Effect of heat/pressure on cyanidin-3-glucoside ethanol model solutions

Margarita Corrales¹, Ralf Lindauer, Peter Butz and Bernhard Tauscher.
Institute of Chemistry and Biology, Federal Research Centre for Nutrition and Food, 76131 Karlsruhe, Germany
E-mail: margarita.corrales@bfel.de

Abstract. The stability of cyanidin-3-glucoside (Cy3gl) in 50% ethanol model solutions under heat/pressure treatments was investigated. Cy3gl was rapidly degraded when solutions were subjected to a heat/pressure treatment. The higher the pressure and the temperature used, the higher the degradation. Moreover, the degradation was increased according to increasing holding times. Parallel to the degradation of Cy3gl several hydrolytic products were formed and identified by LC-DAD/ESI-MS. The degradation of Cy3gl was well fitted to a first order reaction (R=0.99). This study pointed out the rate of susceptibility of Cy3gl in model solutions to degrade when exposed to a heat/pressure treatment and the trigger effect of high hydrostatic pressure to hydrolyse Cy3gl. By contrast, the degradation of anthocyanins in a food matrix (red grape extract solutions) was negligible after a heat/pressure process at 600MPa, 70°C during 1h (P>0.05).

1. Introduction
Anthocyanins (anthos: sprossom, flower, Kyáneos: darkblue) belong to the group of water soluble pigments and are responsible for the red, blue and purple hues of flowers and fruits in nature. Anthocyanins belong to the group of flavanoids whose chemical structure is constituted by 3 aromatic rings with a positive charge on the C-ring and a glucoside moiety at the C3 which confers them certain stability compared to their aglycon forms (anthocyanidins) (Figure 1). Different pH values modify their chemical structure manifesting different colours from yellow to black [1]. Other factors such as oxygen, enzymes, copigments, metal ions, ascorbic acid, sulfur dioxide, sugars and sugar degradation products may influence their stability and colouring properties [2]. Thus, the thermal instability of anthocyanins has been widely reported. Anthocyanin degradation at high temperatures follows a first-order reaction. Hrazdina, [3] pointed out that the decomposition of anthocyanins upon heating led to a chalcone structure which further decomposed to coumarin glucoside derivatives with a loss of the B-ring. Moreover, Adams et al., [4] 1973 reported that the aglycon sugar bonds were highly susceptible to hydrolysis even at acid pH. Later on Simpson, [5] suggested that the thermal degradation of anthocyanins could occur via two mechanisms: (1) hydrolysis of the 3-glucoside linkage to form a labile aglycon; and (2) hydrolytic opening of the pyrillium ring to form a substituted chalcone, which further degrade into brown insoluble compounds. Anthocyanin degradation is generally correlated to a loss or change in color. While the influence of temperature on anthocyanins has been extensively investigated, effects of pressure, also in combination with temperature on anthocyanin model solutions are still un-
known. High hydrostatic pressure has been used in the food industry as pasteurisation and sterilisation method primarily in Japan, United States and in less term in Europe. Pressure pasteurisation is feasible at room temperature since it inactivates micro-organisms avoiding undesirable changes such as vitamin loss and taste or colour modifications. Moreover, the effect of pressure and temperature for sterilisation at pressures up to even more than 1000MPa and temperatures around 80-120°C during very short times has been established yielding high quality foods [6]. The influences of moderate heat/pressure treatments are still under research and suppose a promising field since heat/pressure combined treatments may deliver food products of greater quality than thermal treated ones [7]. Thus, several studies have demonstrated high hydrostatic pressure as an effective pasteurization method for different anthocyanin enriched products which have retain their colour and flavour characteristics during longer time [8-10]. However complex matrices complicate the accurate estimation of the effect of high hydrostatic pressure on individual compounds. For a closer knowledge of the influence of high hydrostatic pressure on anthocyanins, cyanidin-3-glucoside (Cy3gl) in ethanol model solutions were subjected to different heat/pressure treatments and their stability in this work was reported. Cy3gl is the predominant anthocyanin found in red raspberries, blackberries and blood orange [11, 12]. Furthermore, the influence of a heat/pressure treatment was also studied in an anthocyanin enriched matrix.

Figure 1. Chemical structure of anthocyanins and substitution pattern.

2. Material and Methods

2.1. Materials
Cyanidin-3-O-glucoside (Cy3gl) was provided by Extrasynthese, (Genay, France). Dornfelder vitis vinifera red skins were supplied by wineries from the region of Palatine, Germany. Solvents for analysis were purchased by Merck (Darmstadt, Germany).

2.2. Methods

2.2.1. Preparation of Cy3gl ethanol model solutions. The model solutions were prepared with 1.3mM Cy3gl in 50% ethanol solution. Anthocyanins from Dornfelder vitis vinifera red grape skins were extracted weighing 10g of dark grape skins in 100mL 50% ethanol solution assisted by ultrasonics (Bandelin, Sonorex RK 100H, Walldorf, Germany).

2.2.2. Analysis of anthocyanins and degradation products by LC-DAD/ESI-MS. Samples were analysed using an Agilent Technologies LC/MSD Series 1100 (binary solvent delivery, autosampler, UV-Vis Diode Array Detector (DAD), electrospray ionization (ESI); (Agilent Technologies, Palo Alto, CA). The separation was performed with an Aqua column (80A 250 x 4.6mm i.d.; 5µm),
operated at 20°C. Solvents were (A): 0.5% acetic acid/water (v/v) and (B): 0.5% acetic acid/50% water/50% acetonitrile (v/v/v). Conditions were adapted from the method described by Kammerer, Schieberle and Carle, [13]. The elution gradient was: from 10-15% B (10min), 15% (3min), 15-25% (10min), 25-55% (30min) and 55-100% (6min) at a flow rate of 1.0 mL min\(^{-1}\). The injection volume was 20µL and detection was monitored at 280, 320, 370 and 520nm.

For anthocyanin analysis, solution (A) was water/formic acid/acetonitrile (87:10:3; v/v/v) and solution (B): water/formic acid/acetonitrile (40:10:50; v/v/v). The gradient used was: from 10-25% B (10min), 15% B isocratic (3min), from 15-25% B (7min), from 25-55% B (30min), from 55-100% B (1min), 100% B isocratic (5min), from 100-10% B (0.1min) at a flow rate of 1.0mL min\(^{-1}\). The injection volume was 20µL and detection was monitored at 370, 280 and 520nm.

Spectra were recorded in negative ion mode for reaction hydrolytic products and in positive ion mode for anthocyanin analysis 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 a flow rate of 11.0 l min\(^{-1}\), and as nebulising gas at a pressure of 60psi. The nebuliser temperature was 350°C.

2.2.3. Heat/Pressure treatments. A device constituted by a three series of thermostated microautoclaves connected by valves (i.d.=16mm, ~10mL, 700MPa) were used for high hydrostatic pressure treatments (aad GmBH, Frankfurt, Germany). Pressure was generated by an air-driven pump in combination with a pressure intensifier and the pressure-transmitting medium was a mixture of water and glycol (80:20; v/v).

Experiments at 800MPa were carried out in a hydraulic press U 101 Unipress (Polish Academy of Sciences, Warsaw, Poland) which was manually operated by a twin hydraulic piston. The temperature in the vessels was controlled by a thermostat Polystat from Huber (Offenburg, Germany). Samples were pressurized in polyethylene ampoules (250µL) and heat sealed.

Experiments were carried out in triplicate and significant differences among the means were estimated by the analysis of the variance, one way ANOVA (\(P<0.05\)).

3. Results and Discussion

Experiments carried out at different high hydrostatic pressure intensities at 20°C and 30°C did not affect the stability of Cy3gl after 0.5h treatment. Although when temperature was risen up to 50°C a higher degradation of Cy3gl could be observed up to 400MPa (Figure 2). The higher the pressure and the temperature, the higher was the degradation of Cy3gl. In addition, longer holding times also increased Cy3gl degradation rate. The degradation of Cy3gl fitted well a first-order reaction of the form:

\[
\ln(A_t / A_o) = -kt
\]

Where \(A_t\) was the concentration at \(t\) time, \(A_o\) initial concentration, \(k\) reaction constant and \(t\) time. The half life could be determined from the slope by the expression:

\[
T_{1/2} = -\ln(0.5) / k
\]

Cy3gl degradation rate constant in heat-pressurized samples (70°C/600MPa) was 0.33h\(^{-1}\) whereas in heated samples (70°C) was 0.02h\(^{-1}\). The half life (\(T_{1/2}\)) in heat-pressurized samples was 0.38h and 5.77h for heated samples. The latter values are within the range determined by Kirca and Cemeroglu, [11] for blood orange juice at 70°C; 2.0h for 69°Brix concentrate and 6.3h for 11.2°Brix concentrate. By contrast the half life of the reaction in heat-pressurized samples was remarkably shorter indicating a faster degradation.
Figure 2. Effect of high hydrostatic pressure intensity (MPa) and temperature (°C) on Cy3gl degradation. Treatment holding time: 0.5h.

When $\ln(k/k_0)$ was plotted against pressure (Figure 3) the activation volume ($dV$) could be determined from the slope by the following expression:

$$dV = -RT \frac{d \ln(k/k_0)}{dP} \quad (k/k_0 \sim V/V_0)$$

(3)

The activation volume at 70°C was $-2.8 \text{cm}^3 \text{mol}^{-1}$ relatively low compared to chemical reactions mainly influenced by high hydrostatic pressure where activation volumes are often in the range of $-20 \text{cm}^3$ [7]. Hence, the decisive role of the temperature in the results herein achieved.

Figure 3. Effect of high hydrostatic pressure on the degradation rate of Cy3gl in 50% ethanol model solutions. Treatments carried out at 70°C during 0.5h.
According to Cy3gl degradation, some hydrolytic products could be identified by LC-DAD at 280nm. The concentration of hydrolytic products was remarkably higher when samples were treated at 600MPa/70°C/0.5h and higher at 800MPa/70°C/0.5h when the highest disappearance of Cy3gl was also determined. As a result of the degradation, 9 hydrolytic products could be found and identified by LC-DAD/ESI-MS (Figure 4). One of the main components from the degradation had a mass of [195 + H]⁺ which corresponded to a first deglycosidation of the flavilium moiety yielding a chalcone which further transformed into a coumarin derivative with a loss of the B-ring. Quercetin [301 + H]⁺ was also identified which is according to studies reported by Tanchev and Ioncheva, [14]. Other compounds such as phloroglucinaldehyde and protocatechuic acid also indicated in the literature for anthocyanin thermal degradation were not found. Due to the low concentration of formed peaks an accurate identification of further products could not be discerned. Results here achieved suggest the formation of other degradation products different from those obtained in heat treatments. Only NMR experiments might indicate the chemical structure and possible degradation pathways of Cy3gl under a heat/pressure treatment.

![Figure 4. A) LC-DAD Chromatogram of Cy3gl at different heat/pressure conditions (λ=520nm) and B) LC-DAD Chromatogram of Cy3gl derived products at different heat/pressure conditions (λ=280nm). 1. M⁺=195; 2. M⁺=217; 3. M⁺=319; 4. M⁺=unknown; 5. M⁺=545; 6. 464; 7. M⁺=301; 8. M⁺=348; 9. M⁺=unknown.](image)

Moreover the effect of a high hydrostatic pressure treatment was tested in a solution containing red grape extracts from Dornfelder *vitis vinifera*. After a high hydrostatic pressure treatment at 600MPa/70°C/1h only a small loss in the predominant anthocyanin compounds was appreciable (*P*<0.05) (Figure 5). As a result when samples were subjected during a shorter treatment (10min) any anthocyanin concentration loss was detected (data not shown). These results demonstrated that in a matrix anthocyanin degradation did not occur as in model solutions. The presence of other substances with antioxidant properties (e.g: phenolic acids) and the synergistic effects among them may exert a protective effect which avoids anthocyanin rapid degradation and enhances thus product integrity. 
4. Conclusions
Although Cy3gl model solutions were sensitive to different heat/pressure treatments, the treatment of grape extract solutions presented similar characteristics in terms of anthocyanin content as their control patterns. Even heated pressurised extracts at 600MPa, 70°C during 1h did not exhibit drastic losses of anthocyanins probably due to the action of intrinsic protective substances. These results are highly valuable since a moderate pasteurization method may be applied for anthocyanin enriched foods without altering their chemical characteristics and improving product shelf life. Nonetheless, further experiments in reference to the effect of heat and pressure on other compounds present in targeted matrices must be further evaluated. Only the establishment of an appropriate pasteurization method might guarantee the quality and safety of treated products.

![Figure 5. LC-DAD Chromatograms of Dornfelder *vitis vinifera* extracts at different heat/pressure treatments. 1. DI3gl, M⁺=465; 2. Cygl, M⁺=449; 3. Pt3gl, M⁺=479; 4. Pn3gl, M⁺=463; 5. Mv3gl, M⁺=493; 6. DI3acgl, M⁺=507; 7. Pt3acgl, M⁺=521; 8. Pn3acgl, M⁺=505; 9. Mv3acgl, M⁺=535; 10. Cy3pcmgl, M⁺=595; 11. Pt3pcmgl, M⁺=625; 12. Pn3pcmgl, M⁺=609; 13. Mv3pcmgl, M⁺=639. (gl=glucoside; acgl=acetylglucoside; pcmgl=p-coumarylglucoside) Reference]

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