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ScienceDirect
Food Chemistry 110 (2008) 627–635
FOOD CHEMISTRY
www.elsevier.com/locate/foodchem

Anthocyanin condensation reactions under high hydrostatic pressure

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Received 16 July 2007; received in revised form 14 December 2007; accepted 15 February 2008

Abstract

The effects of temperature and/or high hydrostatic pressure on anthocyanin condensation reactions were studied. For this purpose, model solutions containing cyanidin-3-*O*-glucoside (Cy3gl) and pyruvate, in excess, were subjected to different combined temperature/pressure treatments. After a high hydrostatic pressure treatment of 600 MPa, at 70 °C during 30 min, about 25% of Cy3gl was degraded. Parallel to this decrease, a vitisin A-type derivative was formed. By contrast, the rate of condensation was only 5% when samples were heated (70 °C, 30 min). In both cases, the degradation kinetics fitted well to a first order reaction ($R^2 = 0.99$). The decrease in Cy3gl was correlated with a decrease in the antioxidant activity. Moreover, the chemical stability of wine subjected to a temperature/pressure treatment of 600 MPa, at 70 °C during 1 h was investigated. After this treatment, a decrease in the concentration of malvidin-3-*O*-glucoside (Mv3glu) was found. As a consequence, an increase in the concentration of several products of high molecular weight at 370 nm was observed. When wine was subjected to pasteurization conditions (600 MPa, 70 °C, 10 min), no significant changes in the chemical composition were found ($P < 0.05$).

Keywords: Cyanidin-3-*O*-glucoside; High hydrostatic pressure; Vitisin A derivative; First order reaction; Antioxidant capacity; Wine and SO_2

1. Introduction

Anthocyanidins and anthocyanins are plant pigments responsible for the red, blue and purple colours of a great range of flowers and plants in nature. Anthocyanins are the main compounds found in red grapes and are also present in grape derived products such as wines. The predominant anthocyanins in wines are cyanidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, and petunidin-3-O-glucoside and their higher or lower content is influenced by the grape variety, the conditions of the wine cultivar and ripening (Herrmann, 1976). The colour of a young red wine is primarily due to monomeric anthocyanins. However, intrinsic factors, such as temperature, light and pH, can affect wine characteristics, such as colour, bitterness and astringency. These changes are mostly attributed to the conversion of monomeric anthocyanins to more condensed ones during ripening (Somers & Evans, 1986; Somers & Verette, 1988). Anthocyanin condensation-derived adducts and oligomers are responsible for the change of colour in red wines from red to brown–red and are more stable than the monomeric ones (Schwartz, Quast, von Baer, & Winterhalter, 2003). Anthocyanin condensation reactions involve the covalent association of anthocyanins with other flavanols through ethyl bridges or with other small molecules, such as pyruvic acid, vinylphenol and glyoxilic acid (Bakker & Timberlake, 1997; Es-Safi, Fulcrand, Cheynier, & Moutounet, 1999; Francia-Aricha, Guerra, Rivas-Gonzalo, & Santos-Buelga, 1997; Fulcrand, Cameira dos Santos, Sarni-Manchado, Cheynier, & Bonvin, 1996; Liao, Cai, & Haslam, 1992; Remy, Fulcrand, Labarbe, Cheynier, & Moutounet, 2000; Rivas-Gonzalo, Bravo-Haro, & Santos-Buelga, 1995; Santos-Buelga, Bravo Haro, & Rivas-Gonzalo, 1995; Somers, 1971; Timberlake & Bridle, 1976; Vivar-Quintana,

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doi:10.1016/j.foodchem.2008.02.055

Santos-Buelga, Francia-Aricha, & Rivas-Gonzalo 1999). These reactions occur spontaneously in wines, upon ageing but they can also be generated in model solutions with varying temperature, pH and incubation times (Ribéreau-Gayon, 1982; Timberlake & Bridle, 1976). However, the influence of high hydrostatic pressure has not so far been studied. High hydrostatic pressure (HHP) has been used for processing a great range of food products (Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007). Talcott, Brenes, Pires, and Del Pozo Insfran (2003) demonstrated that muscadine grape juices presented a better colour retention when they were subjected to high hydrostatic pressure (HHP) and high temperature. Studies of Mok et al. (2006) and Puig, Vilavella, Daoudi, Guamis, & Minguez (2003), also indicated the feasibility of using HHP to pasteurize red wine. The use of HHP and moderate temperatures can cause microbial inactivation, avoiding undesirable changes such as vitamin loss and taste or colour modifications. Thus, HHP seems a promising method for wine pasteurization since SO_2 can be reduced or replaced. Usually, SO_2 is used as an antiseptic against undesirable microorganisms and as an antioxidant in wines (Amerine, Berg, & Cruess, 1967). SO_2 is commonly added to red wine grapes when they are crushed and destalked, before fermentation–maceration. It inactivates grape enzymes, such as polyphenoloxidases, by reducing their copper cofactor which negatively influences the quality of juices and derivatives. Furthermore, SO_2 can have negative effects on human health (Romano & Suzzi, 1993). Thus, HHP is a promising method for wine pasteurisation. However, as a thermodynamic factor, it is expected to influence anthocyanin condensation reactions. High hydrostatic pressure influences equilibrium of chemical reactions according to reductions in volume (Le Chatelier principle). For this reason, high hydrostatic pressure conditions, under which anthocyanin cyclic derivatives in model solutions are formed, are reported here. This study focussed on studying the formation of vitisin A-type derivatives resulting from the reaction between Cy3glu and pyruvate, as this reaction is prone to occur in wines upon ageing (Alcalde-Eon, Escribano-Bailón, Santos-Buelga, & Rivas Gonzalo, 2006). Moreover, the feasibility of using HHP to pasteurize wines is considered and its effect on the stability of wine anthocyanins reported.

2. Materials and methods

2.1. Chemicals

Cyanidin-3-*O*-glucoside (Cy3gl) was provided by Extrasynthese, (Genay, France) and sodium pyruvate from Sigma (Taufkirchen, Germany). All other reagents and solvents for analysis were purchased from Merck (Darmstadt, Germany). Red wine, Dornfelder, 2004, was provided by Niederkirchener Weinmacher e.G. (Niederkirchen, Germany).

2.2. Experimental procedures

Pure Cy3gl (3.2 mg) and 65.6 mg of sodium pyruvate (~100 equiv.) were mixed in 10 mL of acetate buffer (0.2 M acetic acid, and 0.2 M potassium acetate). Samples subjected to high hydrostatic pressure were adjusted to pH 4.4 and heated samples were adjusted to pH 3.8, since the adiabatic pressure increase causes a decrease in the pH of the buffered solutions due to H^+ loss. This decrease in the pH was at a rate of 0.1 per 100 MPa for acetate buffer (Neuman, Kauzmann, & Zipp, 1973).

2.3. Pressure/temperature treatments

Experiments up to 600 MPa were conducted in a high-pressure device consisting of a series of thermostatted microautoclaves (i.d. = 16 mm, ~10 mL, 700 MPa) connected by valves (aad GmBH, Frankfurt, Germany). Pressure was generated by an air-driven pressure intensifier. The pressure-transmitting medium was a mixture of water and glycol (80:20; v/v).

Samples were pressurized in polyethylene ampoules (250 μL) and heat-sealed. The temperature in the vessels was controlled by a thermostat Polystat from Huber (Offenburg, Germany).

Blank experiments at different temperatures were also conducted, as controls. Samples subjected to heat treatment were previously adjusted to a pH of 3.8 with 0.1 N HCl and heated in a water bath (HBR 4, Digital, Ika-Werke, Staufen, Germany).

2.4. Analysis of anthocyanins by LC-DAD/ESI-MS

Analyses were carried out using an LC-DAD/ESI-MS Agilent instrument (Waldbronn, Germany). The mass spectrometer was equipped with an ESI source in positive mode. The column eluate was recorded in the range m/z 50–1000. The mass spectrometer was programmed to do an MS^2 scan of the most abundant ion in the full mass. Nitrogen was used both as drying gas at flow rates of 11.0–12.0 μL min^{-1} and as nebulising gas at a pressure of 65 psi. The nebuliser temperature was set at 350 °C. The separation was performed with a Phenomenex (Torrance, CA) Aqua C_{18} column, (250 × 4.6 mm i.d; 5 μm particle size) operated at ambient temperature. The DAD was set at different wavelengths: 520, 510, 370 and 280 nm. The mobile phase consisted of water/formic acid/acetonitrile (87:10:3; v/v/v; eluent A) and water/formic acid/acetonitrile (40:10:50; v/v/v; eluent B) using a gradient programme as follows: from 10% to 15% B (10 min), 15% B isocratic (3 min), from 15% to 25% B (7 min), from 25% to 55% B (30 min), from 55% to 10% B (2 min). Total run was of 32 min. The injection volume for all samples was 20 μL. The flow rate was of 1.0 mL min^{-1}.

2.5. Quantification of individual compounds

The losses of Cy3gl and sodium pyruvate were quantified using a calibration curve of the corresponding standard compounds. Products of the reaction were estimated by individual compound peak collection and further analytical calibration with LC-DAD/ESI-MS under the conditions previously reported. Quantification of predominant anthocyanins in wine structurally related to Cy3gl was done by including a molecular weight correction factor (Chandra, Rana, & Li, 2001).

2.6. Antioxidant capacity

The $ABTS^{+}$ method described by Miller, Rice-Evans, Davies, Gopinathan, and Milner (1993) and later improved by Re et al. (1999) was used for the determination of the polar antioxidative capacity. A stock solution of 5 mM $ABTS^{+}$ (2,2′-azinobis [3-ethylbenzothiazoline-6-sulphonic acid]) was diluted in water and preincubated for at least 12 h with 140 mM $K_2S_2O_8$ to produce the radical cation $ABTS^{\bullet+}$. The $ABTS^{\bullet+}$ solution was then diluted in 5 mM saline phosphate buffer pH 7.4 (0.695 g $Na_2HPO_4 \times 2\ H_2O + 0.159$ g $NaH_2PO_4 \times 2H_2O + 4.5$ g NaCl per L) until absorbance readings reached a value of 1.5 at 735 nm. An aliquot of 100 μL of the suitable diluted sample was mixed with 2.9 mL $ABTS^{\bullet+}$ and set 15 min at 30 °C; then absorbance was measured at 735 nm. A calibration curve using TROLOX (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as standard was used to calculate the antioxidant activity of the samples. Results were expressed as μM TROLOX equivalents (TE).

2.7. Determination of total monomeric anthocyanins

Monomeric anthocyanins reversibly change colour according to change in pH. The coloured oxonium form predominates at pH 1.0 whereas the colourless hemiketal form predominates at pH.4.5. By contrast, polymeric or degraded anthocyanins absorb colour at this pH. The difference in the absorbance at 520 nm is proportional to the pigment concentration. Measurements at 700 nm are to correct for haze (Lee, Durst, & Wrolstadt, 2005). Following this method, an aliquot of the clear extract (1 mL) was placed in a 25 mL volumetric flask and made up to the final volume with pH 1.0 buffer (KCl, 0.025 M). The pH was adjusted with HCl (0.2 N). A further 1 mL of extract was also placed in a 25 mL volumetric flask and made up to final volume with pH 4.5 buffer (CH_3CO_2Na, 0.4 M). The pH was adjusted with HCl (0.2 N). Absorbance was measured in a UV-1601 Shimadzu spectrophotometer (Shimadzu, Germany) at 510 nm and at 700 nm. Results were calculated using the following equation and expressed as milligrammes of cyanidin 3-glucoside equivalents per 100 mL ($mg_{Cy3gl}\ L^{-1}$).

$$\text{Total anthocyanins } (mg/L^{-1}) = \frac{A}{eL} \times MW \times D \times \frac{V}{G} \times 100$$

where

$$A = (A_{520\ nm} - A_{700\ nm})_{pH\ 1.0} - (A_{520\ nm} - A_{700\ nm})_{pH\ 4.5},$$

e is cyanidin 3-glucoside molar absorbance (26,900), L is cell path length (1 cm), MW is the molecular weight for cyanidin-3-glucoside (449.2), D is a dilution factor, V is the final volume (mL), and G is the sample weight (mg).

2.8. Statistical analysis

Results were tested for statistical significance by a factorial model Minitab Handout 2 giving 3 grades of significance: $P < 0.05$, $P < 0.01$ and $P < 0.001$. All experiments were carried out in triplicate. The data presented in Tables and Figures represent mean values ± standard deviation ($n = 3$).

3. Results and discussion

3.1. Effect of temperature/pressure treatments on Cy3gl spectra

Anthocyanin condensation reactions involved the formation of a new pyran ring by cycloaddition. Cycloaddition

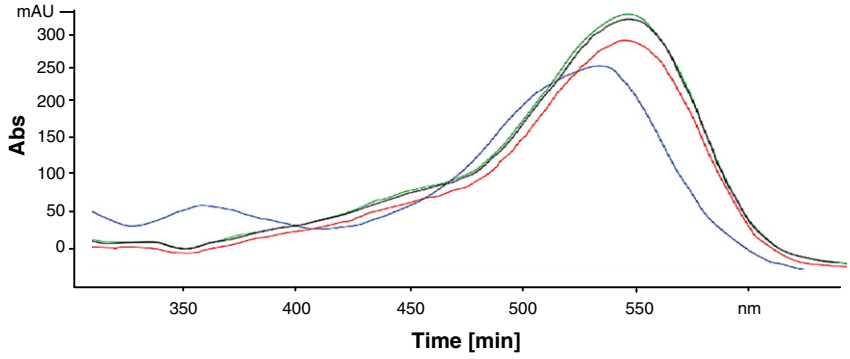

Fig. 1. UV–Vis spectra of Cy3gl and pyruvate in acetate buffer at 20 °C and 0.1 MPa (black), at 70 °C and 0.1 MPa (red) at 20 °C and 600 MPa and (green) at 70 °C and 600 MPa (blue). Treatments holding time 1.5 h. (For interpretation of the references to colour in figure legends, the reader is referred to the web version of this article.)

reactions cause a hypsochromical shift in the visible absorption maxima of the anthocyanins, producing a change in the wine colour towards orange hues (Hayasaka & Asenstorfer, 2002; Rivas-Gonzalo et al., 1995). According to these studies, model solutions containing Cy3gl and pyruvic acid were subjected to different heat and/or pressure processes and the variations in the absorbance spectrum were studied. No changes in the absorbance spectrum of model solutions were found at 25 °C nor under pressures of 0.1, 200 or 600 MPa, even after 6 h of treatment. However, at temperatures of 70 °C, differences in the absorbance spectrum could be observed. Absorbance differences were remarkable, according to an increase in the temperature and the holding time. After a heat and/or pressurized treatment of 1.5 h, the main absorbance peak, in Cy3gl untreated samples at 520 nm (dark violet), slightly decreased when samples were heated at 70 °C. Meanwhile, the absorbance of pressurized samples at 600 MPa and 70 °C showed a high decrease in the shift at 520 nm and an increase in the shift at 350–370 nm (pale-rose) (Fig. 1). According to spectra analysis, the analytical experiments carried out by HPLC-DAD/ESI-MS showed the formation of a new peak, when samples were pressurized at 600 MPa and 70 °C up to 0.5 h, whose mass was $[517 + H^+]$ (Fig. 2). The mass and absorbance here obtained were in agreement with the theoretical mass and absorbance reported for anthocyanin–pyruvic adducts structurally similar to vitisin A. Fragmentation analysis MS^2 showed one major ion $[355 + H^+]$. This ion corresponds to the anthocyanidin by the loss of 94 amu from a pyruvate fragment with water loss. The product obtained matches the features of vitisin A-type derivatives from Rioja wines described by Alcalde-Eon et al. (2006). The cycloadditon reaction in model solutions involved the reaction of the C_4 from the ethylenic bond provided by the pyruvate with the hydroxyl group at C_5 of the anthocyanin followed by the loss of a water molecule and further oxidation (Fig. 3) (Schwartz, Wabnitz, & Winterhalter, 2003). The appearance of this new peak was directly related to a decrease in the initial concentration of Cy3gl when samples were heated under pressure. No significant spectrum changes in samples pressurized at 200 MPa at 70 °C after 3 h compared to heated controls were observed ($P > 0.05$).

3.2. Degradation kinetics of Cy3gl under temperature/pressure treatments

Concentration variations of Cy3gl along the different treatments are represented in Fig. 4. According to spectra results, no significant concentration changes were found when samples were subjected to different high hydrostatic pressure treatments (200, 600 MPa) at 25 °C. In addition, Cy3gl concentration loss was not significantly different between samples subjected to a HHP process at 200 MPa and 70 °C and only heated samples throughout the experiment. On the contrary, when samples were subjected to a pressure of 600 MPa and a temperature of 70 °C, concentration changes were detected as a function of the treatment time and there was significant degradation of Cy3gl ($P < 0.05$). After subjecting samples to holding times of 0.5 h, a loss of ~25% of Cy3gl was determined. Also, Cy3gl was degraded when samples were heated at 70 °C for 0.5 h; however, the degradation was only ~5%. The logarithmic representation of $C(t)/Co$, where C is the concentration

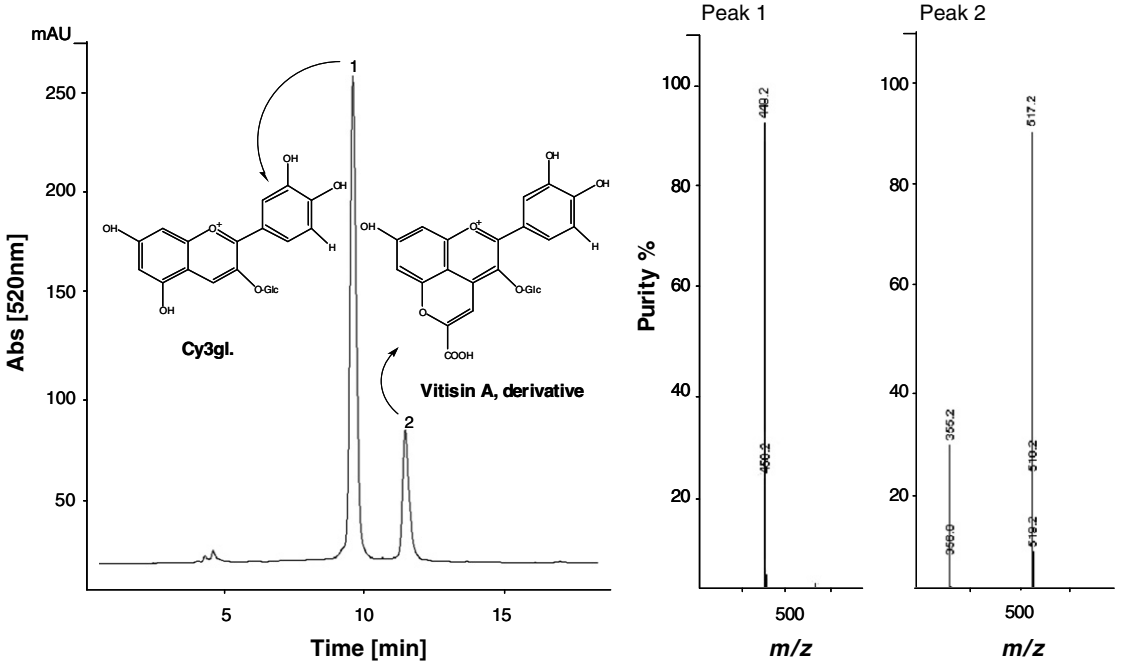

Fig. 2. HPLC and MS^2 spectra of Cy3gl and pyruvate in acetate buffer after a 1.5 h heat/pressure treatment at 70 °C and 600 MPa.

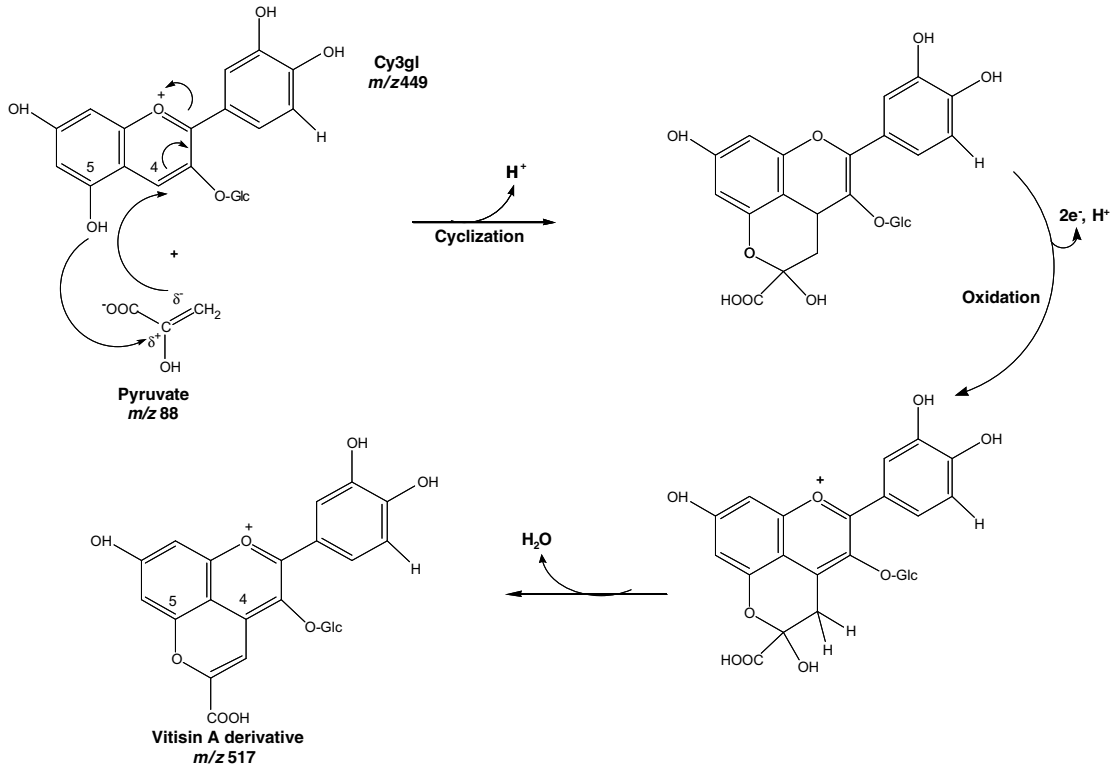

Fig. 3. Condensation reaction of Cy3gl and pyruvate in heated/pressurized samples.

of Cy3gl at time (t) and Co is the cyclic-adduct concentration at time 0 versus time (t, in hours), decayed following a linear function ($R^2 = 0.99$). The reaction constant (k) could be calculated from the slope. Results showed that the use of a combined temperature/pressure treatment accelerated anthocyanin degradation, the constant of the reaction $k = 0.79\ h^{-1}$ was clearly higher than in heated samples where the reaction constant was $k = 0.05\ h^{-1}$. These results are according to previous thermal studies carried out by Cemeroglu, Vlioglu, and Isik (1994), Kirca and Cemeroglu (2003) which showed a first order kinetic thermal degradation of anthocyanins in cherry and blood orange juices. The applied factorial design provided information about how factors jointly influenced the rate of condensation. According to the factorial design, the interaction of high hydrostatic pressure and temperature enhanced reaction yields and their influence was significantly different at all the significance levels studied ($P < 0.05$, $P < 0.01$ and $P < 0.001$). The influences of temperature and time were significantly different when $P < 0.05$ and $P < 0.01$. By contrast, the effect of pressure and time did not show any interaction as their combined effect was not significantly different ($P > 0.05$). The effect of a combined temperature/pressure treatment on anthocyanin condensation reactions was remarkable. When samples were subjected to high hydrostatic pressure and a temperature of 70 °C during longer holding times (6 h), a significant decrease in the concentration of Cy3gl was observed. Cy3gl was degraded by nearly 53%. Simultaneously, a predominant peak under HPLC-DAD/ESI-MS was detected. The molecular ion was

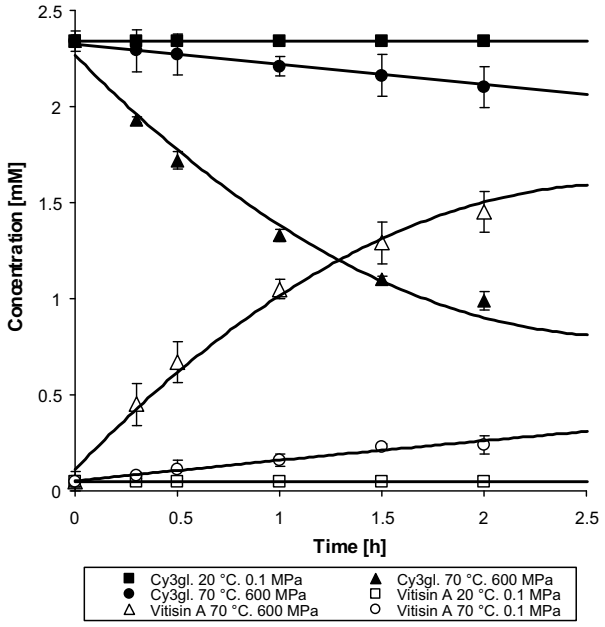

Fig. 4. Degradation and formation rate of Cy3gl and vitisin A, respectively, under different temperature/pressure treatments in acetate buffer.

$[594 + H^+]$, which indicates the formation of a new compound of higher molecular weight. However, its accurate chemical structure could not be discerned by HPLC-DAD/ESI-MS. The degradation of Cy3gl in heated samples during 6 h was around 25%.

3.3. Antioxidant capacity of heated/pressurized samples

The estimation of the antioxidant capacity of the samples subjected to different treatments showed a decrease as a function of treatment holding times (Fig. 5). The formation of cyclic-adducts led to a loss of an active OH-group in the meta-position of the A ring from the flavylium cation molecule mainly responsible for the loss in the antiradical activity (Rice-Evans, Miller, & Paganga, 1996). The 3- and 5-OH groups in the A and C rings with 1-oxo function are required for a maximum radical-scavenging potential. A temperature/pressure combined treatment enhanced the formation of cyclic-adducts, since the loss of Cy3gl was directly correlated with an increase of the formed vitisin A-type derivative (Fig. 4). By contrast, when samples were heated for 1.5 h, a stronger loss of antioxidant capacity was determined (Fig. 5), but a lower concentration of vitisin A-type derivative was found. This may be explained by the instability of anthocyanins at high temperature. The degradation of anthocyanins has been postulated to first involve deglycosylation forming a chalcone, which yields different benzoic acid derivatives (Salidova, Stintzing, & Carle, 2006; Seeram, Bourquin, & Nair, 2001). Also, trihydroxybenzaldehyde has been identified as an end-product of the thermal degradation of anthocyanins (Furtado, Figueiredo, Chaves das Neves, & Pina, 1993) (Fig. 6). The formation of these degradation products causes the rupture of the 3, 4 double bond in conjugation with the 1-oxo function in the C ring, which is responsible for electron delocalization from the B ring. Antioxidant capacity is related to structure in terms of electron delocalization of the aromatic system. When anthocyanins react with free radicals, the phenoxyl radicals produced are stabilized by the resonance effect of the aromatic nucleus (Rice-Evans et al., 1996). This degradation pathway might also explain the decrease in the absorbance at 520 nm, as previously observed in Fig. 1. However, in heated samples, other condensation reactions are also likely to occur. García-Alonso et al. (2005) demonstrated that the antioxidant capacity of old wines decreased according to anthocyanin degradation and formation of more condensation products and oligomers.

3.4. Effect of temperature/pressure treatments on wine

For a better understanding of the influence of combined temperature/pressure treatments on food matrices, wine from the Dornfelder grape variety, was subjected to a pressure of 600 MPa at 70 °C for 1 h. The stability of the predominant anthocyanins in wine was monitored and determined by HPLC-DAD/ESI-MS. Fig. 7A and B represent the chromatograms of wine before and after a heat and heat/pressure treatment at wavelengths of 520 and 370 nm, respectively. A decrease in the concentration of Mv3gl (peak 3) in pressurized samples was detected whereas, in the heated samples, no loss of Mv3gl was observed. Chromatograms taken at a wavelength of

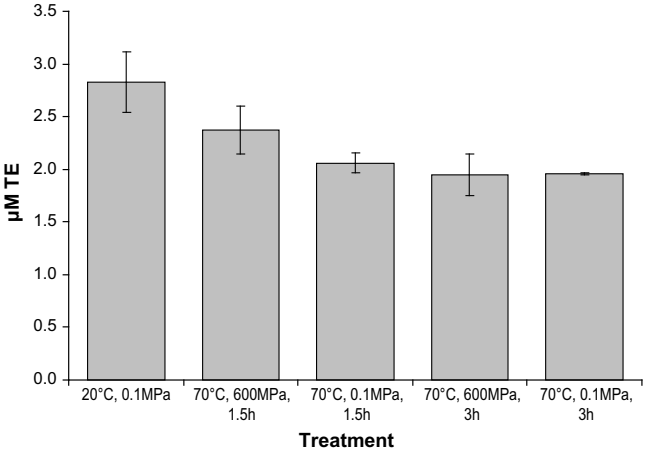

Fig. 5. Antioxidant capacity of Cy3gl and pyruvate in acetate buffer subjected to different temperature/pressure conditions during different holding times.

370 nm showed an increase in the concentration of several peaks of higher molecular weight, such as, a: $[948 + H^+]$, b: $[643 + H^+]$ and c: $[922 + H^+]$ when samples were heated under pressure. The spectrometric estimation of anthocyanin monomer contents in solution tended to decrease when samples were heated and/or pressurized for 1 h. The monomer contents were $21.7 \pm 1.51\ mg_{Cy3gl}\ L^{-1}$ in control samples, $17.9 \pm 0.86\ mg_{Cy3gl}\ L^{-1}$ in samples heated at 70 °C, and $15.9 \pm 0.78\ mg_{Cy3gl}\ L^{-1}$ in heat-pressurized samples (70 °C, 600 MPa). This confirmed that degradation of anthocyanin monomers in heat-pressurized wine samples occurred. However, results were not significantly different ($P > 0.05$).

When samples were subjected to a process at 600 MPa, 70 °C for 10 min, no significant differences in anthocyanin composition or antioxidant activity of the samples were found ($P < 0.05$). These results accord with works of Mok et al. (2006), in which it was demonstrated that physicochemical characteristics of wines did not change after pasteurization at pressures ranging from 100 to 400 MPa at 25 °C during times of 5–30 min. In addition, after HHP treatments at 350 MPa, 25 °C during 20 min, inactivations of aerobic and lactic acid bacteria and yeasts were found. Moreover, studies of Puig et al. (2003), indicated that an HHP treatment of 500 MPa for 5 min reduced wine bacterial population by 99.99% without altering chemical or organoleptic properties of wine. The induction of chemical reactions in wines by high hydrostatic pressure and temperature only occurred after times above 1 h. These reactions are not expected to occur under commercial high hydrostatic pressure pasteurization conditions where pressures range from 400 to 700 MPa, temperatures are not longer than 40–50 °C and holding times are not longer than a few minutes. Long holding times and high temperatures are critical parameters for anthocyanin condensation reactions to occur under pressure. Pasteurization conditions studied here ensure the biochemical and microbial stability

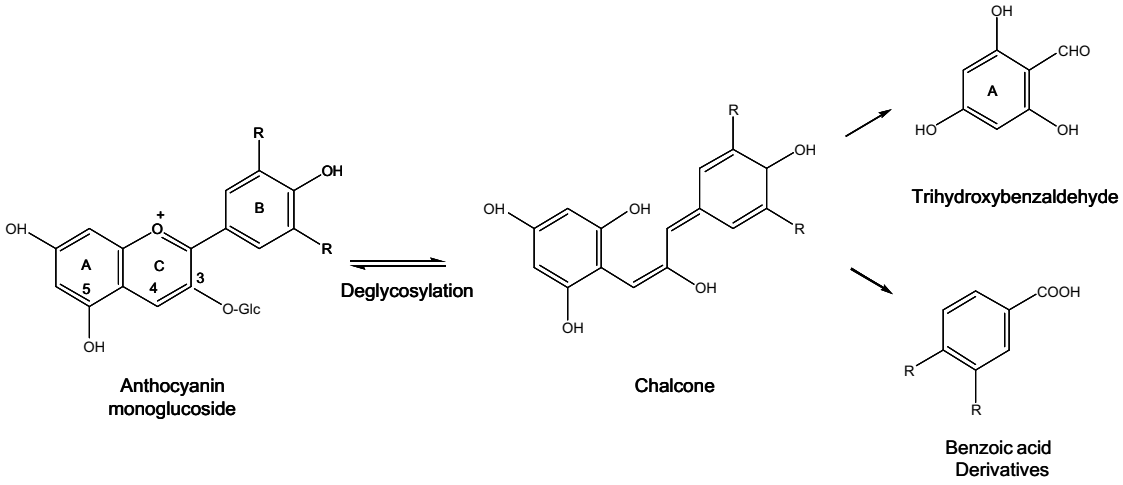

Fig. 6. Degradation of anthocyanin monoglucosides at pH 3.8 accelerated by heat.

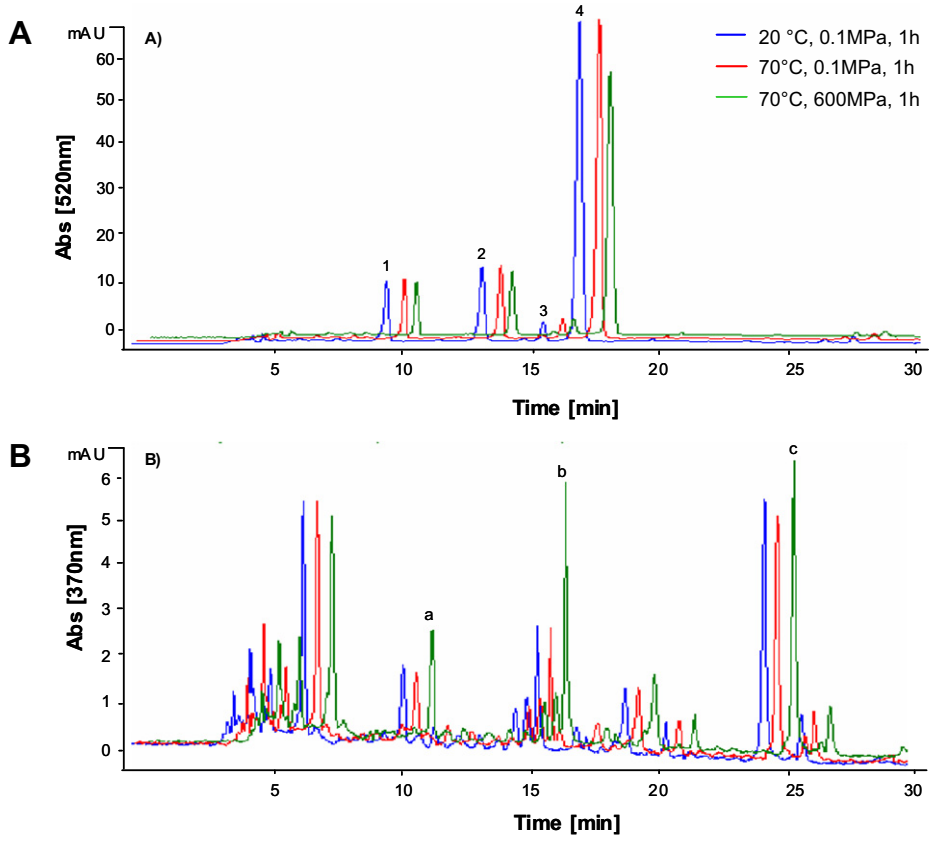

Fig. 7. HPLC-DAD chromatograms of Dornfelder wine samples at 520 and 370 nm. Untreated wine samples at 20 °C and 0.1 MPa (blue). Samples at 70 °C and 0.1 MPa held during 1 h (red) and samples treated at 70 °C and 600 MPa held during 1 h (green). Compounds: **1.** Delphinidin-3-*O*-glucoside, $M^+ = 465$; **2**. Petunidin-3-*O*-glucoside, $M^+ = 479$; **3**. Malvidin-3-*O*-glucoside, $M^+ = 493$; Unidentified products: (a) $M^+ = 948$, (b) $M^+ = 643$, (c) $M^+ = 922$.

of wine and represent an alternative to SO_2 normally applied for vinification. As previously mentioned, SO_2 is widely used in winemaking as an antioxidant and bacteriostatic agent. SO_2 behaves as a powerful nucleophile when dissolved and is capable of bonding covalently with electrophiles such as pyruvate and acetaldehyde to form adducts, e.g. vitisin A. Vitisin A derivatives contribute to the redness of old wines. However, SO_2 can affect wine fermentation by developing resistant and undesired microorganisms which affect organoleptic characteristics of wine (Romano & Suzzi, 1993). Thus, through the use of HHP for wine pasteurisation, the addition of SO_2 could be lowered or even

avoided which may also be desirable for public health (Lueck, 1980).

4. Conclusions

The results reported in this work indicate that temperature/pressure treatments accelerate the synthesis of complex anthocyanin pyruvic acid adducts, such as vitisin A-type derivatives, in acetate buffer. Pyruvic acid adducts, produced from these reactions, presented a different hypsochromic shift from that of genuine anthocyanins. They are also precursors of highly polymerised anthocyanins with different colour ranges which may be of interest from an industrial point of view.

Combined temperature/pressure treatments during long holding times (3–6 h) might enhance degradation and formation of condensation products in wines which contribute to colour, organoleptic and nutritional changes (loss of antioxidant capacity). Nonetheless, the advantages and drawbacks of the influence of heat/pressure treatments during long holding times on organoleptic characteristics of anthocyanin-enriched food products should be further studied. The complexity of the wine matrix offers a plethora of interactions able to occur among wine constituents which complicates an accurate identification and quantification of products. Only NMR characterization of heated/pressurized foods might identify the preference, in synthesis, of one product over another and their influence on product quality. The effect of high hydrostatic pressure on wine matrix constituents was negligible under pasteurization conditions. No chemical changes regarding antioxidant activity and anthocyanin polymerization or condensation reactions were observed. From a chemical point of view, the feasibility of HHP for wine pasteurization was demonstrated. Thus, undesirable effects of SO_2 on wines and on consumer health could be reduced or avoided. However, a complete sensorial analysis of pasteurized wines has to be further investigated.

Acknowledgments

We thank the DAAD (German Academical Exchange Programme) and La-Caixa for a Doctoral Grant.

References

Alcalde-Eon, C., Escribano-Bailón, M. T., Santos-Buelga, C., & Rivas Gonzalo, J. C. (2006). Changes in detailed pigment composition of red wine during maturity and ageing. A comprehensive study. *Analytica Chimica Acta, 563*, 238–254.

Amerine, M. A., Berg, H. W., & Cruess, W. V. (1967). In *The technology* of winemaking. Westport, CT, USA: AVI Publishing Company Inc.

Bakker, J., & Timberlake, C. F. (1997). Isolation, identification, and characterization of new colour-stable anthocyanins occurring in some red wines. *Journal of Agriculture and Food Chemistry, 45*, 35–43.

Cemeroglu, B., Vlioglu, S., & Isik, S. (1994). Degradation kinetics of anthocyanins in sour cherry juice and concentration. *Journal of Food Science, 59*, 1216–1218.

Chandra, A., Rana, J., & Li, Y. (2001). Separation, identification, quantification and method validation of anthocyanins in botanical supplement raw materials by HPLC and HPLC-MS. *Journal of Agriculture and Food Chemistry, 49*(8), 3515–3521.

Es-Safi, N. E., Fulcrand, H., Cheynier, V., & Moutounet, M. (1999). Studies on the acetaldehyde-induced condensation of (−) epicatechin and malvidin-3-*O*-glucoside. *Journal of Agriculture and Food Chemistry, 47*, 2096–2102.

Francia-Aricha, E. M., Guerra, M. T., Rivas-Gonzalo, J. C., & Santos-Buelga, C. (1997). New anthocyanin pigments formed after condensation with flavonols. *Journal of Agriculture and Food Chemistry, 45*, 2262–2265.

Fulcrand, H., Cameira dos Santos, P. J., Sarni-Manchado, P., Cheynier, V., & Bonvin, J. F. (1996). Structure of new anthocyanin-derived wine pigments. *Journal of Chemical Society, 1*, 735–739.

Furtado, P., Figueiredo, P., Chaves das Neves, H., & Pina, F. (1993). Photochemical and thermal degradation of anthocyanidins. *Journal of Photochemistry and Photobiology, 75*, 113–118.

García-Alonso, M., Rimbach, G., Sasai, M., Nakahara, M., Matsugo, S., Uchida, Y., et al. (2005). Electron spin resonance spectroscopy studies on the free radical scavenging activity of wine anthocyanins and pyranoanthocyanins. *Molecular Nutrition & Food Research, 49*(12), 1112–1119.

Hayasaka, Y., & Asenstorfer, R. E. (2002). Screening for potential pigments derived from anthocyanins in red wine using nanoelectrospray tandem mass spectrometry. *Journal of Agriculture and Food Chemistry, 50*(4), 756–761.

Herrmann, K. (1976). Flavonols and flavones in food plants: A review. *Journal of Food Technology, 11*, 433–448.

Kirca, A., & Cemeroglu, B. (2003). Degradation kinetics of anthocyanin in blood orange juice and concentrate. *Food Chemistry, 81*, 583–587.

Lee, J., Durst, R. W., & Wrolstadt, E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colourants, and wines by the pH differential method: Collaborative study. *Journal of AOAC International, 88*(5), 1269–1278.

Liao, H., Cai, Y., & Haslam, E. (1992). Polyphenol interactions. Antocyanins: Copigmentation and colour changes in red wines. *Journal of the Science of Food and Agriculture, 59*, 299–305.

Lueck, E. (1980). Sulfur dioxide. In Antimicrobial food additives. (pp. 115–131). Berlin: Springer.

Miller, N. J., Rice-Evans, C., Davies, M. J., Gopinathan, V., & Milner, A. A. (1993). Novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical Science, 84*(4), 407–412.

Mok, C., Song, K. T., Park, Y. S., Lim, S., Ruan, R., & Chen, P. (2006). High hydrostatic pressure pasteurization of red wine. *Journal of Food Science, 71*(8), 265–269.

Neuman, R. C., Kauzmann, W., & Zipp, A. (1973). Pressure dependence of weak acid ionization in aqueous buffers. *Journal of Physical Chemistry, 77*, 2687–2691.

Puig, A., Vilavella, M., Daoudi, L., Guamis, B., & Minguez, S. (2003). Microbiological and biochemical stabilization of wines using the high pressure technique. *Bulletin del I'OIV, 76*(869–870), 596–617.

Rastogi, N. K., Raghavarao, K. S. M. S., Balasubramaniam, V. M., Niranjan, K., & Knorr, D. (2007). Opportunities and challenges in high pressure processing of foods. *Critical Reviews in Food Science and Nutrition, 47*, 69–112.

Re, R., Pellegrini, N., proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolourization assay. *Free Radical Biological Medicine, 26*(9–10), 1231–1237.

Remy, S., Fulcrand, H., Labarbe, B., Cheynier, V., & Moutounet, M. (2000). First confirmation in red wine of products resulting from direct anthocyanin–tannin reactions. *Journal of Science of Food and Agriculture, 80*, 745–751.

Ribéreau-Gayon, P. (1982). In P. Markakis (Ed.), Anthocyanins as food colours (pp. 192–207). NY, USA: Academic Press.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology & Medicine, 20*(7), 933–956.

Rivas-Gonzalo, J. C., Bravo-Haro, S., & Santos-Buelga, C. (1995). Detection of compounds formed through the reaction of malvidin-3-monoglucoside and catechin in the presence of acetaldehyde. *Journal of Agriculture and Food Chemistry, 43*, 1444–1449.

Romano, P., & Suzzi, G. (1993). Sulfur dioxide and wine microorganisms. In G. H. Fleet (Ed.), *Wine microbiology and biotechnology* (pp. 373–393). Victoria, Canada: Harwood Academic.

Salidova, E., Stintzing, F. C., & Carle, R. (2006). Thermal degradation of acylated and nonacylated anthocyanins. *Journal of food Science, 71*(8), 504–510.

Santos-Buelga, C., Bravo Haro, S., & Rivas-Gonzalo, J. C. (1995). Interactions between catechin and malvidin-3-monoglucoside in model solutions. *Zeitschrift für Lebensmitteluntersuchung und Forschung, 201*(3), 269–274.

Schwartz, M., Quast, P., von Baer, D., & Winterhalter, P. (2003). Vitisin A content of chilean wines from *vitis vinifera Cv.* Cabernet Sauvignon and contribution to the colour of aged red wines. *Journal of Agricultural and Food Chemistry, 51*, 6261–6267.

Schwartz, M., Wabnitz, T. C., & Winterhalter, P. (2003). Pathway leading to the formation of anthocyanin–vinylphenol adducts and related pigments in red wines. *Journal of Agricultural and Food Chemistry, 51*, 3682–3687.

Seeram, N. P., Bourquin, L. D., & Nair, M. G. (2001). Degradation products of cyanidin glycosides from tart cherries and their bioactivities. *Journal of Agriculture and Food Chemistry, 49*, 4924–4929.

Somers, T. C. (1971). The polymeric nature of red pigments. *Phytochemistry, 10*, 2175–2186.

Somers, T. C., & Evans, M. E. (1986). Evolution of red wines I. Ambient influences on colour composition during early maturation. *Vitis Journal, 25*, 31–39.

Somers, T. C., & Verette, E. (1988). *Phenolic composition of natural wine types*. In H. F. Linskens & J. F. Jackson (Eds.), *Modern methods of plant analysis, wine analysis* (pp. 219–257). Berlin, Germany: Springer-Verlag.

Talcott, S. T., Brenes, C. H., Pires, D. M., & Del Pozo Insfran, D. (2003). Phytochemical stability and colour retention of copigmented and processed muscadine grape juice. *Journal of Agriculture and Food Chemistry, 51*, 957–963.

Timberlake, C. F., & Bridle, P. (1976). Interactions between anthocyanins, phenolic compounds and acetaldehyde and their significance in red wines. *American Journal of Enology and Viticulture, 27*, 97–105.

Vivar-Quintana, A. M., Santos-Buelga, C., Francia-Aricha, E., & Rivas-Gonzalo, J. C. (1999). Formation of anthocyanin-derived pigments in experimental red wines. *Food Science and Technology International, 5*, 347–352.