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ABSTRACT

Protein haze development in bottled white wines is attributed to the slow denaturation of unstable proteins, which results in their aggregation and flocculation. These protein fractions can be removed by using bentonite; however, a disadvantage of this technique is its cost. The effects of high hydrostatic pressure (HHP) on wine stability were studied. Fourier transform infrared spectroscopy experiments were performed to analyse the secondary structure of protein, thermal stability was evaluated with differential scanning calorimetry, while a heat test was performed to determine wine protein thermal stability. The results confirmed that high pressure treatments modified the α-helical and β-sheet structures of wine proteins. Throughout the 60 days storage period the α-helix structure in HHP samples decreased. Structural changes by HHP (450 MPa for 3 and 5 min) improve thermal stability of wine proteins and thus delay haze formation in wine during storage.

1. Introduction

Proteins play a significant role in the colloidal stability and clarity of white wine (Sauvage, Bach, Moutounet, & Vernhet, 2010). The formation of an unattractive deposit in bottled wine, caused by protein aggregation during storage, is a common defect of commercial wines, which makes them unacceptable to consumers. Although turbid wines do not constitute a health risk, protein haze formation is an important issue in oenology (Marangon et al., 2011; Pocock, Hayasaka, McCarthy, & Waters, 2000; Sauvage et al., 2010). Such precipitates commonly result from denaturation of grape proteins in wine and haze-forming proteins have been identified as pathogenesis-related (PR) grape proteins; thaumatin-like (TL) proteins and chitinases are the predominant proteins found in must and wine (Marangon, Van Sluyter, Haynes, & Waters, 2009; Marangon et al., 2011). Both have low-molecular weight, ranging from 20 to 35 kDa; being resistant to proteolysis and low wine pH, they are able to survive fermentation and remain in wine and potentially form haze (Marangon et al., 2011). The haze formation phenomenon is a multifactorial process and the presence of proteins is a pre-requisite. However, the wine matrix consisting of polyphenols, polysaccharides, sulphate, pH, and ethanol also facilitates the complex interplay that results in haze (Batista, Monteiro, Loureiro, Teixeira, & Ferreira, 2009; Batista, Monteiro, Loureiro, Teixeira, & Ferreira, 2010; Pocock, Alexander, Hayasaka, Jones, & Waters, 2007). In fact, to mitigate the risk of haze formation in white wines due to thermolabile proteins, wine producers can use several organic and inorganic fining agents. Bentonite is one of the inorganic fining agents commonly used to prevent haze formation by removing wine proteins among other components and stabilising the wine (Versari, Laghi, Thornagate, & Boulton, 2011). Bentonite interacts electrostatically with proteins and causes flocculation of the fractions because the clay surface is negatively charged at wine pH (3.0–3.5) and positively adsorbs charged proteins (Vanrell et al., 2007). Although effective in preventing haze formation, bentonite treatment causes a significant loss of aroma and colour, alters taste by removing polyphenols and reducing the volume of the wine (between 3% and 10%). For these reasons, increasing attention is given today to developing alternative practises to stabilise wine proteins that would maintain its quality and be economically viable and sustainable (Vanrell et al., 2007).

High hydrostatic pressure (HHP) technology has been used in the field of food processing because it offers several advantages related to traditional methods of food conservation and hygiene

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(Dzwolak, Kato, & Taniguchi, 2002). Among the effects that it has on food components, HHP affects the non-covalent bonds (ionic, hydrophobic, and hydrogen bridges) of proteins; therefore, the primary structure remains unchanged, whereas the secondary, tertiary, and quaternary structures may unfold and disassociate (Dzwolak et al., 2002). High hydrostatic pressure is already being used in winemaking, predominantly for microbiological control; Morata, Benito, González, Tesfaye, and Suárez-Lepe (2012) used HHP (100 MPa for 24 h) in red wines (DO Mancha) to control *Dekkera/Brettanomyces* using *Dekkera bruxellensis* D37 as a model member. The HHP treatment controlled *D. bruxellensis* D37 very well, and prevented to a large extent negative effects often caused by filtration, pasteurisation, and flash pasteurisation. We suggest that HHP can be used not only to attain microbiological stability but also to stabilise proteins.

To the best of our knowledge, no research has yet been published that describes the effect of HHP on wine protein stability. In the present study, we evaluated the effect of HHP on the secondary structure and thermal stability of wine proteins.

2. Materials and methods

2.1. Materials

2.1.1. Wine samples

The Sauvignon blanc wines used in this study were acquired from the 2012 and 2013 harvest in the Casablanca Valley, Chile. The wine contained 15–20 ppm of free sulphur dioxide (SO_2). None of the wines contained bentonite. Once fermentation was complete, wines were packaged in 60 L polyethylene drums and stored until the HHP treatment.

2.2. Application of high hydrostatic pressure

Samples of packaged wines were pressurised in a 2 L pressure unit (Avure Technologies Incorporated, Kent, WA, USA) with a 700 × 600 mm cylinder. In all cases, water was used as the pressure-transmitting medium; Sauvignon blanc samples were treated at 400, 450 and 500 MPa for 3, 5, 8, and 10 min at ambient temperature and compared to untreated samples. The pressurised and untreated samples were stored at 16 °C until protein characterisation was conducted. The control samples did not contain bentonite.

2.3. Physical–chemical properties of Sauvignon blanc wine

The values of physical–chemical parameters, such as pH, total acidity, volatile acidity, free and bound sulphur, percentage of alcohol by volume, and chromatic characteristics, were determined according to the Official Newspaper of the European Communities. (1990). Total protein concentration was determined by Bradford's method with Coomassie brilliant blue reagent measure absorbance at 595 nm with a spectrophotometer (Cecil CE2021, England) after 5 min of incubation (Salazar, Brujin, Seminario, Güell, & López, 2007). Total protein content was expressed as mg/L in reference to a standard of bovine serum albumin (Sigma, cat. No. A-3803). Total phenolic content was determined by Folin–Ciocalteu's method (Singleton & Rossi, 1965). The test sample (1 mL) was mixed with 50 mL distilled water, 5 mL Folin–Ciocalteu's reagent, and 20 mL 20% sodium carbonate solution. After 30 min, the absorbance at 750 nm was recorded. Results were expressed as Gallic acid equivalents. All determinations were triplicated.

2.4. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of Sauvignon blanc wine were recorded with an IRPrestige-21 spectrometer (Shimadzu 21 Corporation, Kyoto, Japan). Samples were spotted at the centre of an IR transparent ZnSe optical disc. For each measurement, a total of 128 scans were collected at 4 cm^{-1} resolution. The FTIR spectra of Sauvignon blanc wine were determined at wave lengths ranging from 1600 to 1700 cm^{-1}. The spectroscopy experiments were conducted at 15 d intervals during the 60 d storage period (0, 15, 30, 45, 60 d). Measurements were repeated six times and values were averaged to reduce baseline effects. The spectra were manipulated by the algorithms of the IRSolution-21 version 1.50 software, smoothed (9 points) and normalised, the second derivative was then applied (9 points) and values were expressed in terms of energy absorption units.

2.5. Differential scanning calorimetry (DSC)

Differential scanning calorimetry experiments were carried out with a DSC1 instrument (Mettler Toledo AG, Analytical, Schwerzebach, Switzerland). The instrument was first calibrated for temperature and enthalpy using indium as standard (T_m: 156.6 °C; ΔH_m: 28.45 J/g). Wine protein fraction was previously concentrated (64-fold) with Amicon Ultra 15 10 kDa filter devices. Model wine (artificial wine) was prepared with 2 g/L malic acid, 12% (v/v) ethanol, and pH reference of 3.0. Wine concentrate, 70 µl, was put in a 100 µl aluminium pan and sealed. The sample was subjected to an isothermal phase (25 °C for 3 min) and then scanned at 10 °C/min from 25 to 95 °C. A pan with 70 µl of model wine was used as reference. Baselines obtained from model wine were finally subtracted from sample data (Marangon et al., 2011). The melting temperature (T_m) and melting enthalpy (ΔH_m) were calculated from the peak area using the software provided by the manufacturer (Stareware, v.10.01 Mettler Toledo). All measurements were duplicated.

2.6. Heat stability tests

Heat tests were conducted according to Pocock, Salazar, and Waters (2011) at 80 °C for 2 h. After heating, samples were

Table 1
Effect of HHP on physical–chemical properties of Sauvignon blanc wine.

Physical–chemical properties	Treatments			
	Control	500 MPa × 3 min	500 MPa × 5 min	500 MPa × 10 min
pH	3.52 ± 0.01	3.51 ± 0.01	3.55 ± 0.01	3.57 ± 0.01
Total acidity (g/L of tartaric acid)	3.18 ± 0.18	3.01 ± 0.15	3.10 ± 0.13	3.13 ± 0.21
Volatile acidity (g/L of acetic acid)	2.03 ± 0.38	1.42 ± 0.34	1.16 ± 0.16	1.39 ± 0.15
Free sulphur dioxide, mg/L	34.13 ± 0.74	23.47 ± 1.96	20.48 ± 0.00	22.19 ± 1.48
Total sulphur dioxide, mg/L	72.96 ± 1.28	44.37 ± 1.48	44.37 ± 1.48	46.08 ± 2.56
Alcohol (% v/v)	13.1 ± 0.3	13.2 ± 0.1	13.1 ± 0.3	13.0 ± 0.3
Colour (420 nm)	0.147 ± 0.002	0.169 ± 0.003	0.155 ± 0.004	0.163 ± 0.005
Polyphenols (GAE, mg/L)	475 ± 12	468 ± 14	480 ± 18	481 ± 19
Proteins (BSA, mg/L)	20.8 ± 0.1	21.4 ± 0.4	18.1 ± 1.3	19.3 ± 1.4

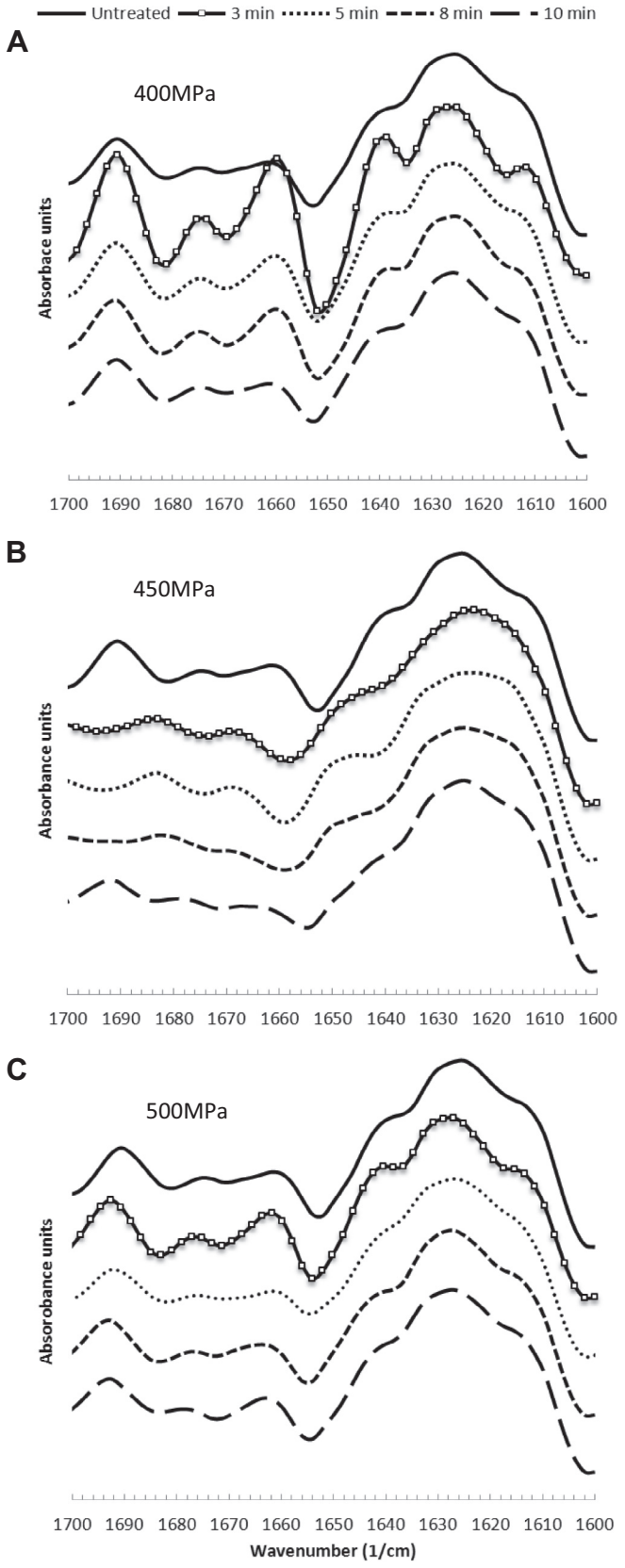

Fig. 1. Second derivative of the FT-IR spectra in the amide I region (1700–1600 cm^{-1}) of Sauvignon blanc wine pressurized at: (A) 400 MPa, (B) 450 MPa, (C) 500 MPa.

immediately cooled in tap water and placed on ice for another 2 h. The amount of haze produced was measured at room temperature with a nephelometer (Hanna Instruments, HI 83749, RI, USA). Samples were considered to contain unstable proteins when the difference between heated samples and unheated control samples was greater than 2 nephelometry turbidity units (NTU).

2.7. Statistical analysis

A completely randomised block design with factorial arrangement was constructed to test the hypothesis. An analysis of variance (ANOVA) was performed using Statgraphics Centurion XVI (Statistical Graphics Corp., Herdon, USA) and SPSS v.17 software to detect significant differences among the treatments. Significance testing was performed by Scheffe's test and Tukey's test; differences were statistically significant when $p < 0.05$. Analysed parameters include physical–chemical properties, total protein content, secondary structures, enthalpy, melting temperature, and turbidity.

3. Results and discussion

3.1. Physical–chemical properties of Sauvignon blanc wine

The conventional oenological parameters that were analysed include the following: alcohol (%), colour, protein content, pH, volatile acidity, total acidity, protein content, and free, combined, and total SO_2 contents. Control wine values were similar and comparable to those published in the literature (Dufrechou, Poncet-Legrand, Sauvage, & Vernhet, 2012). Wines that were pressure-treated at 450 and 500 MPa exhibited physical–chemical properties similar to those of untreated wine (control). An example of the effect of HHP at 500 MPa on the physical–chemical properties of Sauvignon blanc wine is shown in Table 1. An unchanged percentage of alcohol (v/v) is especially advantageous because wines and other alcoholic beverages are commercialised and categorised in accordance with this property. However, results indicated a difference in colour ($p < 0.05$). Our results showed that the colour value of the control sample is lower than that of the treated samples (Table 1). The difference between the colour of the treated samples and control wine is due to the effect of HHP on the wine matrix, which reduces entropy and thus increases the size of absorbent particles. These results are comparable to those described by Mok et al. (2006), Lonvaud-Funel, Dupont, Demazeau, and Bignon (1994). Their results demonstrated that these parameters experienced little change caused by applying HHP, which is similar to the results of the present study (Table 1). The total protein content was not changed ($p < 0.05$) by HHP treatment. The fact that the wine maintained its physical–chemical properties despite HHP treatment is advantageous because these properties might affect the product's sensory quality.

3.2. Evaluation of spectroscopic properties of Sauvignon blanc wine

The spectroscopic properties of the wine proteins were evaluated to ascertain the effect of HHP on secondary structure of proteins. Fourier transform infrared spectroscopy (FTIR) provides information about the chemical composition and conformational structure of food components, and it is a particularly useful tool to monitor changes in secondary structures of protein (Jackson & Mantsch, 1995; Willard, Merrit, Dean, & Settle, 1981). A second derivative procedure was applied to the spectra to resolve the overlapping to the secondary structural elements that contributed to the amide I band, whereby the band position is preserved (Pelton & McLean, 2000). The amide I band, located in the region between 1600 and 1700 cm^{-1}, arises predominantly from C≡O stretching vibrations, which is weakly coupled with in-plane

N—H bending and C—N stretching vibrations. (Ojagh, Nuñez-Flores, López-Caballero, Montero, & Gómez-Guillen, 2011).

Fig. 1 shows the second derivative of the FT-IR spectra in the amide I region of untreated wine sample and pressurized samples at 400, 450 and 500 MPa for 3, 5, 8, and 10 min (Fig. 1A–C, respectively). The minima in the second derivatives refer to maxima in the original spectra. In each graph, spectra of treated samples are shown below the spectrum of the untreated sample to comparatively evaluate the changes undergone during the high pressure treatment. In the present study, the IR second derivative spectra reveal five bands at the 1615, 1635, 1654, 1669, and 1681 cm^{-1} wavenumbers that mostly coincided with those described previously for protein secondary structure in water samples (Table 2). It has been reported that four different types of proteins made up of different isoforms are present in wine: thaumatin-like proteins (TL), chitinases, β-glucanases, and invertases (Cilindre et al., 2008; Esteruelas et al., 2009; Falconer et al., 2010; Monteiro et al., 1999; Sauvage et al., 2010; Waters, Shirley, & Williams, 1996). Therefore, the major peak at 1654 cm^{-1} was indicative of the higher contribution of α-helical structure of total wine proteins in the matrix and the lower contribution of intramolecular and intermolecular β-sheet at 1615 and 1635 cm^{-1}, respectively.

When 400 MPa pressure is applied for 5, 8, and 10 min on wine samples (Fig. 1A), the secondary spectra are similar to the untreated sample and indicate that there is no pressure effect on protein secondary structure. However, at 400 MPa for 3 min (Fig. 1A), the α-helix (1654 cm^{-1}) and turn (1669 and 1681 cm^{-1}) structures increased the most, while β-sheet (1635 cm^{-1}) had a moderate increase. The increase of α-helix and β-sheet structures has been attributed to the denaturing effect of high pressure, which leads to protein unfolding (Ojagh et al., 2011).

The comparison of the second derivative spectra of the untreated samples with pressure-treated samples at 450 MPa (Fig. 1B) suggests an interconversion of structures contributing to absorptions at 1691, 1658, and 1641 cm^{-1} of samples treated for 3, 5, 8, and 10 min. The absorption band at 1654 cm^{-1} shifted towards 1658 cm^{-1}, this indicates a loss of α-helix and an increase of turn, intermolecular, and intramolecular β-sheet structures (Table 2). The changes in electrostatic interactions and hydrogen bond stability could in turn contribute to the loss of α-helix content under 450 MPa. These findings are similar to the results obtained by previous studies related to salmon muscle myofibers proteins with higher salt concentrations (Carton, Bocker, Ofstad, Sørheim, & Kohler, 2009).

A modification of the protein spectrum can indicate a loss of the native state. To highlight the evolution of the secondary structure on wine according to time after high pressure treatment, the height of the peak of the second derivatives for the bands located at 1615–1693 cm^{-1} (intermolecular β-sheet), 1641 cm^{-1} (β-sheet), 1654 cm^{-1} (α-helix) and 1662–1685 cm^{-1} (turn) were used (Astruc et al., 2012). Secondary structures of untreated samples maintain their conformation for 2 weeks after which α-helix and turn structures increase significantly, while β-sheet and intermolecular β-sheet decrease. Recent studies suggest that this behaviour is due to strong hydrogen bonding and electrostatic interactions, but weaken hydrophobic interactions. Therefore, wine proteins evolve over time, which may support the formation of local hydrogen-bonded structures such as α-helices (Fatima, Sharma, & Guptasarma, 2010). The effect of HHP treatment on the evolution of the secondary structure of wine proteins stored at 16 °C for 60 d is shown in Fig. 2. A pressure of 400 MPa for 5 and 8 min after 45 d decreases α-helix (Fig. 2B–E, respectively). Moreover, wine samples treated at 450 MPa, regardless of pressurisation time (3, 5, 8, or 10 min) (Fig. 2G–I, respectively) decrease α-helix structure, which is maintained for up to 45 d; this favours hydrophobic interaction and subsequent aggregation phenomena (Fatima et al., 2010). The changes in electrostatic interactions and hydrogen bond stability could in turn contribute to the loss of α-helix structure under high pressure conditions (Liu, Zhao, Xiong, Xie, & Qin, 2008). A recovery of the α-helix structure is observed after 60 d, which suggests a reversible behaviour of the wine proteins. According to Falconer et al. (2010), this behaviour could be associated with the presence of thaumatin-like proteins.

3.3. Evaluation of the thermal properties of Sauvignon blanc wine

The thermal stability of wine proteins measured by DSC as influenced by different HHP treatments is shown in Fig. 2. Melting temperature (T_m) and melting enthalpy (ΔH_m) values are shown in Table 3.

A main broad peak ranging from 63 °C to 65 °C was observed in all samples regardless of treatment applied (Fig. 3). In accordance with previous studies about thermal stability of wine proteins in Sauvignon blanc wine (Falconer et al., 2010), this peak could be attributed to thaumatin-like proteins. These authors found that the melting temperature of 55 °C, 62 °C, and 81 °C, corresponded to chitinases, thaumatin-like proteins, and invertases, respectively. They also noted that the most abundant protein species in this type of wine was thaumatin-like proteins. Due to the low sensitivity of DSC, the wine samples were concentrated and measured at a high heating rate to observe thermal transitions. Under these conditions, protein aggregation may have occurred because both wine proteins and all the components with molecular weight >10 kDa were concentrated. For this reason, it was not possible to detect unfolding of chitinase and invertase proteins as in other studies (Dufrechou et al., 2012), and only one major endothermic peak was observed (Fig. 3). This could suggest that the various existing protein domains are held together by interdependent forces as one single cooperative unit. Given this situation, it is also possible that unfolding of chitinases could be overlapped with the broad peak found for thaumatin-like proteins.

Table 2
Tentative assignment of protein secondary structures in water.

Absorption wavenumber (cm^{-1})	Tentative assignments	References
1614–1624	Intermolecular β-sheet	Matheus, Friess, and Mahler (2006) and Pikal, Rigsbee, and Roy (2008)
1627–1639	Intramolecular β-sheet	Matheus et al. (2006) and Kong and Yu (2007)
1650–1657	α-helix	Barth (2007), Matheus et al. (2006), Dzwolak et al. (2002), Kong and Yu (2007), Takekiyo, Takeda, Isogai, Kato, and Taniguchi (2007) and Pikal et al. (2008)
1658–1670	Turn structures	Barth (2007), Matheus et al. (2006) and Kong and Yu (2007)
1678–1684	Turn structures	Barth (2007), Matheus et al. (2006) and Kong and Yu (2007)
1690	Intermolecular β-sheet	Kong and Yu (2007)

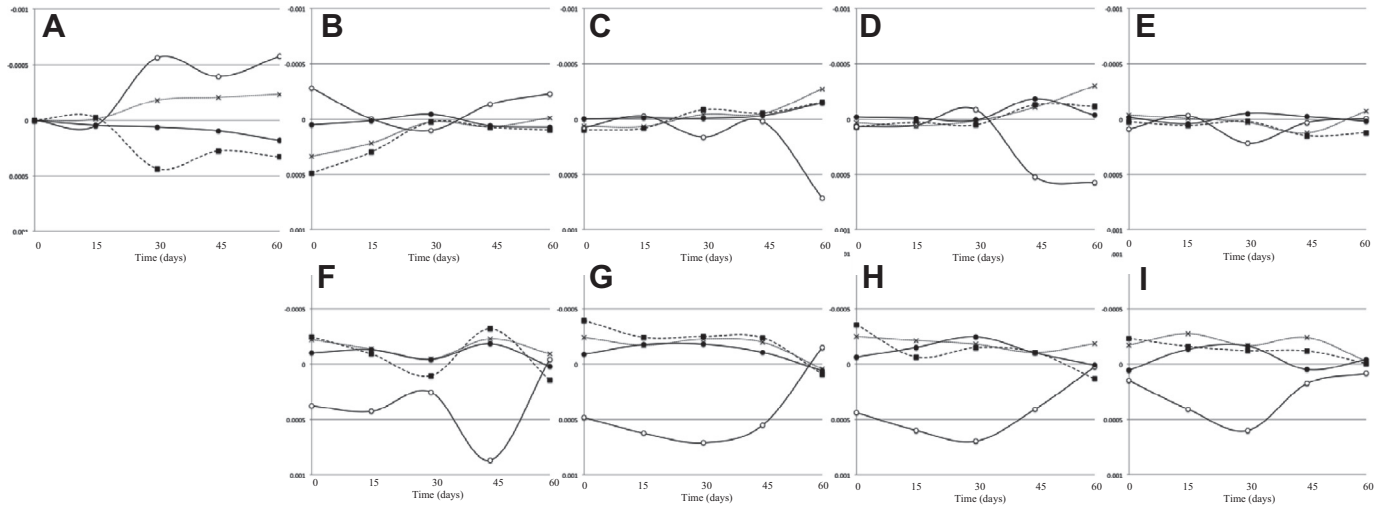

Fig. 2. Evolution of the secondary structure of wine proteins HPP-treated Sauvignon blanc wine stored at 16 °C up to 60 days: (A) Untreated wine; (B) wine treated at 400 MPa × 3 min, (C) wine treated at 400 MPa × 5 min, (D) wine treated at 400 MPa × 8 min, (E) wine treated at 400 MPa × 10 min, (F) wine treated at 450 MPa × 3 min, (G) wine treated at 450 MPa × 5 min, (H) wine treated at 450 MPa × 8 min, (I) wine treated at 450 MPa × 10 min, -■- β-sheet, -○- α-helix, -●- intermolecular β-sheet, -×- turn.

Table 3
Melting temperatures (T_m) and melting enthalpy (ΔH_m) of wine proteins subjected to different HHP treatments.

Treatments		Thermodynamic parameters		Protein instability (ΔNTU)
Pressure (MPa)	Time (min)	T_m (°C)	ΔH_m (J/g)	
Untreated		63.54 ± 0.02^{b}	0.20 ± 0.03^{a}	103.3 ± 2.9^{a}
400	3	64.88 ± 0.15^{ab}	0.10 ± 0.00^{cd}	99.6 ± 1.0^{a}
	5	63.94 ± 0.07^{ab}	0.09 ± 0.00^{cd}	100.3 ± 1.0^{a}
	10	64.74 ± 0.19^{ab}	0.10 ± 0.01^{cd}	100.1 ± 2.2^{a}
450	3	65.20 ± 0.14^{a}	0.08 ± 0.00^{d}	91.5 ± 2.1^{b}
	5	65.10 ± 1.05^{a}	0.07 ± 0.00^{d}	82.4 ± 4.6^{b}
	10	64.36 ± 0.04^{ab}	0.13 ± 0.01^{bc}	88.4 ± 1.5^{b}
500	3	63.91 ± 0.14^{ab}	0.11 ± 0.01^{cd}	88.9 ± 2.5^{b}
	5	63.92 ± 0.14^{ab}	0.13 ± 0.01^{bc}	89.9 ± 1.6^{b}
	10	63.60 ± 0.01^{b}	0.17 ± 0.01^{ab}	89.0 ± 3.2^{b}

$^{a–d}$ Different superscripted letters in each column indicate significant differences ($p < 0.05$).

The influence of HHP treatments on melting temperature and melting enthalpy is shown in Table 3. Applying HHP on wine samples did not significantly change melting temperature of wine proteins, independently of time. A significant increase was observed only in samples treated at 450 MPa for 3 and 5 min when compared with unpressurized control samples. According to Weiss, Young, and Roberts (2009), T_m values can be used to qualitatively rank a protein's unfolding propensity. These results might therefore suggest that applying high pressure at 450 MPa for 3 and 5 min produced a partial wine protein unfolding and improved their thermal stability. Thus, the production of haze proteins in wines could be delayed. Consistent with the results obtained for melting temperatures, enthalpy values of wine proteins were significantly decreased by pressure as compared with the untreated sample (Table 3). The effect of pressurisation time on the unfolding enthalpy was not observed. Structural modifications of proteins by high pressure are related to the rupture of non-covalent interactions and the following reformation of intra- and inter-molecular bonds within or between protein molecules (Messens, Van Camp, & Huyghebaert, 1997). The largest decrease in enthalpy values was observed in the wine samples processed at 450 MPa for 3 and 5 min. This means that these treatments produced a greater degree wine protein of unfolding, because the residual enthalpy value (ΔH_d), calculated from the area under the endothermic peak, is correlated with the content of ordered protein secondary structure. It is actually a net value from a combination of endothermic reactions, such as the disruption of hydrogen bonds, and exothermic reactions, such as the breakup of hydrophobic interactions and protein aggregations (Ma & Harwalkar, 1991; Privalov & Khechinashvili, 1974). It should be emphasised that the higher thermal stability and major structural changes observed in wine proteins were obtained by 450 MPa pressure for 3 and 5 min. With this pressure–time combination, the structural conformations achieved by the wine proteins could provide higher thermal stability and thus delay haze formation in wine during storage.

3.4. Heat stability tests of Sauvignon blanc wine

Heat stability tests performed on Sauvignon blanc wine (Table 3) showed that its nephelometric turbidity units (ΔNTU) were above the accepted norm of 2NTU identified by Moine-Ledoux and Dubourdieu (1999); this suggested that both the untreated and treated wine could form visible turbidity in bottles when exposed to elevated temperatures during storage or transport.

Results indicated that heat stability of wines pressurised at 400 MPa did not differ from that of the control and was less than for wines treated at 450 MPa and 500 MPa. This is consistent with the findings in the FTIR spectra and DSC data (Fig. 2 and Table 3) in which wines treated at 400 MPa × 3 min (Fig. 2B) had undergone a structural evolution similar to that of untreated wine on day 60. Additionally, wines treated at 500 MPa (Fig. 2F–I) retained structures similar to those of untreated wine as it matures. These results

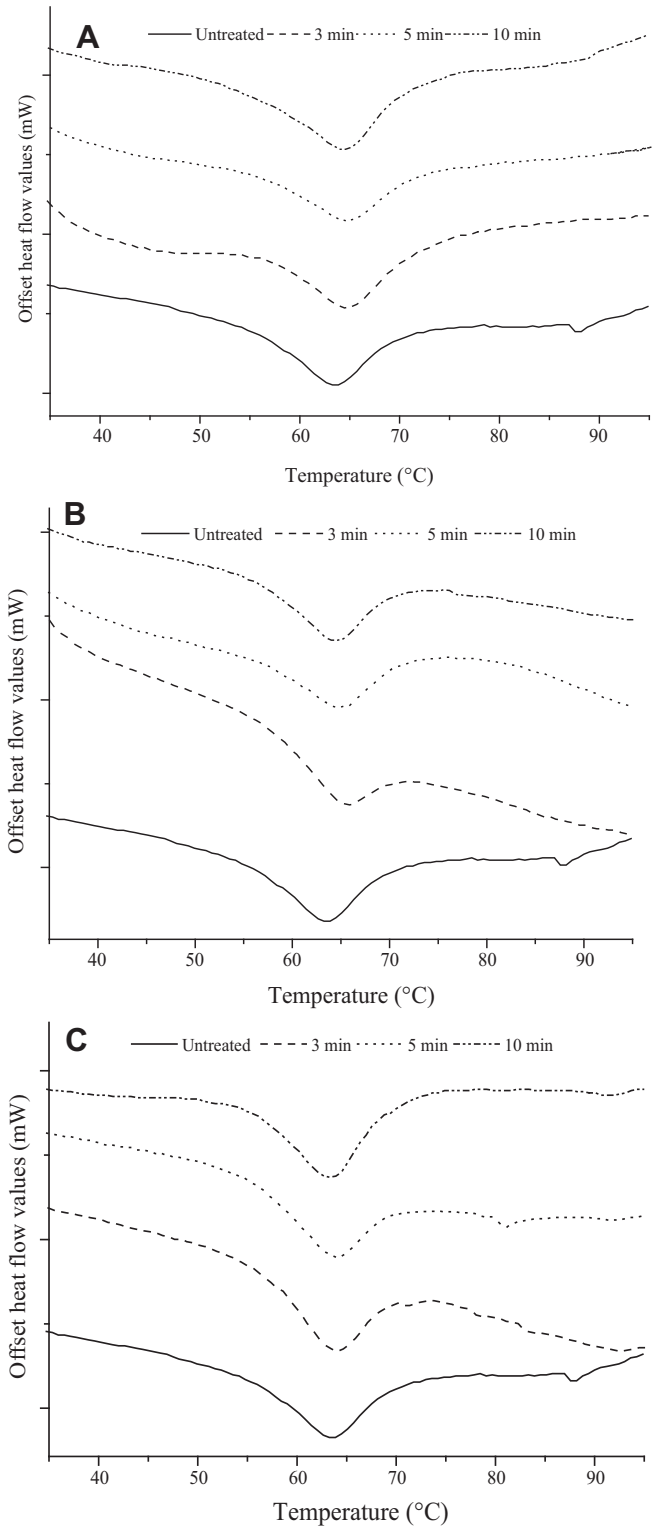

Fig. 3. Thermograms of wine proteins measured by DSC as influenced by high pressure treatments: (A) 400 MPa, (B) 450 MPa, and (C) 500 MPa.

are also supported by study findings using DSC (Table 3). The major structural changes experienced in wine proteins were produced at 450 MPa because enthalpy associated with residual native structure (ΔH_m) was significantly reduced as melting temperature (T_m) increased and thus caused an increase in wine protein thermal stability.

The type of protein present in the wine matrix may also account forthe heat stability of wines. Falconer et al. (2010) demonstrated that individual proteins have different sensitivities to denaturation and therefore contribute differently to haze formation; haze therefore arises from part of the protein mixture and not necessarily the entire protein mixture. Chitinases are the least thermally stable protein components; they therefore unfold and form aggregates more readily than do other major PR proteins, such as types of thaumatin-like proteins (Falconer et al., 2010). In their study of the role of grape thaumatin-like proteins and chitinases in white wine haze formation, Marangon et al. (2011) found a linear relationship between chitinase concentration and haze formation in model wine and unfiltered wines containing different types of protein.

According to the FTIR results, the HHP treatment can change the native structure of wine proteins, most likely by altering intramolecular interactions. A modification of the protein chain may cause an increase in the exposure of active sites to which other molecules in the wine, such as polyphenols, may bind. The hydrophobic groups tend to be concentrated in the internal cavity and are the most likely sites for protein–polyphenol interactions after removing water by hydrophobicity and protein–ligand interactions (Grigera & McCarthy, 2010). Protein–polyphenol interactions are a common cause of turbidity in beer and wine, and the intensity of turbidity is affected by both their concentration and ratio (Siebert, Troukhanova, & Lynn, 1996). A protein has limited sites to which polyphenols may bind. When the number of polyphenols present equals the number of protein active sites, a large network is formed which corresponds to large particles. Each polyphenol can link two proteins when there is excess protein; however, with a low concentration of polyphenols in the matrix, hydrophobic unions are formed between polyphenols and proteins, and the particles formed are therefore small. Furthermore, in the case of an excess of polyphenols, all of the protein active sites become saturated and some cannot attach, which also results in small particles (Siebert et al., 1996).

4. Conclusion

Wine is a complex matrix where the haze formation phenomenon is a multifactorial process. After high pressure treatment there is a reduction of sulphur dioxide (free and total) as compared with the traditional technique to stabilize wine. At 450 MPa, the α-helical structure decreases in wine proteins, which could be related to an increase of intermolecular interactions between proteins and other non-protein compounds in the wine matrix. Furthermore, a decrease of melting enthalpy and an increase of melting temperature might improve the thermal stability of wine proteins and delay haze formation. Our findings suggest that pressurisation at 450 MPa may significantly contribute to stabilising proteins in Sauvignon blanc wine. Finally, the modifications in protein structure, protein thermal stability, and wine stability depend on the intensity of the HHP treatment.

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