggesting the chemical treatments impacted the two concentrate types in a similar manner. There was no significant difference between malvidin-3-*O*-glucoside degradation in CMH and FD concentrates.

Concentrate storage temperature and acetaldehyde or acid treatment affected anthocyanin loss. After nine days of heating at 50, 60, and 70 °C, low pH and acetaldehyde-treated concentrates had similarly lower malvidin-3,5-*O*-diglucoside concentrations compared to the control, likely due to a faster rate of conversion of anthocyanins into pyranoanthocyanins and polymeric pigments [32]. As noted above, seed tannin had no effect on color parameters (Table 3). Pigmented polymer concentrations showed an opposing trend to anthocyanins, with acetaldehyde treatment giving significantly higher concentrations after nine days of accelerated aging at all three temperatures, compared to the low pH and seed tannin treatments and the control (Table 4).

Unlike treatments involving acetaldehyde addition only, treatments with low pH only did not increase polymeric pigment concentration to the same extent, despite being as effective in preserving red color at 50 and 60 °C (Table 4). This suggests that the mechanism for color stabilization due to low pH was different from acetaldehyde color stabilization. Anthocyanin conversion at low pH could be attributable to direct condensation with flavan-3-ols and tannins, whereas acetaldehyde treatment could permit both direct condensation and acetaldehyde-mediated condensation reactions. At 70 °C, anthocyanin hydrolysis at low pH appeared to be the cause of significant anthocyanin and red color loss in low pH only treatments, compared to the control (Table 4).

Malvidin-3-*O*-glucoside was present at a much lower concentration in Rubired concentrate, approximating 5% that of malvidin-3,5-*O*-diglucoside. The concentration of malvidin-3-*O*- glucoside showed different trends to the diglucoside at 50 and 60 °C. Low pH increased malvidin-3,5-*O*-diglucoside reactivity while decreasing reactivity of the less stable malvidin-3-*O*-glucoside. All treatments at low pH had significantly higher malvidin-3-*O*-glucoside concentrations compared to the control and seed tannin treatment, with the acetaldehyde treatment giving the lowest concentration, likely due to pyranoanthocyanin-forming reactions (Table 4).

The increased pigmented polymer concentration in FD concentrate (~160%) compared to CMH concentrate (~130%) after heat treatment (Table 2) could be attributed to FD concentrate having a higher proportion of color in anthocyanin form. The difference in pigmented polymer increase between the two concentrates cannot be explained by malvidin-3,5-*O*-diglucoside and malvidin-3-*O*-glucoside loss, as these were fairly similar. However, conversion of other major anthocyanins in Rubired such as 3,5-diglucosides of peonidin and petunidin, and delphinidin mono and diglucosides [39,40] may possibly explain the differences in pigmented polymer formation. Indeed, the concentrate type by treatment interaction for pigmented polymer formation was statistically significant (Table 2). All treatments involving acetaldehyde addition had about double the pigmented polymer concentration of the control, seed tannin, and low pH treatments. In addition, acetaldehyde treatments had greater increases in pigmented polymer concentrations in FD concentrate compared to CMH concentrate (Figure 2). The FD concentrate initially had higher anthocyanin and lower pigmented polymer concentrations compared to CMH concentrate, and the lower pigmented polymer formation in CMH concentrate may point to equilibrium effects limiting anthocyanin conversion into more stable pigments.

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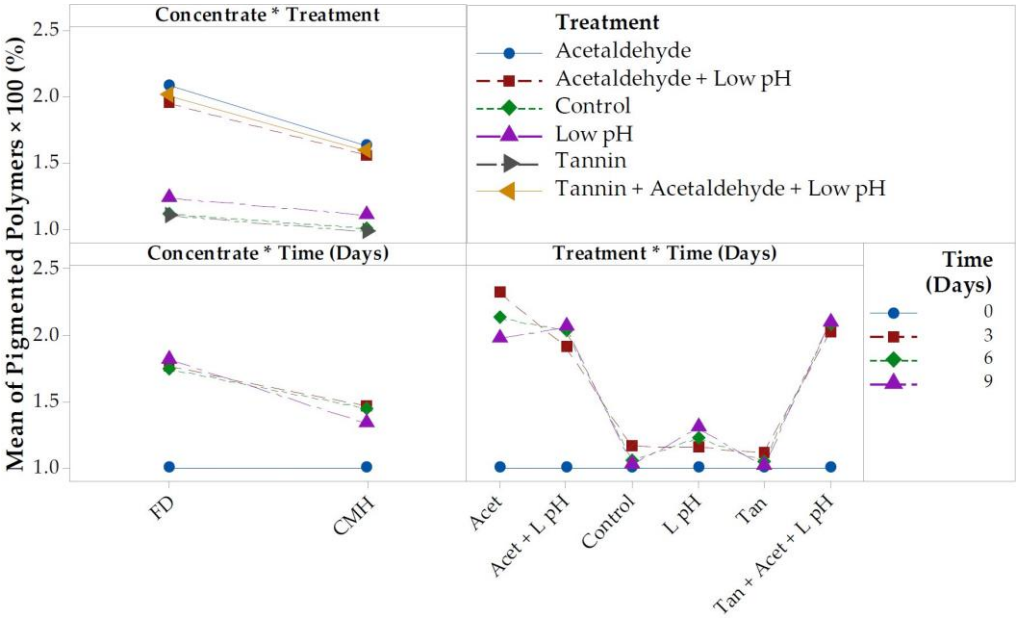

Figure 2. Fitted means for interaction plot for pigmented polymers in Rubired concentrate from conventional must heating (CMH) vs. flash détente (FD) heated at 50 ˚C; $\alpha = 0.05$; $n = 2$. All data normalized to 68 ˚Brix and to the initial concentration to enable calculation of percentage change.

3.4. Brown Color Evolution

Brown color formation during accelerated aging was greatly influenced by storage temperature (Table 1), acetaldehyde treatment, and pH, whereas seed tannin and concentrate type had no effect (Tables 2 and 3). Brown color units exhibited different trends depending on storage temperature. After nine days of accelerated aging, control CMH and FD concentrates had decreased in brown color at 50 ˚C by 17–23%, whereas they had increased by 75–77% at 70 ˚C (Table 1).

All treatments involving acetaldehyde addition significantly increased brown color units in both CMH and FD concentrates after nine days of heating at 50 or 60 ˚C, but not at 70 ˚C, where only treatment with acetaldehyde alone gave a similar effect (Table 3). This was consistent with findings in model wine showing that acetaldehyde reacted with flavan-3-ols, which are known to be present in grape concentrate, to form yellowish flavanol-ethyl-flavanol adducts [41]. In addition to brown color arising from such adducts, anthocyanins can react with acetaldehyde to form pyranoanthocyanins such as vitisins [37] as indicated earlier, which are red pigments with a higher proportion of brown color compared to anthocyanins [38]. At 50 or 60 ˚C, the observed increase in brown color of low pH concentrate (Table 3) potentially arose from the reaction of flavan-3-ols with glyoxylic acid arising from oxidation of tartaric acid to form a carboxymethine bridge-linked adduct that can undergo further reaction to form a yellowish xanthylium compound [42]. This resulted in intermediate brown color units compared to seed tannin treatment and control that had the lowest brown color units after nine days of accelerated aging.

Compared to the initial color, brown color units increased in all treatments involving acetaldehyde addition and low pH but decreased in control and tannin treated concentrates after heating at 50 and 60 ˚C (Figure 3). At 70 ˚C, brown color units in concentrates from all low pH treatments initially increased, but then started to decrease after three days of accelerated aging, resulting in significantly lower brown coloration (Table 3). It is postulated that brown color loss in all low pH treatments heated at 70 ˚C was due to acid hydrolysis of brown colored flavanol-ethyl-flavanol adducts that have previously been reported to be susceptible to acid hydrolysis [43].

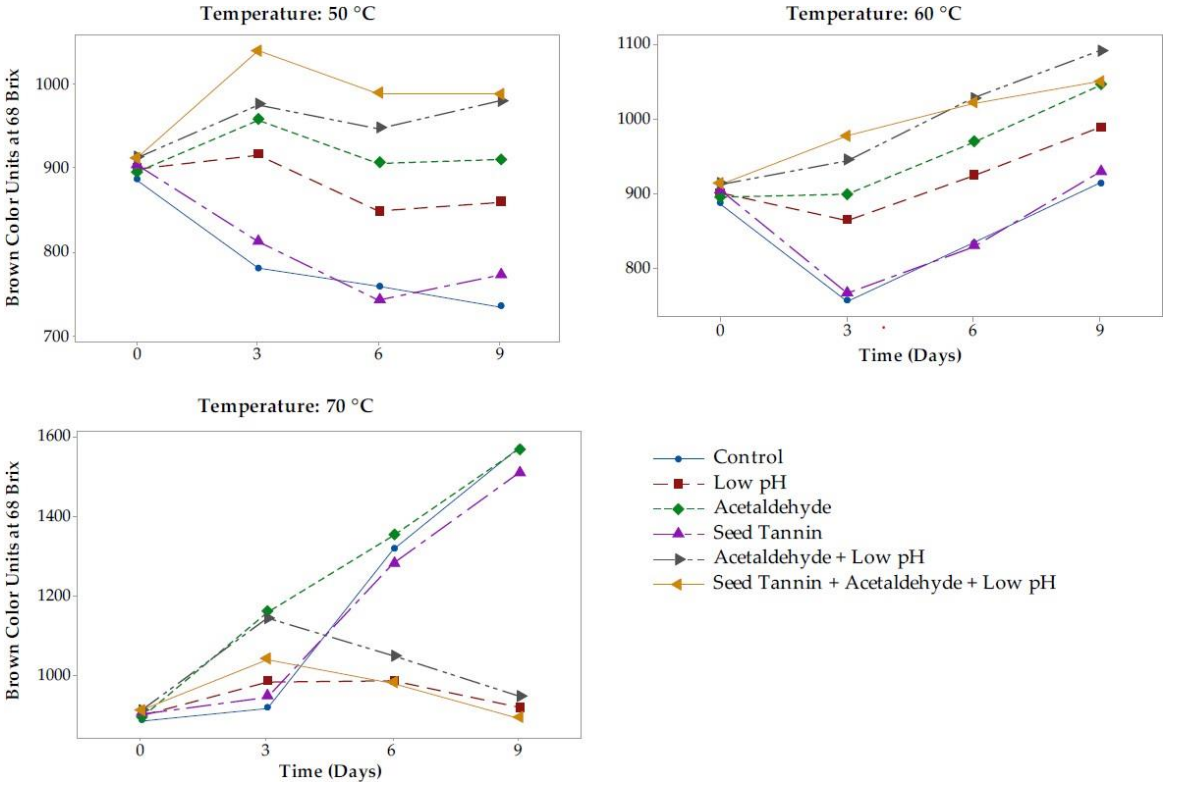

Figure 3. Effect of seed tannin, low pH, and acetaldehyde on brown color evolution in Rubired concentrate from conventional must heating (CMH) at different temperatures. All data normalized to 68 °Brix.

The trend for the brown index (ratio of brown to red color) was very consistent for the two types of concentrates at each of the temperatures studied (Table 3). Seed tannin treatments and controls gave the highest brown index, followed by acetaldehyde addition only, with low pH treatments giving the lowest ratios. A significantly greater decrease in red color (compared to brown color) had the effect of increasing the brown index in tannin treatments and controls during accelerated aging.

Low pH treatments with acetaldehyde addition had a significantly lower brown index, despite having significantly higher brown color units at 50 and 60 °C (Table 3). This was due to the increase in brown color units in combined low pH and acetaldehyde treatments being counteracted by a greater increase in red color units, due to the increased formation of the more stable pyranoanthocyanins and pigmented polymers (Table 4). On the other hand, the lower brown index in all low pH treatments at 70 °C was mainly associated with the loss of brown color.

Different browning mechanisms appear to be at play, depending on temperature and pH. Control and seed tannin treatments, which were at a normal Rubired concentrate pH of 4.0, developed less brown color after heating at 50 and 60 °C compared to other treatments involving acetaldehyde addition or low pH. This suggests that brown color formation at these temperatures was driven by acid-catalyzed pyranoanthocyanin and xanthylium compound formation and not by caramelization or the Maillard reaction, since the rate of brown color formation for the two latter reactions increases with pH [44–46]. The two-fold increases in brown color observed in concentrates at their original pH and after heating at 70 °C may be largely due to caramelization of sugars at the higher temperature, whereas no significant caramelization appears to take place below 55 °C [47].

In addition to the brown pigments formed via the Maillard reaction and caramelization (i.e., melanoidins), furfural and 5-hydroxymethylfurfural (5-MHF) derived from these reactions may have further contributed to browning due to their reaction with the flavanols present in the concentrate, forming yellow-orange xanthylium salts [48].

The complexity of grape juice means that some inferences have to be made, and the broader food chemistry knowledge needs to be drawn upon to rationalize the outcomes. To further understand the extraction process and color stabilization phenomena, work was undertaken to assess the impact of treatments on other concentrate constituents that may potentially affect color, quality, and product safety. This involved quantifying 5-HMF, caftaric acid, grape reaction product (GRP), proanthocyanidins, gallic acid, and quercetin glycosides during aging.

3.5. Impact of Treatments on Concentrate Quality Indicators

3.5.1. 5-Hydroxymethylfurfural (5-HMF) Formation

5-HMF concentrations increased to a significantly greater extent in CMH concentrate compared to FD concentrate during accelerated aging (Table 2). It was hypothesized that the longer heat exposure during CMH concentrate production generated more 5-HMF precursors, leading to a much faster rate of 5-HMF formation during aging (Figure S2). Low pH significantly increased 5-HMF formation at all temperatures studied, whether from caramelization or the Maillard reaction, in agreement with previous research [49]. In contrast, acetaldehyde and seed tannin had no effect on 5-HMF formation (Table S1).

All low pH concentrates had on average approximately two times the concentration of 5-HMF than concentrates with unadjusted pH, following accelerated aging at 50, 60, and 70 °C (Figure S3). For all low pH treatments, 5-HMF formation was positively correlated with brown color at 50 and 60 °C, but negatively correlated at 70 °C, with the latter being consistent with previous research [50].

Although the combined treatment involving low pH and acetaldehyde addition was most effective at preserving red color, this also resulted in significantly higher 5-HMF concentrations irrespective of temperature, and at 50 and 60 °C, higher brown color units as outlined above in Section 3.4. High brown color units are detrimental to concentrate quality, and higher 5-HMF concentrations are undesirable because of potential in vivo conversion to 5-sulfoxymethylfurfural, which is a known genotoxin [51]. Additionally, some countries (e.g., in the European Union) impose limits on the allowable concentration of 5-HMF in grape juice concentrate. Formation of 5-HMF will likely be a concern where concentrate is stored for extended periods of time before use. Further research is therefore needed to determine methods for stabilizing red grape color without increasing the brown color or 5-HMF concentration of concentrate.

3.5.2. *trans*-Caftaric Acid and 2-*S*-Glutathionyl Caftaric Acid (Grape Reaction Product)

Caftaric acid concentrations were monitored to compare the oxidative state of the two concentrates, as well as to determine if enzymatic oxidation played a role in brown color formation during aging. FD concentrate initially contained ~four-fold higher caftaric acid concentrations than CMH-derived concentrate, suggesting that the higher temperature treatment for a shorter duration of time used to produce FD resulted in less oxidation than the lower temperature treatment for a longer duration of time employed during CMH production. The caftaric acid levels in the control concentrate generally showed an increasing trend during accelerated aging, suggesting that enzymatic oxidation by polyphenol oxidase did not play a role in brown color formation, as previously reported [52], due to heat inactivation of oxidative enzymes [53].

Seed tannin and acetaldehyde treatments did not significantly impact caftaric acid concentrations (Table 4), while the low pH treatments gave lower caftaric acid concentrations (compared to controls) after nine days of accelerated aging, possibly due to acid hydrolysis to give caffeic and tartaric acids [54]. GRP concentrations were not affected by any of the treatments after heating at 50 and 60 °C, but the combination of low pH and high temperature decreased the GRP concentration at 70 °C (Table 4), likely due to acid hydrolysis of 2-*S*-glutathionyl caftaric acid into 2-*S*-glutathionyl caffeic acid [54].

The caftaric acid trend for all low pH treatments at 70 °C (Figure S4) was similar to that observed for brown color. Although the caftaric acid concentration initially increased (i.e., up until day three), the concentration dropped to its initial concentration by day nine. This suggested a similar reaction mechanism, i.e., acid hydrolysis, may have contributed to the decrease in both brown color and caftaric acid concentrations.

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3.5.3. Proanthocyanidin, Gallic Acid, and Quercetin Glycosides

Proanthocyanidin and gallic acid concentrations increased during accelerated aging (Figures S5, S6), potentially due to polymerization of monomeric and oligomeric flavan-3-ols [55] and hydrolysis of gallate esters [56], respectively. There were no significant differences between the proanthocyanidin concentrations of CMH and FD concentrates, but there was a general increase with increasing temperature for all treatments (Figure S5). Low pH and acetaldehyde treatments, either individually or in combination, gave higher proanthocyanidin concentrations after nine days of heating at 50 or 60 °C, while seed tannin additions had less impact (Table 4). Proanthocyanidin concentrations in the low pH treatments were lower than the control after nine days of heating at 70 °C, possibly due to increased acid hydrolysis at the higher temperature [57]. Seed tannin treatments gave higher or similar gallic acid concentrations compared to the control (Table 4), whereas low pH treatments gave lower gallic acid concentrations at 50 and 60 °C. At 70 °C, gallic acid concentrations increased and plateaued in all concentrates after three days of heating, decreasing thereafter possibly due to thermal degradation to yield no significant difference amongst treatments (Table 4, Figure S6). The concentration of quercetin glycosides, a co-pigmentation cofactor with the potential to influence red color intensity and stability [58,59], was monitored during aging. Quercetin glycoside concentrations decreased during accelerated aging of all treatments, possibly due to acid hydrolysis (Table 2).

3.5.4. Sediment Formation

Heating the concentrates to accelerate the aging process resulted in sediment formation in all concentrates. The quantity and composition of sediment was therefore measured to determine if precipitation accounted for some of the losses in color and phenolic compounds that were observed during heat treatment. The sediments were insoluble in water, but soluble in 30% aqueous ethanol. Significantly higher sediment formation was observed at 70 °C, compared to 50 and 60 °C, with treatments involving low pH giving three times the quantity of solids as that for control, seed tannin, and acetaldehyde treatments (Figure 4).

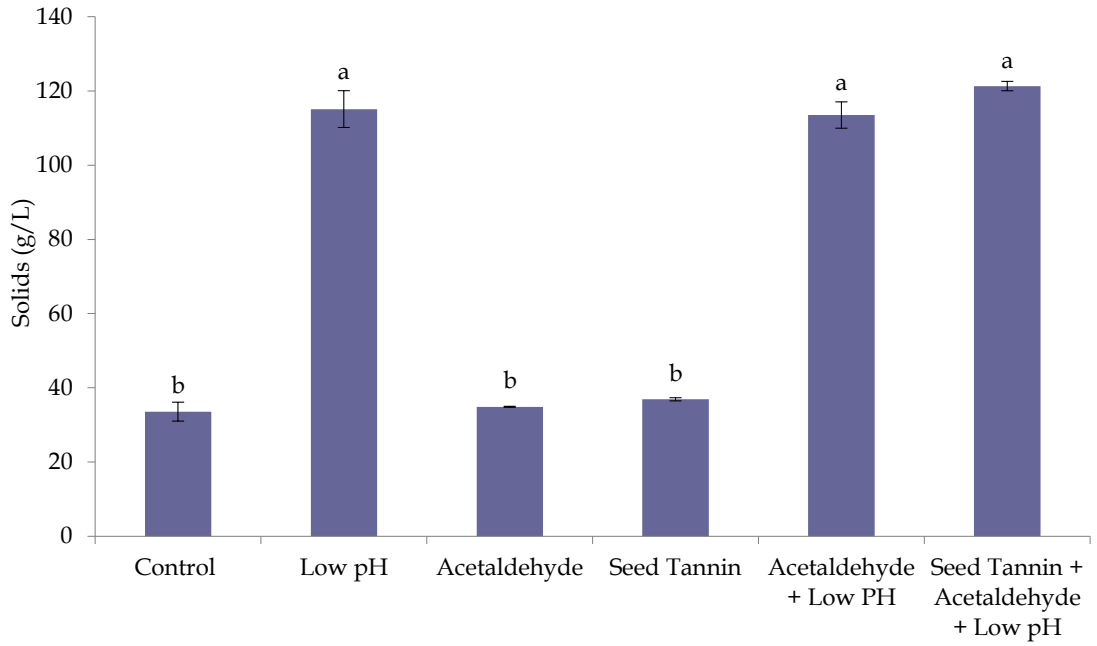

Figure 4. Precipitate formation in Rubired concentrate derived from conventional must heating (CMH) after 12 days of accelerated aging at 70 °C. Values are means of two replicates ± standard deviation; bars sharing the same letter are not significantly different; $\alpha = 0.05$; $n = 2$; Tukey pairwise comparisons.

There was no significant difference in sediment red and brown coloration amongst treatments (Table S2), suggesting precipitation did not account for the color losses observed for concentrates (Table 3). The brown color of sediments was positively correlated to sediment mass (Pearson's r = 0.79). Together these results suggest that the brown color of sediments was due to adsorption to solid particles and not the result of brown color precipitation driving sediment formation.

Phenolic compounds in sediments represented less than 10% of the amount determined for the concentrates. Sediment collected from 50 mL samples of concentrate after 12 days of heating at 70 °C had relatively small amounts of total tannin (27–65 mg), proanthocyanidins (7–18 mg), and pigmented polymers (1 mg), with the lowest amounts observed for control concentrate, and the highest amounts for treatments involving both low pH and acetaldehyde addition (Table S2). Concentrations of malvidin-3-*O*-glucoside, catechin, epicatechin, gallic acid, caftaric acid, and quercetin glycosides were below the detection limits in all sediments. The higher amounts of phenolic compounds from all low pH treatments that had higher red color stability suggested that phenolic precipitation had no effect on color retention in concentrates. The presence of tannin in precipitates was consistent with findings reported by other researchers [55], who showed acetaldehyde-mediated crosslinking of catechin increased the mean degree of polymerization (MDP) of catechin-catechin polymers, leading to some tannin precipitation. Similar to findings from the current study, the precipitates were reported to be ethanol soluble, but water insoluble [50].

The concentration of 5-HMF in sediment was positively correlated (Pearson's r = 0.99) to sediment mass, and ranged from 12–81 mg (Table S2), with seed tannin and acetaldehyde treatments having 5-HMF masses that were ~1.5 times that of the control, whereas all low pH treatments had amounts that were ~6.4 times greater, on average. As a result, sediments from low pH concentrates had around twice the 5-HMF concentration (i.e., g of 5-HMF/g of sediment) of control sediment, despite having ~3 times the mass of sediment than the control. It is therefore likely that sediment formation was linked to caramelization and the Maillard reaction, whose mechanisms follow different pathways depending on pH and temperature [49].

3.6. Color Degradation Kinetics

Reaction orders, rate constants, calculated activation energies, half-lives, and Q10 values were determined during accelerating aging (Table 5). Reactions were first order for red color, malvidin-3,5-*O*-diglucoside and malvidin-3-*O*-glucoside degradation, and zero order for browning index and violet color formation. The reaction order determined for anthocyanins was consistent with the reaction order that has been reported for malvidin-3-*O*-glucoside in model systems [59]. Proanthocyanidin formation followed zero order kinetics while quercetin glycoside degradation followed first order reaction kinetics. Additionally, red color in CMH concentrate was more stable at 50 °C, as shown by a half-life of 233.9 h and activation energy of 65.2 kJ/mol compared with 203.3 h and 59.2 kJ/mol for FD concentrate (Table 5).

The reaction orders for brown color and pigmented polymer formation were indeterminate, as differing trends were observed at the different temperatures studied. Brown color units decreased at 50 and 60 °C but showed an increasing trend at 70 °C. On the other hand, pigmented polymer concentration was unchanged at 50 and 60 °C, but increased at 70 °C. As a result, pigmented polymer formation did not conform to any simple reaction order. Brown index followed zero order kinetics. Other researchers [47] have also reported zero order kinetics for brown color formation in pekmez (a molasses-like syrup made with grape juice) stored at 55, 65, and 75 °C for 10 days at pH4.0.

Table 5. Kinetic parameter data for color, phenolic compounds, and 5-hydroxymethylfurfural (5-HMF) for concentrates derived from conventional must heating (CMH) and flash détente (FD) concentrates after nine days of accelerated aging at 50 °C.

	Concentrate Type	Reaction Order	Rate Constant (k) at 50 °C	Half Life (h) at 50 °C	Activation Energy (kJ/mol)	Q10
Red color	CMH	1	4.9×10^{-5}	233.9	65.2	1.61
	FD		5.7×10^{-5}	203.3	59.2	1.58
Brown index	CMH	0	2.0×10^{-5}	187.5	224.7	3.26
	FD		1.9×10^{-5}	175.5	229.3	3.25
Violet color	CMH	0	5.4×10^{-3}	992.4	141.3	2.02
	FD		5.1×10^{-3}	1012.0	138.4	2.27
Malvidin-3,5-O-diglucoside	CMH	1	7.2×10^{-5}	161.2	171.7	2.87
	FD		6.6×10^{-5}	175.1	177.8	2.85
Malvidin-3-O-glucoside	CMH	1	1.8×10^{-4}	65.4	-	2.93
	FD		1.8×10^{-4}	63.6	-	2.66
Quercetin glycosides	CMH	1	2.6×10^{-5}	437.1	213.3	2.70
	FD		2.2×10^{-5}	531.2	233.5	3.17
Proanthocyanidins	CMH	0	2.5×10^{-2}	421.8	240.3	2.86
	FD		1.8×10^{-2}	509.3	291.9	3.61

4. Conclusions

This research showed that FD concentrate had lower color stability compared to CMH concentrate, making it less desirable for applications where high color stability is a key requirement. On the other hand, FD concentrate had significantly lower 5-HMF formation, which might make it more suitable in low pH, long shelf-life food and beverage applications where 5-HMF formation may be a concern. The study also demonstrated beneficial red and violet color stabilization effects due to treatments involving low pH and acetaldehyde addition to Rubired concentrate, with an additive effect observed, which appeared to follow different mechanisms when these treatments were combined. However, the downside to low pH and acetaldehyde addition was increased brown color, with the former also increasing 5-HMF and sediment formation. The net effect to color quality was positive, because these treatments decreased the ratio of brown to red color. In contrast, seed tannin had no effect on red or violet color stability or brown color formation, regardless of the temperature used to accelerate aging.

It was evident from this work that the ideal treatment conditions for Rubired color stabilization are temperature dependent. When deciding on a treatment, consideration should be given to the level of heating that juice and concentrate will be subjected to during production, or as an ingredient in a food or beverage product during subsequent processing. Consideration should also be given to the acidity of foods and beverages, and to the temperature and duration of storage of the concentrate, as well as that of foods and beverages after colorant addition.

Whereas some mechanisms have been proposed for red color loss to explain how low pH and acetaldehyde might increase red color stability and brown color formation, the mechanism of brown color loss is not well understood. Research is therefore needed with the aim of developing solutions for remediating browning in concentrates, which is generally considered detrimental to quality. This is in addition to studies that provide red color stabilization without increasing brown coloration in juice concentrates to determine if findings from this research can be implemented under normal juice and concentrate production and storage conditions. Finally, research will be required to understand consumer acceptability of concentrates and juices with a higher violet to red color ratio due to acetaldehyde treatment.

Supplementary Materials: The following are available online at http://www.mdpi.com/2304-8158/9/9/1270/s1, Figure S1. Flowchart of conventional must heating vs. flash détente processes for production of Rubired concentrate; Figure S2. Fitted means for interaction plot for 5-hydroxymethylfurfural (5-HMF) in Rubired concentrate from conventional must heating (CMH) vs. flash détente (FD), heated at 50 °C; $\alpha = 0.05$; $n = 2$. All data normalized to 68 °Brix and to initial concentration to enable calculation of percentage change; Figure S3. Effect of seed tannin, low pH, and acetaldehyde on 5-hydroxymethylfurfural (5-HMF) formation in Rubired concentrate from conventional must heating (CMH) at different temperatures. All data normalized to 68 °Brix. Note the different y-axis scales; Figure S4. Effect of seed tannin, low pH, and acetaldehyde on caftaric acid concentration in Rubired concentrate from conventional must heating (CMH) at different temperatures. All data normalized to 68 °Brix; Figure S5. Change in proanthocyanidin concentration during accelerated aging of Rubired concentrate from conventional must heating (CMH) at different temperatures. Note the different y-axis scales; Figure S6. Change in gallic acid concentration during accelerated aging of Rubired concentrate from conventional must heating (CMH) at different temperatures. All data normalized to 68 °Brix. Note the different y-axis scales; Table S1. Comparison of 5-hydroxymethylfurfural (5-HMF) concentrations in Rubired concentrate from conventional must heating (CMH) vs. flash détente (FD), after nine days of accelerated aging at different temperatures; Table S2. Sediment composition of concentrate from conventional must heating after 12 days of accelerated aging at 70 °C.

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Supplementary Materials For

Impact of Juice Extraction Method (Flash Détente vs. Conventional Must Heating) and Chemical Treatments on Color Stability of Rubired Juice Concentrates under Accelerated Aging Conditions

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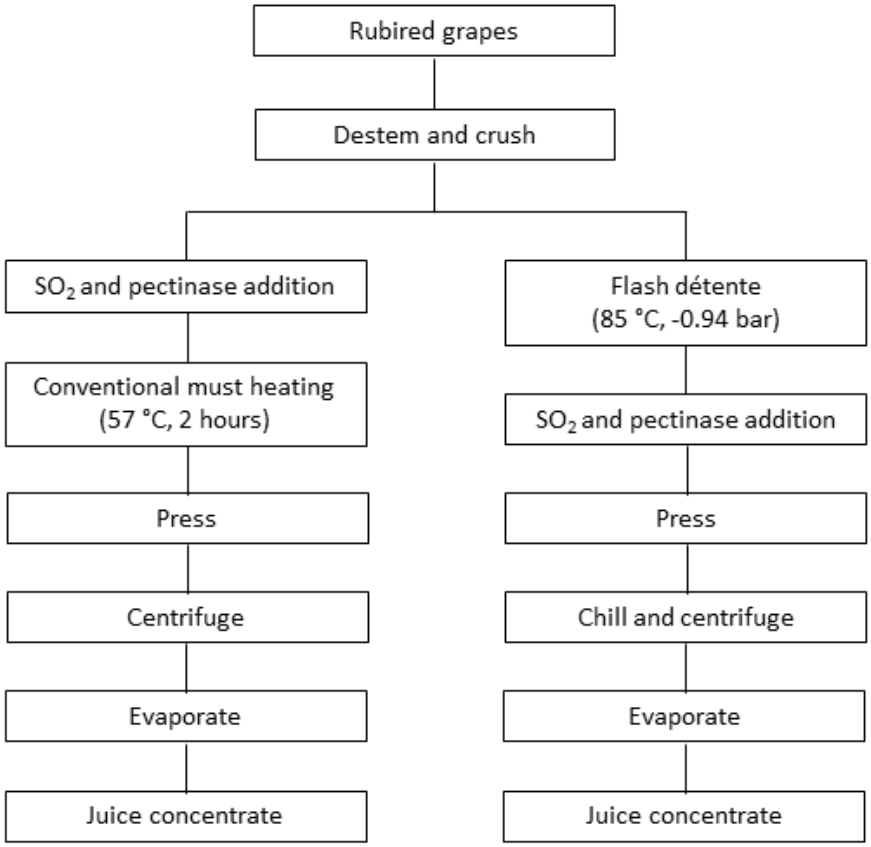
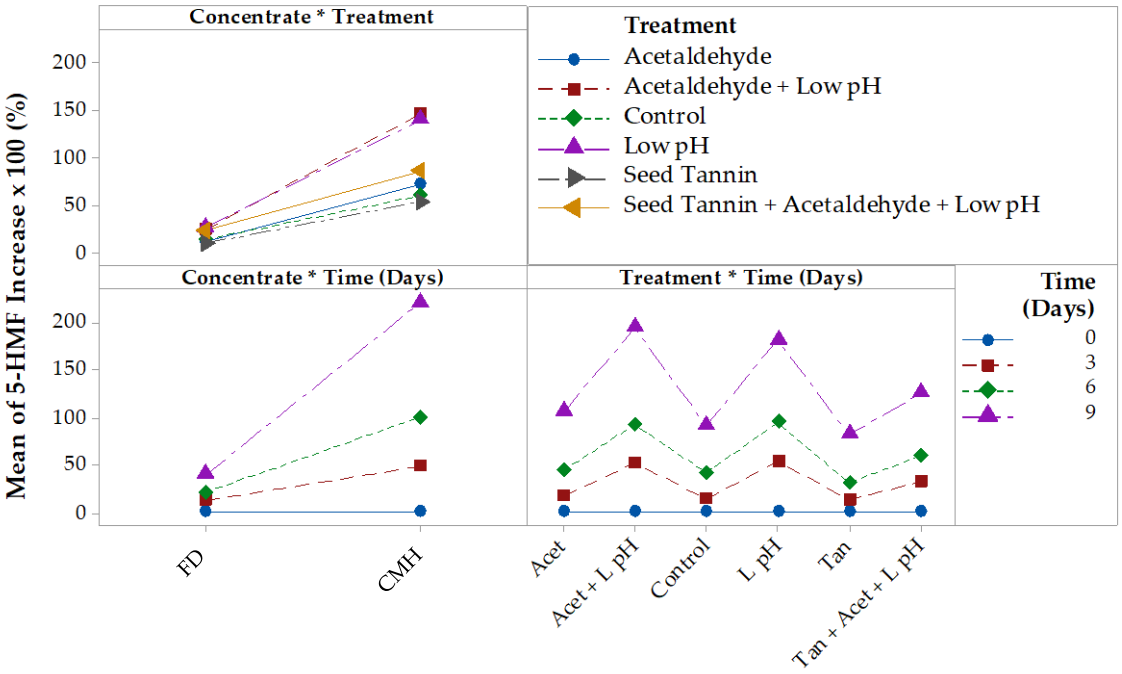


Figure S1. Flowchart of conventional must heating vs. flash détente processes for production of Rubired concentrate.



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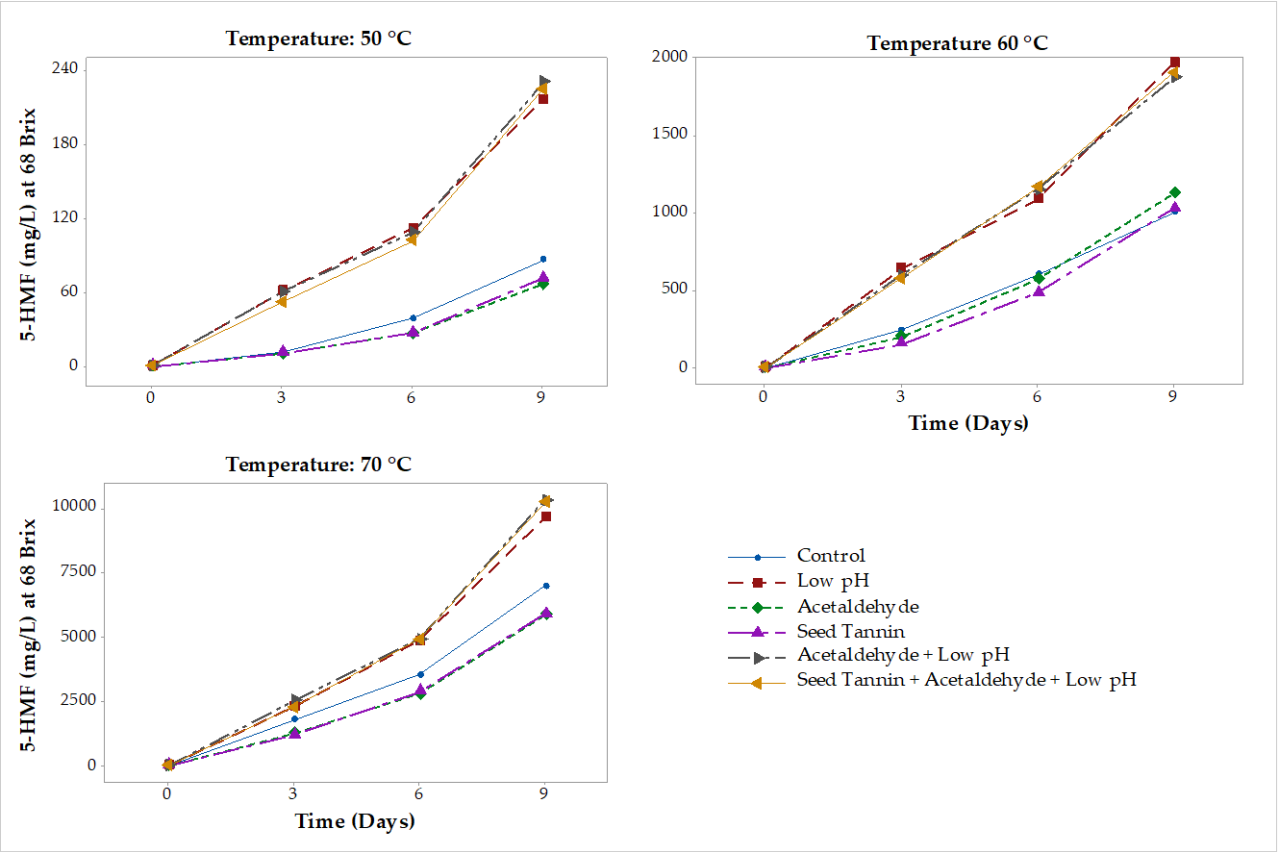

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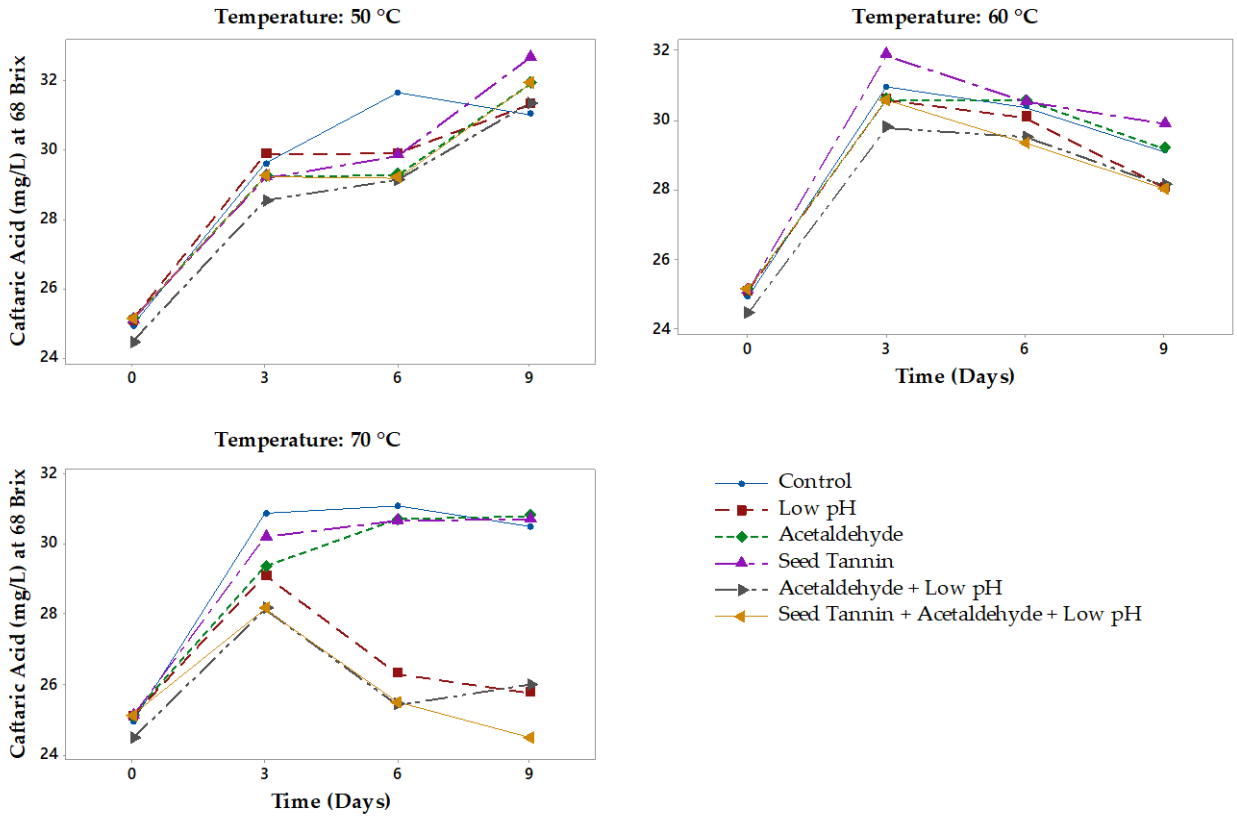

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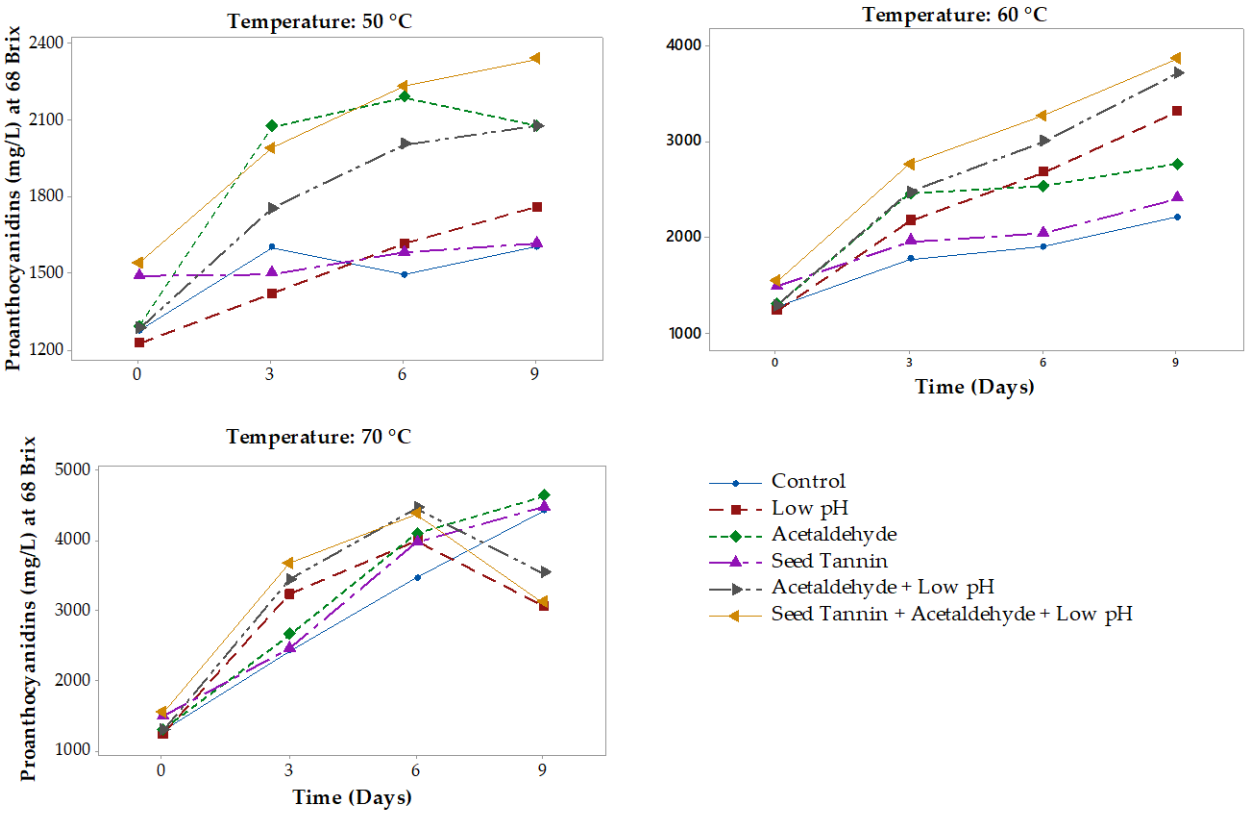

Figure S5. Change in proanthocyanidin concentration during accelerated aging of Rubired concentrate from conventional must heating (CMH) at different temperatures. All data normalized to 68 °Brix. Note the different y-axis scales.

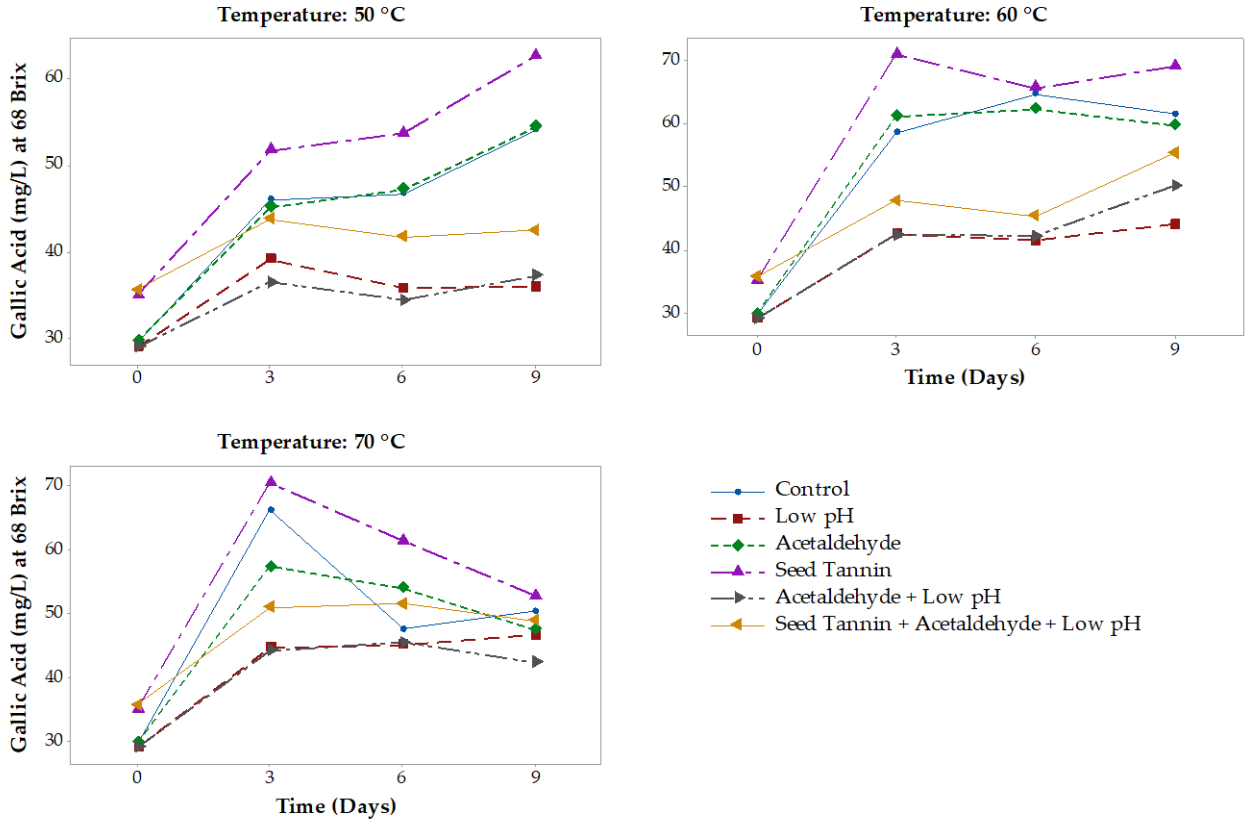

Figure S6. Change in gallic acid concentration during accelerated aging of Rubired concentrate from conventional must heating (CMH) at different temperatures. All data normalized to 68 °Brix. Note the different y-axis scales.

Table S1. Comparison of 5-hydroxymethylfurfural (5-HMF) concentrations in Rubired concentrate from conventional must heating (CMH) vs flash détente (FD), after nine days of accelerated aging at different temperatures.

Treatment	Temperature	CMH		FD	
		5-HMF (mg/L)	%RSD	5-HMF (mg/L)	%RSD
Seed Tannin + Acetaldehyde + Low pH	50 °C	225 a	9.5	176 a	12.3
Acetaldehyde + Low pH		230 a	19.5	186 a	20.9
Low pH		217 a	1.7	168 ab	13.4
Acetaldehyde		67 b	24.0	73 c	5.0
Seed Tannin		72 b	17.3	59 c	13.3
Control		87 b	23.2	87 bc	4.3
Seed Tannin + Acetaldehyde + Low pH	60 °C	1909 a	5.4	1580 a	2.5
Acetaldehyde + Low pH		1875 a	11.9	1769 a	4.8
Low pH		1969 a	1.3	1626 a	16.3
Acetaldehyde		1127 b	9.3	914 b	1.3
Seed Tannin		1030 b	3.8	858 b	6.3
Control		1005 b	7.8	874 b	0.1
Seed Tannin + Acetaldehyde + Low pH	70 °C	10234 a	6.9	8054 a	1.5
Acetaldehyde + Low pH		10317 a	3.8	7618 a	3.6
Low pH		9665 a	1.6	7056 ab	7.8
Acetaldehyde		5860 b	4.6	4694 c	4.5
Seed Tannin		5887 b	3.7	4676 c	0.8
Control		6975 b	6.2	6290 b	3.0

Data are means of two replicates (n = 2), normalized to 68 °Brix; %RSD = percentage relative standard deviation. Means followed by different letters (within columns, by temperature) are significantly different (α = 0.05, Tukey pairwise comparisons).

Table S2. Sediment composition of concentrate from conventional must heating after 12 days of accelerated aging at 70 °C.

Treatment	A420	A520	5-HMF (mg)	Proantho-cyanidins (mg)	Pigmented Polymers (mg)	Tannin (mg)	Sediment (g)
Control	3.12	1.14	11.91 b	7.00 b	0.86 b	26.6	3.36 b
Seed Tannin	6.66	2.64	20.17 b	12.38 ab	0.99 ab	52.9	3.69 b
Acetaldehyde	4.3	1.73	14.53 b	10.68 ab	0.97 ab	32.9	3.49 b
Low pH	6.91	2.79	72.02 a	16.68 ab	1.07 ab	62.1	11.51 a
Acetaldehyde + Low pH	7.41	3.21	80.86 a	17.61 a	1.16 a	64.8	11.35 a
Seed Tannin + Acetaldehyde + Low pH	7.51	3.25	75.3 a	17.86 a	1.16 a	64.6	12.13 a
p-value	ns	ns	0.0001	0.025	0.026	ns	0.0001
Pearson's Coefficient r with Sediment Mass	0.79	0.82	0.99	0.92	0.89	0.87	–

Data are means of two replicates (n = 2), normalized to 68 °Brix. Means followed by different letters (within columns) are significantly different (α = 0.05, Tukey pairwise comparisons); ns = no significance.

Chapter 4.

Impact of Fermentation Temperature and Grape Solids Content on Chemical Composition and Sensory Profile of Flash-treated Cabernet Sauvignon Wines Fermented Off Skins

Statement of Authorship

Title of Paper	Impact of Fermentation Temperature and Grape Solids Content on Chemical Composition and Sensory Profile of Flash-treated Cabernet Sauvignon Wines Fermented Off Skins
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Principal Author

Name of Principal Author (Candidate)	Richard G. Ntuli		
Contribution to the Paper	Designed experiments, planned and executed production and laboratory scale trials, performed statistical analyses on data sets, interpreted the data, and wrote 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	25/07/2020

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	Yaelle Saltman		
Contribution to the Paper	Assisted with sensory evaluation of wines and edited the manuscript.		
Signature		Date	13/12/2020

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Contribution to the Paper	Contributed to the research idea and experimental design, supervised experimental work, assisted with data interpretation, and edited the manuscript.

Signature		Date	13/12/2020

Name of Co-Author	Keren Bindon		
Contribution to the Paper	Assisted with chemical analysis of wines and data interpretation, and edited the manuscript.		
Signature		Date	14/12/2020

Name of Co-Author	David W. Jeffery		
Contribution to the Paper	Contributed to the research idea and experimental design, and assisted with data interpretation. Edited and revised the manuscript.		
Signature		Date	15/12/2020

Name of Co-Author	Kerry L. Wilkinson		
Contribution to the Paper	Contributed to the research idea and experimental design, and assisted with data interpretation. Edited and revised the manuscript and acted as the corresponding author.		
Signature		Date	27/07/2020

Impact of Fermentation Temperature and Grape Solids Content on Chemical Composition and Sensory Profile of Flash-Treated Cabernet Sauvignon Wines Fermented Off Skins

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Highlights

- The effect of flash détente and red juice fermentation conditions were studied
- Temperature and solids content affected wine composition and sensory profiles
- Fermentation with low solids enhanced red fruit and confectionery attributes
- Flash détente treatment mitigated the intensity of green and savory attributes
- Fermentation conditions affected wine aroma, and polysaccharide and glycerol levels

Abstract

This study investigated the color, phenolic, polysaccharide, volatile and sensory profiles of Cabernet Sauvignon wines made from flash détente (FD) treated musts fermented at different temperatures (16, 24 or 32 °C), with and without suspended grape solids. Low fermentation temperature and low solids content increased the concentration of esters, whereas the opposite conditions increased the concentration of fusel alcohols, polysaccharides and glycerol. Higher fermentation temperatures also increased linalool concentration independent of solids content. Traditional maceration fermentation conditions gave the highest concentration of fusel alcohols and 1-hexanol relative to FD treatments. Pre-fermentation removal of grape solids from FD juice created wines with increased red fruit and confectionery attributes, whereas inclusion of 3.5% grape solids increased dark fruit notes. In comparison, control wines had significantly higher green and savory attributes compared to wines from FD treatments. Research findings demonstrated the potential for FD to be used to create differentiated red wine styles.

Keywords: flash détente, flash release, thermovinification, color stability, liquid phase fermentation, *Vitis vinifera*

1. Introduction

Consumer demand for different red wine styles is constantly evolving due to changing consumer demographics, taste preferences and consumption occasions, as well as marketing influences and other associated food and beverage trends. Winemakers are therefore increasingly interested in new techniques and efficient processes that can modify wine composition to create differentiated wine styles that satisfy consumer needs and expectations.

Red wine is predominantly made using a traditional maceration process whereby the skins and seeds of crushed grapes are left to soak in the so-called 'must' to facilitate the extraction of phenolic compounds (Lerno, Reichwage, Ponangi, Hearne, Block, & Oberholster, 2015) and other macromolecules, along with the volatile compounds (and their precursors) responsible for wine aroma and flavor (Escudero, Campo, Fariña, Cacho, & Ferreira, 2007). The factors influencing extraction include must heating, fermentation temperature, maceration time, the use of pectolytic enzymes, cap management, must or grape freezing, saigneé, must nutrient status, yeast selection and oxygen availability. In contrast to white winemaking, traditional maceration approaches during red winemaking require skin contact (including seeds and pulp associated with the pomace) and therefore do not enable experimentation with different levels of suspended grape solids to potentially influence wine style. Additionally, since higher fermentation temperatures aid the extraction process during traditional maceration, there is not much leeway to lower the fermentation temperature as a means of influencing wine style.

In order to study the impact of suspended grape solids and fermentation temperature on red wine composition and sensory profiles, a must pre-treatment step such as flash détente (FD) can conceivably

be employed to generate a highly-extracted, phenolic-rich juice that could be subjected to different experimental conditions. FD is still a relatively new process and limited research has been undertaken to investigate its impact on wine composition (Doco, Williams, & Cheynier, 2007; Morel-Salmi, Souquet, Bes, & Cheynier, 2006). Furthermore, few published studies have explored the impact of fermentation temperature on the composition of wine made from conventional heat-extracted red grape juice (Geffroy et al., 2015; Girard, Kopp, Reynolds, & Cliff, 1997) and one study experimented with unheated simulated grape juice (Molina, Swiegers, Varela, Pretorius, & Agosin, 2007). Molina and colleagues reported higher concentrations of ethyl esters associated with fruity characters in model wines derived from fermentations at 15 °C compared to 28 °C.

Geffroy et al. (2015) reported losses of grape-derived aroma compounds such as norisoprenoids, terpenols and some phenols, in heated musts that were fermented on-skins, but an increase in ethyl esters when must heating was followed by fermentation off-skins. In contrast, more intense fruit aromas and flavors, and higher concentrations of esters were found for heat treated musts fermented off-skins at 15 °C, compared to traditional maceration fermentations at 20 °C and 30 °C (Girard et al., 1997). The effect of thermovinification followed by off-skins fermentation was not distinguished from that of fermentation temperature in either of those studies so the extent to which heat treatment and/or fermentation temperature explain the changes in volatile composition remain unclear. Furthermore, the effects of fermentation temperature on the composition and sensory properties of red wine arising from flash détente are yet to be reported.

The impact of suspended grape solids concentration on wine composition is typically considered to be more relevant to white wine production, hence most of the research undertaken to date has focused

on white juice or synthetic model juice without skin contact (Casalta, Vernhet, Sablayrolles, Tesniere, & Salmon, 2016). These studies are unlikely to mimic the outcomes of heat-extracted red juice fermentations conducted without skin contact, however, due to the marked compositional differences between solids from heat-treated red musts and white grape solids (Vernhet, Bes, Bouissou, Carrillo, & Brillouet, 2016). Despite the potential practical and enological significance, the influence of suspended solids derived from heat-treated red musts (such as FD) on the composition and style of red wines fermented without further skin contact has seemingly not been investigated.

Given the gaps in existing research, the present study aimed to determine the impact of fermentation temperature and the inclusion of different levels of grape suspended solids on the chemical and sensory profiles of red wines produced from juice obtained after FD-treatment of Cabernet Sauvignon grape must. Whereas previous studies used turbidity measurements to estimate the concentration of suspended solids, more recent research suggests there is not good correlation between turbidity measurements and the concentration of suspended solids (Casalta et al., 2016). In the current study, total wet suspended solids measurements were used to accurately determine solids content. Compositional analyses including basic wine chemistry, phenolics, polysaccharides and volatile composition, and sensory outcomes for FD wines were compared with wines made via a conventional maceration approach, to better understand the impact of FD treatments.

2. Material and methods

2.1. Must and juice preparation

Cabernet Sauvignon grapes (90 tonnes were machine harvested at commercial maturity from a vineyard located in the Central Valley region of California. Fruit was sampled at the winery for

compositional analysis with 2 × 10 kg samples taken from each truck using a zone sampler (Yuba City Steel, Yuba City, CA, USA) being pooled and lightly pressed, and 500 mL of the resulting juice analyzed using a WineScan FT120 interferometer (Foss Electric, Hillerød, Denmark) for determination of basic grape chemical parameters (Table S1 of the Supplementary Materials). Grapes were received at the Gallo Livingston winery, destemmed with a Diemme destemmer (Diemme Enologia, Lugo, Italy), and ~87 tons of must was reserved for FD processing (described in section 2.2.). Separately, 1.5 tons of must was collected (in duplicate), 8% w/v aqueous potassium metabisulfite solution was added to give 60 mg/L of total SO_2, and a subsample of must (from each replicate) was transferred to 40 L stainless steel cans (one per replicate), with mixing to ensure homogeneity. Containers were transported to the Gallo research winery for fermentation using traditional maceration (control) (Fig. S1 of the Supplementary Materials).

2.2. Flash détente and juice processing conditions

Approximately 87 tons of must was treated using a commercial flash détente unit (Della Tofolla, Trevignano, Italy) running in a continuous processing mode at 27 tons/hour. Must was heated to 85 °C while maintaining the vacuum chamber at −0.94 bar. On entering the vacuum chamber, the must temperature instantaneously dropped to 32 °C due to absorption of the latent heat of vaporization as the heated must was flashed off under vacuum. Approximately 10% of the must volume was lost as condensate from the vacuum chamber, which increased the total soluble solids from 24.4 °Brix in grapes to 28.2 °Brix in the must following FD treatment. Must exiting the flash chamber then had potassium metabisulfite solution (8% w/v) added to attain 60 mg/L of total SO_2. Must was then processed using a pneumatic press (Diemme Enologia, Lugo, Italy), operating at 2 bars. Potable water was then added to the juice to adjust the reducing sugar concentration to 23.4 g/100 mL. Juice was then mixed and divided equally into two tanks. One tank had solids removed by centrifugation (GEA Westfalia Northvale, NJ,

USA). The clarified (i.e., low solids 'LS' treatment series) and unclarified (i.e., high solids 'HS' treatment series) juices were transferred into 40 L stainless steel cans (six per solids level, Fig. S1 of the Supplementary Materials) with mixing to assure homogeneity, and transported to the Gallo research winery for fermentation.

2.3. Measurement of suspended solids in juice

The suspended solids content of fermentation-ready juice was measured using specialized 15 mL conical-bottom graduated polycarbonate centrifuge tubes (Nalgene, Rochester, NY, USA) and a swinging bucket centrifuge (Beckman Coulter, Brea, CA, USA). Four samples of clarified and unclarified juice were collected and immediately transferred into centrifuge tubes before settling. Juice samples were centrifuged at 4000 × *g* for 15 min; clarified and unclarified juice were found to have < 0.5% and 3.5 % v/v total wet suspended solids, respectively.

2.4. Fermentations

Fermentations were carried out using purpose-built 40 L fermentors with temperature control and mixing capabilities. Must (control) and juices (treatments) were transferred from cans into fermentors, ensuring all suspended solids were also transferred. Yeast assimilable nitrogen was adjusted to 300 mg/L with diammonium phosphate and tartaric acid was added to attain a titratable acidity (TA) of 6 g/L (as tartaric acid equivalents). Rohavin MX enzyme (AB Enzymes, Darmstadt, Germany) was added at a rate of 0.04 mL/L and fermentors were then inoculated with Lalvin ICV D254 yeast (Lallemand, Canada) at 0.18 g/L. FD juice fermentations were carried out at 16, 24 and 32 °C (hereafter 'LT', 'MT' and 'HT', respectively) with and without suspended solids (i.e., HS and LS, respectively), giving a total of six different fermentation treatments (in duplicate, Table S1 of the Supplementary Materials). A traditional

maceration fermentation (control) was conducted (in duplicate) at 30 °C. All wines were fermented until dry (reducing sugars ≤ 0.5 g/L), after which free sulfur dioxide was adjusted to 35 mg/L using potassium metabisulfite solution, before being filtered through 1 µm cellulose filter pads (Gusmer Enterprises, Fresno, CA, USA) followed by a Vitipore II Plus 0.45 µm polyvinylidene fluoride filter (Millipore Sigma, Burlington, MA, USA), prior to bottling in 750 mL glass bottles with screw cap closures, using a GAI 1006 monoblock filler (Prospero International, Perugia, Italy).

2.5. Basic chemical analysis of juice and wine

Juice and wine analyses included total soluble solids (TSS), TA, pH, free and total sulfur dioxide, alcohol (as % v/v) and reducing sugars, determined by fourier transform infrared spectroscopy using a WineScan FT120 interferometer (Foss Electric, Hillerød, Denmark). Absorbance at 420, 520 and 620 nm was measured in 1 mm path length cuvettes (and normalized to 1 cm path length) using a UV/visible Lambda 35 spectrophotometer (Perkin Elmer, Waltham, MA, USA), and tristimulus color was measured using an Ultrascan Pro spectrophotometer (Hunter Lab, Reston, VA, USA). Chemical parameters for FD-derived juice following dilution with water (as in section 2.2.) are summarized in Table S1 of the Supplementary Materials.

2.6. Analysis of phenolic compounds

Total phenolics was measured by the Folin–Ciocalteu method (Singleton & Rossi, 1965) with results reported in mg/L gallic acid equivalents (GAE).

Phenolic profiling of juice and wine samples was undertaken by HPLC analysis as previously described (Ntuli, Ponangi, Jeffery, & Wilkinson, 2020). Samples were filtered through 0.45 µm cellulose

acetate syringe filters (Whatman, Maidstone, Kent, UK) and analyzed by reversed-phase chromatography using an Agilent 1200 HPLC (Agilent Technologies, Palo Alto, CA) equipped with a quaternary pump, diode array detector (DAD), and Varian PLRP-S column (250 × 4.6 mm, 5 µm particle size, Varian Inc, Palo Alto, CA) fitted with a PLRP-S guard cartridge. A binary solvent gradient was used consisting of water with 0.5% v/v orthophosphoric acid (85% w/v) for mobile phase A and acetonitrile with 0.5% v/v phosphoric acid (85% w/v) for mobile phase B. The column thermostat was set at 50 °C and the injection volume was 20 µL. Standards and wavelengths used for quantifying individual phenolic compounds were as previously described (Ntuli et al., 2020). Proanthocyanidins were quantified at 230 nm instead of the traditionally used 280 nm to eliminate interference with co-eluting anthocyanins that appeared as shoulder peaks on proanthocyanidin signals at 280 nm. The co-eluting anthocyanins had similar absorption peaks at 532 and 230 nm, whereas proanthocyanidins had no absorption at 532 nm, making it possible to eliminate anthocyanin interference.

Wine proanthocyanidins were measured again one year after bottling, together with color properties, according to published methods (Mercurio, Dambergs, Herderich, & Smith, 2007). For proanthocyanidin analysis, a 25 µL sample of wine was combined with 300 µL of a 0.04% w/v solution of methyl cellulose (Sigma Aldrich, Castle Hill, NSW, Australia) in a 96-well plate, mixed on an automated plate shaker and left to stand for 3 min. A control sample was included where water was added in place of the methyl cellulose solution. A 200 µL aliquot of saturated ammonium sulfate (Sigma Aldrich) was added, followed by 475 µL of water. Samples were mixed on an automated plate shaker and left to stand for 10 min. Plates were sealed and centrifuged at 2885 × *g*, using a Hettich Universal 32 R centrifuge equipped with a SX4750 rotor with microplate carriers. A 300 µL aliquot of supernatant from each sample was transferred into a 370 µL 96 well UV plate and the absorbance was measured at 280 nm using a

SpectraMax M2 microplate reader (Molecular Devices, Berkshire, UK). Quantification was performed by determining the difference in absorbance at 280 nm between control and MCP-treated samples, using (-)-epicatechin (Sigma Aldrich) as the quantitative standard. To ensure standardization of proanthocyanidin analyses across wine sampling time points, a purified commercial seed extract (Tarac Technologies, Nuriootpa, SA, Australia) was included for each 96 well plate assayed.

2.7. Polysaccharide preparation and quantification

Wine polysaccharides were quantified as previously described (Bindon et al., 2019). A 1 mL aliquot of wine was added to 5 mL of absolute ethanol and allowed to precipitate at 4 °C for 18 h. Samples were centrifuged at 8000 × *g* for 5 min and the supernatant discarded. The resulting pellets were reconstituted in 800 µL of Milli-Q water, transferred to a 1 mL Pur-a-Lyzer dialysis tube of molecular weight cut-off 3500 Da (Sigma-Aldrich) and dialyzed against three changes of Milli-Q water at 4 °C. After dialysis, each sample was transferred to a fresh screw-cap centrifuge tube, frozen at −80 °C and freeze-dried. Lyophilized samples were reconstituted in 2 M TFA and hydrolyzed at 100 °C for 3 h. Hydrolysates were cooled on ice, and thereafter concentrated under vacuum at 30 °C in a Heto vacuum centrifuge (Heto-Holten A/S, Allerod, Denmark) before being resuspended in 300 µL of Milli-Q water. Internal standard was added to the sample to give a final concentration of 0.3 M of ribose and deoxy-glucose (Sigma-Aldrich). The derivatising reagent was 0.5 M of methanolic 1-phenyl-3-methyl-5-pyrazolone (PMP) (Sigma-Aldrich) in 1 M NH_4OH. For the derivatization, 25 µL of sample containing internal standard was mixed with 96.2 µL of derivatizing reagent and placed in a heating block at 70 °C for 1 h. After this step, the samples were cooled on ice and neutralized with formic acid. The samples were extracted twice with dibutyl ether (Sigma-Aldrich) and the aqueous upper layer discarded. The dibutyl ether was removed under vacuum at room temperature. PMP-monosaccharide derivatives were then

quantified at 250 nm by HPLC using a C18 column (Kinetex, 2.6 μm, 100 Å, 100 × 3.0 mm) protected with an in-line filter (KrudKatcher Ultra HPLC in-line filter, 0.5 μm; Phenomenex, Lane Cove, NSW, Australia). The mobile phases were solvent A, 10% v/v acetonitrile in 40 mM aqueous ammonium acetate, and solvent B, 70% v/v acetonitrile in water. The linear gradient used was: 92% solvent A at 0 min; 84% at 12 min; 0% at 12.5 min, then returning to starting conditions (92% solvent A) from 14.5 to 18.5 min. A flow rate of 0.6 mL/min was used with a column temperature of 30 °C. The PMP-monosaccharide derivatives were identified using commercial standards (Sigma-Aldrich).

2.8. Wine volatile compounds

Aroma compounds in wine samples were extracted using headspace solid-phase microextraction (SPME) with a divinylbenzene/carboxen/polydimethylsiloxane (DVD/CARC/PDMS) fiber assembly (Supelco, Bellefonte, PA, USA) and analyzed by GC-MS as described by Siebert and colleagues (2005), with some modifications. Analyses were performed using a Hewlett Packard 6890 gas chromatograph (Agilent Technologies) equipped with a HP 5975 mass selective detector, CTC Combi Pal autosampler and a DB-5ms DG 30 m × 0.250 mm I.D., 0.5 μm film thickness (J&W Scientific, Santa Clara, CA). The oven temperature program was modified as follows: 40 °C for 5 min, increased by 3 °C/min to 170 °C, and then 30 °C/min to a final temperature of 275 °C. The carrier gas used was helium at 2 mL/min. Calibration standards comprised ethyl hexanoate, ethyl octanoate, 1-octen-3-ol, ethyl isovalerate, ethyl butanoate, ethyl dihydrocinnamate, ethyl isobutyrate, 2-methylbutyl acetate, and 2-phenylethanol (Sigma-Aldrich); linalool, 2-phenylethyl acetate (Fluka, St. Louis, MO, USA); β-damascenone (Advanced Biotech, Inc., Totowa, NJ); and isoamyl acetate (Mallickrodt, St. Louis, MO, USA).

2.9. Sensory analysis of wine

Wine sensory profiles were determined by descriptive analysis (DA) with a trained panel of 9 judges (7 female and 2 male, aged between 18 and 50 years), comprising University of Adelaide staff and students. Panelists were recruited based on their availability and prior wine sensory experience, and completed 12 hours of training (6 × 2 h weekly sessions) prior to formal evaluation. Training sessions involved identification of descriptive terms, familiarization in recognizing and rating the intensity of attributes, and practice evaluation sessions conducted in isolated sensory booths under the controlled conditions used during formal evaluation (i.e., red lighting and a temperature of 22–23 °C). This also enabled evaluation of panel performance.

The DA panel generated 17 attributes including: red fruit, dark fruit, dried fruit, confectionery, green and savory aromas and flavors; overall aroma intensity; acidity, alcohol heat, astringency and body. Reference standards were prepared (Table S2 of the Supplementary Materials) for use in training sessions and during formal evaluations. Two formal evaluation sessions were held (on two different days), with 14 wines (i.e., duplicates from the seven fermentation treatments) presented at each session. Wines (30 mL) were presented in a randomized order, in four digit-coded XL5 215 mL stemmed wine glasses (covered with lids), at ambient temperature, in brackets of seven wines. Breaks were enforced between each bracket (5 min) and between each wine (1 min) to avoid sensory fatigue. Distilled water and plain crackers were provided as palate cleaners. Panelists rated the intensity of each sensory attributed using 15-cm unstructured line scales, with anchor points of ‘low’ and ‘high’ placed at 10 and 90% on the scale, respectively. Data were acquired with Red Jade software (Redwood Shores, CA, USA). DA panelists gave informed consent before participating in the study, which was approved by the Human Research Ethics Committee of the University of Adelaide (HREC-2018-067).

2.10. Statistical analysis

Chemical and sensory data were analyzed by one and two-way analysis of variance (ANOVA) using Minitab (State College, PA). Panel performance during training was checked using PanelCheck software. Principal component analysis (PCA) of sensory data was performed using XLSTAT (Addinsoft, Paris, France). Partial least squares regression (PLSR) analysis was performed using UnScrambler 11 software (Camo Analytics, Oslo, Norway) with full cross-validation and the use of an uncertainty test to identify significant variables. Tukey-HSD was used for mean comparisons of treatments with significant differences, setting $\alpha = 0.05$.

3. Results and discussion

3.1. Effect of treatments on basic wine composition

Analysis of wines showed that fermentation temperature and the concentration of suspended solids during fermentation had an effect on ethanol, malic acid, TA, volatile acidity (VA) and glycerol concentrations (Table 1). Fermentation temperature impacted fermentation kinetics whereas suspended solids content had no effect. Off-skins ferments at 16, 24 and 32 °C took 16, 7 and 5 days respectively to complete fermentation, whereas control ferment at 30 °C took 8 days. Fermentations conducted at higher temperatures produced wines with lower ethanol content and higher TA, VA and glycerol. The decrease in TA at lower fermentation temperature was possibly due to the combination of the higher ethanol concentration and cold-induced potassium bitartrate precipitation, while the higher titratable acidity in high suspended solids wines might be due to inhibition of tartrate crystallization by polysaccharides that were present at higher concentrations in these wines (section 3.4). Ethanol concentration had an inverse relationship with fermentation temperature, similar to findings reported for white juice fermentations (Torija et al., 2003), but contrary to a study that found no significant effect of

fermentation temperature on ethanol concentration, where differences amongst treatments were ≤ 7 °C (Pérez, Assof, Bolcato, Sari, & Fanzone, 2018). The significantly lower ethanol concentration in higher temperature fermentations was likely due to higher evaporative losses. Fermentations with a higher concentration of suspended solids produced wines with lower ethanol levels, possibly due to stripping that was aided by greater carbon dioxide nucleation on sludge particles (Casalta et al., 2016), along with higher malic acid levels. Liquid phase fermentations conducted at higher temperatures produced wines with higher glycerol and VA, consistent with earlier studies using synthetic or white grape juices (Beltran, Novo, Guillamón, Mas, & Rozès, 2008; Rollero et al., 2015). Fermentations with higher concentrations of suspended solids also produced wines with higher glycerol content but lower VA. The increased glycerol concentration in high solids fermentations was consistent with previous research on *Saccharomyces cerevisiae* that investigated the effect of phytosterol concentration (Luparia, Soubeyrand, Berges, Julien, & Salmon, 2004) on glycerol formation. Suspended grape solids in unclarified fermentations have been shown to act as a rich source of phytosterols for fermenting yeasts (Casalta et al., 2016). A significant interaction was observed between fermentation temperature and suspended grape solids content on the concentration of glycerol ($P = 0.044$) and malic acid ($P = 0.003$), and on titratable acidity ($P = 0.007$) (Table 1). According to the interaction plots (data not shown), the highest increases in TA and glycerol concentration were seen in higher temperature fermentations with solids, whereas the highest malic acid concentrations were in low temperature fermentations with solids.

Traditional maceration fermentations were carried out at 30 °C and gave wines with similar ethanol and glycerol concentrations as the liquid phase wines fermented at higher temperature (32 °C) with suspended solids (Table 2). All the liquid phase fermentations resulted in wines with significantly lower

lactic acid levels and VA than the traditional maceration control wine, possibly due to inactivation of native lactic acid and acetic acid bacteria as a consequence of thermal treatment.

3.2. Effect of treatments on wine color

Wine color was affected by both fermentation temperature and the concentration of suspended solids during fermentation (Table 1). Wines fermented at higher temperatures (24 and 32 °C) and those fermented with suspended solids had significantly higher red color (A520) and color intensity compared to wines fermented at the lowest temperature (16 °C) or with only trace levels of solids. On the other hand, wine chemical age 2 (Mercurio et al., 2007) and non-bleachable pigments increased with fermentation temperature but were not impacted by solids concentration. It is likely that the higher fermentation temperatures initially accelerated condensation reactions between anthocyanins and other relevant wine constituents (acetaldehyde, phenolics) resulting in greater color stability. However, color intensity measured one year after bottling showed no significant differences between the treatments, whereas wine color density (WCD SO_2) was generally similar among treatments. It was hypothesized that the higher color in fermentations with suspended solids was due to leaching of color adsorbed to suspended grape solids as the ethanol concentration increased during fermentation. It is also possible that lower tartrate crystallization in fermentations with high solids due to inhibition by polysaccharides reduced color loss arising from co-precipitation of color compounds and tartrates. Similar trends to those for red color were seen for brown color and lightness (A420, Hunter B and Hunter L values, Table 1). As could be expected, higher fermentation temperature promoted brown color formation, possibly via chemical oxidation as well as reaction of some phenolic compounds with other phenolic or non-phenolic compounds to yield orange colored pigments. A significant ($P < 0.05$) interaction between fermentation temperature and suspended grape solids content was observed for red and brown color, color intensity,

hue, and Hunter lightness in liquid phase fermentations. The interaction showed that suspended solids increased brown and red color concentrations to a greater extent at 24 °C, compared to fermentation at 16 and 32 °C.

Traditional maceration control fermentations gave significantly higher wine hue values compared to all liquid phase FD treated fermentations (Table 2). The combination of low red color extraction and higher fermentation temperature, which increased brown color formation, had the effect of increasing hue in control wines. Wine chemical age 2 was significantly higher in wines from traditional macerated fermentations after one year of bottle aging, although this did not increase wine color density.

3.3. Effect of treatments on phenolic composition

Fermentation temperature had an effect on the concentration of catechin, epicatechin, malvidin-3-glucoside and non-bleachable pigments such as pigmented polymers, but had no effect on caftaric acid, quercetin glycosides and the total phenolics content of wine (Table 1). As expected, fermentation temperature was negatively correlated with the monomeric flavan-3-ol and anthocyanin concentrations of finished wines (Table 1) due to a greater conversion to derived pigments at higher fermentation temperatures such as pigmented polymers (Gao, Girard, Mazza, & Reynolds, 1997), anthocyanin-flavanol adducts, and pyranoanthocyanins (de Freitas, Fernandes, Oliveira, Teixeira, & Mateus, 2017). The increased reaction at higher temperature was reflected in the increase in total pigmented polymers and wine chemical age 2, indicating the presence of a greater proportion of non-bleachable pigments relative to monomeric and/or ionized anthocyanins. Unlike for classic maceration fermentations where an increase in fermentation temperature leads to increased tannin extraction from seeds (Lerno et al., 2015), no temperature effect was seen for liquid phase ferments (Table 1). Incorporation of suspended

solids during fermentation had no effect on proanthocyanidin concentration but had the effect of decreasing the total phenolics, caftaric acid and catechin concentrations of finished wines (Table 1), possibly due to adsorption phenomena.

All FD treated fermentations had significantly higher caftaric acid concentrations while most also had elevated quercetin glycosides compared to the traditional maceration controls (Table 2). These observed trends may have been due to heat inactivation of polyphenol oxidase preventing the enzymatic oxidation of caftaric acid and to increased quercetin glycoside extraction as a result of the FD treatment. Compared with liquid phase fermentations, wines prepared via traditional maceration had a higher monomeric flavan-3-ol concentration, indicative of seed extraction during fermentation (Lerno et al., 2015). Proanthocyanidin and total phenolics concentrations of wines from traditional maceration fermentations were generally comparable to those from liquid phase fermentations, although concentrations were higher than some liquid phase fermentations with inclusion of solids. Traditional maceration at the relatively high temperature of 30 °C resulted in a significantly lower malvidin-3-glucoside concentration compared to liquid phase fermentations performed at lower temperatures likely due to increased conversion to derived pigments.

3.4. Effect of treatments on polysaccharide composition

Pectic polysaccharides such as rhamnogalacturonans (RGI and RGII), type II arabinogalactan-proteins (AGP), and arabinans are derived from grape berry cell walls whereas mannoproteins (MP) are released from yeast autolysis during fermentation (Vidal, Williams, Doco, Moutounet, & Pellerin, 2003) or aging on lees (Doco et al., 2003). In the current study, two-way ANOVA revealed that fermentation temperature and solids content impacted polysaccharide concentration and composition, but an

interactive effect between the two factors was not observed (Table 1). As fermentation temperature increased, the concentration of both grape- and yeast-derived polysaccharides also increased. Proportional changes in the characteristic sugars, glucuronic acid, galacturonic acid and arabinose, were indicative of increased grape-derived polysaccharides (AGP and pectic material) extraction, whereas elevated mannose was indicative of increased extraction of mannoproteins (MP) derived from yeast autolysis. Polysaccharides rich in arabinose, together with the calculated ratio of arabinose to galactose, increased with temperature, likely due to increased extraction of arabinans. The concentration of polysaccharides rich in rhamnose and galactose was not impacted (Table 1). The observed effects may have been due to increased extraction at high temperature as well as relative differences in polysaccharide solubility or yeast biomass (Sener, 2007).

In comparison with liquid phase fermentations containing trace levels of suspended solids, incorporation of 3.5% solids increased polysaccharides rich in mannose, suggesting increases in yeast MPs from increased yeast activity and associated biomass, possibly due to the improved availability of nutrients and sterols (Casalta et al., 2016). The increase in MPs was accompanied by an increment in grape-derived pectic material rich in galacturonic acid (homogalacturonan) extracted from cell wall fragments associated with the solids (Table 1). Glucose, rhamnose (rhamnogalacturonans), and sugars indicative of PRAGs were not affected by the level of suspended solids.

When the total polysaccharide concentrations of wines from traditional maceration and liquid-phase fermentation of FD-treated must were compared, significantly higher concentrations were observed in control wines (Table 2). This was attributed to the additional skin contact associated with traditional maceration. In this case, polysaccharide-derived mannose suggestive of yeast MPs was unchanged, but

all classes of pectic polysaccharides, including the associated rhamnose, galacturonic acid, galactose and arabinose sugars, increased with skin contact. This finding was consistent with previous research (Doco et al., 2007) and confirmed that significant skin contact during fermentation is necessary for substantial grape-derived polysaccharide extraction to take place, in particular the extraction of PRAGs and RG II. There were greater increases in arabinose than galactose with skin contact during control fermentations relative to liquid-phase fermentations (Table 2). The Ara:Gal ratio for traditional maceration fermentations was close to the previously reported ratio (>1) for red wine (Doco et al., 2003), whereas FD liquid phase fermentations all had much lower ratios, ranging from 0.43 to 0.60. This can potentially be ascribed to heat-facilitated enzymatic degradation of polysaccharides rich in arabinose such as arabinans, and/or dearabinosylation of AGPs (Doco et al., 2003).

Collectively, these findings demonstrate that fermentation temperature and the must preparation technique (FD vs. traditional maceration) differentially modified wine polysaccharide composition. It is worth noting that traditional maceration fermentations had markedly higher total polysaccharide concentrations than FD treatments fermented in liquid phase. The inference that additional skin contact may be required to optimize polysaccharide extraction from grapes warrants further research.

3.5. Effect of treatments on aroma composition

3.5.1. Ethyl esters

Volatile esters are important to wine aroma as they impart desirable fruit, confectionery and floral characters. Fermentation temperature and suspended grape solids strongly impacted grape- and yeast-derived volatiles in wine as shown in Table 3. Yeast-derived aroma compounds impacted by both factors included ethyl esters, acetate esters, and higher alcohols. In the FD ferments, the concentrations of ethyl

butanoate, hexanoate, octanoate and decanoate were significantly increased as fermentation temperature decreased and in response to a decrease in suspended solids. Based on odor activity values (OAV) shown in Table 4, the changes in ethyl ester composition would be expected to alter the sensory profile of the respective wines based on the three most impactful ethyl esters (ethyl octanoate, ethyl hexanoate and ethyl butanoate) being greatly influenced by the treatments.

These findings were consistent with previous research (Beltran et al., 2008; Molina et al., 2007) that showed similar effects of fermentation temperature on ethyl ester formation in synthetic juice and white juice ferments. Ester production has been suggested to arise from detoxification mechanisms as part of a yeast stress response (Mason & Dufour, 2000). Stressful conditions may arise in clarified ferments due to lack of phytosterols (Casalta et al., 2016), which may explain the higher ester production under these conditions. On the other hand, upregulation of genes involved in short-chain (C_4-C_8) fatty acid synthesis (Beltran et al., 2006) and changes in membrane fatty acid composition (Torija et al., 2003) may also explain the higher ethyl ester formation at lower fermentation temperature. It should also be noted that evaporative losses under higher fermentation temperature conditions may also contribute to lower ethyl ester concentrations.

Ethyl isobutyrate and ethyl isovalerate showed opposing trends similar to acetate esters with respect to both fermentation temperature and suspended solids content whereas ethyl dihydrocinnamate was not affected by either of the factors studied. Of all the ethyl esters quantified, only ethyl hexanoate and ethyl octanoate had significant fermentation temperature by suspended solids interactions, showing a synergistic effect of low temperature and trace solids according to 2-way ANOVA (Table 3). Interestingly, there was no significant difference in ethyl ester composition between traditional

maceration fermentations and the liquid phase FD wine fermented at 32 °C with 3.5% suspended solids, most likely due to the comparable fermentation temperature and high concentration of suspended solids (Table 4). These two treatments yielded the lowest ethyl ester concentrations.

3.5.2. Acetate esters

The concentration of most acetate esters was impacted by both fermentation temperature and suspended grape solids (Table 3). Isoamyl acetate, with an OAV up to 315, was by far the most impactful acetate ester detected in wines, followed by 2-methylbutyl acetate (OAV 68) and 2-phenylethyl acetate (OAV 6) (Table 4). The concentrations of isoamyl acetate, isobutyl acetate, 2-methylbutyl acetate, 2-phenylethyl acetate and ethyl acetate increased with fermentation temperature, which accounted for the observed trend in total esters, but opposed the trend for ethyl esters. Other researchers (Rollero et al., 2015) have reported differences in the regulation of ethyl and acetate ester synthesis, which may explain the differing response to fermentation temperature observed between the two classes of esters in the current study.

The lower suspended solids content of liquid phase FD fermentations resulted in significant increases in the concentrations of isoamyl acetate, isobutyl acetate and ethyl acetate esters, whereas changes in other acetate esters were not statistically significant (Table 3). A significant interaction between the two factors studied was observed by 2-way ANOVA for isobutyl acetate, whereby increasing fermentation temperature together with clarification appeared to have a synergistic effect on its formation. In contrast, the traditional maceration control had significantly lower isoamyl acetate and total ester concentrations compared with each of the liquid-phase FD treatments, with the exception of the 16 °C treatment containing 3.5% solids (Table 4). Contrary to what was observed for ethyl esters,

the two higher temperature liquid phase fermentations, with or without suspended solids, had significantly higher concentrations of acetate esters compared with the traditional maceration control. This was of interest, since these respective treatments had a similar fermentation temperature range and solids concentration. Must heating of liquid phase fermentations and/or skin contact in traditional maceration ferments may account for the observed differences.

3.5.3. C_{13}-norisoprenoids, terpenes and alcohols

Of the volatile compounds that were grape-derived, β-damascenone which imparts a cooked apple note, was the most impactful based on OAVs shown in Table 4. The concentration of β-damascenone and linalool increased as fermentation temperature increased whereas the concentrations of 1-hexanol, which imparts a characteristic green aroma note, and methanol were not impacted (Table 3). Fermenting FD juice with suspended solids increased the concentration of 1-hexanol and methanol, but had no effect on the concentration of linalool and β-damascenone. The higher polysaccharide concentration of fermentations with suspended solids was likely responsible for the higher methanol concentration arising from demethylation of pectic polysaccharides. The concentration of 1-octen-3-ol decreased as both fermentation temperature and suspended solids content increased. According to the 2-way ANOVA, a synergistic effect was observed for 1-octen-3-ol concentrations, whereby a decrease in temperature for clarified fermentations led to higher concentration. It is hypothesized that losses were minimized due to less volatilization and less adsorption to suspended solids.

The traditional maceration control treatment was found to have a significantly higher 1-hexanol concentration compared to all of the liquid-phase FD wines (Table 4). Heat inactivation of lipoxygenase enzymes responsible for converting fatty acids extracted from grape skins into C_6-alcohols, and loss in

the condensate during flash détente processing likely explain the lower concentrations observed in FD wines. Conversely, increased skin contact in traditional maceration fermentations would be expected to enhance extraction of fatty acid precursors, leading to more 1-hexanol formation. In addition, the traditionally macerated control wine had a significantly lower 1-octen-3-ol concentration compared to all low to medium temperature off-skins ferments. The control wines also had significantly lower concentrations of linalool and β-damascenone compared to FD wines fermented at a similar temperature, suggesting increased adsorption by bulk grape solids.

3.5.4. Fusel alcohols, aldehydes and sulfides

Both fermentation temperature and suspended solids content had a significant effect on the concentrations of acetaldehyde, active amyl alcohol, isoamyl alcohol, isobutyl alcohol, 2-phenylethanol, and total fusel alcohol in the wines (Table 3). Isoamyl alcohol, likely the most sensorially impactful fusel alcohol (based on OAV) was found to increase with fermentation temperature together with active amyl alcohol, isobutyl alcohol, 2-phenylethanol, and total fusel alcohols, while acetaldehyde concentrations showed the opposite trend, likely due to enhanced reaction with phenolic compounds and aroma precursors at higher temperature (Aleixandre-Tudo et al., 2016). Previous research into the effects of fermentation temperature on acetaldehyde formation has produced inconsistent results, with some studies reporting no effects, while others reported either positive or negative correlations with temperature (Liu & Pilone, 2000). Fermentation temperature and suspended solids content had no effect on dimethyl sulfide concentration (Table 3).

Increasing the concentration of suspended solids in liquid-phase fermentations increased active amyl alcohol, isoamyl alcohol, isobutyl alcohol, 2-phenylethanol, and total fusel alcohol concentrations,

while acetaldehyde showed the opposite trend and *n*-propyl alcohol was unaffected (Table 3). The trend for fusel alcohols can be rationalized based on research (Rollero et al., 2015) that showed a positive correlation between isoamyl alcohol and isobutyl alcohol concentration with phytosterol concentration in synthetic juice ferments. Comparing traditional maceration controls with the liquid-phase FD treatments, control wines were found to have significantly higher isoamyl alcohol, isobutyl alcohol, 2-phenylethanol, dimethyl sulfide, and total fusel alcohol concentrations (Table 4). It is postulated that both grape skins and suspended solids increased amino acid concentrations during fermentation, which favored fusel alcohol formation via the Ehrlich pathway. Although heating of must was expected to increase amino acid extraction in liquid phase fermentations (Geffroy et al., 2015), oxygen uptake from cap irrigation during traditional maceration may have played a greater role in increasing fusel alcohol formation (Crowell & Guymon, 1963) relative to liquid phase treatment with suspended solids fermented at a similar temperature that did not require cap management..

3.6. Sensory evaluation

The inclusion of suspended solids in juice had a greater effect on wine aroma and flavor than fermentation temperature (Table S3 of the Supplementary Materials). The FD wines fermented in liquid phase with only trace levels of solids had significantly higher ratings for aroma intensity, red fruit (raspberry and strawberry), and confectionery (candied fruit) attributes, and significantly lower ratings for dark fruit (blueberry and plum) compared to the 3.5% solids treatments. These results were consistent with the observed increase in total ester concentration in response to the decrease in suspended solids during fermentation. Other researchers reported that fruit character was enhanced in white wines made with limited solids contact, and that increases in flavor and complexity were perceived when solids contact was extended (Williams, Ough, & Berg, 1978). In the current study, liquid-phase fermentations

conducted at 32 °C produced wines with lower ratings for dark fruit aroma and flavor, savory aroma, body and astringency, relative to the other liquid phase treatments. The body decreased despite increases in glycerol concentration compared to low temperature ferments. Although glycerol concentration was relatively high in the wines, i.e., up to 10.6 g/L (Table 2), the 4.5 g/L variation amongst wines suggest that perceived body or viscosity might not be variable amongst the wines (Gawel & Waters, 2008). Dried fruit and green aroma and flavor, alcohol intensity, and acidity were not impacted by either fermentation temperature or solids content. According to the two-way ANOVA results, no significant interactions between fermentation temperature and solids content were observed (Table S3 of the Supplementary Materials).

Results from the one-way ANOVA showed that control wines made via a traditional maceration technique with no FD treatment had significantly lower red fruit and confectionery aroma and flavor, and significantly higher green and savory aroma and flavor than any of the liquid-phase FD treatments (Table S4 of the Supplementary Materials). Ratings for dried fruit aroma and flavor, alcohol heat and acidity were not significantly different amongst the wines. Although control wine had higher concentrations of grape derived polysaccharides that was expected to increase body or fullness based on studies in model wines (Vidal et al., 2004), no significant difference was detected in the complex wine matrices.

Principal component analysis (PCA) of sensory data revealed that the seven treatments could be defined as three distinct groups (Fig. 1), with principal components 1 and 2 explaining ~91% of variability in sensory attributes amongst treatments. Wines made from clarified liquid-phase FD musts were perceived as having intense confectionery and red fruit aroma and flavor, regardless of the

fermentation temperature. Wines from liquid-phase FD treated musts fermented at low to medium temperatures with 3.5% grape solids were perceived as having more intense dark fruit characters and clustered in the top quadrants of the PCA biplot (Fig. 1). In contrast, control wines clustered in the lower right quadrant and were clearly separated from FD wines.

The control wines had a distinctive sensory profile characterized by intense green and savory characters (Tables S2 & S4 of the Supplementary Materials), with high astringency. Based on their concentrations in wines, proanthocyanidins, quercetin glycosides and caftaric acid would be expected to impact astringency, while catechin and epicatechin would not (Hufnagel & Hofmann, 2008). Although 1-hexanol was significantly higher in traditional maceration control fermentations, its calculated OAV suggested that it did not impact green aroma perception in the wines. Dimethyl sulfide, which was higher in control wine, has a characteristic cooked cabbage or asparagus note which might have increased green attribute perception. In addition, other more potent C_6 compounds (Ferreira & Lopez, 2019) not analyzed in the present work may have followed a similar trend to 1-hexanol and could have been responsible for the higher perceived green aroma and flavor in the control wines. The concentration of 3-isobutyl-2-methoxypyrazine (IBMP), a grape derived compound that can also impart intense green aroma attributes, was below the detection limit of the method in all of the wines so would not explain the higher green attribute rating in control wine. The higher savory rating for traditional control ferments was consistent with the higher concentration of dimethyl sulfide, a compound reported to impart a ‘green olive’ attribute (Escudero et al., 2007)**.**

In order to better qualify the relationship between wine chemical composition and wine sensory properties introduced by the treatments, PLSR analysis was performed (Fig. 2). Using data from all of

the winemaking treatments, all of the sensory attributes (except dried fruit aroma and flavor, acidity, body, astringency and alcohol heat) were well-modelled by the chemical data, as indicated by the location of the variables in the outer quadrant of correlation loadings, corresponding to an explained variance of ≥ 50% (Fig. 2B). Replicate ferments for each treatment were positioned closely within the scores plot (Fig. 2A), indicating that fermentations from the respective treatments performed similarly. The R^2 values for calibration and validation of well-described sensory attributes are shown in Table S5 of the Supplementary Materials, together with the corresponding weighted regression coefficients (Fig. S2).

The primary separation of the winemaking treatments was on the first factor (Figure 2a), which distinguished the control from the FD treatments. As already described for the PCA, the control wines were clearly separated on the basis of having higher green and savory aromas and flavors, as well as a higher overall aroma intensity. The separation between the control and FD treatments was mainly due to higher concentrations of polysaccharides and associated sugar constituents, monomeric phenolics (catechin, epicatechin and gallic acid), dimethyl sulfide and lactic acid, as well as higher alcohols. Of the higher alcohols, only 1-hexanol could be described as having a 'green' attribute, and was well correlated with green aroma and flavor (Fig. S2 of the Supplementary Materials). Conversely, all FD wines were associated with elevated red fruit, confectionery and dark fruit attributes relative to the control wines, and were lower in green and savory attributes. The main separation of the FD treatments from the control was due to the presence of higher concentrations of ethyl esters and total esters, acetaldehyde, alcohol, malvidin-3-glucoside and quercetin glucosides. However, separation of FD treatments was evident from the second factor (Figure 2a), based on solids contact. Solids contact in the FD treatments had a greater influence on wine sensory properties and chemical composition than

temperature, as discussed previously. The PLSR revealed that increases in dark fruit attributes and lowered red fruit and confection attributes with higher levels of solids contact were strongly associated with lowered concentrations of multiple ethyl esters, and increased 2-phenyl ethanol together with higher alcohols, namely isoamyl alcohol, methanol and total fusel alcohols.

4. Conclusions

The present study has shed light on how fermentation temperature and grape solids content can be manipulated to influence the chemical composition and sensory profiles of Cabernet Sauvignon wine, revealing potential impacts on the style of red wines prepared from liquid-phase fermentation of FD treated must. Fermentation temperature and suspended solids were shown to impact the general wine chemical composition, as well as color, phenolics and polysaccharides, with important changes to aroma-relevant volatiles. Furthermore, the inclusion of suspended solids in liquid-phase fermentations was clearly shown to have a major effect on both wine chemical composition and the resultant sensory profile. This research has highlighted the contribution of esters such as ethyl octanoate, ethyl hexanoate and isoamyl acetate to the flavor profile of red wines made via liquid phase fermentation of FD treated must. On the other hand, fusel alcohols were thought to play a lesser role. Higher fermentation temperatures and the inclusion of suspended solids were shown to aid polysaccharide extraction into wine, and are suggested to influence mouthfeel, but this requires further investigation. The sensory profiles of Cabernet Sauvignon wine made from liquid-phase FD musts, with or without suspended solids, were clearly distinguishable from one another. More relevant were the clear differences between the FD wines and wines made via traditional maceration, indicating that there is potential for FD technology to be used in the development of novel wine styles. Findings from this research suggest FD offers new opportunities for creating lighter-bodied, fruit-driven red wines with less apparent ‘green’

notes, via off-skins fermentations under non-traditional conditions. These differentiated wines might be used as bases for non-traditional red wine styles, including spritzers, or as blending components for traditional styles of red wines with enhanced fruit profiles.

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Supporting Materials: Figure S1 Flowchart of Cabernet Sauvignon must and FD juice preparation; **Figure S2** Heat map showing weighted regression coefficients of the PLSR model developed to show the relationship between key sensory attributes and significant wine chemical data (identified using an uncertainty test and high correlation loadings); **Table S1** Composition of grapes at harvest and juice following processing by flash détente (FD); **Table S2** Attributes and standards used in descriptive analysis of Cabernet Sauvignon wines; **Table S3** Effect of fermentation temperature and suspended solids on sensory profiles of flash détente derived Cabernet Sauvignon wines.; **Table S4** Mean intensity

ratings for sensory attributes of Cabernet Sauvignon wines made via traditional maceration (Control) vs fermentation of flash détente derived juice (at different temperatures, with and without suspended solids). **Table S5** Partial least squares regression (PLSR) parameters for sensory attributes which were well-modelled using either all the chemical data (all data) or a sub-set of variables identified using an uncertainty test and high correlation loadings (sig var)[§].

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Figure Captions

Fig. 1. Principal component analysis biplot of sensory attributes with statistically significant ratings for Cabernet Sauvignon wines made via traditional maceration (Control) vs. fermentation of flash détente derived juice (at different temperatures, with and without suspended solids). A = aroma attribute; F = flavor attribute; LT, MT and HT denote low (16 °C), medium (24 °C) and high (32 °C) fermentation temperatures, respectively; LS and HS denote low (<0.5%) and high (3.5%) suspended solids, respectively.

Fig. 2. PLS regression results for sensory attributes using significant wine chemical compositional data identified using an uncertainty test and high correlation loadings. **2A.** Scores plot for wines from traditional maceration (Control, green triangle) and flash-détente treatments with different solids content where high solids (HS, red) and low solids (LS, blue) are shown, in combination with temperature regimes low (LT, 16ºC), medium (MT, 24ºC) and high (HT, 32ºC) and Control (30ºC); **2B**. X and Y loadings for PLS2 regression. X loadings (chemical variables) are shown in black, Y loadings (sensory attributes, A, aroma; F, flavor) are shown in red. Codes for wine chemical measures are as follows: **A – Basic wine measurements**: A1, alcohol (%); A2, lactic acid; A3, malic acid; A4, glycerol; A5, pH; A6, titratable acidity; A7, volatile acidity; **B – Wine volatiles and higher alcohols**: B1, dimethyl sulfide; B2, isoamyl acetate; B3, ethyl acetate; B4, ethyl butanoate; B5, ethyl decanoate; B6, ethyl hexanoate; B7, ethyl isobutyrate; B8, ethyl isovalerate; B9, ethyl octanoate; B10, total esters; B11, 2-phenylethanol; B12, 1-hexanol; B13,1-octen-3-ol, B14, acetaldehyde; B15, active amyl alcohol; B16, isoamyl alcohol; B17, isobutyl alcohol; B18, methanol; B19, total fusel alcohols; **C – Wine phenolics and color measures**: C1, caftaric acid; C2, catechin; C3, epicatechin; C4, gallic acid; C5, malvidin-3-glucoside; C6, quercetin glycosides; C7, pigmented polymers; C8, proanthocyanidin (HPLC); C9,

proanthocyanidin (MCP); C10, chemage2 (no units); C11, wine color density; C12, color intensity; C13, Hunter A; C14, Hunter B; C15, Hunter L; C16, A420; C17, A520; C18, hue; C19, total anthocyanin; C20, total phenolics; C21, non-bleachable pigments; **D – Wine polysaccharides and constituent sugar residues**: D1, mannose; D2, rhamnose; D3, glucuronic acid; D4, galacturonic acid; D5, galactose; D6, arabinose; D7, total polysaccharides.

Table 1 Effect of fermentation temperature and suspended solids content on basic chemistry, color, and phenolic and polysaccharide composition of flash détente derived Cabernet Sauvignon wines.

Parameter	Mean Comparisons					*P*-values			Model Adjusted R^2
	Fermentation Temperature			Solids Content		Fermentation Temperature	Solids Content	Fermentation Temperature × Solids Content	
	16 °C	24 °C	32 °C	<0.5%	3.5%				
Basic Chemistry									
ethanol (% v/v)	14.1a	13.8b	13.5c	13.9a	13.7b	*0.0001*	*0.0001*	*0.261*	97.6%
lactic acid (mg/L)	70.0a	60.3b	58.5b	64.2a	61.7a	*0.001*	*0.134*	*0.354*	81.9%
malic acid (g/L)	1.88a	1.87a	1.54b	1.70b	1.83a	*0.0001*	*0.0001*	*0.003*	97.2%
pH	3.51b	3.54ab	3.58a	3.55a	3.53a	*0.034*	*0.262*	*0.147*	56.8%
TA (g/L)	5.3b	6.0a	5.9a	5.4b	6.1a	*0.0001*	*0.0001*	*0.007*	95.6%
VA (g/L)	0.9b	1.5a	1.6a	1.6a	1.1b	*0.0001*	*0.001*	*0.286*	91.0%
glycerol (g/L)	6.6b	11.0a	10.1a	7.9b	10.6a	*0.006*	*0.010*	*0.044*	80.7%
Color									
A420	1.01b	1.21ab	1.33a	1.12a	1.25a	*0.009*	*0.054*	*0.027*	77.4%
A520	1.54b	1.96a	2.07a	1.67b	2.05a	*0.017*	*0.014*	*0.023*	78.0%
A620	0.38b	0.43ab	0.46a	0.42a	0.43a	*0.037*	*0.612*	*0.036*	63.8%
color intensity	2.56b	3.18a	3.41a	2.79b	3.30a	*0.014*	*0.021*	*0.024*	77.9%
WCD SO_2 CORR (au)*	6.06a	6.12a	6.37a	6.35a	6.02a	*0.248*	*0.058*	*0.057*	55.2%
chemical age 2*	0.065b	0.066b	0.081a	0.070a	0.071a	*0.015*	*0.881*	*0.132*	63.7%
non-bleachable pigments (au)*	0.97b	1.01b	1.19a	1.07a	1.04a	*0.005*	*0.429*	*0.017*	79.2%
hue	0.66a	0.63a	0.64a	0.67a	0.61b	*0.125*	*0.001*	*0.013*	84.7%
hue*	0.58b	0.59b	0.61a	0.59a	0.59a	*0.004*	*0.449*	*0.638*	72.6%
Hunter A	56.8a	58.3a	59.0a	56.5b	59.5a	*0.142*	*0.008*	*0.048*	70.5%
Hunter B	15.2b	20.1a	23.4a	17.8b	21.3a	*0.005*	*0.033*	*0.021*	81.0%
Hunter L	45.6a	40.5b	37.3b	43.3a	39.1b	*0.007*	*0.021*	*0.031*	79.9%

Table 1 Contd.

Parameter	Mean Comparisons					*P*-values			Model Adjusted R^2
	Fermentation Temperature			Solids Content		Fermentation Temperature	Solids Content	Fermentation Temperature × Solids Content	
	16 °C	24 °C	32 °C	<0.5%	3.5%				
Phenolic Compounds									
total phenolics (mg/L)	965a	1,010a	982a	1,021a	950b	*0.403*	*0.031*	*0.020*	65.5%
caftaric acid (mg/L)	25.0a	26.8a	25.3a	26.3a	25.0b	*0.046*	*0.030*	*0.273*	60.7%
catechin (mg/L)	21.5a	21.8a	19.5b	21.3a	20.5b	*0.0001*	*0.002*	*0.422*	93.9%
epicatechin (mg/L)	7.25a	6.75a	5.25b	6.67a	6.17a	*0.003*	*0.134*	*1.000*	74.8%
malvidin-3-*O*-glucoside (mg/L)	143.5a	142.3a	133.5b	141.8a	137.7a	*0.022*	*0.114*	*0.708*	57.2%
pigmented polymers (mg/L)	11.8b	12.8ab	13.3a	12.7a	12.5a	*0.014*	*0.585*	*0.014*	74.8%
proanthocyanidins (mg/L)[a]	110.8a	120.3a	116.5a	121.5a	110.2a	*0.443*	*0.095*	*0.042*	52.25%
quercetin glycosides (mg/L)	11.3a	12.0a	10.8a	11.7a	11.0a	*0.229*	*0.253*	*0.03*	55.7%
degree of ionization (%)*	14.7a	14.3a	14.2a	14.4a	14.4a	*0.234*	*0.970*	*0.502*	2.6%
total anthocyanin (mg/L)*	296.5a	305.7a	295.0a	305.2a	293.0a	*0.642*	*0.250*	*0.644*	0.00%
tannin (mg/L)[b]*	377.0a	409.6a	415.8a	471.6a	330.1a	*0.859*	*0.059*	*0.363*	22.2%
Polysaccharides									
mannose (mg/L)	60.1b	61.9b	82.1a	58.1b	78.0a	*0.001*	*0.0001*	*0.225*	90.0%
rhamnose (mg/L)	18.5a	20.3a	20.2a	19.6a	19.7a	*0.240*	*0.947*	*0.172*	23.9%
glucuronic acid (mg/L)	3.90c	4.27b	4.98a	4.29b	4.47a	*0.0001*	*0.024*	*0.061*	95.3%
galacturonic acid (mg/L)	49.2b	54.5ab	62.6a	48.8b	62.1a	*0.036*	*0.006*	*0.520*	70.4%
glucose (mg/L)	24.8b	30.4a	32.5a	29.3a	29.2a	*0.011*	*0.949*	*0.392*	62.8%
galactose (mg/L)	47.8a	46.4a	49.2a	47.8a	47.7a	*0.531*	*0.959*	*0.877*	0.0%
arabinose (mg/L)	20.9b	26.7a	28.5a	25.0a	25.7a	*0.003*	*0.531*	*0.884*	73.9%
arabinose:galactose ratio	0.44b	0.58a	0.58a	0.52a	0.54a	*0.0001*	*0.337*	*0.443*	87.0%
total polysaccharides (mg/L)	225.3b	244.4b	280.0a	232.9b	266.9a	*0.009*	*0.011*	*0.906*	74.1%

Data are means of two replicates (n = 2). Means followed by different letters (within rows) are statistically different (two-way ANOVA, Tukey's LSD post hoc, $\alpha = 0.05$). Polysaccharides were determined as monosaccharide sugars; [a]Proanthocyanidins (as catechin units) determined by HPLC-DAD; [b]proanthocyanidins (as epicatechin units) determined by the methyl cellulose precipitation (MCP) assay. *Measured 1 year after bottling.

Table 2 Basic chemistry, color, and phenolic and polysaccharide composition of Cabernet Sauvignon wines made via traditional maceration (Control) vs fermentation of flash détente derived juice (at different temperatures, with and without suspended solids).

Parameter	Control	LTLS	LTHS	MTLS	MTHS	HTLS	HTHS	*P*-value	Model Adjusted R^2
Basic Chemistry									
ethanol (% v/v)	13.4d	14.2a	14.0b	13.9b	13.6c	13.7c	13.4d	*0.0001*	98.2%
lactic acid (mg/L)	123.5a	71.5b	68.5b	60.0b	60.5b	61.0b	56.0b	*0.001*	86.6%
malic acid (mg/L)	1,904ab	1,754c	2,013a	1,799bc	1,937a	1,546d	1,535d	*0.0001*	96.9%
pH	3.75a	3.54bc	3.53bc	3.54bc	3.53bc	3.57bc	3.59b	*0.0001*	89.5%
TA (g/L)	6.2ab	4.9e	5.7cd	5.5d	6.6a	5.7cd	6.0bc	*0.0001*	96.1%
VA (g/L)	2.6a	1.1cd	0.8d	1.8b	1.2cd	1.8b	1.3c	*0.0001*	96.0%
glycerol (g/L)	10.4ab	6.1b	7.1b	7.9b	14.1a	9.6ab	10.6ab	*0.004*	80.9%
Color									
A420	1.38ab	0.99d	1.03bcd	1.00cd	1.42a	1.36abc	1.30abcd	*0.005*	79.3%
A520	1.71b	1.41b	1.68b	1.49b	2.44a	2.12ab	2.03ab	*0.006*	77.9%
A620	0.43a	0.40a	0.36a	0.38a	0.49a	0.49a	0.44a	*0.03*	63.4%
color intensity	3.09abc	2.41c	2.71bc	2.49bc	3.87a	3.48ab	3.34abc	*0.006*	77.6%
WCD SO_2 CORR (au)*	5.72b	6.39ab	5.73b	5.97ab	6.26ab	6.67a	6.06ab	*0.03*	62.6%
chemical age 2*	0.11a	0.07bc	0.06bc	0.06c	0.07bc	0.08b	0.08bc	*0.001*	88.5%
non-bleachable pigments (au)*	1.23a	1.04abc	0.89c	0.92bc	1.10abc	1.26a	1.13ab	*0.003*	82.8%
hue	0.80a	0.70b	0.61bc	0.67bc	0.59c	0.64bc	0.64bc	*0.001*	87.8%
hue*	0.74a	0.58b	0.58b	0.58b	0.59b	0.61b	0.61b	*0.0001*	94.9%
Hunter A	54.6b	54.3b	59.3ab	55.9ab	60.6a	59.2ab	58.7ab	*0.01*	74.1%
Hunter B	24.7a	14.9c	15.6bc	14.8c	25.3a	23.7a	23.0ab	*0.002*	83.4%
Hunter L	39.8abcd	47.1a	44.2abc	45.9ab	35.1d	36.8cd	37.8bcd	*0.004*	79.8%

Table 2 Contd.

Parameter	Control	LTLS	LTHS	MTLS	MTHS	HTLS	HTHS	*P*-value	Model Adjusted R^2
Phenolic Compounds									
total phenolics (mg/L)	1,083a	1,024ab	907b	975ab	1,044ab	1,063ab	900b	*0.019*	68.6%
caftaric acid (mg/L)	9.50b	25.5a	24.5a	27.0a	26.5a	26.5a	24.0a	*0.0001*	96.7%
catechin (mg/L)	38.0a	22.0b	21.0b	22.0b	21.50b	20.0b	19.0b	*0.0001*	93.6%
epicatechin (mg/L)	12.0a	7.5ab	7.0b	7.0b	6.5b	5.5b	5.0b	*0.008*	75.6%
malvidin-3-*O*-glucoside (mg/L)	122.0c	146.0a	141.0ab	143.0ab	141.5ab	136.5abc	130.5bc	*0.004*	80.4%
pigmented polymers (mg/L)	12.0ab	12.5ab	11.0b	12.0ab	13.5a	13.5a	13.0a	*0.008*	75.8%
proanthocyanidins (mg/L)[a]	149.0a	121.5ab	100.0b	112.5ab	128.0ab	130.5ab	102.5b	*0.035*	61.6%
quercetin glycosides (mg/L)	7.0b	12.0a	10.5ab	11.0a	13.0a	12.0a	9.5ab	*0.007*	76.3%
degree of ionization (%)*	13.93	14.65	14.81	14.20	14.4	14.4	14.0	*0.329*	16.0%
total anthocyanin (mg/L)*	224.96b	308.65a	284.4ab	306.34a	305.0a	300.6a	289.4a	*0.011*	73.4%
tannin (mg/L)[b]*	661.0	472.0	282.0	414.3	404.9	528.3	303.3	*0.106*	44.6%
Polysaccharides									
mannose (mg/L)	84.6a	46.4c	73.9a	53.0bc	70.8ab	74.9a	89.4a	*0.0001*	90.2%
rhamnose (mg/L)	43.5a	18.5b	18.5b	19.0b	21.5b	21.3b	19.1b	*0.0001*	94.8%
glucuronic acid (mg/L)	8.66a	3.93d	3.86d	4.08d	4.46cd	4.85bc	5.10b	*0.0001*	99.0%
galacturonic acid (mg/L)	153.8a	43.9b	54.5b	49.2b	59.7b	53.2b	71.9b	*0.001*	87.1%
glucose (mg/L)	31.0	24.9	24.7	29.2	31.7	33.8	31.2	*0.073*	51.3%
galactose (mg/L)	60.9a	47.5b	48.2ab	46.1b	46.7b	49.9ab	48.4ab	*0.027*	64.5%
arabinose (mg/L)	51.4a	21.0b	20.9b	26.1b	27.2b	27.9b	29.1b	*0.0001*	95.7%
arabinose:galactose ratio	0.84a	0.44c	0.43c	0.57b	0.58b	0.56b	0.60b	*0.0001*	96.5%
total polysaccharides (mg/L)	433.9a	206.1b	244.5b	226.8b	262.0b	265.9b	294.2b	*0.001*	88.9%

Data are means of two replicates (n = 2). Means followed by different letters (within rows) are statistically different (one-way ANOVA, Tukey's LSD post hoc, $\alpha = 0.05$). LT, MT and HT denote low (16 °C), medium (24 °C) and high (32 °C) fermentation temperatures respectively; LS and HS denote low (<0.5%) and high (3.5%) suspended solids, respectively. Polysaccharides were determined as monosaccharide sugars; [a]proanthocyanidins (as catechin units) determined by HPLC-DAD; [b]proanthocyanidins (as epicatechin units) determined by the methyl cellulose precipitation (MCP) assay. *Measured 1 year after bottling.

Table 3 Effect of fermentation temperature and suspended solids content on the volatile composition of flash détente derived Cabernet Sauvignon wines.

Volatile Compound (µg/L)	Mean Comparisons					*P*-values			Model Adjusted R^2
	Fermentation Temperature			Solids Content		Fermentation Temperature	Solids Content	Fermentation Temperature × Solids Content	
	16 °C	24 °C	32 °C	<0.5%	3.5%				
Ethyl Esters									
ethyl butanoate	717a	343b	244b	523a	346b	*0.0001*	*0.001*	*0.104*	96.3%
ethyl decanoate	353a	357a	275b	399a	258b	*0.012*	*0.0001*	*0.384*	89.0%
ethyl dihydrocinnamate	2.73a	2.78a	3.08a	2.77a	2.95a	*0.066*	*0.129*	*0.052*	60.7%
ethyl hexanoate	747a	545b	362c	635a	467b	*0.0001*	*0.0001*	*0.003*	99.3%
ethyl isobutyrate	28.0c	41.5b	62.0a	41.0a	46.7a	*0.0001*	*0.080*	*0.900*	90.8%
ethyl isovalerate	4.75b	5.00b	7.75a	4.83b	6.83a	*0.0001*	*0.0001*	*0.296*	94.9%
ethyl octanoate	1477a	1095b	793c	1357a	886b	*0.0001*	*0.0001*	*0.033*	98.7%
Acetate Esters									
isoamyl acetate	6,066b	7,003b	8,530a	7,976a	6,423b	*0.001*	*0.001*	*0.258*	89.3%
isobutyl acetate	99.3c	161.3b	266.5a	213.5a	137.8b	*0.0001*	*0.0001*	*0.007*	96.6%
2-methylbutyl acetate	158.0b	229.5ab	283.0a	237.8a	209.2a	*0.052*	*0.409*	*0.176*	49.0%
2-phenylethyl acetate	309c	935b	1473a	947a	864a	*0.0001*	*0.404*	*0.581*	90.1%
ethyl acetate	37.3b	38.3b	44.8a	51.0a	29.2b	*0.005*	*0.0001*	*0.241*	96.9%
total esters	13,178b	14,264b	16,867a	16,558a	12,981b	*0.001*	*0.0001*	*0.078*	92.9%
Alcohols, C_{13}-Norisoprenoids and Terpenes									
1-hexanol	261.8a	263.2a	192.4a	183.1b	295.1a	*0.036*	*0.001*	*0.106*	81.5%
1-octen-3-ol	100.0a	62.9b	37.7c	80.6a	53.2b	*0.0001*	*0.0001*	*0.001*	99.1%
methanol (×1000)	21.3a	21.0a	21.0a	20.2b	22.0a	*0.422*	*0.0001*	*0.422*	91.6%
β-damascenone	5.15c	5.95b	6.45a	5.85a	5.85a	*0.0001*	*1.000*	*0.068*	87.2%
linalool	4.25c	8.75b	18.3a	10.3a	10.5a	*0.0001*	*0.842*	*0.046*	95.2%

Table 3 Contd.

Volatile Compound (µg/L)	Mean Comparisons					*P*-values			Model Adjusted R^2
	Fermentation Temperature			Solids Content		Fermentation Temperature	Solids Content	Fermentation Temperature × Solids Content	
	16 °C	24 °C	32 °C	<0.5%	3.5%				
Fusel Alcohols and Aldehydes (×1000)									
active amyl alcohol	37.8c	67.3a	63.5b	36.8b	75.5a	*0.0001*	*0.0001*	*0.0001*	99.7%
isoamyl alcohol	154c	206b	232a	150b	245a	*0.0001*	*0.0001*	*0.001*	99.3%
isobutyl alcohol	23.5c	34.0b	43.8a	26.8b	40.7a	*0.0001*	*0.0001*	*0.0001*	99.5%
2-phenylethanol	13.3b	26.0a	27.8a	16.7b	28.0a	*0.0001*	*0.0001*	*0.001*	98.8%
n-propyl alcohol	36.3a	25.8b	34.5a	31.7a	32.7a	*0.007*	*0.607*	*0.127*	70.4%
total fusel alcohols	265c	359b	402a	262b	422a	*0.0001*	*0.0001*	*0.0001*	99.5%
acetaldehyde	19a	13b	13b	16a	14b	*0.001*	*0.015*	*0.009*	89.5%
Sulfur Compounds									
dimethyl sulfide	7.0	6.3	6.0	6.5	6.3	*0.467*	*0.805*	*0.648*	0.0%

Data are means of two replicates (n = 2). Means followed by different letters (within rows) are statistically different (two-way ANOVA, Tukey's LSD post hoc, $\alpha = 0.05$).

Table 4 Volatile composition of Cabernet Sauvignon wines made via traditional maceration (Control) vs fermentation of flash détente derived juice (at different temperatures, with and without suspended solids).

Volatile Compound (µg/L)	Control	LTLS	LTHS	MTLS	MTHS	HTLS	HTHS	P-value	Detection Threshold (µg/L)	OAV Min	OAV Max	Model Adjusted R^2
Ethyl Esters												
ethyl butanoate	211d	854a	581b	412c	274cd	304cd	184d	*0.0001*	20 [a]	9.2	42.7	96.7%
ethyl decanoate	142d	409ab	297bc	427a	287bc	362ab	189cd	*0.0001*	200 [a]	0.7	2.0	91.0%
ethyl dihydrocinnamate	3.05a	2.75a	2.70a	2.80a	2.75a	2.75a	3.40a	*0.183*	2 [d]	1.4	1.7	32.9%
ethyl hexanoate	338d	870a	624b	617b	473c	420c	305d	*0.0001*	5 [a]	61	174	99.4%
ethyl isobutyrate	63a	25c	31c	38bc	45abc	60ab	64a	*0.001*	15 [d]	1.7	4.3	87.2%
ethyl isovalerate	12.0a	4.0b	5.5ab	4.0b	6.0ab	6.5ab	9.0ab	*0.016*	3 [d]	1.3	4.0	70.3%
ethyl octanoate	543d	1,778a	1,177b	1,298b	892c	996c	590d	*0.001*	2 [a]	272	889	99.0%
Acetate Esters												
isoamyl acetate	4,037d	7,056bc	5,077cd	7,436ab	6,569bc	9,437a	7,623ab	*0.0001*	30 [a]	135	315	90.3%
isobutyl acetate	120c	111c	88c	196b	127c	334a	199b	*0.0001*	1,600 [c]	0.1	0.2	95.8%
2-methylbutyl acetate	188a	169a	148a	203a	256a	342a	224a	*0.098*	5 [b]	29.6	68.4	46.0%
2-phenylethyl acetate	519b	326b	292b	931ab	939ab	1,586a	1,360a	*0.001*	250 [a]	1.2	6.3	87.3%
ethyl acetate	36.0c	49.0ab	25.5d	47.5b	29.0cd	56.5a	33.0cd	*0.0001*	7,500 [a]	<0.1	<0.1	96.7%
total esters	8,425d	15,288b	11,067cd	15,259b	13,268bc	19,126a	14,608b	*0.0001*	–	–	–	94.5%
Alcohols, C_{13}-Norisoprenoids and Terpenes												
1-hexanol	1,912a	200b	324b	180b	346b	169b	216b	*0.0001*	8,000 [a]	0.02	0.2	95.1%
1-octen-3-ol	34.5de	120.7a	79.3b	77.5b	48.4c	43.6cd	31.9e	*0.0001*	40 [e]	0.8	3.0	99.1%
methanol (×1000)	95.5a	20.5b	22.0b	20.0b	22.0b	20.0b	22.0b	*0.0001*	100 *	0.2	1.0	99.5%
β-damascenone	4.9c	4.9c	5.4bc	6.2ab	5.8abc	6.5a	6.4ab	*0.003*	0.05 [a]	98	130	82.8%
linalool	8.0bc	3.5c	5.0c	10.5b	7.0bc	17.0a	19.5a	*0.0001*	25 [d]	0.1	0.8	95.3%

Table 3 Contd.

Volatile Compound (µg/L)	Control	LTLS	LTHS	MTLS	MTHS	HTLS	HTHS	P-value	Detection Threshold (µg/L)	OAV Min	OAV Max	Model Adjusted R^2
Fusel Alcohols and Aldehydes (× 1000)												
active amyl alcohol	87b	23f	53d	39e	96a	49d	79c	*0.0001*	30[c]	0.8	3.2	99.7%
isoamyl alcohol	337a	101e	207c	148d	264b	200c	265b	*0.0001*	30[a]	3.4	11.2	99.0%
isobutyl alcohol	72.0a	13.5f	33.5d	26.5e	41.5c	40.5c	47.0b	*0.0001*	40[a]	0.3	1.8	99.4%
2-phenylethanol	39.1a	10.2e	16.3d	17.4d	34.6b	22.5c	33.2b	*0.0001*	10a	1.0	3.9	99.0%
n-propyl alcohol	37a	39a	34ab	23b	29ab	34ab	35ab	*0.035*	306 [f]	0.1	0.1	61.6%
total fusel alcohols	572a	186e	343c	253d	465b	345c	459b	*0.0001*	–	–	–	99.1%
acetaldehyde	13b	22a	16b	14b	13b	13b	14b	*0.0001*	100 [g]	0.1	0.2	89.9%
Sulfur Compounds												
dimethyl sulfide	11.5a	7.5ab	6.5b	6.0b	6.5b	6.0b	6.0b	*0.011*	10 [a]	0.6	1.2	73.3%
methyl thioacetate	4c	37a	34ab	49a	26abc	10bc	4c	*0.001*	30 *	0.1	1.2	87.5%

Data are means of two replicates (n = 2). Means followed by different letters (within rows) are statistically different (one-way ANOVA, Tukey's LSD post hoc, α = 0.05). LT, MT and HT denote low (16 °C), medium (24 °C) and high (32 °C) fermentation temperatures respectively; LS and HS denote low (<0.5%) and high (3.5%) suspended solids, respectively. *Unpublished E & J Gallo data. [a](Guth, 1997); [b](Teranishi, Flath, Guadagni, Lundin, Mon, & Stevens, 1966); [c](Etievant, 1991); [d](Ferreira, López, & Cacho, 2000); [e](Boutou & Chatonnet, 2007); [f](Peinado, Moreno, Bueno, Moreno, & Mauricio, 2004); [g](Liu et al., 2000).

Fig. 1

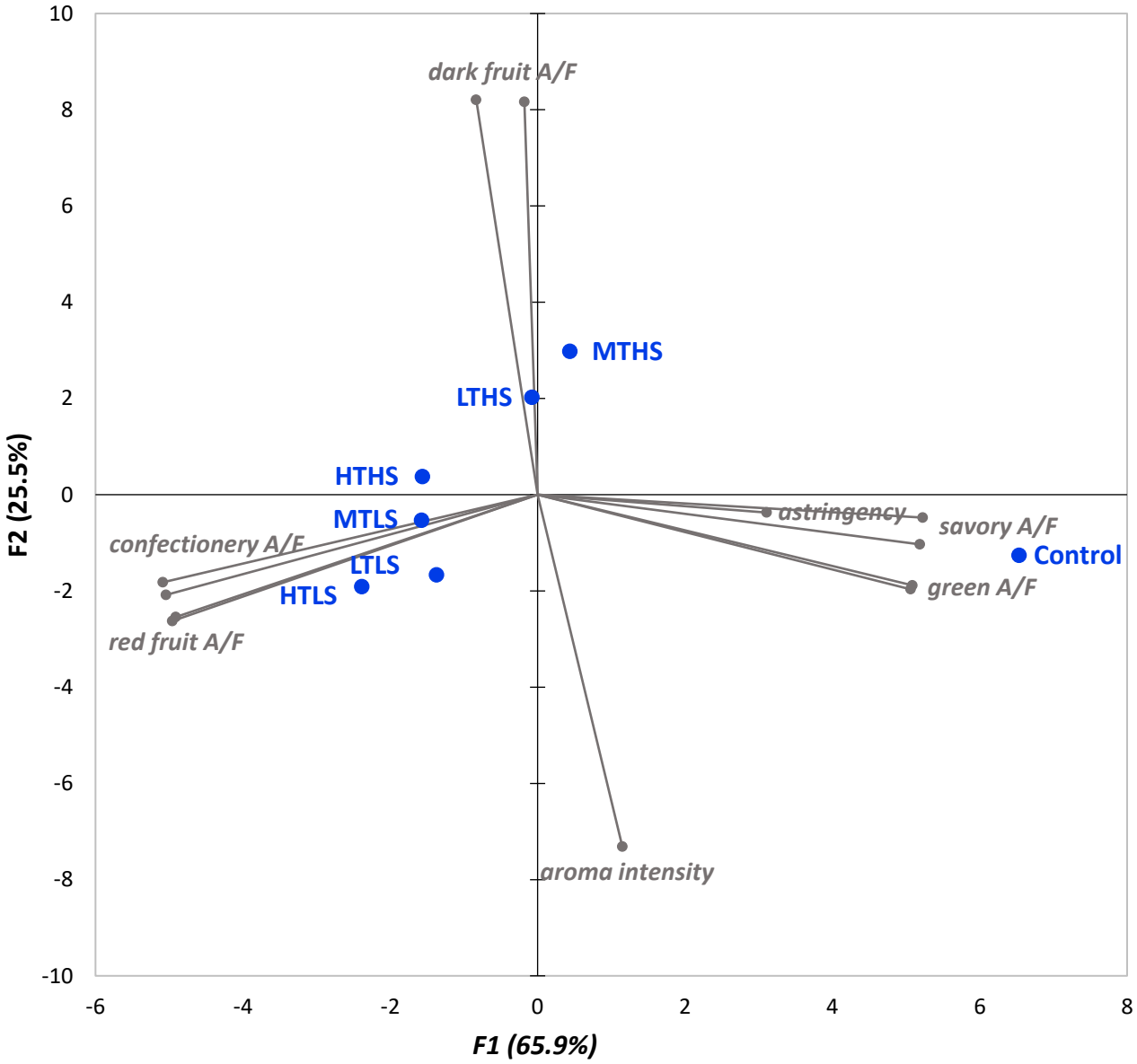
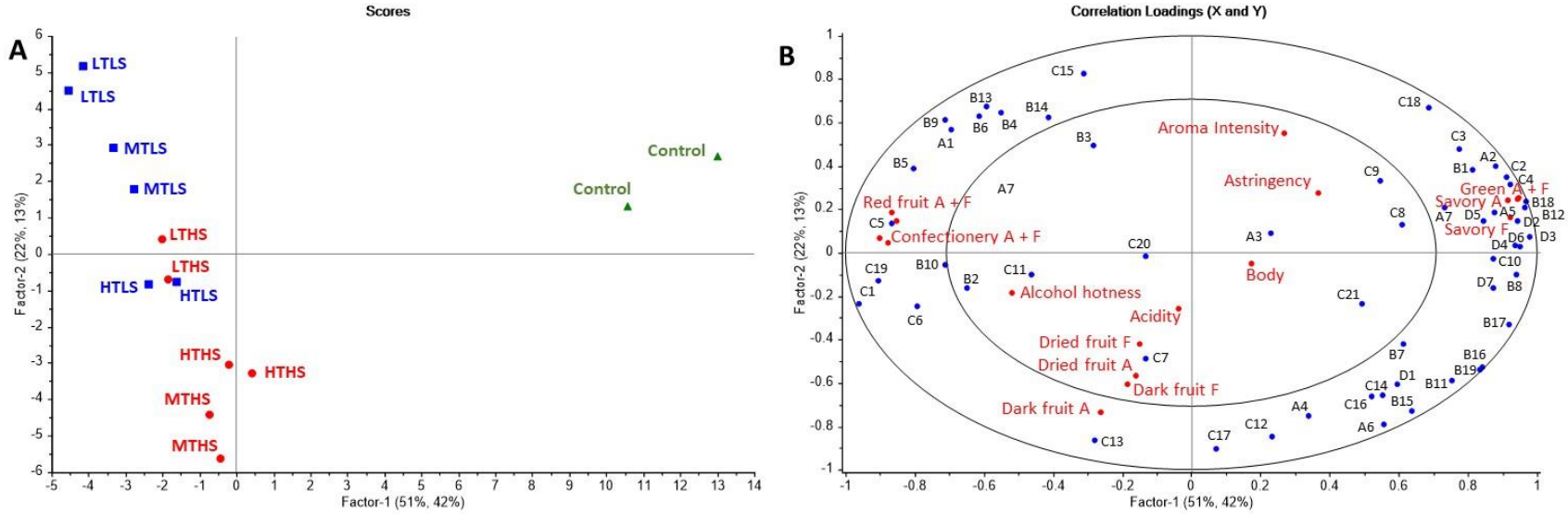

dark fruit A/F
MTHS
LTHS
HTHS
MTLS
LTLS
HTLS
astringency
savory A/F
Control
green A/F
confectionery A/F
red fruit A/F
aroma intensity
F2 (25.5%)
F1 (65.9%)
10
8
6
4
2
0
-2
-4
-6
-8
-10
-6
-4
-2
0
2
4
6
8

Fig. 2


A
Scores
LTLS
LTLS
MTLS
MTLS
LTHS
LTHS
HTLS
HTLS
HTHS
HTHS
MTHS
MTHS
Control
Control
Factor-2 (22%, 13%)
Factor-1 (51%, 42%)
B
Correlation Loadings (X and Y)
Aroma Intensity
Astringency
Red fruit A + F
Confectionery A + F
Alcohol hotness
Body
Acidity
Dried fruit F
Dried fruit A
Dark fruit F
Dark fruit A
Green A + F
Savory A
Savory F
Factor-2 (22%, 13%)
Factor-1 (51%, 42%)

Supplementary Materials

Table S1 Composition of Cabernet Sauvignon grapes at harvest and juice following processing by flash détente (FD).

Parameter	Grapes	FD Juice[a]
total soluble solids (°Brix)	24.4	-
reducing sugars (g/100 mL)	-	23.4
TA (g/L)	2.5	3.2
pH	3.8	4.0
yeast assimilable nitrogen (mg/L)	115	-
glycerol (mg/L)	295	-
gluconic acid (mg/L)	127	-
matter other than grapes (MOG), %	0.4	-
A420 nm	-	6.31
A520 nm	-	9.12
A620 nm	-	2.08
phenolic compounds (mg/L)		
total phenolics	-	1,434
caftaric acid	-	30
catechin	-	24
epicatechin	-	10
grape reaction product	-	6
gallic acid	-	2
malvidin-3-*O*-glucoside	-	194
pigmented polymers	-	11
polymeric tannins	-	106
quercetin glucosides	-	18

[a]Juice was analyzed after water additions were made.

Table S2 Attributes and standards used in descriptive analysis of Cabernet Sauvignon wines.

Attribute	Reference Standards
red fruit	¼ fresh strawberry and ¼ fresh raspberry thinly sliced
dark berry fruit	30 mL of blueberry juice (Bickford's) and 5 fresh blueberries thinly sliced
dark tree fruit	1 tsp of plum jam (Cottee's) and 30 mL of prune juice (Sunraysia)
confectionery	4.5 g each of diced strawberry and cream and raspberry lollies (Natural Confectionery Co.)
bubble gum	1.8 g of thinly sliced bubble gum (Wrigley's Hubba Bubba)
green	3 g of diced rachis (sourced fresh from the vineyard)
dried fruit	1 dried apricot and 1 dried peach diced (Yummy Snack Foods fruit and nut mix)
savory	2 chopped black olives and 15 mL of the juice of the black olive
sweat	unpolished natural leather
alcohol	2 mL of 96% ethanol
acid	tartaric acid; 0.5, 1.0 and 1.5 g/L for low, medium and high
body	xanthan gum: 0.04, 0.08 and 0.12 g/L as low, low–med and medium body
tannin	assorted materials (e.g. silk, velvet, sandpaper)

Standards were prepared in 30 mL of Cabernet Sauvignon wine, except sweat and dark berry fruit standards (which were served as is).

Table S3 Effect of fermentation temperature and suspended solids on sensory profiles of flash détente derived Cabernet Sauvignon wines.

Attribute	**Fermentation Temperature**			***P-values***	**Solids Content**		***P-values***
	16 °C	**24 °C**	**32 °C**		**<0.5%**	**3.5%**	
overall intensity A	65.9	61.8	67.3	*ns*	69.0a	61.0b	*0.003*
red fruit A	52.2	51.7	58.9	*ns*	64.6a	44.0b	*0.0001*
dark fruit A	49.0ab	57.0a	44.4b	*0.049*	40.0b	60.2a	*0.0001*
dried fruit A	20.7	24.9	24.9	*ns*	23.0	23.9	*ns*
confectionery A	51.6	48.1	58.0	*ns*	60.7a	44.5b	*0.0001*
green A	18.8	16.8	13.0	*ns*	14.7	17.7	*ns*
savory A	19.3a	17.5ab	10.6b	*0.016*	13.7	17.8	*ns*
red fruit F	50.3	48.0	58.2	*ns*	60.6a	43.8b	*0.0001*
dark fruit F	48.4ab	52.3a	40.1b	*0.045*	38.7b	55.1a	*0.0001*
dried fruit F	19.0	20.7	20.2	*ns*	19.4	20.6	*ns*
confectionery F	45.0	42.2	52.8	*ns*	53.8a	39.5b	*0.001*
green F	22.9	19.4	18.4	*ns*	18.4	22.0	*ns*
savory F	23.0	24.2	16.6	*ns*	18.3	24.3	*ns*
alcohol	33.3	39.3	31.6	*ns*	34.5	35.0	*ns*
acid	44.8	52.3	44.9	*ns*	47.3	47.4	*ns*
body	45.6ab	52.1a	41.8b	*0.034*	46.8	46.2	*ns*
astringency	48.2ab	52.6a	41.7b	*0.026*	49.4	45.6	*ns*

Data are means of two wine replicates presented to 9 judges during two formal sensory evaluation sessions. Means followed by different letters (within rows) are statistically different (two-way ANOVA, Tukey's LSD post hoc, $\alpha = 0.05$); ns = not significant; A = aroma attribute; F = flavor attribute. No statistically significant two-way interactions (fermentation temperature × solids content) were observed.

Table S4 Mean intensity ratings for sensory attributes of Cabernet Sauvignon wines made via traditional maceration (Control) vs fermentation of flash détente derived juice (at different temperatures, with and without suspended solids).

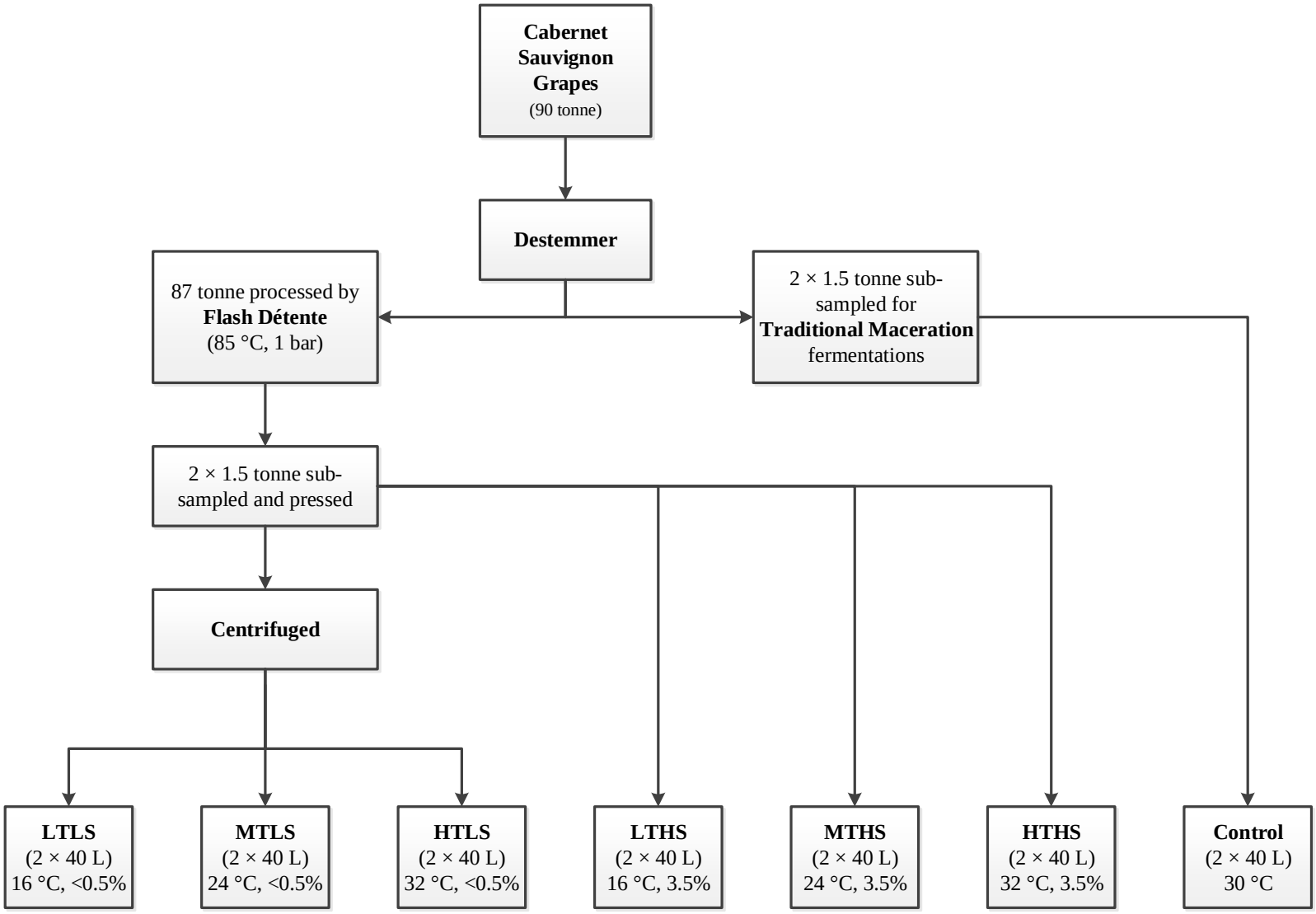
Attribute	Control	LTLS	LTHS	MTLS	MTHS	HTLS	HTHS	*P*
overall intensity A	72.0a	73.8a	57.9b	62.9ab	60.8ab	70.1ab	64.4ab	*0.002*
red fruit A	13.6	62.2ab	42.3bc	65.9a	37.5c	65.7a	52.1abc	*0.0001*
dark berry fruit A	34.8c	39.4bc	58.5ab	43.2bc	70.8a	37.3bc	51.4abc	*0.0001*
dried fruit A	20.6	21.6	19.8	20.5	29.3	26.9	22.8	*ns*
confectionery A	10.9d	59.4ab	43.7bc	56.9abc	39.3c	65.6a	50.4abc	*0.0001*
green A	87.3a	16.8b	20.8b	16.2b	17.4b	11.0b	15.0b	*0.0001*
savory A	63.9a	16.5b	22.1b	14.8b	20.1b	9.9b	11.2b	*0.0001*
red fruit F	16.5d	58.8ab	41.9bc	60.0ab	36.0cd	62.9a	53.5abc	*0.0001*
dark berry fruit F	37.3bc	41.6bc	55.2ab	40.6bc	63.9a	33.9c	46.3abc	*0.0001*
dried fruit F	17.7	20.4	17.6	16.6	24.8	21.1	19.3	*ns*
confectionery F	10.7c	53.7ab	36.3b	48.8ab	35.6b	58.9a	46.6ab	*0.0001*
green F	84.9a	22.7b	23.1b	17.8b	20.9b	14.7b	22.0b	*0.0001*
savory F	64.1a	21.0b	25.0b	20.2b	28.3b	13.7b	19.5b	*0.0001*
alcohol	27.1	35.5	31.1	38.4	40.1	29.6	33.7	*ns*
acid	46.2	42.1	47.6	51.3	53.4	48.4	41.4	*ns*
body	50.4	48.4	42.8	48.0	56.3	43.9	39.6	*ns*
astringency	56.6a	49.9ab	46.5ab	50.8ab	54.3a	47.5ab	36.0b	*0.014*

Data are means of two wine replicates presented to 9 judges during two formal sensory evaluation sessions. Means followed by different letters (within rows) are statistically different (one-way ANOVA, Tukey's LSD post hoc, $\alpha = 0.05$); ns = not significant; A = aroma attribute; F = flavor attribute. LT, MT and HT denote low (16 °C), medium (24 °C) and high (32 °C) fermentation temperatures respectively; LS and HS denote low (<0.5%) and high (3.5%) suspended solids, respectively.

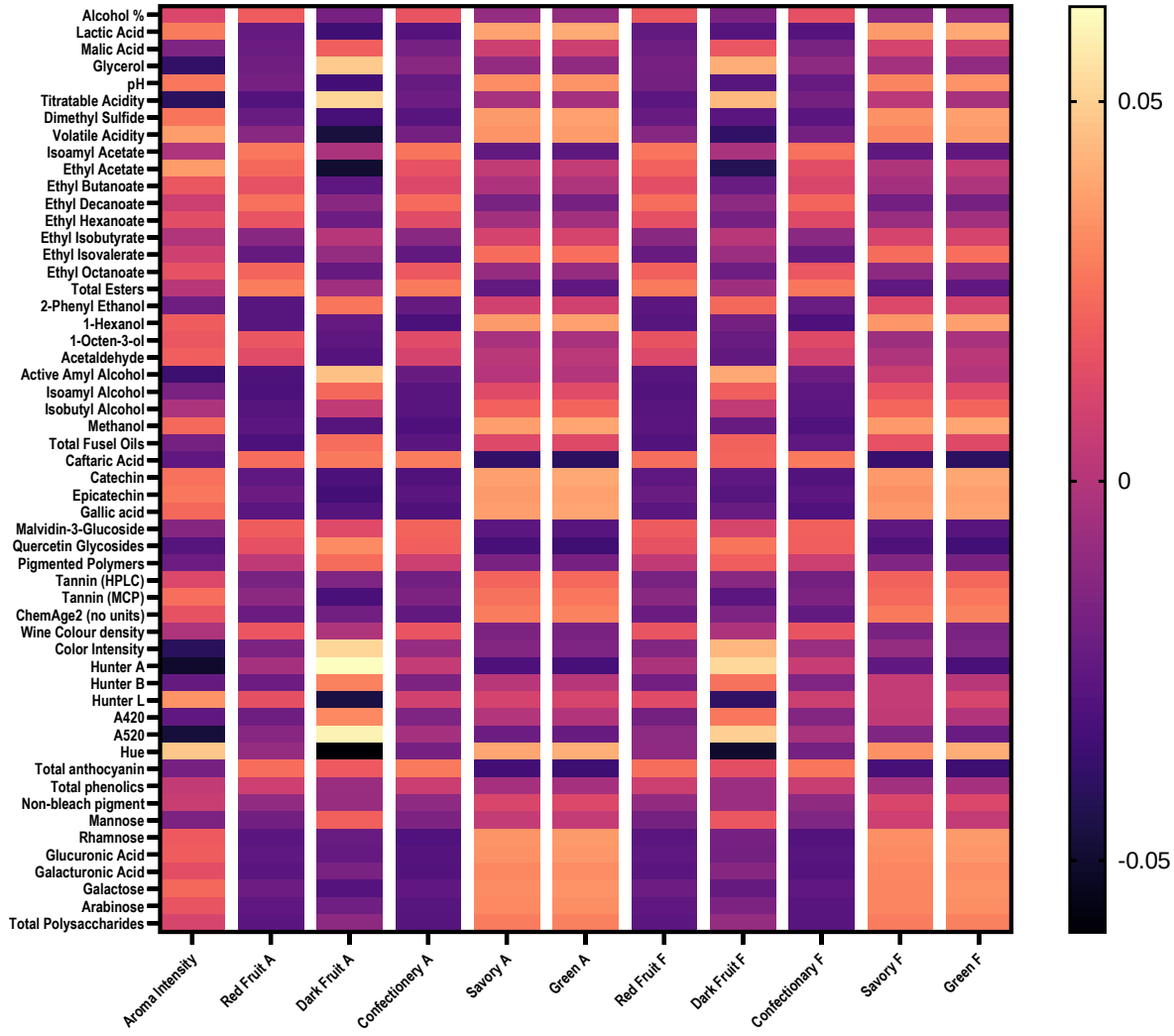
Table S5 Partial least squares regression (PLSR) parameters for sensory attributes which were well-modelled using either all the chemical data (all data) or a sub-set of variables identified using an uncertainty test and high correlation loadings (sig var)[§].

Attribute	Data	PLS Model Parameters				
		Factor no.	R^2_{cal}[†]	R^2_{val}[†]	$RMSE_{cal}$[‡]	$RMSE_{val}$[‡]
overall intensity A	all data	6	0.95	0.81	1.31	2.78
	sig var	4	0.80	0.56	2.68	4.19
red fruit A	all data	6	0.99	0.96	1.67	3.88
	sig var	4	0.97	0.93	3.31	5.16
dark berry fruit A	all data	6	0.95	0.82	2.90	5.84
	sig var	4	0.93	0.82	3.28	5.69
confectionery A	all data	6	0.98	0.92	2.50	5.12
	sig var	4	0.98	0.94	2.52	4.67
green A	all data	6	0.97	0.91	2.86	5.58
	sig var	4	0.98	0.94	2.72	4.67
savory A	all data	6	0.98	0.96	3.26	5.40
	sig var	4	0.98	0.97	3.21	4.97
red fruit F	all data	6	0.99	0.95	1.66	3.65
	sig var	4	0.97	0.94	2.64	4.23
dark berry fruit F	all data	6	0.92	0.71	2.90	6.00
	sig var	4	0.89	0.70	3.46	6.05
confectionery F	all data	6	0.98	0.92	2.19	4.69
	sig var	4	0.96	0.92	2.93	4.64
green F	all data	6	0.96	0.86	3.06	6.36
	sig var	4	0.96	0.90	3.10	5.41
savory F	all data	6	0.97	0.90	4.11	7.96
	sig var	4	0.97	0.91	4.34	7.57

[§] sig var indicates a subset of significant variables selected using an uncertainty test and high correlation loadings; [†] PLSR model parameters where R2 values of cal = calibration, val = validation for the number of factors used are shown; [‡] RSME = root mean square error of prediction for cal and val.



Supplementary Figure S1 Flowchart of Cabernet Sauvignon must and FD juice preparation.



Supplementary Figure S2 Heat map showing weighted regression coefficients of the PLSR model developed to show the relationship between key sensory attributes and significant wine chemical data (identified using an uncertainty test and high correlation loadings).

Chapter 5.

Impact of Skin Contact Time, Oak and Tannin Treatments on Chemical Composition, Color Stability and Sensory Profile of Flash-treated Merlot Wines

Statement of Authorship

Title of Paper	Impact of Skin Contact Time, Oak and Tannin Treatments on Chemical Composition, Color Stability and Sensory Profile of Flash-treated Merlot Wines
Publication Status	☐ Published ☐ Accepted for Publication ☐ Submitted for Publication ☑ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Ntuli, R. G., Saltman Y. Ponangi, R., Bindon K., Jeffery D. W., & Wilkinson, K. L. (2020). Impact of Skin Contact Time, Oak and Tannin Treatments on Chemical Composition, Color Stability and Sensory Profile of Flash-treated Merlot Wines. Prepared for submission to Food Chemistry

Principal Author

Name of Principal Author (Candidate)	Richard G. Ntuli		
Contribution to the Paper	Designed experiments, planned and executed production and laboratory scale trials, performed statistical analyses on data sets, interpreted the data and wrote 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	25/07/2020

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.

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Impact of Skin Contact Time, Oak and Tannin Addition on the Chemical Composition, Color Stability and Sensory Profile of Flash-Détente Merlot Wines

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Highlights

- The effects of flash détente, on vs off skins fermentation and adjuncts were studied
- Fermenting off skins enhanced red fruit and confection aromas but lessened mouthfeel
- Fermenting on or off skins with oak additives, enhanced dark fruit attributes
- Flash détente mitigated the intensity of green, savory and dusty attributes
- Oak and tannin addition pre-fermentation did not improve wine color stability

ABSTRACT

This study investigated the impact on wine composition and style of fermenting flash détente (FD) treated Merlot must with different levels of bulk and fine-settleable grape solids, along with pre-fermentation addition of enological tannin or toasted oak chips for stabilizing the color of Merlot wine made from FD juice. Treatments varied from juice-only fermentations to fermentations which were drained and pressed off skins at residual sugar concentrations of 17, 7 and 0 °Brix. Off-skins fermentations with or without 0.4 g/L of enological tannin produced wines with significantly higher red fruit and confectionery ratings, whereas those with 4 g/L addition of toasted oak chips produced wines with significantly higher dark fruit, vanilla, toasty and allspice ratings. Macerative fermentation of FD must produced wines with significantly higher dark fruit, body and astringency ratings. All wines from FD processing had significantly lower green, savory and dusty ratings. Pre-fermentation addition of enological tannin or toasted oak chips did not improve wine color stability.

Keywords: flash release, flash expansion, on-skins, off-skins, liquid phase, bulk solids, fine solids

1. Introduction

The ability to derive different red wine styles is key to meeting the diverse sensory preferences of different consumer groups and wine producers constantly adapt viticultural and winemaking practices to achieve this. Extraction of grape components, which can be undertaken in a variety of ways, is a main focal point when producing quality red wine. One innovative approach utilizes flash detenté (FD, also known as flash release), where the combination of heat and vacuum treatment during must processing causes grape cell membranes to rupture, rendering phenolics and other grape components such as polysaccharides more extractable into juice. Fermenting flash détente treated juice with different levels of skin contact has the potential to produce differentiated wines styles compared to traditional red winemaking, thereby offering flexibility when crafting wines for certain market segments.

Different styles can be attained by influencing mouthfeel and/or aroma and flavor attributes of wine. Mouthfeel encompasses body or viscosity, astringency and other tactile oral sensations. Body in wine has been reported to be influenced by polysaccharides, glycerol and alcohol whereas astringency is impacted by phenolic compounds and organic acids as well as interaction between polyphenolic compounds and polysaccharides (Quijada-Morín, Williams, Rivas-Gonzalo, Doco, & Escribano-Bailón, 2014). Factors that drive extraction of phenolic compounds into wine (Sacchi, Bisson, & Adams, 2005), and those that impact concentration of volatile compounds in red wines derived from traditional classic maceration (Longo et al., 2020) are well documented. The factors influencing phenolic and volatile compounds composition include must heating, fermentation temperature, maceration time, the use of enzymes, cap management, must or grape freezing, saigneé, cold soak, must nutrient status, yeast selection and oxygen availability. Although some studies (Geffroy, Lopez, Serrano, Dufourcq, Gracia-Moreno, Cacho, et al., 2015; Maza et al., 2019) have investigated the impact of thermovinification on

wine phenolic and volatile composition, few studies have investigated the effect of FD on phenolic (Samoticha, Wojdyło, Chmielewska, & Oszmiański, 2017; Morel-Salmi, Souquet, Bes, & Cheynier, 2006) and polysaccharide (Doco, Williams, & Cheynier, 2007) extraction, or wine volatile composition and sensory profiles. Thus, despite the relative maturity of FD as a technology, the effect of different fermentation conditions on the chemical and sensory profiles of wines derived from FD treated red grape must warrants further investigation.

Classic maceration is a relatively slow and inefficient process such that only about 40% of anthocyanins and 20% of grape skin tannins will have been extracted by the end of fermentation (Cerpa-Calderón & Kennedy, 2008). In comparison, fermentations of FD treated must have been shown to achieve up to 60% proanthocyanidin extraction (Morel-Salmi, Souquet, Bes, & Cheynier, 2006), making early pressing a potentially viable tool for the modulation of wine composition and sensory properties. The heating associated with FD also degrades oxidative enzymes while diminishing the concentration of some grape derived aroma compounds in wine (Geffroy, et al. (2015). The enhanced extraction occurs earlier than normal during winemaking, offering the potential to implement different winemaking regimes. As such, FD red must could be fermented ‘on skins’ more like a traditional macerative fermentation (where the level of skin contact during fermentation is dictated by the need to attain a certain level of extraction), or pressed and fermented ‘off skins’ in a similar approach to white wine production.. The enhanced early extraction with FD allows the contact time for grape skins and suspended fine solids to be varied, thus offering an efficient means to control wine composition and sensory properties without negatively impacting phenolic extraction. Limiting skin contact time through earlier draining and pressing has the added benefit of potentially mitigating the need for capital expenditure to increase red fermentor capacity. Taking this one step further, eliminating skin contact

during fermentation would make it possible to ferment red wines in less expensive white wine fermentors, thereby circumventing the need for more complex and costly red fermentors.

Color loss during and after fermentation is a major challenge during liquid phase red fermentations (Gao, Girard, Mazza, & Reynolds, 1997; Morel-Salmi, Souquet, Bes, & Cheynier, 2006). Gao, Girard, Mazza, and Reynolds (1997) noted that the anthocyanin concentration of heat extracted juice was at its maximum concentration at the start of fermentation, and approximately three times that of classic maceration. During fermentation, the anthocyanin concentration of heated juice decreased at a faster rate compared with that of classic maceration, however, once peak anthocyanin concentrations had been attained. Finding a way to preserve the color extracted into juice during fermentation would overcome one of the key drawbacks of liquid phase red fermentations.

The addition of commercial tannin or oak, either pre- or post-alcoholic fermentation, has been explored as a means of stabilizing wine color. Inconsistent results have been reported in literature, however Pre-fermentation addition of enological tannins derived from grape skin, seed and oak (Chen, Escott, Loira, del Fresno, Morata, Tesfaye, et al., 2016) yielded a positive effect on red wine color stabilization, although some of the enological tannins might have contributed color to the wines, and research involving model wines (Vignault, Gombau, Pascual, Jourdes, Moine, Canals, et al., 2019) suggested a beneficial copigmentation effect, with hydrolyzable tannins being more effective compared to condensed tannins. In contrast, Ghanem, Taillandier, Rizk, Rizk, Nehme, Souchard, et al. (2017) found no effect when enological tannins derived from grapes were added post-fermentation and a number of studies (Gómez García-Carpintero, Gómez Gallego, Sánchez-Palomo, & González Viñas, 2012); (Rodríguez-Bencomo, 2010) have investigated pre-fermentation addition of oak chips, but rarely

considered the impact on color stability. Some studies reported pre-fermentation oak addition can improve short term color stability (Gordillo, Cejudo-Bastante, Rodríguez-Pulido, González-Miret, & Heredia, 2013), while others (Soto Vázquez, 2010) reported no increase in co-pigmentation or improvement in color stability in wines after bottling. To date, no studies have reported the impact of tannin or oak treatment on the long term color stability of bottled wines.

Given the possibilities of FD during winemaking but the relative lack of research, the aim of the study was to investigate the impact on chemical composition and sensory profiles of maceration time on skins during fermentation for flash détente derived red wine. The study also aimed to investigate whether pre-fermentation addition of enological tannin or toasted oak chips could be used for long term color stabilization of red wines made via off-skins fermentation of flash détente derived juice.

2. Materials and methods

2.1. Must and juice preparation

Merlot grapes (90 tonnes) were machine harvested from a vineyard located in the Lodi area in Central Valley region of California. Fruit was sampled from each truck using a zone sampler (Yuba City Steel, Yuba City, CA, USA) and lightly pressed to obtain juice that was analyzed by fourier transform infrared spectroscopy (FTIR) using a WineScan FT120 interferometer (Foss Electric, Hillerød, Denmark) for determination of basic chemical parameters (Table S1 of the Supplementary Materials). Grapes were received at the Gallo Livingston winery, destemmed and crushed, and must (1.5 tonnes, in duplicate) was collected for traditional maceration (i.e. control) fermentation. Potassium metabisulfite was added to attain 60 mg/L of total SO_2, before must was transferred to 40 L cans (one per 1.5 tonne

replicate), with mixing to assure homogeneity, and transported to the Gallo research winery for fermentation (Figure S1 of the Supplementary Materials).

2.2. Flash détente processing conditions

The remaining must (~87 tonnes) was flash treated using a Della Toffola commercial flash détente unit (Della Toffola, Trevignano, Italy) running in a continuous processing mode at 27 tonnes of must per hour. Must was heated to 85 °C while maintaining the vacuum chamber at -0.94 bar. On entering the vacuum chamber, must temperature instantaneously dropped to 32 °C due to absorption of the latent heat of vaporization resulting from the flashing off of heated must under vacuum. Approximately 10% of the must volume was lost as condensate from the vacuum chamber. This increased total soluble solids from 25.2 °Brix in the grapes, to 27.9 °Brix in the must post-flash treatment (Table S1 of the Supplementary Materials).

The flashed must (FM) was collected in three 1.5 tonne bins upon exiting the flash chamber and potassium metabisulfite was added to attain 60 mg/L of total SO_2. Must from one bin was transferred into 6 × 40 L cans while mixing to assure homogeneity and shipped to Gallo research winery for fermentation. The must in the two remaining bins were transferred into a Diemme pneumatic press (Diemme Enologia, Lugo, Italy) and pressed at 2 bars at the Livingston pilot winery. Flashed juice was collected from the press, mixed and centrifuged to reduce solids to less than 1% v/v wet suspended solids (measured by centrifuging juices (4000 × *g* for 15 min) using a swinging bucket centrifuge (Beckman Coulter, Brea, CA)).

Clarified flashed juice was transferred into 6 × 40 L cans and transported to the Gallo research winery for fermentation. Basic juice chemistry composition was measured as described above for grapes. Phenolic compounds were analyzed as described in Section 2.6 below. Chemical parameters for diluted juice are summarized in Table S1 of the Supplementary Materials.

2.3. Fermentations

Fermentations were carried out using 40 L fermentors with temperature control and mixing capabilities. All fermentations had water added pre-ferment to adjust reducing sugar concentration to 23.4 g/100 mL of juice. The YAN target was set at 300 mg/L and diammonium phosphate was used as a nitrogen supplement. Tartaric acid was added to attain a pH of 3.70 at the start of fermentation. Rohavin MX enzyme (AB Enzymes, Darmstadt, Germany) was added to all ferments at a dose rate of 0.04 mL/L. Each fermentation was inoculated with Lalvin ICV D254 yeast (Lallemand, Canada) added at 0.18 g/L, and fermented at 30 °C. A total of 7 fermentation treatments (in duplicate) were performed (Figure S1 of the Supplementary Materials). Three were off-skins fermentations using FD-derived juice with: no additives (FJ); the addition of 0.4 g/L of commercial enological tannin (FJ + Tannin); or the addition of 4 g/L of medium toasted oak chips (FJ + Oak), representing an intermediate addition rate. The enological tannin, which comprised a mixture of grape and oak tannins, and was added above the maximum recommended dose of 0.3 g/L, was dissolved 1:10 w/v in warm water and added directly to the FD juice. The total phenolics content of the tannin (based on Folin–Ciocalteu analysis) was 51% on a dry mass basis. Three on-skins fermentations were also performed, using FD-derived must pressed at 17, 7 and 0 °Brix, representing 1, 2 and 5 days of fermentative skin contact, respectively (FM Day 1, FM Day 2, and FM Day 5). Additionally, a control involving traditional fermentative maceration (pressed at 0 °Brix after 5 days of skin contact) was included (TM Control). Cap management for all on-skins fermentations

was via pump-over at a rate of 4 tank volumes per day. All wines were fermented to dryness (i.e., reducing sugars < 0.5 g/L), after which free sulfur dioxide was adjusted to 35 mg/L using potassium metabisulfite, and wines filtered through 1 µm cellulose filter pads (Gusmer Enterprises, Fresno, CA, USA) followed by a Vitipore II Plus 0.45 µm polyvinylidene fluoride filter, prior to bottling in 750 mL glass bottles with screw cap closures using a GAI 1006 monoblock filler (Prospero International, Perugia, Italy).

2.4. Basic chemical analysis of juice and wine

Juice and wine analyses by FTIR included total soluble solids, titratable acidity (TA), pH, free and total sulfur dioxide, alcohol (as % v/v), and reducing sugars. Absorbances at 420, 520 and 620 nm were measured using a UV/visible spectrophotometer (Perkin Elmer, Waltham, MA, USA).

2.5. Analysis of total phenolics and proanthocyanidins

Total phenolics was measured by the Folin–Ciocalteu method (Singleton and Rossi (1965) and reported in gallic acid equivalents (GAE). Proanthocyanidins were determined in wine after one year in the bottle using the methyl cellulose precipitation (MCP) assay as described previously (Kassara & Kennedy, 2011; Mercurio, Dambergs, Herderich, & Smith, 2007).

2.6. Analysis of phenolic compounds by HPLC

Juice and wine samples were filtered through 0.45 µm cellulose acetate syringe filters (Whatman, Maidstone, UK) and analyzed by reversed-phase chromatography using an Agilent 1200 HPLC (Agilent Technologies, CA, USA) equipped with a quaternary pump, diode array detector (DAD) and Varian

PLRP-S column (250 × 4.6 mm, 5 µm, Varian Inc., Palo Alto, CA, USA) protected by a PLRP-S guard cartridge. A binary solvent gradient consisted of water with 0.5% (v/v) orthophosphoric acid (85% w/v) for mobile phase A, and acetonitrile with 0.5% (v/v) orthophosphoric acid (85% w/v) for mobile phase B. The column thermostat was set at 50 °C and injection volume was 20 µL. Standards and wavelengths used for quantifying individual phenolic compounds were as previously described (Ntuli, Ponangi, Jeffery, & Wilkinson, 2020). Proanthocyanidins were quantified at 230 nm instead of 280 nm to eliminate interference with co-eluting anthocyanins that would show up as shoulder peaks on proanthocyanidin hump at 280 nm. The co-eluting anthocyanins had similar absorption peaks at 532 nm and 230 nm whereas proanthocyanidins had no absorption peak at 532 nm, making it possible to eliminate anthocyanin interference.

2.7. Total anthocyanins by spectrophotometry

Total anthocyanins in wines were determined by the modified Somer's Assay as described by Mercurio, Dambergs, Herderich, and Smith (2007). A 20 µL aliquot of wine was added to 980 µL of 1 M HCl and mixed thoroughly. Samples were left to stand in the dark for 1 hour. A further 100 µL of wine was added to 900 µL of buffer solution containing 0.5% (w/v) tartaric acid, 12% (v/v) ethanol and 0.375% (w/v) sodium metabisulfite, mixed and left to stand in the dark for 1 hour. A 300 µL aliquot of pH adjusted sample was transferred into a 370 µL 96 well UV plate (Greiner Bio-One, Kremsmünster, Austria) and absorbances at 520 nm measured using a SpectraMax M2 Microplate Reader (Molecular Devices, San Jose, CA, USA). The instrument was corrected using an appropriate sample blank and a water constant correct function to approximate a 1 cm path length.

2.8. Tannin composition analysis by phloroglucinolysis and gel permeation chromatography

Wine tannin was isolated using SPE on Oasis HLB (3 mL, 60 mg, 30 μm) cartridges (Waters, Rydalmere, NSW, Australia). The method was a variation of a published method (Jeffery, Mercurio, Herderich, Hayasaka, & Smith, 2008) modified for the isolation of total tannin as described previously (Kassara & Kennedy, 2011). SPE isolates were reconstituted in methanol and analyzed using phloroglucinolysis to determine tannin subunit composition and mean degree of polymerization (mDP) according to the micro-scale approach described by (Kassara & Kennedy, 2011). Samples were also analyzed by gel permeation chromatography (GPC) to determine tannin size distribution. The GPC approach was adapted to allow for increased size distribution resolution of high molecular mass material as described previously (Bindon & Kennedy, 2011). Briefly, two PL gel columns were connected in series: 500 Å followed by 10^4 Å (both 300 × 7.5 mm, 5 μm; Varian Inc., Mulgrave, Vic., Australia) protected by a guard column (50 × 7.5 mm, 5 μm) comprising the same material. The isocratic method used mobile phase of *N*,*N*-dimethylformamide containing 1% v/v glacial acetic acid, 5% v/v water and 0.15 M lithium chloride. Prior to GPC analysis, SPE isolates in methanol were diluted with 4 volumes of mobile phase and the injection volume was 20 μL. The flow rate was maintained at 1 mL/min with a column temperature of 60 °C, where elution was monitored at 280 nm and 520 nm. For calibration, a second order polynomial was fitted with the cumulative mass distribution at 50% for each standard, which were the same as those used previously (Bindon & Kennedy, 2011).

2.9. Polysaccharide preparation and quantification by HPLC

Polysaccharides in wines were quantified as previously described (Bindon, Kassara, Solomon, Bartel, Smith, Barker, et al., 2019). Briefly, A 1 mL aliquot of wine was added to 5 mL of absolute ethanol, mixed and held at 4 °C to precipitate for 18 h. Samples were centrifuged at 8000 × *g* for 5 min,

the supernatant was discarded and the pellet recovered. Pellets were air dried briefly to remove excess ethanol, and reconstituted in 800 µL of Milli-Q water. Samples were then dialyzed at 4 °C in a 1 mL Pur-a-Lyzer (Sigma-Aldrich, St. Louis, MO, USA) tube (3500 Da cut off) against 3 changes of Milli-Q water. Thereafter, samples were frozen at −80 °C and lyophilized. Dry material was then hydrolyzed in 2 M TFA at 100 °C for 3 h, cooled on ice, and concentrated under vacuum at 30 °C (Heto-Holten A/S, Allerød, Denmark). After being resuspended in Milli-Q water, monosaccharide liberated by hydrolysis were quantified as described previously (Bindon, et al., 2019).

2.10. Analysis of wine volatile compounds by GC-MS

Volatile compounds comprising esters, fusel alcohols, terpenes, C_{13}-norisoprenoids and C_6 compounds were extracted using headspace solid-phase microextraction (SPME) with a divinylbenzene/carboxen on polydimethylsiloxane coating fiber assembly (Supelco, Bellefonte, PA, USA) and analyzed by GC-MS using published methodology (Siebert, Smyth, Capone, Neuwöhner, Pardon, Skouroumounis, et al., 2005) with some modifications. Analysis was performed on a Hewlett Packard 6890 gas chromatograph (Agilent Technologies) equipped with an HP 5975 mass selective detector (MS), CTC Combi Pal autosampler and a DB-5ms+ DG 30 m × 0.250 mm I.D., 0.5 µm film thickness (J&W Scientific, Santa Clara, CA, USA). The carrier gas used was helium, operating at 2 mL/min. The oven temperature program was modified as follows: 40 °C for 5 min, increased by 3 °C/min to 170 °C, and then 30 °C/min to a final temperature of 275 °C. Calibration standards were ethyl hexanoate, ethyl octanoate, 1-octen-3-ol, ethyl isovalerate, ethyl butanoate, ethyl dihydrocinnamate, ethyl isobutyrate, 2-methyl butyl acetate, and 2-phenylethanol (Sigma-Aldrich); linalool, 2-phenylethyl acetate (Fluka, St. Louis, MO, USA); β-damascenone (Advanced Biotech, Inc., Totowa, NJ, USA); and isoamyl acetate (Mallickrodt, St. Louis, MO, USA). For determination of oak volatiles, wine samples

underwent liquid-liquid extraction and analysis by GC-MS as previously described (Prida & Chatonnet, 2010).

2.11. Sensory analysis of wine

Wine sensory profiles were determined by descriptive analysis (DA) with a trained panel of 10 judges (8 female and 2 male, aged between 18 and 50 years), comprising University of Adelaide wine science staff and students. Panelists were recruited based on their availability and prior wine sensory experience, and completed 12 hours of training (6 × 2 hours sessions held weekly) prior to formal evaluation. Training sessions involved identification of descriptive terms, familiarization in recognizing and rating the intensity of attributes, and practice evaluation sessions conducted in isolated sensory booths under the controlled conditions used during formal evaluation (i.e. red lighting and a temperature of 22–23 °C) This also enabled evaluation of panel performance.

The DA panel generated 23 attributes comprising aroma and flavors of red fruit, fresh dark fruit, jam dark fruit, confectionery, vanilla, toasty, allspice, green, savory, and dusty, as well as overall aroma intensity, astringency, and body. Reference standards were prepared (Supplementary Table S2) for use in training sessions and during formal evaluations.

Two formal evaluation sessions were held (on two different days), with 14 wines (i.e., duplicates from the seven fermentation treatments) presented at each session. Wines were presented in a randomized order, in four digit-coded XL5 215 mL stemmed wine glasses (covered with lids), at ambient temperature in brackets of seven wines. Breaks were enforced between each bracket (5 min) and between each wine (1 min) to avoid sensory fatigue. Distilled water and plain crackers were also provided as

palate cleaners. Panelists rated the intensity of each sensory attributed using 15 cm unstructured line scales, with anchor points of 'low' and 'high' placed at 10 and 90% on the scale, respectively. Data were acquired with Red Jade software (Redwood Shores, CA, USA). DA panelists gave informed consent before participating in the study, which was approved by the Human Research Ethics Committee of the University of Adelaide (HREC-2018-067).

2.12. Statistical analysis

Chemical data were analyzed by one-way analysis of variance (ANOVA) and repeated measures ANOVA using Minitab (State College, Pennsylvania). Sensory data were analyzed by one-way ANOVA using Minitab. Principal component analysis (PCA) of sensory data was performed using XLSTAT (Addinsoft, Paris, France). Tukey-HSD post hoc test was used for mean comparisons of treatments with significant differences ($\alpha = 0.05$).

3. Results and discussion

3.1. Effect of treatments on basic wine composition

One of the key objectives of the present study was to assess the impact of skin contact of FD treated must on wine chemical composition. Wines made from FD treated must fermented on skins (FM), and wines made via traditional maceration of untreated must (TM Control) had significantly higher malic acid ($P = 0.0001$), titratable acidity ($P = 0.0001$), and glycerol ($P = 0.0001$) compared to wines from pressed-off FD treated must that was fermented off-skins (FJ treatments) (Table 1). This suggests that skin contact might have facilitated extraction of organic acids during fermentation or mitigated their precipitation as salts due to increased polysaccharide extraction. On the other hand, phytosterols in grape

solids might have increased glycerol formation by yeasts. Traditional maceration of untreated must produced wines that had a significantly higher concentration of volatile acidity ($P = 0.0001$) compared to ferments that underwent flash treatment, likely due to the presence of native lactic acid and acetic acid bacteria in the former.

3.2. Effect of treatments on phenolic composition

The wines from FD treated musts, fermented either on- or off-skins, all had significantly higher concentrations of caftaric acid, malvidin-3-glucoside, and quercetin glycosides compared to TM Control wine (Table 1). There was no significant difference in the concentration of caftaric acid and malvidin-3-glucoside, nor in mDP, between FD treatments fermented either off or on-skins, indicating that a rapid extraction of these two compounds occurred in response to flash treatment. It was expected that heat induced inactivation of polyphenol oxidase prevented enzymatic oxidation of caftaric acid, facilitating its retention in wine, while FD treatment increased the extraction of malvidin-3-glucoside and quercetin glycosides in musts. Increasing the skin contact time following FD treatment significantly increased both total phenolics and proanthocyanidin concentrations, as well as the proportion of epicatechin gallate subunits (based on percent galloylation, Table 1), which is considered to be due to increased tannin extraction from seeds. Increasing the skin contact time of flash détente treated must (FM treatments) did not significantly affect the concentration of caftaric acid, proanthocyanidins, malvidin-3-glucoside, pigmented polymers, and quercetin glycosides, nor mDP. By comparison, FD treated musts fermented off-skins (FJ treatments) had significantly lower total phenolics, galloylated tannin, flavan-3-ol monomers, pigmented polymers, proanthocyanidins and quercetin glycosides than their on-skins counterparts. Notably, enological tannin addition did not remediate the lower extraction of phenolics, thereby resulting in a wine with ~70% of the total phenolics concentration of the ferment with highest

level of extraction (FM Day 5). Tannin composition in the traditional maceration treatments was more similar to the FJ treatments, having higher trihydroxylation and lower galloylation than FM treatments. Unlike in the TM Control fermentations where both seed and skin tannin extraction can be anticipated to increase during fermentation (Cerpa-Calderón & Kennedy, 2008), these findings indicated that FD favored pre-fermentation skin tannin extraction (higher tri-OH %), whereas extraction of seed in FM treatments may have occurred to a greater extent (higher galloylation %) compared to TM Control.

3.3. Effect of enological treatments on wine color stability

Unlike for classic maceration ferments where color extraction takes place during fermentation, the flash détente approach favors the rapid pre-fermentation extraction of color into juice. Approximately 40% of red color that was extracted into juice through flash treatment was lost during fermentation (Table 2; Supplementary Table S1). Adding a commercially available enological tannin at 0.4 g/L or adding toasted oak at 4 g/L as a source of hydrolyzable tannins did not improve wine color stability or impact anthocyanin and pigmented polymer concentration despite increasing proanthocyanidin concentration in wine by ~30% and ~20%, respectively compared to FJ only ferment (Tables 1 and 2). At the end of primary fermentation there was no significant difference in A520, A420, A620, color intensity and hue between FJ + Tannin or FJ + Oak wines and FJ wine without additives. Furthermore, repeated measures ANOVA showed no significant difference in color among the wines even after 12 months of bottle aging. Other researchers (Harbertson, Parpinello, Heymann, & Downey, 2012) have reported similar results, with no increase in color observed following addition of enological tannin prior to barrel aging for 3 months.

Immediately after bottling, FM wines had significantly higher A520, A420, A620 and color intensity compared to either TM Control wines or FJ wine series. Increasing the skin contact time of the FM ferments, however, did not significantly influence color parameters of the wine. These results indicated that with FM ferments, the plateau for color extraction was reached early on during fermentation, such that no further increase in color was found from pressing when sugar was below 17 °Brix. All wines had no significant difference in hue at the time of bottling and after 12 months of bottle aging (Table 2). Interestingly, FM wines lost more red color (~60%) during 12 months of aging compared to FJ wine series and TM Control, which lost ~50% of their initial color. This outcome may be due to greater complexation of anthocyanins and derived pigments with wine polysaccharides that are present at higher concentrations in FM wines.

3.4. Effect of treatments on wine polysaccharide composition

Polysaccharides found in wine are either mannoproteins, derived from yeast autolysis during fermentation (Ayestarán, Guadalupe, & León, 2004; Vidal, Williams, Doco, Moutounet, & Pellerin, 2003) and aging on lees (Doco, Vuchot, Cheynier, & Moutounet, 2003), or polysaccharides rich in arabinose and galactose (PRAGs) such as type II arabinogalactan-proteins and arabinans, and acidic pectic polysaccharides like rhamnogalacturonans (RGI and GRII), derived from grape berry cell walls. FM wines had a significantly higher concentration of polysaccharides compared to FJ wine series (Table 1), with an extension of time on skins during fermentation notably increasing glucuronic and galacturonic acid-rich polysaccharides as well as total polysaccharide concentration while having no effect on mannose, galactose and arabinose-rich polysaccharides. Wine polysaccharides from the TM Control ferment were compositionally very similar to those from FM ferments, with the exception that galacturonic acid-rich polysaccharides increased substantially in the flash treated musts pressed after

sugar dropped below 7 °Brix (day 2). The concentration of polysaccharides rich in glucose, xylose and fucose was not significantly impacted by the treatments. Other research using flash détente treatments has shown that it favors the selective extraction of PRAGs and RGII **(**Doco, Williams, & Cheynier, 2007), while the present results indicated that homogalacturonan was primarily extracted in the Merlot wines as skin contact time increased following flash treatment, because there was no proportional enrichment in arabinose, fucose or rhamnose (Table 1). Differences in heating temperature and duration of high temperature exposure prior to vacuum flash treatment and different grape varieties may possibly explain the different outcomes between these studies.

3.5. Effect of treatments on aroma composition

3.5.1. Esters

FJ wine series had ~60% higher concentration of ethyl esters compared with FM treatments that had some contact with solids during fermentation. (Table 3). On the other hand, skin contact time during fermentation for the flash-treated musts had no significant effect on ester concentration in wine.

Grape bulk and fine solids are a rich source of exogenous amino acids, lipids and phytosterols among other nutrients, and would therefore be expected to influence ethyl ester formation by yeasts which is impacted by nutrient status of juice or must. The lower concentration of ethyl esters in ferments with fine grape suspended solids may have been due to suppressed synthesis of the lipids needed for ethyl ester production by yeasts (Rollero, Bloem, Camarasa, Sanchez, Ortiz-Julien, Sablayrolles, et al., 2015). On the other hand, the lower concentration of acetate esters may have been due to a diminished activity of alcohol acetyltransferases in the presence of exogenous lipids contained in the grape solids (Rollero, et al., 2015), since these enzymes catalyze the conversion of higher alcohols into acetate esters.

FJ wine series had significantly higher concentrations of ethyl butanoate, ethyl decanoate, ethyl hexanoate, ethyl octanoate, isoamyl acetate, isobutyl acetate, and ethyl acetate when compared with wines from any treatment which had some skin contact during fermentation. The high odor activity value (OAV) for esters as summarized in Table 3 suggested that presence or absence of grape derived solids would be expected to impact wine aroma and flavor profile.

Based on this assessment, the three most odor-active esters in decreasing order were ethyl octanoate, isoamyl acetate, ethyl hexanoate and ethyl butanoate. Ethyl octanoate would be expected to increase fruity characters, which when present together with 1-octen-3-ol (also present at significantly higher concentration in FJ wine series), have been shown to positively impact wine flavor. The most impactful acetate ester, isoamyl acetate is generally considered to impart a negative effect on sensory quality of red wine (Liu, Xing, Li, Yang, & Pan, 2016) although it is considered to positively enhance Pinotage bouquet. The concentration of ethyl dihydrocinnamate, 2-methylbutyl acetate, and 2-phenylethyl acetate was not impacted by skin contact in the FD series of treatments, while ethyl isovalerate was significantly higher in traditional maceration ferments compared with off-skins ferments.

The similarity in ester composition between wines made with any form of skin contact suggested that flash treatment alone did not have a significant impact on wine ester composition. Rather, the present study demonstrated that removal of grape fine settleable solids, skins, pulp and seeds prior to fermentation as in the FJ treatments played a much greater role in increasing ester composition in wine and could potentially create the most differentiated wine style.

3.5.2. C_{13}-norisoprenoids, terpenes and alcohols

Flash détente treatment followed by fermentation off-skins significantly decreased the concentration of 1-hexanol while increasing that of 1-octen-3-ol, β-damascenone and linalool compared with treatments that had some skin contact during fermentation (Table 3). An evaluation of OAVs showed that β-damascenone was expected to influence wine aroma and flavor profile although other research suggest this compound would not directly impact wine aroma at levels reported in current study. Increasing skin contact time during fermentation reduced β-damascenone concentration while having no significant effect on 1-hexanol, 1-octen-3-ol and linalool.

3.5.3. Fusel alcohols and aldehydes

FJ wine series had a significantly lower concentration of the five fusel alcohols that were monitored in the study, while concomitantly increasing acetaldehyde concentration compared with all fermentations with skin contact (Table 3). The clear distinction in isoamyl alcohol concentration between off-skins ferments and all ferments conducted with skin contact, in combination with high OAVs in the wines, suggested that this compound may play an important role in differentiating the fruit profile of wines from these two treatments and control. The negative correlation between isoamyl alcohol and isoamyl acetate between the 2 treatment groups (FJ, FM) and TM control may also help differentiate the three wine groups.

Flash détente treatment followed by fermentation on-skins did not significantly impact isoamyl alcohol, active amyl alcohol, n-propyl alcohol and acetaldehyde concentration compared to traditional maceration ferments with unheated must. The flash treatment in combination with on-skins fermentation, however, significantly increased the concentration of the impact odorant 2-phenylethanol, which has a

rose flower note, while lowering the concentration of the less impactful compounds with lower OAVs, like isobutyl alcohol and methanol, as well as total fusel alcohols, when compared with wines derived from traditional maceration.

3.5.4. Oak volatile compounds

Expectedly, the ferments that received 4 g/L oak treatment had significantly higher concentrations of *cis*-oak lactone, *trans*-oak lactone, 4-methyl guaiacol, and acetovanillone compared to other treatments (Table 3). Flash treatment followed by fermentation on-skins significantly lowered benzyl alcohol while increasing furfuryl alcohol, *cis*-oak lactone, *trans*-oak lactone, guaiacol, 4-methylguaiacol, and acetovanillone when compared with traditional maceration. Almost all wines from FD treated must had significantly higher concentration of 5-hydroxymenthylfurfural (5-HMF) compared to TM Control, likely due to caramelization of sugars. An assessment of OAVs suggested that only *cis*-oak lactone and guaiacol, which were present at concentrations at or above sensory detection threshold, were likely to impact oak aroma and flavor perception, with oak-treated wines unsurprisingly having significantly higher oak related attribute ratings. These results indicate that increasing oak add rate to try and achieve color stabilization is not a viable option if aiming to not alter wine sensory profile.

3.6. Sensory evaluation

There were distinct sensory differences between FM, FJ series, and TM Control wines. FJ wine series and TM Control wine were perceived to have significantly higher aroma intensity ratings compared to FM wines (Table 4). The higher overall aroma intensity rating for off-skins ferments with or without commercial tannin addition of ~70% was deemed to be especially due to high intensity ratings for red fruit (raspberry and strawberry) (~70%), and confectionery (candied fruit) (~60%). On the other

hand, the high aroma intensity rating for flash-treated off-skins ferment treated with oak was attributed to high ratings for toasty, vanilla and allspice attributes, whereas for traditional maceration control ferment it was due to high ratings for green, savory, and dusty aroma and flavor. The significantly higher concentration of C_6 compounds (Table 3) and volatile acidity (Table 1) in TM Control wine may explain these high attribute ratings, although the calculated OAVs (Table 3) suggested that 1-hexanol would not be expected to impact the perception of green aroma in the wines. The trend with 1-hexanol may have possibly been reflected in the concentrations of other more potent C_6 compounds that were not analyzed in this study. The high red fruit and confectionery ratings for flash treated musts fermented off-skins were consistent with the observed higher ester concentrations in these wines (Table 3).

Wines from on-skins ferments generally had higher ratings for fresh and jam dark fruit compared to off-skins ferments with or without added enological tannin (Table 4). The lower red fruit ratings for ferments conducted with skin contact may potentially have resulted from red fruit suppression by fusel alcohols (Ferreira, Sáenz-Navajas, Campo, Herrero, de la Fuente, & Fernández-Zurbano, 2016) which may have also resulted in greater expression of dark fruit. Oak addition to off-skins ferments significantly reduced red fruit and confectionery attribute ratings while increasing jam dark fruit flavor compared to the other conditions. Enological tannin addition had no significant effect on wine aroma, flavor or mouthfeel.

Increasing skin contact time during fermentation of flash détente treated musts increased wine body and astringency, likely due to increased extraction of polysaccharides (Vidal, Francis, Williams, Kwiatkowski, Gawel, Cheynier, et al., 2004) and proanthocyanidins (Hufnagel & Hofmann, 2008), respectively. On the other hand, flash détente treated off-skins ferments with or without enological tannin

addition were perceived to have lower body and astringency. The higher body in on-skins ferments was potentially due to enhanced extraction during fermentation due to longer skin contact time. Glycerol may also impact wine body (Gawel & Waters, 2008) but the relatively small difference in concentration between the wines (up to 1.7 g/L) suggested it was not responsible for the perceived differences in body.

Although differences in astringency could be attributable to tannin concentration alone, the influence of polysaccharides is unclear. Concomitant increases of tannin and pectic polysaccharides have been observed to elicit higher astringency in Shiraz wines (Bindon, et al., 2019), whereas other research (Vidal, et al., 2004) reported an opposing trend, showing that polysaccharides modulate tannin astringency. Wine tannin (puckering astringency) and quercetin glycosides (velvety astringency) were the only phenolic compounds that were present in wines at a concentration above their sensory threshold for astringency (Hufnagel & Hofmann, 2008). As reported in section 3.2, off-skins flash détente treatments presumably favored extraction of lower molecular weight skin tannins with a higher proportion of trihydroxylation while reducing seed tannin extraction as evidenced by lower galloylation, which may have contributed to the lower perceived astringency ratings. Adding toasted oak to off-skins ferments significantly increased both body and astringency to match that of on-skins ferments likely due to extraction of hydrolyzable tannins. Although TM Control wines had a significantly lower concentration of quercetin glycosides and proanthocyanidins than FM wines they had similar astringency ratings, suggesting that the concentration differences were not significant enough to elicit sensory differences, or that compositional differences were more important. Alternatively, the higher volatile acidity in wines from traditional maceration ferments may have increased the perception of astringency (Lawless, Horne, & Giasi, 1996) in these wines.

Principal component analysis done to give an overview of treatments and their sensory attributes grouped the 7 wines into 4 distinct groups (Fig. 1). Principal component 1 (PC 1) explained 63% of variability in sensory attributes between the wines and PC 2 explained an additional 22%. FJ and FJ + Tannin wines were similar and identified by intense red fruit and confectionery attributes (top right quadrant). On the other hand, FJ + Oak wine was positioned separately (in the bottom right quadrant), distinguished by vanilla, toasty and allspice attributes. FM wines that had different skin contact times were clustered together (bottom left quadrant) and characterized by high ratings for fresh dark and jam dark fruit, and astringency. Conversely, the TM Control wine, which had a distinctive sensory profile characterized by intense green, savory and dusty characters was positioned in the top left quadrant, separate to the FD wines.

4. Conclusions

This study demonstrated that flash détente treated must can be used to create differentiated wine styles by fermenting under different conditions, defined by the contact period, or lack thereof, of skins, seeds, pulp and fine settleable solids during fermentation. Contact with grape solids during fermentation was shown to impact chemical composition and sensory profile of red wines made from flash détente treated must. Whether or not wine had contact with grape solids during fermentation had greater impact on wine aroma composition and flavor profile compared to contact time with solids (skins, seeds, pulp), which mainly impacted phenolic extraction and mouthfeel attributes.

Off-skins ferments with or without 0.4 g/L commercial tannin addition produced wines that had significantly higher red fruit and confectionery attribute ratings while oak addition reduced these attributes, and increased jam dark fruit, toasty, vanilla and allspice attributes. The increase in red fruit

and confectionery attributes in flash-treated off-skins ferments was deemed due to the combination of increased ester formation and greater extraction of β-damascenone and linalool from must as well as diminished fusel alcohol formation. The study also showed that flash détente can be used to influence wine body and astringency by increasing the extraction of polysaccharide, quercetin glycosides and proanthocyanidin. Pre-fermentation addition of enological tannin or addition of toasted oak chips at a level that was perceivable with respect to aroma and flavor by a panel of trained tasters did not improve short or long-term color stability of FD wines. More research is therefore needed to explore treatments that help stabilize color after FD processing. This might entail screening a wider range of enological tannin and oak products to better understand the efficacy and value of the diverse range of adjuncts that are commercially available.

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Supplementary Materials: Figure S1 Flowchart of Merlot must and FD juice preparation. **Figure S2** Principal component analysis biplot of statistically significant chemical parameters for Merlot wines derived from traditional maceration (TM Control) vs. fermentations of flash détente juice (FJ) with oak (+ Oak) or tannin (+ Tannin) addition, and must (FM) with pressing to remove skins at TSS levels of 17, 7 or 0 °Brix (Day 1, Day 2 and Day 5, respectively); **Table S1** Basic chemistry of Merlot grapes and flash détente (FD) treated juice analyzed before and after water addition to aid fermentation (WAF); **Table S2** Attributes and standards used in descriptive analysis of Merlot wines.

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Figure Captions

Fig. 1. Principal component analysis biplot of sensory attributes with statistically significant ratings for Merlot wines derived from traditional maceration (TM Control) vs. fermentations of flash détente juice (FJ) with oak (+ Oak) or tannin (+ Tannin) addition, and must (FM) with pressing to remove skins at TSS levels of 17, 7 or 0 °Brix (Day 1, Day 2 and Day 5, respectively).

Table 1 Comparison of basic chemistry, and phenolic and polysaccharide composition of Merlot wines derived from traditional maceration (TM Control) vs. fermentations of flash détente juice (FJ) with oak (+ Oak) or tannin (+ Tannin) addition, and must (FM) with pressing to remove skins at TSS levels of 17, 7 or 0 °Brix (Day 1, Day 2 and Day 5, respectively).

Parameter	FJ	FJ + Tannin	FJ + Oak	FM Day 1	FM Day 2	FM Day 5	TM Control	*P*-value	Model Adjusted R^2
Basic Chemistry									
alcohol % v/v	13.9	14.0	13.9	14.2	14.0	13.9	13.9	*0.181*	33.1%
malic acid (g/L)	1.41c	1.43c	1.42c	1.80ab	1.71b	1.99a	1.58bc	*0.0001*	92.4%
pH	3.55c	3.55c	3.54c	3.56c	3.59bc	3.65a	3.64ab	*0.001*	89.1%
TA g/L	6.5c	6.6c	6.6c	7.4a	7.1ab	7.1ab	7.1b	*0.0001*	95.0%
VA (g/L)	1.9bc	2.0b	2.1b	1.9bc	2.0bc	1.5c	3.0a	*0.0001*	93.4%
glycerol (g/L)	8.0b	8.0b	8.3b	9.7a	9.5a	9.7a	9.5a	*0.0001*	89.5%
Phenolic Compounds									
total phenolics (mg/L)	1,114e	1,334d	1,208e	1,622c	1,840b	1,970a	1,140e	*0.0001*	99.4%
proanthocyanidins (mg/L)[a]*	460d	456d	570d	966bc	1111b	1458a	755cd	*0.0001*	95.3%
total anthocyanin (mg/L)*	247a	246a	244a	265a	264a	267a	178b	*0.001*	85.9%
caftaric acid (mg/L)	29.0a	29.0a	29.0a	30.0a	30.5a	28.5a	18.5b	*0.001*	88.6%
catechin (mg/L)	22.0de	22.0de	21.0e	35.5c	48.0b	62.0a	28.0d	*0.0001*	98.7%
epicatechin (mg/L)	19.5e	20.0e	20.5e	37.0c	50.5b	62.0a	28.5d	*0.0001*	98.6%
malvidin-3-*O*-glucoside (mg/L)	82.0ab	82.0ab	80.5b	82.0ab	91.0a	83.0ab	70.0c	*0.002*	84.8%
pigmented polymers (mg/L)	11.5b	12.0b	12.0b	14.5a	16.5a	15.5a	11.0b	*0.0001*	93.7%
proanthocyanidins (mg/L)[b]	118.5b	152.0b	140.0b	197.5ab	277.0a	256.0a	151.5b	*0.003*	82.9%
quercetin glycosides (mg/L)	24.0cd	24.5cd	23.0d	27.5ab	29.0a	26.0bc	13.0e	*0.0001*	98.3%
Tannin Composition*									
MM by 50% GPC (g/mol)[c]	1721cd	1653d	1756bcd	1997abc	2100a	2099a	2050ab	*0.002*	85.0%
MM by subunit (g/mol)[c]	1432b	1467b	1527ab	1579ab	1632ab	1583ab	1908a	*0.043*	59.1%
mDP [d]	4.79b	4.90b	5.10ab	5.24ab	5.38ab	5.18ab	6.37a	*0.038*	60.6%
Tri-OH (%)[e]	18.39abc	18.42abc	20.00a	16.70cd	17.41bcd	15.15d	19.33ab	*0.001*	85.8%
galloylation (%)[e]	3.74c	4.26c	4.00c	5.46bc	6.90ab	8.48a	4.20c	*0.0001*	93.2%
mass conversion (%)[f]	36.85a	48.17a	35.16a	29.79a	49.57a	47.85a	49.68a	*0.094*	46.9%

Table 1 Contd.

Parameter	FJ	FJ + Tannin	FJ + Oak	FM Day 1	FM Day 2	FM Day 5	TM Control	*P*-value	Model Adjusted R^2
Polysaccharides (mg/L)									
total polysaccharides	82.8c	85.4c	85.0c	144.7b	177.5ab	211.8a	165.0ab	*0.0001*	92.6%
mannose	25.9b	25.3b	26.1b	33.8ab	35.4a	36.3a	40.7a	*0.001*	86.3%
rhamnose	2.4d	3.0d	3.4cd	7.8bc	8.7b	11.1ab	13.5a	*0.0001*	92.2%
glucuronic acid	1.7c	1.6c	1.7c	3.1b	3.7ab	4.6a	3.9ab	*0.0001*	94.7%
galacturonic acid	9.5cd	9.1d	9.3cd	31.7bcd	53.3ab	68.0a	33.6bc	*0.0001*	92.7%
glucose	13.6a	13.8a	13.8a	16.4a	16.1a	17.1a	13.3a	*0.058*	54.8%
galactose	18.4b	19.6b	19.4b	30.2a	32.3a	37.1a	31.0a	*0.0001*	89.9%
xylose	1.39	1.45	1.166	1.57	1.82	2.73	0.83	*0.277*	21.5%
arabinose	8.8c	10.5bc	9.0c	19.0abc	24.8abc	33.5a	26.8ab	*0.003*	81.3%
fucose	1.11a	1.13a	1.14a	1.26a	1.38a	1.45a	1.31a	*0.067*	52.5%
arabinose:galactose ratio	0.48b	0.54ab	0.47b	0.64ab	0.77ab	0.89a	0.87ab	*0.015*	70.7%

Data are means of two replicates (n = 2). Means followed by different letters (within rows) are statistically different (one-way ANOVA, Tukey's LSD post hoc, $\alpha = 0.05$). Polysaccharides were determined as monosaccharide sugars; [a]proanthocyanidins (as epicatechin units) determined by the methyl cellulose precipitation (MCP) assay; [b]proanthocyanidins (as catechin units) determined by HPLC-DAD; [c]tannin molecular mass determined using 50% elution by gel permeation chromatography (50% GPC) or subunit composition from phloroglucinolysis; [d]mean degree of polymerization; [e] proportion of trihydroxylation (Tri-OH) or galloylation; [f] mass conversion based on % recovery of tannin subunits by phloroglucinolysis as a proportion of tannin concentration by MCP assay. *Measured 1 year after bottling.

Table 2 Comparison of color metrics of Merlot wines derived from traditional maceration (TM Control) vs. fermentations of flash détente juice (FJ) with oak (+ Oak) or tannin (+ Tannin) addition, and must (FM) with pressing to remove skins at TSS levels of 17, 7 or 0 °Brix (Day 1, Day 2 and Day 5, respectively), after bottling and 1 year after bottling.

Color Metrics	Mean Comparisons After Bottling							Mean Comparisons 1 Year After Bottling							*P*-value		
	FJ	FJ + Oak	FJ + Tannin	FM Day 1	FM Day 2	FM Day 5	TM Control	FJ	FJ + Oak	FJ + Tannin	FM Day 1	FM Day 2	FM Day 5	TM Control	Treatment	Time	Treatment × Time
A520	3.99bc	4.06bc	4.25b	6.21a	6.62a	6.33a	3.11bcd	2.02de	2.16de	2.05de	2.39de	2.66cde	2.64cde	1.62e	*0.0001*	*0.0001*	*0.0001*
A420	2.19c	2.35c	2.45bc	4.75a	4.62ab	4.80a	2.00c	1.45c	1.58c	1.48c	1.88c	2.01c	2.05c	1.48c	*0.001*	*0.0001*	*0.022*
A620	0.62c	0.69bc	0.73bc	1.64ab	1.61ab	1.80a	0.61c	0.35c	0.39c	0.35c	0.46c	0.51c	0.52c	0.31c	*0.002*	*0.0001*	*0.021*
intensity	6.19b	6.41b	6.70b	10.95a	11.25a	11.14a	5.11b	3.47b	3.74b	3.53b	4.27b	4.67b	4.70b	3.10b	*0.0001*	*0.0001*	*0.005*
hue	0.55d	0.58cd	0.58cd	0.76abc	0.70bcd	0.75abcd	0.64bcd	0.72abcd	0.73abcd	0.72abcd	0.79ab	0.76abc	0.78abc	0.91a	*0.003*	*0.0001*	*0.043*

Data are means of two replicates (n = 2). Means followed by different letters (within rows) are statistically different (one-way ANOVA, Tukey's LSD post hoc, $\alpha = 0.05$).

Table 3 Comparison of volatile composition of Merlot wines derived from traditional maceration (TM Control) vs. fermentations of flash détente juice (FJ) with oak (+ Oak) or tannin (+ Tannin) addition, and must (FM) with pressing to remove skins at TSS levels of 17, 7 or 0 °Brix (Day 1, Day 2 and Day 5, respectively).

Volatile Compound (µg/L)	*P*-value	Mean Comparisons for All Treatments							Detection Threshold (µg/L)	OAV Range		Model Adjusted R^2
		FJ	FJ + Tannin	FJ + Oak	FM Day 1	FM Day 2	FM Day 5	TM Control		Min	Max	
Ethyl Esters												
ethyl butanoate	*0.002*	371.0ab	529.0a	526.5a	298.0b	324.5b	268.5b	268.0b	20 [a]	13.4	26.5	85.2%
ethyl decanoate	*0.0001*	244.0ab	294.5a	303.0a	152.0bc	130.5c	116.5c	124.0c	200 [a]	0.6	1.5	90.1%
ethyl dihydrocinnamate	*0.121*	4.05a	3.65a	3.70a	3.00a	3.50a	3.30a	3.30a	2 [d]	1.5	2.0	42.1%
ethyl hexanoate	*0.0001*	454.5a	455.5a	458.5a	375.5b	354.0b	362.0b	317.0b	5 [a]	63.4	91.7	90.7%
ethyl isobutyrate	*0.032*	38.0b	54.5ab	63.5a	55.5ab	66.0a	62.5ab	49.0ab	15 [d]	2.5	4.4	62.9%
ethyl isovalerate	*0.006*	4.5c	6.0bc	6.0bc	10.0abc	13.0ab	11.0abc	14.0a	3 [d]	1.5	4.7	78.1%
ethyl octanoate	*0.0001*	1096a	1231a	1166a	680b	665b	569b	537b	2 [a]	268.5	615.5	94.9%
total ethyl esters	*0.0001*	2212a	2574a	2527a	1574b	1556b	1393b	1312b	–	–	–	94.8%
Acetate Esters												
isoamyl acetate	*0.001*	7768a	8429a	8830a	6151ab	7528ab	4300bc	2302c	30 [a]	76.7	294.3	87.7%
isobutyl acetate	*0.0001*	407.0a	385.5a	446.0a	184.0b	188.0b	120.0b	97.0b	1600 [c]	0.1	0.3	91.9%
n-butyl acetate	*0.001*	20.0ab	20.5ab	23.5a	11.5c	14.0bc	11.5c	10.0c	1800 [f]	<0.1	<0.1	87.0%
2-methylbutyl acetate	*0.19*	202.0a	234.0a	219.0a	206a	394.5a	195.0a	104.5a	5 [b]	20.9	78.9	32.0%
2-phenylethyl acetate	*0.027*	758ab	837ab	1057a	775ab	871ab	441ab	198b	250 [a]	0.8	4.2	64.9%
ethyl acetate	*0.0001*	74.0a	78.0a	84.5a	45.0b	46.0b	42.5b	39.0b	7500 [a]	<0.1	<0.1	97.2%
total acetate esters	*0.001*	9229ab	9984a	10660a	7372ab	9041ab	5110bc	2750c	–	–	–	86.4%
total esters	*0.001*	11440a	12558a	13187a	8946ab	10597ab	6502bc	4062c	–	–	–	87.9%
Alcohols, C_{13}-Norisoprenoids and Terpenes												
1-hexanol	*0.0001*	169c	169c	169c	645ab	526bc	594b	1004a	8000 [a]	<0.1	0.1	90.2%
1-octen-3-ol	*0.0001*	50.2a	52.8a	53.1a	38.8b	35.5b	39.0b	33.0b	40 [e]	0.8	1.3	93.6%
methanol	*0.0001*	24500d	24000d	26500d	56000c	68500bc	85000b	133000a	100000*	0.2	1.3	96.6%
β-damascenone	*0.007*	2.45a	2.35ab	2.50a	2.45a	2.35ab	1.90b	1.85b	0.05 [a]	37.0	50.0	77.1%
linalool	*0.004*	11.0ab	13.0a	12.5a	11.0ab	9.5abc	8.0bc	6.0c	25 [d]	0.2	0.5	80.0%

Table 3 Contd.

Fusel Alcohols and Aldehydes											
active amyl alcohol	*0.0001*	38500b	38500b	42000b	66000a	65500a	68000a	63000a	30000 [c]	1.3	2.3	95.7%
isoamyl alcohol	*0.0001*	158500b	158500b	174500b	284000a	272500a	272500a	296000a	30000 [a]	5.3	9.9	93.6%
isobutyl alcohol	*0.0001*	31000d	32000d	35000cd	46500b	44500b	41500bc	63000a	40000 [a]	0.8	1.6	95.4%
n-propyl alcohol	*0.016*	24000b	24500b	25500b	34500a	31000ab	30000ab	30000ab	306000 [f]	0.1	0.1	70.0%
2-phenyl ethanol	*0.0001*	19743c	19441c	19667c	37351ab	40193a	40422a	34074b	10000 [a]	1.9	4.0	97.7%
total fusel alcohols	*0.0001*	296243c	296940c	323170c	524350b	522200b	537420b	619100a	–	–	–	97.7%
acetaldehyde	*0.002*	19500a	18500a	18000ab	16000abc	14000bc	13500c	13000c	100000 [c]	0.1	0.2	84.7%
Oak Aroma Compounds												
hexyl acetate	*0.002*	36.4a	41.5a	35.0a	22.3ab	24.3ab	11.2b	10.6b	670 [k]	<0.1	<0.1	83.9%
furfural	*0.002*	79.6bc	75.8c	82.35bc	127.0ab	100.5bc	95.6bc	161.1a	14100 [d]	<0.1	<0.1	83.7%
furfuryl alcohol	*0.0001*	739cd	825bcd	1519abc	1387abc	1606ab	1850a	67d	2000 [i]	<0.1	0.9	89.8%
cis-oak lactone	*0.0001*	48.7d	50.6d	169.4a	69.5c	79.2bc	91.7b	9.7e	24 [h]	0.4	7.1	99.4%
trans-oak lactone	*0.0001*	7.0e	7.3e	23.9a	11.7d	13.8c	17.2b	5.0f	172 [h]	<0.1	0.1	99.6%
guaiacol	*0.0001*	6.6d	6.3d	11.4ab	8.6c	10.3b	12.5a	5.0e	9.5 [d]	0.5	1.3	98.6%
benzyl alcohol	*0.0001*	302b	308b	355b	363b	328b	322b	1048a	200000 [i]	<0.1	<0.1	98.7%
4-methylguaiacol	*0.0001*	10.2d	9.8d	23.7a	11.6d	15.5c	18.8b	5.0e	65 [l]	<0.1	0.4	98.2%
maltol	*0.004*	99.1bc	109.6bc	207.9a	132.0abc	131.7abc	138.8ab	50.0c	Unknown	–	–	80.1%
5-HMF	*0.003*	1358ab	1390ab	1739ab	1965a	1302abc	987bc	538c	Unknown	–	–	81.9%
vanillin	*0.024*	15.4ab	12.0b	31.9a	17.5ab	14.8ab	14.5b	11.0b	200 [a]	0.1	0.2	65.8%
acetovanillone	*0.0001*	72.6d	72.4d	135.6a	83.5c	88.2bc	97.5b	50.0e	1000 [j]	0.1	0.1	99.0%
ethyl vanillate	–	BDL	BDL	BDL	BDL	BDL	BDL	76.7	990 [j]	<0.1	<0.1	–
syringaldehyde	–	BDL	BDL	78.1	BDL	BDL	BDL	BDL	Unknown	–	–	–

Data are means of two replicates (n = 2). Means followed by different letters (within rows) are statistically different (one-way ANOVA, Tukey's LSD post hoc, $\alpha = 0.05$). [a](Guth, 1997); [b](Teranishi, Flath, Guadagni, Lundin, Mon, & Stevens, 1966); [c](Etievant, 1991); [d](Ferreira, López, & Cacho, 2000); [e](Boutou & Chatonnet, 2007); [f](Peinado, Moreno, Bueno, Moreno, & Mauricio, 2004); [g]; [h](Brown et al 2006); [i](Gomez-Miguez et al 2007); [j](Lopez et al 2002); [k](Ugliano et al 2008); [l](Mayr et al 2014). BDL = below detection limit; *Unpublished data.

Table 4 Comparison of sensory profile of Merlot wines derived from traditional maceration (TM Control) vs. fermentations of flash détente juice (FJ) with oak (+ Oak) or tannin (+ Tannin) addition, and must (FM) with pressing to remove skins at TSS levels of 17, 7 or 0 °Brix (Day 1, Day 2 and Day 5, respectively).

Parameter	*P*-value	Mean Comparisons for All Treatments						
		FJ	FJ + Tannin	FJ + Oak	FM Day 1	FM Day 2	FM Day 5	TM Control
aroma intensity	*0.0001*	73.4a	71.6ab	72.0ab	56.2c	58.0bc	54.0c	79.8a
red fruit A	*0.0001*	76.2a	69.9a	39.1b	32.9b	21.7bc	14.3c	12.2c
red fruit F	*0.0001*	72.6a	69.9a	41.7b	30.0bc	25.8bc	14.3c	15.3c
confectionery A	*0.0001*	67.4a	57.4a	28.3b	21.9bc	18.8bc	10.0c	5.3c
confectionery F	*0.0001*	62.3a	55.3a	31.3b	18.2bc	20.2bc	7.5c	6.8c
fresh dark fruit A	*0.0001*	34.1cd	27.5d	37.5bcd	49.7abc	56.2ab	60.0a	41.5abcd
fresh dark fruit F	*0.0001*	35.0bc	30.5c	38.4bc	53.5ab	58.7a	60.3a	44.5abc
jam dark fruit A	*0.0001*	29.7b	29.5b	47.0ab	45.7ab	57.6a	52.3a	29.3b
jam dark fruit F	*0.0001*	25.5c	33.6bc	46.9ab	50.7ab	53.7a	50.7ab	28.0c
toasty A	*0.0001*	13.2b	18.7b	60.2a	23.3b	20.3b	17.8b	17.2b
toasty F	*0.0001*	15.1b	17.7b	59.6a	23.8b	18.6b	15.4b	16.1b
vanilla A	*0.0001*	15.4b	19.5b	50.0a	19.4b	17.3b	8.8b	5.4b
vanilla F	*0.0001*	15.1b	16.7b	47.6a	14.9b	11.7b	6.9b	4.6b
allspice A	*0.001*	5.5b	4.4b	21.4a	10.8ab	10.6ab	13.1ab	16.6ab
allspice F	*0.0001*	5.9c	7.2c	22.0a	12.2abc	7.6bc	11.3abc	19.4ab
green A	*0.0001*	5.0d	11.5cd	11.3cd	20.3bc	20.6bc	27.0b	79.6a
green F	*0.0001*	12.4c	16.2bc	17.6bc	25.2bc	27.0bc	32.7b	78.5a
savory A	*0.0001*	10.1c	14.1bc	20.4bc	17.6bc	18.1bc	24.9b	74.6a
savory F	*0.0001*	15.4b	19.2b	25.5b	22.5b	24.7b	28.8b	74.3a
dusty A	*0.0001*	9.9c	11.6bc	23.0bc	15.0bc	21.6bc	24.3b	41.9a
dusty F	*0.0001*	14.8c	14.5c	26.7bc	19.3bc	24.1bc	33.2ab	43.9a
body	*0.0001*	42.1c	47.5bc	64.1ab	51.9abc	59.5ab	66.4a	56.8abc
astringency	*0.0001*	36.92d	46.52cd	62.75abc	58.00bc	69.58ab	76.28a	70.42ab

Data are means of two wine replicates presented to 9 judges during two formal sensory evaluation sessions. Means followed by different letters (within rows) are statistically different (one-way ANOVA, Tukey's LSD post hoc, $\alpha = 0.05$); A = aroma attribute; F = flavor attribute.

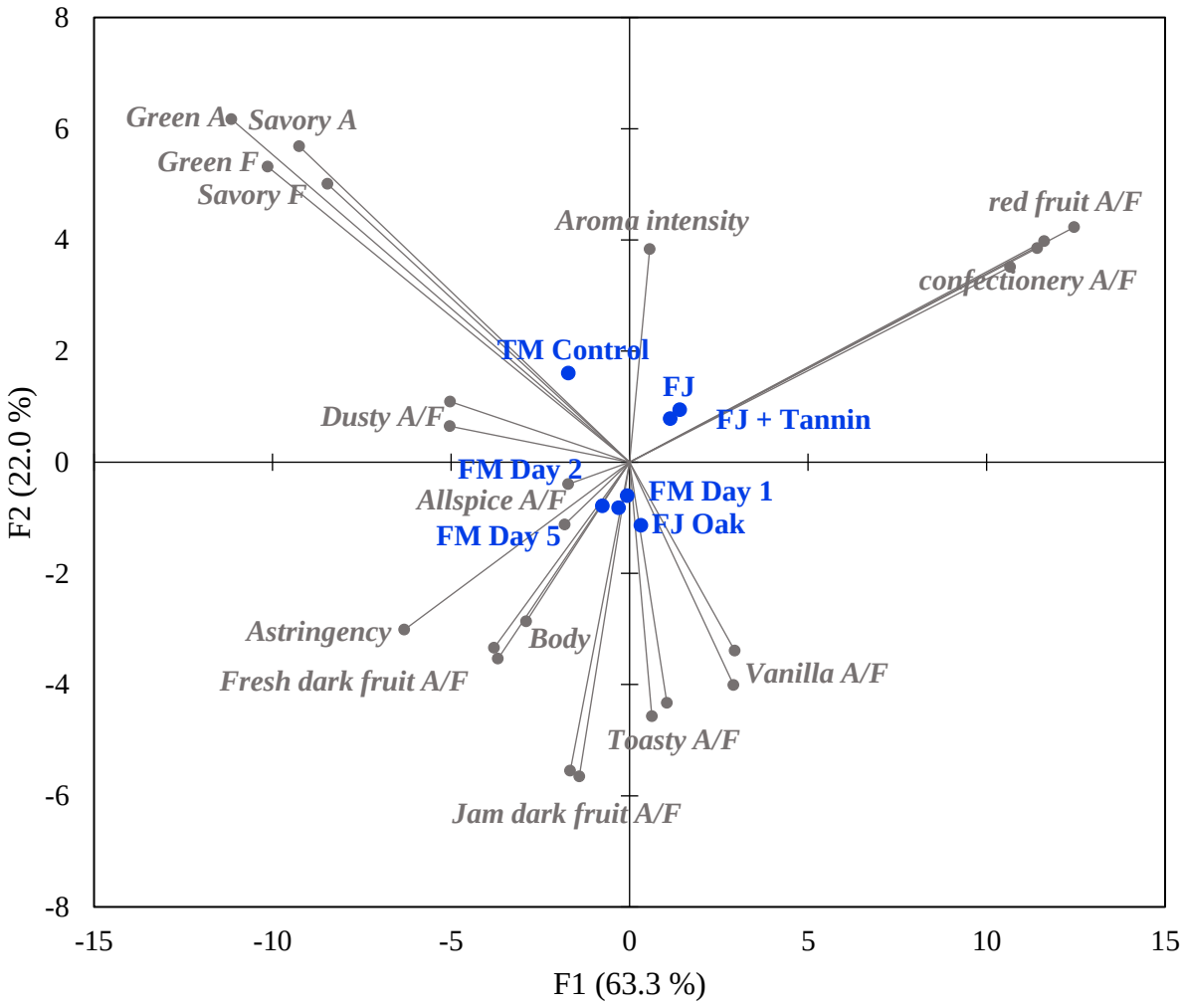
Green A
Savory A
Green F
Savory F
Aroma intensity
red fruit A/F
confectionery A/F
TM Control
FJ
FJ + Tannin
Dusty A/F
FM Day 2
FM Day 1
Allspice A/F
FJ Oak
FM Day 5
Astringency
Body
Vanilla A/F
Fresh dark fruit A/F
Toasty A/F
Jam dark fruit A/F
F2 (22.0 %)
F1 (63.3 %)
8
6
4
2
0
-2
-4
-6
-8
-15
-10
-5
0
5
10
15

Fig. 1

Supplementary Materials

Table S1 Basic chemistry of Merlot grapes and flash détente (FD) treated juice analyzed before and after water addition to aid fermentation (WAF).

Parameter	Grapes	FD Juice (pre-WAF)	FD Juice (post-WAF)
Total soluble solids (°Brix)	25.2	27.9	–
Reducing sugars (g/100 mL)	–	28.52	23.37
Titratable acidity (g/L)	3.4	3.8	3.11
pH	3.75	3.91	3.70
Yeast assimilable nitrogen (mg/L)	244	–	–
Glycerol (mg/L)	282	–	–
Gluconic acid (mg/L)	169	–	–
Matter other than grapes (MOG), %	0.7	–	–
A420 nm	–	6.43	5.27
A520 nm	–	8.61	7.06
A620 nm	–	2.57	2.11
Phenolic compounds (mg/L)			
Total phenolics	–	1791	1468
Caftaric acid	–	38	31
Catechin	–	29	24
Epicatechin	–	25	20
Grape reaction product	–	12	10
Gallic acid	–	3	2
Malvidin-3-glucoside	–	180	147
Pigmented polymers	–	11	9
Polymeric tannins	–	133	109
Quercetin glucosides	–	33	27

Supplementary Table S2 Attributes and standards used in descriptive analysis of Merlot wines.

Attribute	Reference Standards
red fruit	1 fresh strawberry and ½ fresh raspberry thinly sliced
confectionary	4 g of diced strawberry and cream lolly (The Natural Confectionery Co.)
fresh dark fruit	30 mL blueberry Juice (Bickford's) and 5 fresh blueberries sliced into thin layers
jam dark fruit	2 tinned black cherries (Riviana Foods), 2 Morello cherries (Global Food Distributors), 3 tsp forest fruit jam (Menora Foods), 3 tsp plum jam (Cottee's) and 2 tinned plums (SPC Ardmona)
toasty	0.2 g American oak chips (large grade, heavy toast, O.C. Inc.)
vanilla	5 drops vanilla essence (Queen Fine Foods)
allspice	pinch of ground allspice (Masterfoods)
green	1 g of sliced rachis (sourced fresh from the vineyard), 2 g of sliced capsicum (green bell pepper) and 1 g of sliced tomato stems
savory	2 tinned black olives (Siena Foods), 2 tinned green olives (Siena Foods) and ½ tsp soy sauce (Cerebos Foods)
dusty	unpolished natural leather
body	xantham gum: 0.04, 0.08 and 0.16 g/L as low, low–med and medium body
astringency	1 and 1.8 g/L of grape seed extract (as low and high)

Standards were prepared in 30 mL of Merlot wine, except dusty and dark fruit standards (which were sniffed as is).

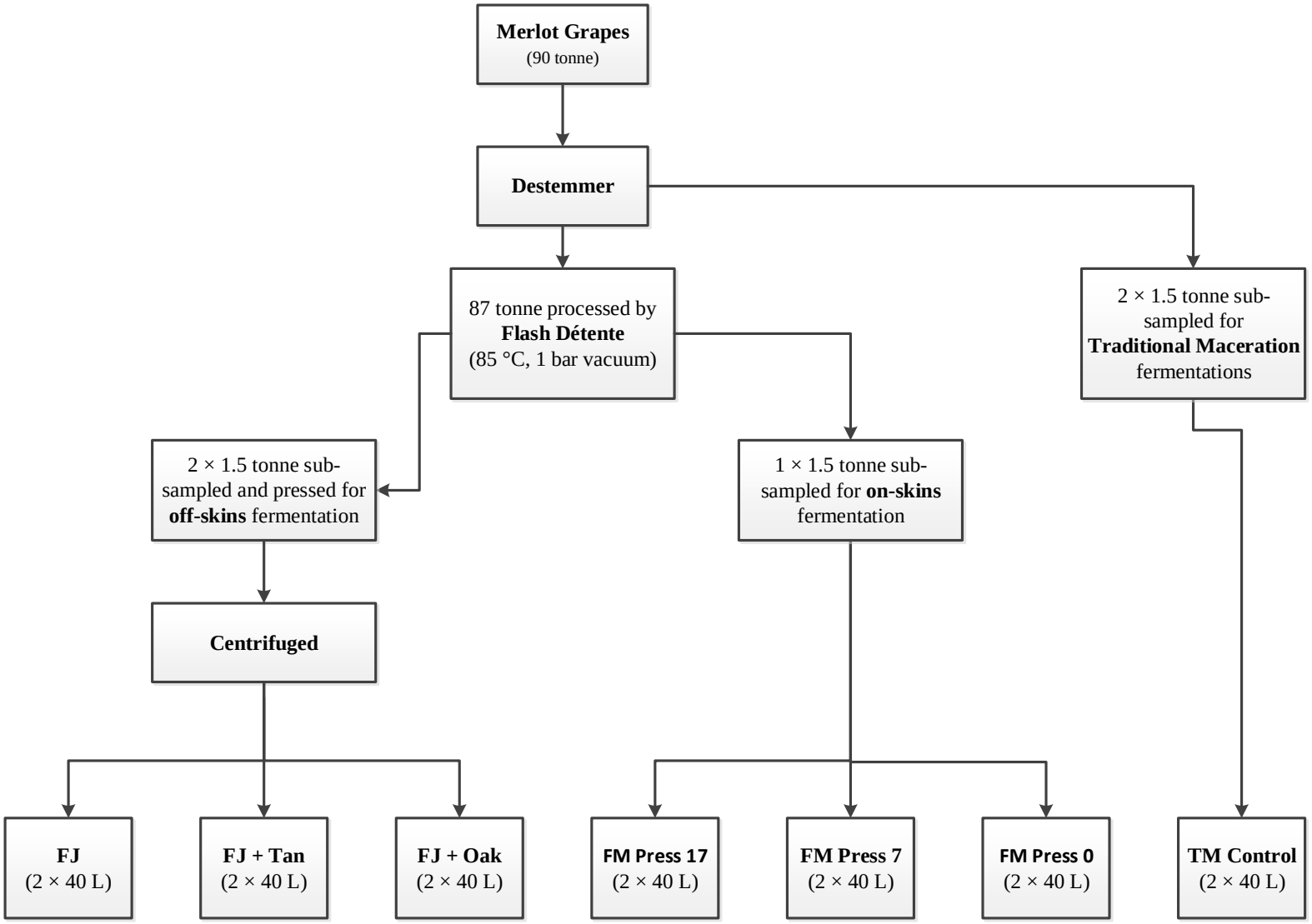

Figure S1 Flowchart of Merlot must and FD juice preparation.

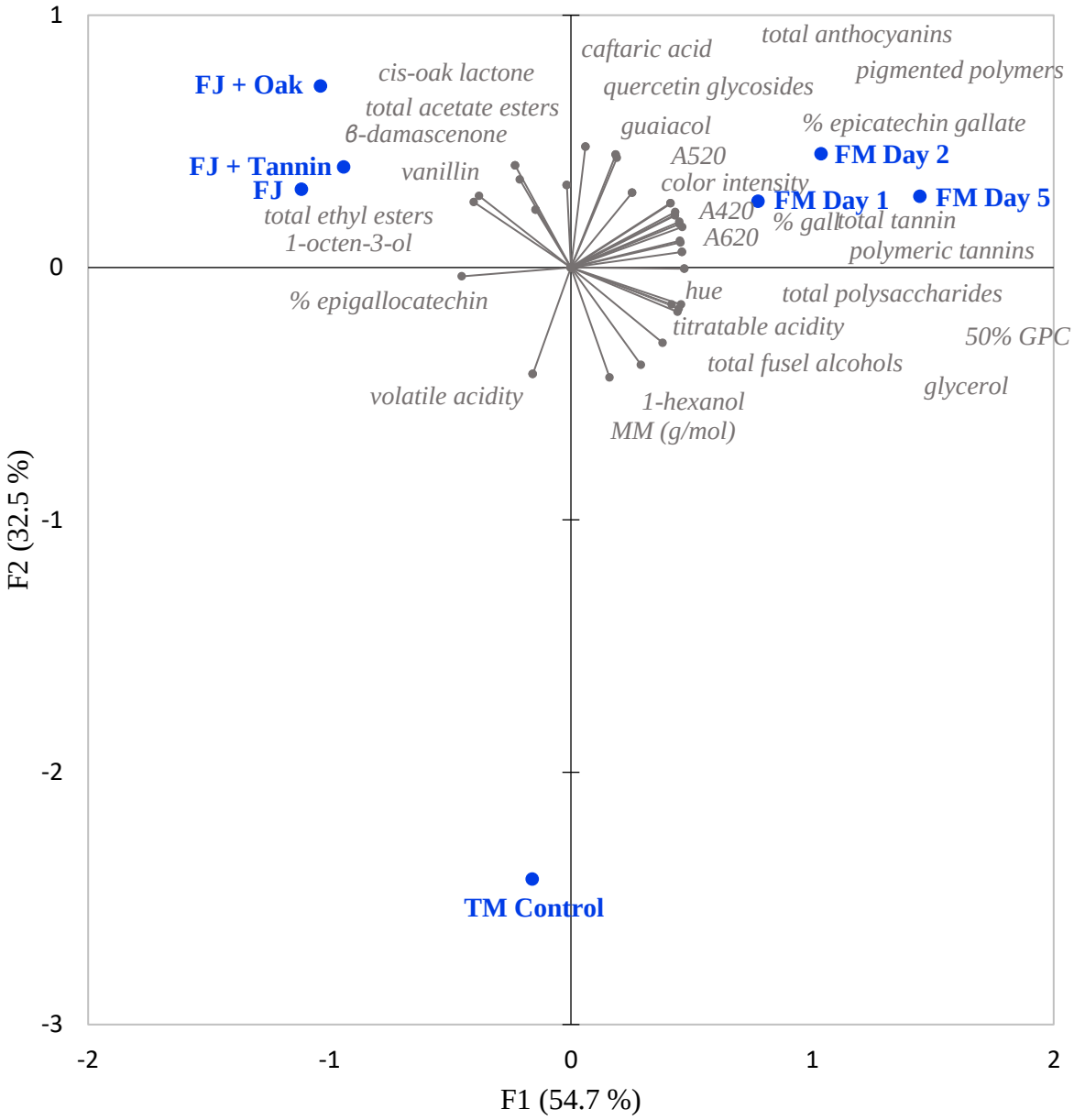

Figure S2 Principal component analysis biplot of statistically significant chemical parameters for Merlot wines derived from traditional maceration (TM Control) vs. fermentations of flash détente juice (FJ) with oak (+ Oak) or tannin (+ Tannin) addition, and must (FM) with pressing to remove skins at TSS levels of 17, 7 or 0 °Brix (Day 1, Day 2 and Day 5, respectively).

Chapter 6

Concluding Remarks and Future Directions

6.1 Conclusions

6.1.1 Colour Extraction and Stability of Rubired Juice Concentrate produced via Conventional Must Heating or Flash Détente Processing

Colour is an important quality parameter for foods and beverages that influence consumer preference and taste perception of juice [28]. A significant amount of colour remains in grape skins even after extraction during traditional alcoholic fermentation [29] and colour loss during production and ageing is a major challenge for Rubired concentrate producers. The objective of chapter 2 study was to increase the yield, quality and stability of Rubired red colour through the use of FD, in lieu of traditional extraction via CMH. FD did not significantly increase red colour yield but reduced the ratio of brown colour to improve the overall colour quality. Increasing the FD extraction time did not significantly impact the concentration of red, violet and brown colour in juice or concentrate. However, FD processing generated higher concentrations of suspended grape solids, and therefore produced juices with 4-fold lower flux through diatomaceous earth filtration medium, potentially increasing the cost of juice processing. FD concentrate also had lower colour stability under normal cold temperature storage conditions. In order to make FD an attractive option for red concentrate production, further research is needed to find ways to increase FD red colour extraction and stability while also finding ways to mitigate poor juice filterability which would increase processing costs.

6.1.2 Impact of Juice Extraction Method (Flash Détente vs. Conventional Must Heating) and Chemical Treatments on Colour Stability of Rubired Juice Concentrates under Accelerated Ageing Conditions

Low colour stability negatively impacts colour yield during production and storage, while also restricting concentrate usage in applications where colour stability is a key requirement. Natural

food colourings are increasingly being adopted as replacements for synthetic food dyes but adoption is limited by the poor colour stability of natural colourings, among other factors. In agreement with results under normal cold storage conditions, accelerated ageing showed that FD produced Rubired grape concentrate with significantly lower colour stability, with a half-life of 203.3 hours and activation energy of 59.2 kJ/mol at 50 °C, compared to the CMH concentrate with 233.9 hours and 65.2 kJ/mol. However, the current research also demonstrated beneficial red and violet colour stabilisation as a result of pH adjustment, acetaldehyde addition, and a combination of these two treatments. Notably, the addition of seed tannin had no effect on red and violet colour stability or brown colour formation. The downside to the low pH and acetaldehyde treatments was increased brown colour formation, but the net effect on overall colour quality was nevertheless positive, given these treatments decreased the ratio of brown to red colour. The combined low pH and acetaldehyde treatment was the most effective at preserving red colour, whilst increasing violet colour and minimising the brown colour ratio, but had the negative effect of increasing concentration of 5-hydroxymethylfurfural which has potential to be converted to 5-sulfoxymethylfurfural, a known genotoxin. Using FD concentrate which was shown in this research to generate less 5-HMF compared to CMH concentrate under accelerated ageing conditions, may help mitigate this health risk. In contrast to combined low pH and acetaldehyde treatment, addition of acetaldehyde alone increased red colour stability and enhanced violet colour formation, without increasing the 5-hydroxymethylfurfural concentration.

6.1.3 Impact of Fermentation Temperature and Grape Solids Content on the Chemical Composition and Sensory Profile of Flash-treated Cabernet Sauvignon Wines Fermented off skins

This study sheds light on how fermentation temperature and fine grape solids content can be manipulated to influence the chemical composition and sensory profiles of red wines, in order to target specific styles of red wine made without prolonged skin contact (i.e. 'off-skins') by fermenting juice derived from flash détente treated must. To the best of the authors' knowledge, no prior research has investigated the impact of incorporating suspended solids from flash détente treated red musts in combination with different fermentation temperatures on the chemical composition and sensory profiles of red wines fermented 'off-skins'. Fermentation temperature and suspended solids were shown to affect both general wine chemical composition, i.e. colour, phenolics and polysaccharide compositions, as well as wine aroma composition. The suspended solids present in 'off-skin' red fermentations were found to have a major effect on wine chemical and sensory profiles, likely due to grape solids being a rich source of lipids and phytosterols [15] The current research highlights the critical contribution of esters such as ethyl octanoate, ethyl hexanoate and isoamyl acetate in defining the flavour profile of red wines made via liquid phase fermentation of flash détente treated must, whereas fusel alcohols were shown to play a lesser role.

The sensory profiles of wines made via off-skins fermentations, with or without the removal of suspended fine solids, were clearly distinguishable from each other, and from wines made using a classic maceration approach. Increased fermentation temperatures and concentrations of suspended solids were shown to facilitate polysaccharide extraction into wine, potentially modulating astringency [30]. Descriptive analysis results showed that removal of grape solids from flash détente derived Cabernet Sauvignon juice prior to fermentation gave wines with increased

red fruit and confectionery attributes, whereas fermentations with addition of 3.5% grape solids enhanced the intensity of dark fruit notes. Wines made using classic maceration approach had significantly higher green and savoury characters compared to wines from the flash treatments irrespective of their solids content. These research findings identify opportunities for potentially creating differentiated light bodied, fruity and refreshing styles of red wine, made from fermentations without prolonged skin contact under different winemaking conditions. These differentiated wines may also be used as blending options in more traditional style red wines, giving winemakers a broader range of blending components with which to attain their desired wine styles.

6.1.4 Impact of Skin Contact Time, Oak or Tannin Addition on Chemical Composition, Colour Stability and Sensory Profile of Flash-Detente Merlot Wines

This study demonstrated that flash détente can be used to create differentiated styles of Merlot wine, by conducting fermentations with different levels of skins, seeds, pulp, stems and suspended solids contact. Bulk and fine suspended grape solids contact during fermentation influenced the chemical composition and sensory profiles of red wines made from flash détente treated Merlot must. Having no contact with bulk and fine suspended grape solids during fermentation had a greater impact on wine aroma composition and flavour profiles compared to skin contact time, which mainly impacted phenolic extraction and mouthfeel attributes. Oenological tannin addition to ‘off-skins’ fermentations (with no suspended fine solids) produced wines that were characterised by significantly more intense red fruit and confectionery attribute ratings, while oak addition decreased the intensity of these attributes and enhanced jam, dark fruit, toasty, vanilla and allspice attribute ratings. The increase in red fruit and confectionery attributes in off-skins fermentations was attributed to the combination of increased ester formation, the higher

concentration of 1-octen-3-ol, which in the presence of ethyl octanoate positively impacts wine flavor [31], and increased extraction of β-damascenone and linalool from must, as well as decreased fusel alcohol formation, as was observed with Cabernet Sauvignon.

Interactions between phenolic compounds and polysaccharides have been reported to influence body and astringency [32, 33]. This study showed that flash détente can be used to influence wine body and astringency by increasing polysaccharide, quercetin glycoside and polymeric tannin extraction. However, pre-fermentation addition of oenological tannin at higher than recommended dosage rates or addition of toasted oak chips at a level that is perceivable by a panel of trained tasters, did not improve short or long-term colour stability of wines.

6.2 Future Directions

6.2.1 Colour Extraction and Stability of Rubired Juice Concentrate produced via Conventional Must Heating or Flash Détente Processing

FD did not significantly increase colour extraction compared to CMH extraction; increasing the extraction time did not result in any significant increase in colour extraction. This was most likely due to extraction being limited by equilibrium effects. The loss of water as condensate during FD processing increased colour concentration in juice, purportedly limiting further colour transfer from grape cells. The addition of condensate water back into the feed must or re-extraction of pressed skins could be explored as a means for further increasing colour yield. The addition of condensate water, which is known to contain 'green' C_6 aroma compounds will likely increase the concentration of these undesirable compounds in juice but should not negatively impact the concentrate aroma profile, since the evaporation of juice into concentrate was shown in this study to effectively remove such compounds from concentrate.

Further research is needed to mitigate the two main drawbacks of flash détente processing, being lower colour stability and poor juice flux during filtration. This thesis explored conversion of Rubired anthocyanins into more stable derivatives using acetaldehyde, tannin and low pH treatments. Other approaches such as microencapsulation [34], co-pigmentation [20] and anthocyanin self-association [35], warrant further investigation. Efforts to improve filterability should include the investigation of enzyme and fining treatments to hydrolyse or remove polysaccharides, proteins and other compounds that negatively affect filterability.

6.2.2 Impact of Juice Extraction Method (Flash Détente vs. Conventional Must Heating) and Chemical Treatments on Colour Stability of Rubired Juice Concentrate under Accelerated Ageing Conditions

This research demonstrated that treating Rubired concentrate with acetaldehyde or low pH (applied individually or in combination) increased red colour stability but also resulted in an increase of violet colour. Further research will be needed to understand consumer acceptability of concentrates and juices with a higher violet to red colour ratio compared to concentrates or juices with no acetaldehyde or low pH treatment.

Addition of a commercial seed tannin appears to have mixed results with some researchers [36, 37] reporting similar results in wine, while others [38] have reported positive colour stabilisation following the addition of grape derived tannins to wines. Further research is needed to screen a broader range of commercially available tannins to determine their effectiveness. Since the composition of most commercial tannins may not be well defined, a separate line of research should focus on evaluating tannins isolated from grape seeds, grape skins and oak wood, as well as blends of these tannins.

This research demonstrated that brown colour can decrease in Rubired concentrate during ageing under certain conditions; however, the reasons for this are not well understood. Further research is therefore needed to understand mechanisms associated with brown colour phenomena, with the goal of developing solutions for remediating browning in concentrates, which is detrimental to quality. Strategies that achieve red colour stabilisation without increasing the brown colour such as addition of antioxidants that don't bind with sugars making them ineffective, or removing amino acids from concentrate to prevent browning from Maillard reaction should also be investigated.

Concentration of 5-HMF was shown to increase to a significantly greater extent in CMH concentrate compared to FD concentrate during accelerated aging. In addition, increasing storage temperature and lowering pH markedly increased 5-HMF formation. Future research is needed to understand how well accelerating aging trends model behavior under normal concentrate cold storage conditions.

6.2.3 Impact of Fermentation Temperature and Grape Solids Content on the Chemical Composition and Sensory Profile of Flash-treated Cabernet Sauvignon Wines Fermented off skins

This study investigated the effect of grape solids and temperature during fermentation, without oxygen supplementation, although most commercial winemaking now involves macro-oxygenation during fermentation as standard practice. It has been reported [15] that yeasts will metabolise lipids from external sources (e.g. grape solids) to produce sterols when fermenting in the absence of oxygen, whereas in the presence of oxygen, phytosterol synthesis will take place in the absence of external lipid sources. Previous research [39] suggested that oxygenation of wines prior to malolactic fermentation would be expected to enhance aroma by increasing red fruit and

spicy attributes while causing the emergence of new nutty and sweet fruit attributes that were not present prior to oxygen treatment.

In addition to improving wine aroma by diminishing vegetal notes [40] and enhancing fruit notes, macro-oxygenation would be expected to promote polymerisation of tannins and pigments to modify mouthfeel properties [41] and increase colour stability [42] due to acetaldehyde mediated formation of pyranoanthocyanins and ethyl-bridged tannins and derived pigments. Acetaldehyde formed due to macro-oxygenation could crosslink tannins via ethylene bridges to either form less astringent polymers or polymers that precipitate, thereby decreasing astringency [43, 44]. Future research should therefore investigate the combined effect of macro-oxygenation and removal vs supplementation of fine grape solids, on red wine composition and sensory profiles.

The banana aroma/flavour elicited by isoamyl acetate, which is characteristic of varietals such as Pinotage [45, 46], but often perceived as a negative attribute, is sometimes associated with liquid phase red grape fermentations . In addition to fermenting juice on suspended grape solids at lower temperatures, which this research has shown to lower isoamyl acetate concentrations, micro-oxygenation has also been reported to induce the same effect [46, 47]. Nevertheless, future research should investigate strategies that reduce the isoamyl acetate concentration of fruity wines produced from liquid phase fermentations without diminishing fruit quality. Focus of this research should include yeast selection, to identify strains that produce less isoamyl acetate, as well research to optimize oxygen add rate and timing to minimize loss of desirable aroma compounds.

Future work should also consider liquid phase red fermentations with higher concentrations of suspended solids, to help define the cut-off point beyond which wine quality is negatively impacted. Whereas in white winemaking it is recommended to keep fine suspended solids within the 50–150

NTU range [15] during fermentation (to ensure an adequate supply of lipids to yeast and prevent development of off-notes), no guidance or recommendation is similarly available for liquid phase red fermentations.

6.2.4 Impact of Skin Contact Time, Oak or Tannin Addition on the Chemical Composition, Colour Stability and Sensory Profile of Flash-Detente Merlot Wines

Despite numerous claims from vendors suggesting beneficial colour stabilisation effect of oenotannins, no comprehensive study has been undertaken to investigate the chemical composition and colour stabilisation of the diverse range of commercial tannins in use within the wine industry. The present study involved one commercial oenological tannin, while other studies [48] have assessed only a handful. With no standard oenotannin definition or standardised preparation method, it is not surprising that findings differ among the studies with regard to the colour stabilisation effects of tannins [36-38]. The oenotannin used in the present study had 50% total tannin on a dry mass basis, with composition of the remaining material being unknown. Additional research is therefore needed to screen a wider range of oenological tannins, representing both hydrolysable and condensed tannins from various sources, to better understand their chemical composition and colour stabilisation efficacies, and to determine if their usage improves wine sensory quality.

Conducting liquid phase fermentations, with or without suspended grape solids, was shown to have greater effect on wine aroma composition and flavour profiles compared to the effects of bulk grape solids, which mainly impacted phenolic extraction and mouthfeel. The effect of pre-fermentation removal of suspended solids from classic maceration red fermentations or flash détente treated red must fermented on skins warrants investigation. Based on findings from the present work, such fermentations could potentially increase the fruity aroma and flavour intensity

while improving mouthfeel properties, to create wines of greater sensory appeal. Pre-fermentation removal of suspended solids could be achieved by centrifuging juice once the cap has formed during cold maceration. Centrifuged juice would then be returned to the fermenter prior to inoculation and primary fermentation. In addition, the effect of different sizes and concentrations of suspended grape solids in traditional classic maceration fermentations on wine composition and sensory profiles warrant investigation.

6.3 Summary

This research highlighted the benefits and drawbacks of using flash détente to enhance the extraction, quality and stability of colour in Rubired grape concentrate while also exploring chemical treatments for stabilizing Rubired concentrate colour (chapters 2 and 3). The main benefits of using FD extraction for concentrate production were increased colour quality and slower rate of 5-HMF formation during ageing, but the associated drawbacks were poor colour stability and juice filterability, which might negate any financial gain associated with enhanced colour quality. Addition of acetaldehyde and acid (to lower pH), either individually or in combination, can be used to stabilise Rubired colour, but addition of seed tannin had no effect on colour stability.

The research also demonstrated how conditions such as temperature, the percentage of suspended grape solids and the duration of skin contact time during fermentation of flash détente treated juice or must can be used to create differentiated styles of red wine while improving the efficiency of red winemaking by overcoming the need for prolonged skin contact (and associated cap management) during fermentation (Chapter 4 and 5). Both studies demonstrated increased concentrations of esters, β-damascenone, linalool, quercetin glycosides, and total anthocyanins (after 1 year in bottle) and lower concentrations of fusel alcohols, 1-hexanol, and polysaccharides

from FD-derived juices fermented without suspended solids, compared to traditional maceration ferments. This created FD-derived wines that had high ratings for red fruit and confectionery attributes together with low ratings for green and savoury attributes compared to traditional maceration ferments. However, the pre-fermentation addition of oenological tannin or oak chips did not affect the colour stability of red wine made from FD treated juice. Overall, this research suggests that there is a place for FD in red juice concentrate and red wine production, but wider adoption of technology for concentrate production requires addressing some of the key drawbacks identified in this research.

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