

Stability of the anthocyanins extracted from residues of the wine industry

Estabilidade de antocianinas extraídas do resíduo de indústrias de vinho

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Abstract

Anthocyanins are highly important due to their antioxidant capacity. They are the most important among the phenolic compounds and one of the main natural dyes used in the food industry. In this research, residue of processed grapes was used to investigate the presence of anthocyanins, the possibility of their extraction from the residue, and their stability. The extraction solution consisted of 70 mL of ethanol 70% and 30 mL of HCl 0.1% at pH 2.0. The results found for the processed grapes residue was 26.20 mg.100 g⁻¹. In order to evaluate stability, caffeic acid was added at 0.5:1 w/v; 0.8:1 w/v; and 1:1 w/v concentrations. Anthocyanins extract reached the greatest stability at 0.5:1 w/v concentration, with 82.47% color retention and a half-life period of 15 days. Therefore, the use of this organic acid as a stabilizer for anthocyanins is feasible.

Keywords: anthocyanins; co-pigment; determination; stability.

Resumo

As antocianinas têm grande importância pelo seu potencial antioxidante, sendo, dos compostos fenólicos, o mais importante e um dos principais corantes naturais que pode ser usado na indústria alimentícia. Neste trabalho, foi utilizado resíduo de uva processada, com o objetivo de verificar a presença de antocianinas e a possibilidade de extração destas do resíduo, bem como estudar a sua estabilidade. A solução extratora utilizada foi 70 mL de etanol 70% e 30 mL de HCl 0,1% em pH 2,0. O resultado encontrado no resíduo de uvas processadas foi de 26,20 mg.100 g⁻¹. Na avaliação da estabilidade, foi utilizado ácido cafeico, adicionado nas concentrações (0,5: 1 p/v ; 0,8:1 p/v ;1:1 p/v). O extrato de antocianinas apresentou maior estabilidade na concentração de (0,5:1 p/v), com uma retenção na cor de 82,47% e um tempo de meia vida de 15 dias. Sendo, portanto viável a utilização deste ácido orgânico como um estabilizante das antocianinas.

Palavras-chave: antocianinas; copigmento; determinação; estabilidade.

1 Introduction

Found in nature, color is one of the main food qualities accountable for the blue, violet and red pigments of flowers and fruits. It highly affects their aesthetic value and foregrounds the acceptability of a great variety of food products, especially as a natural pigment (STRINGHETA, 1991). The antioxidant potential of phenolic compounds reduces singlet oxygen in lipid oxidation reactions and chelates metal with a wide range of pharmacological, anti-allergenic, anti-arteriogenic, anti-inflammatory, anti-microbial, anti-thrombosis properties, cardio-protection and vasodilator effects. (PUUPPONEN-PIMIÄ et al., 2001; MANACH; MAZUR; SCALBERT, 2005). In natural products, most pigment-causing compounds belong to the flavonoid group. Classification of flavonoid types is initially based on the analysis of their solubility and pigment reactions. Grapes are a source of several phenolic compounds in high concentrations and wine sub-products may maintain amounts of phenolic compounds that belong to the flavonoid group.

Free anthocyanins are seldom found in plants since they occur with the glycosylated sugars that stabilize the molecule (FRANCIS, 2000). Although glycosylation frequency occurs in position 3, it may also occur in positions 7, 3', 4' and 5' (BROUILLARD, 1982). Glucose, arabinose, xylose, rhamnose, galactose, fructose and disaccharides make up the most common sugars linked to

anthocyanins, forming glycosylated mono-glycosides, di-glycosides and tri-glycosides as aglycone (Figure 1).

Anthocyanins sugars are acylated by ferulic p-cumaric acid, caffeic acid, p-hydroxybenzoic acid, synapic acid, malonic acid, acetic acid, succinic acid and malic acid. Co-pigmentation reaction is probably the main molecular interaction mechanism involved in color variations and astringency during wine production and aging (MAZZA, 1995).

Increase in stability occurs because the pigment, competing with water and interacting with the anthocyanins, mixes with colored forms and modifies the pigments essence (GRIS et al., 2007).

In experiments with red wine, Darías-Martín et al. (2001) investigated the effect of adding caffeic acid and catechine to wine during the pre-fermentation stage. After 90 days, the caffeic acid increased wine absorbance by 60%, whereas wine with catechine showed lower absorbance rates when compared to those of control.

Current technology favored a greater use of wine-making secondary products which consist of several products obtained during grape processing. Some 31.7 kg of sub-products are

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resulted of 100 L of wine (CAMPOS, 2005). Consequently, there is great interest in exploring such residues in the wine-making process. In fact, these have been mainly discarded in the several processes of extraction, especially anthocyanins (ARVANITOYANNIS; LADAS; MAVROMATIS, 2006).

Residue is mainly consisted of water, approximately 60-70% (FAMUYIWA; OUCH, 1982; SASTRE; SINOVA; PAUNERO, 1994), and also can be obtained, ethanol, methanol, higher alcohols, aldehydes, esters, volatile acids, polyphenols, tannins, proteins, cellulose, pectines, mineral salts and sugar depending on the pressing process (ORRIOLS, 1994).

The objective of this research was to evaluate and quantify the total anthocyanins rates in processed grapes residue and their stability with organic acid.

2 Materials and methods

2.1 Materials

Samples of processed grapes residue (20% of Bordô grape and 80% of Isabel grape) 2008 crop, were obtained from Agro-industrial Cooperative of Winemakers (COAVITI), in Marialva, northern region of the State of Parana, Brazil, at 23° 29' 06" S and 51° 29' 31" W geographic location. They were conditioned in thermal boxes with ice and sent to the Food Biochemistry Laboratory, Department of Chemistry at the State University of Maringa - UEM. They were then conditioned in plastic bags (with 0.5 mm density) and stored at -18 ± 0.5 °C for further analysis.

2.2 Methods

Preparation of anthocyanins extract

Extraction was performed from the agro-industrial residue of processed grapes. In a beaker containing 100 g of residue sample was added 200 mL of extraction solution (70 mL ethanol 70% and 30 mL HCl 0.1% at pH 2.0); the mixture was mechanically stirred and kept in the dark for 2 minutes (JU; HOWARD, 2003). It was kept overnight in the dark at a refrigeration temperature of 4 ± 1 °C, covered with cellophane film. The beaker was wrapped in aluminum paper to avoid degradation of anthocyanins. Crude extract was then filtered in

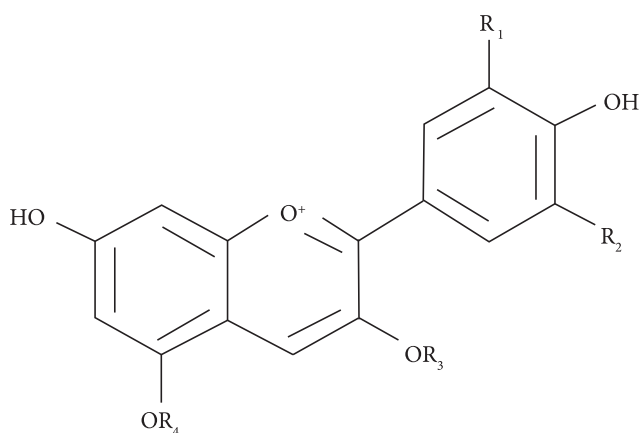


Figure 1. Basic structure of anthocyanin. Source (MAZZA; MINIATI, 1993).

nylon tissue and the residue was washed in 100 mL of extractor and adjusted to 250 mL. A second vacuum filtration with Whatman n. 2 was performed later. The extract was then filtered in a 0.45 µm membrane (Millipore, Bedford, MA), maintained at 4.0 ± 1 °C in amber flasks, and employed for analyses during the experiments. The crude extract pH was 2.0.

Determination of total anthocyanins

Prior to determining the total anthocyanins in the extract, the crude extract solution was scanned to measure maximum absorbance wavelength as shown in Figure 2. Analysis of total anthocyanins was carried out by retrieving 2.0 mL aliquot from the stored solution at 4 ± 1 °C and completing a 25 mL volumetric balloon with the extraction solution (70 mL ethanol 70% and 30 mL HCl 0.1% at pH 2.0). The mixture was kept at rest for 2 hours. Absorbance of diluted extract was measured by maximum absorption wavelength for total anthocyanins $\text{mg} \cdot 100 \text{ g}^{-1}$. Equation 1 was employed to calculate the total anthocyanins, following Vanini et al. (2009).

$$\text{FD} = \text{VEO}/\text{VA} \times \text{VS} \quad (1)$$

$$\text{AT} (\text{mg} \cdot 100 \text{ g}^{-1}) = \text{A} \times \text{FD} / \text{E}_{1\text{cm}}^{1\%}$$

where: FD = dilution factor; VEO = volume of original crude extract (250 mL); VA = volume of extract aliquot used for dilution in extraction solvent (2 mL); VS = volume of solution used for extract dilution (25 mL); AT = total anthocyanins (mg) per 100 g of sample; A = absorbance of diluted extract at the maximum absorption wavelength.

$\text{E}_{1\text{cm}}^{1\%} = 98.20$; coefficient of molar absorption capacity for the mixture.

Study of stability

The contents of total solids of crude extract were calculated by drying 3 mL in a buffer at 105 °C (AOAC, 1998) to determine the concentration of caffeic acid to be added to the anthocyanins. The result obtained ($\text{mg} \cdot \text{mL}^{-1}$) was the initial parameter for the addition of co-pigments in different weight/volume (w/v) proportions. Control (without caffeic acid) and test samples (with caffeic acid in the following concentrations: 0.5:1w/v;

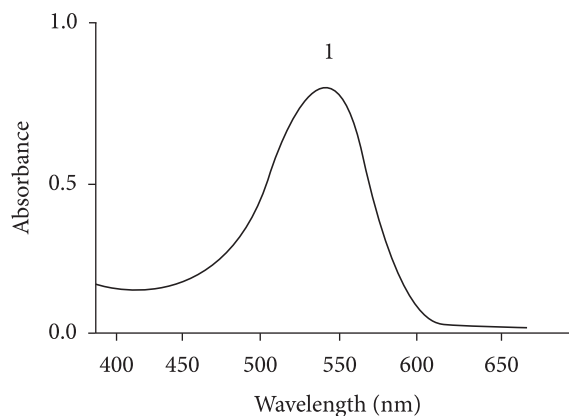


Figure 2. Absorbance spectrum in UV-Vis region of processed grapes residue under conditions of the extraction solution (70 mL ethanol 70% and 30 mL HCl 0.1% at pH 2.0), at room temperature in the dark.

0.8:1 w/v; and 1:1 w/v) were prepared. Preparation of solutions was performed by methodology according to Ju and Howard (2003). Solutions were prepared in 25 mL volumetric balloons and left at rest for 2 hours in the dark at room temperature for 100 days.

2.3 Statistical analysis

Data analysis was carried out with ANOVA and Tukey' test focusing on significant differences in means. Statistic 6.0 software program was employed with significance level between mean differences at 5% ($p < 0.05$). All analyses were made in triplicates and the results were given as means.

3 Results and discussion

Table 1 shows the determination of total anthocyanins: the minimum concentration of grape mixture, 20% Bordô grapes and 80% Isabel grapes, was $43.2 \text{ mg} \cdot 100 \text{ g}^{-1}$ of the total anthocyanins, and the maximum concentration was $49.2 \text{ mg} \cdot 100 \text{ g}^{-1}$ of the total anthocyanins. Regarding the concentration of the processed grapes residue, the minimum concentration was $23.2 \text{ mg} \cdot 100 \text{ g}^{-1}$ and the maximum was $28.2 \text{ mg} \cdot 100 \text{ g}^{-1}$. Extraction, which depends on the type of residue, may have a recuperation of up to 52.5%, what explains the increasing interest in exploiting sub-products of processed grapes.

Caffeic acid in the molecule increases the stability of anthocyanins. Dangles, Saito and Brouillard (1993) suggested an interaction between pelargonidine chromophore and caffeil groups of anthocyanins extracted from petals of *Pharbits nil* (red-purple cultivars). An increase in values of absorbance (hyperchromic effect) in the length of maximum absorption (Figure 3), and with the addition of caffeic acid at the concentrations: 2-test (0.5: 1 w/v), 3-test (0.8:1 w/v), 4-test (1:1 w/v), at wavelength 535 nm, as shown in Figure 4. According to Darias-Martín et al., (2001), when caffeic acid is added to wine, there is a 60% increase in the wine absorbance rates by the end of a 90-day period.

Pigment/co-pigment complex depends on the concentration of both according to an increase in the co-pigment/anthocyanins relationship. There was an increase at the absorbance in the first day, with hyperchromic effect, because its coefficient of absorption increased the concentrations of molecules in the colored wavelength of maximum absorption (BROUILLARD, 1982; OSAWA, 1982) in treatments with no additions of organic acid and with presence of caffeic acid in concentrations 0.5:1; 0.8:1; 1:1 (w/v caffeic acid to anthocyanins), as shown in Figure 4. There was a decrease in absorbance compared to control after

Table 1. Concentration of the total anthocyanins in residues of processed grapes.

Assays	Mixture of grapes (80% Isabel and 20% Bordô) $\text{mg} \cdot 100 \text{ g}^{-1}$	Residues of processed grapes in $\text{mg} \cdot 100 \text{ g}^{-1}$
1	49.2 ^a	28.2 ^b
2	47.7 ^a	27.2 ^b
3	43.2 ^b	23.2 ^a
mean	46.7	26.2

Concentrations as mean; different letters in the same column show different statistics among themselves (Tukey HSD, $p < 0.05$).

7 days. The bathochromic effect may be explained by the local reduction in the polarity of the chromophore flavilium caused by the involvement of the latter with co-pigment through hydrophobic associations (DANGLES; SAITO; BROUILLARD, 1993). Hereafter, samples had a constant linearity in absorbance up to the 100th day.

In Table 2 as shown the results with the experiments using caffeic acid 1:1 and 0.8:1 had the least color retentions: 69.62 and 69.11% respectively and with ratio of 0.5:1 (w/v) showed a color retention of 82.47%, half-life period of 15.04 days, when compared to other concentrations. The addition of caffeic acid increased the absorbance rates compared to control, and, according to Darias-Martín et al. (2001), showed hyperchromic effects.

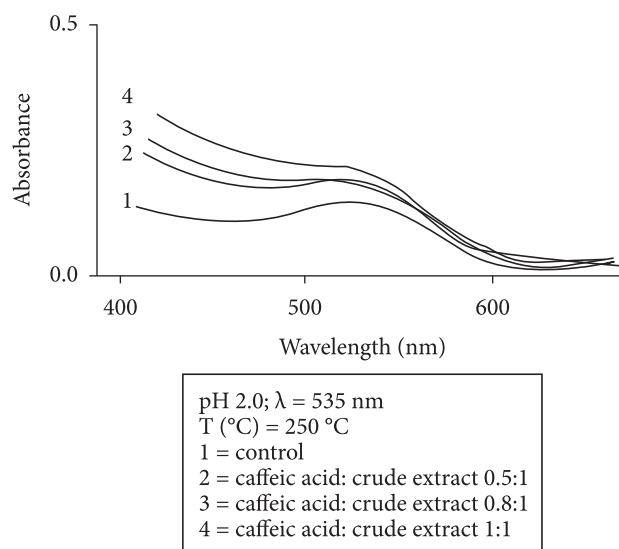


Figure 3. Absorption spectrum in UV-Vis region of processed grapes residue (*Vitis Labrusca*), with caffeic acid, at room temperature in the absence of light.

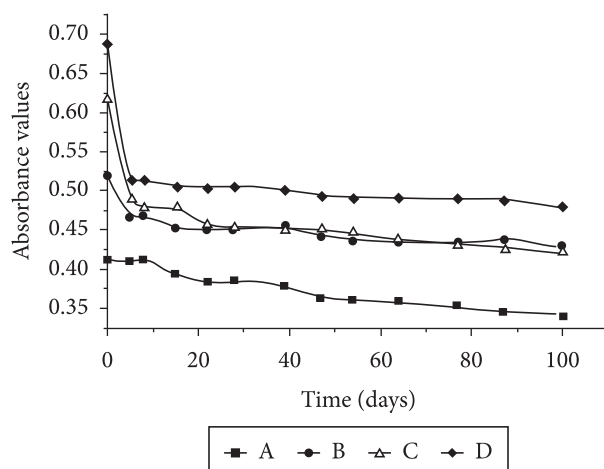


Figure 4. Absorbance, 535 nm, in the extraction of processed grapes residue with the addition of caffeic acid: a) control; b) concentration (0.5:1 w/v); c) concentration (0.8:1 w/v); d) concentration (1:1 w/v), at room temperature in dark.

Table 2. Color retention (%R) and half-life period ($t_{1/2}$) of anthocyanins in the mixture of residue of processed grapes, with the addition of caffeic acid.

Treatments	Control	0.5:1	0.8:1	1:1
R(%)	82.52	82.47	69.11	69.62
T 1/2	15.03	15.04	7.81	7.97

4 Conclusions

Residue determination of processed grapes at 26.20 mg.100 g⁻¹ of total anthocyanins showed feasibility of extraction. As anthocyanins degrade themselves through several factors, the addition of organic compounds, such as caffeic acid at 0.5: 1 w:v concentration, produces a retention time of 82.47% and a life increase of anthocyanins of 15.04 days. Depending on the processed grapes residue, 52.5% of anthocyanins may be recovered. It can be used as natural pigment by the food industry.

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