Ciência e Tecnologia de Alimentos
ISSN: 0101-2061
revista@sbcta.org.br
Sociedade Brasileira de Ciência e Tecnologia de Alimentos
Brasil

CLEMENTE, Edmar; GALLI, Dirseu
Stability of the anthocyanins extracted from residues of the wine industry
Ciência e Tecnologia de Alimentos, vol. 31, núm. 3, julio-septiembre, 2011, pp. 765-768
Sociedade Brasileira de Ciência e Tecnologia de Alimentos
Campinas, Brasil

Stability of the anthocyanins extracted from residues of the wine industry

Estabilidade de antocianinas extraídas do resíduo de indústrias de vinho

Edmar CLEMENTE[1]*, Dirseu GALLI[1]

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- thombosis properties, cardio-protection and vasodilator effects. (PUUPPONEN-PIMIÃ 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 glycated sugars that stabilize the molecule (FRANCIS, 2000). Although glycation frequency occurs in position 3, it may also occur in positions 7, 3', 4' and 5' (BROUILLARD, 1982). Glucose, arabinose, xylose, ramnose, galactose, frutose and disaccharides make up the most common sugars linked to anthocyanins, forming glycated mono-glycosides, di-glycosides and tri-glycosides as aglicone (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

---

Received 28/1/2010
Accepted 18/5/2010 (004635)

[1] *Laboratório de Bioquímica de Alimentos, Departamento de Química – DQI, Universidade Estadual de Maringá – UEM, CEP 87020-900, Maringá, PR, Brasil, e-mail: eclemente@uem.br*
**Corresponding author*

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 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 mg.100 g^{-1}. Equation 1 was employed to calculate the total anthocyanins, following Vanini et al. (2009).

$$FD = VEO/VA \times VS$$
$$AT\ (mg.100\ g^{-1}) = A\ X\ FD\ /\ E^{1\%}_{1\ cm} \qquad (1)$$

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.

$E^{1\%}_{1\ cm}$ = 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 (mg.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;

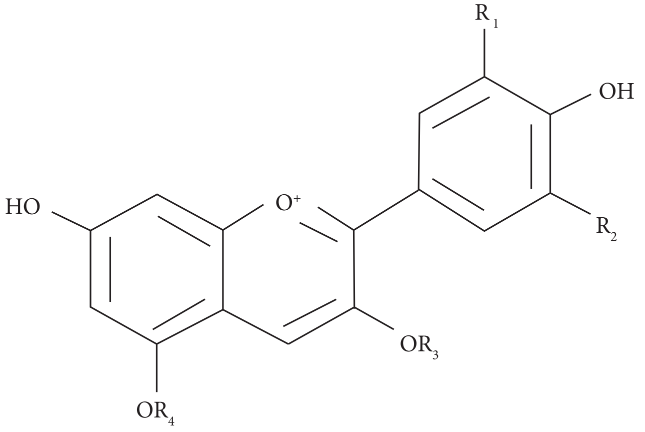

Figure 1. Basic structure of anthocyanin. Source (MAZZA; MINIATI, 1993).

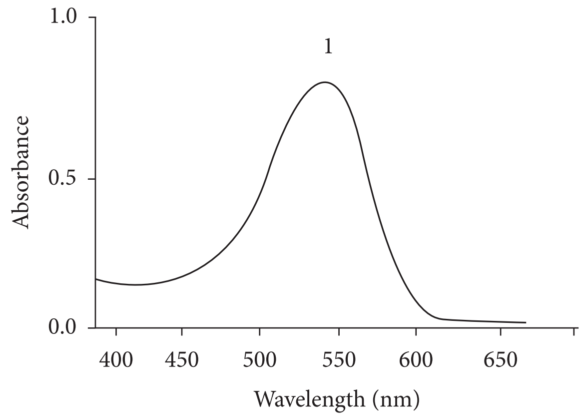

Figure 2. Absorbance spectrum in UV-Vis region of processed grapes residue under conditions of the extraction solution (70 mL ethanol 70% and 30 mL HCl 0.1% at pH 2.0), at room temperature in the dark.

0.8:1w/v; and 1:1w/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 mg.100 g^{-1} of the total anthocyanins, and the maximum concentration was 49.2 mg.100 g^{-1} of the total anthocyanins. Regarding the concentration of the processed grapes residue, the minimum concentration was 23.2 mg.100 g^{-1} and the maximum was 28.2 mg.100 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 cafeic 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 Darías-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 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.

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

Assays	Mixture of grapes (80% Isabel and 20% Bordô) mg.100 g^{-1}	Residues of processed grapes in mg.100 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, $p < 0.05$).

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 Darías-Martín et al. (2001), showed hyperchromic effects.

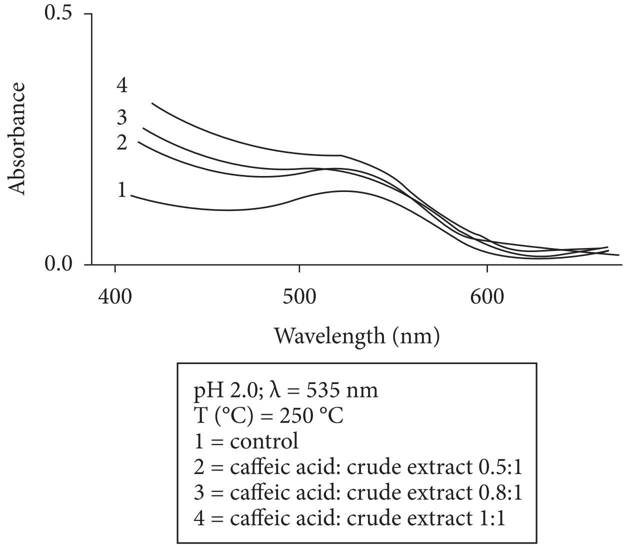

Figure 3. Absorption spectrum in UV-Vis region of processed grapes residue (*Vitis Labrusca*), with caffeic acid, at room temperature in the absence of light.

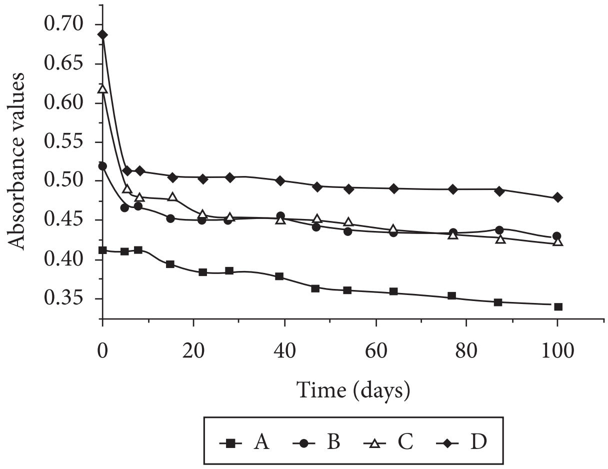

Figure 4. Absorbance, 535 nm, in the extraction of processed grapes residue with the addition of caffeic acid: a) control; b) concentration (0.5:1 w/v); c) concentration (0.8:1 w/v); d) concentration (1:1 w/v), at room temperature in dark.

Table 2. Color retention (%R) and half-life period (t½) 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.

Acknowledgements

The authors are greatful to the Agro-industrial Cooperative of Winemakers (COAVITI), Marialva, State of Parana, Brazil.

References

ARVANITOYANNIS, I. S.; LADAS, D.; MAVROMATIS, A. Potential uses and applications of treated wine waste: a review. **International Journal of Food Science and Technology**, v. 41, p. 475-487, 2006. http://dx.doi.org/10.1111/j.1365-2621.2005.01111.x

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS - AOAC. **Official methods of analysis**. 65th ed. Washington: AOAC, 1998. 1070 p.

BROUILLARD, R. Chemical structure of anthocyanins. In: MARKAKIS, P. **Anthocyanins as Food Colors**. New York: Academic Press, 1982. p. 1-39.

CAMPOS, L. **Obtenção de extratos de bagaço de uva Cabernet Sauvignon (Vitis vinifera)**: Parâmetros de processo e modelagem matemática. 2005. Dissertação (Mestrado em Engenharia de Alimentos)-Universidade Federal de Santa Catarina, Florianópolis, 2005.

DANGLES, O.; SAITO, N.; BROUILLARD, R. Anthocyanin intramolecular copigment effect. **Phytochemistry**, v. 34, n. 1, p. 119-124, 1993. http://dx.doi.org/10.1016/S0031-9422(00)90792-1

DARÍAS-MARTÍN, J. et al. Enhancement of red wine colour by pre-fermentation addition of copigments. **Food Chemistry**, v. 73, p. 217-220, 2001. http://dx.doi.org/10.1016/S0308-8146(00)00286-7

FAMUYIWA, O.; OUGH, C. S. Grape pomace possibilities as animal feed. **American Journal of Enology and Viticulture**, v. 39, n. 2, p. 44-46, 1982.

FRANCIS, F. J. Anthocyanins and betalanais: composition and applications. **Cereal Foods World**, v. 45, p. 208-213, 2000.

GRIS, E. F. et al. Caffeic acid copigmentation of anthocyanins from Cabernet Sauvignon grape extracts in model systems. **Food Chemistry**, v. 100, p. 1289-1296, 2007. http://dx.doi.org/10.1016/j.foodchem.2005.10.014

JU, Z. Y.; HOWARD, L. R. Effects of Solvent and Temperature on Pressurized Liquid Extraction of Anthocyanins and Total Phenolics from Dried Red Grape Skin. **Journal of Agricultural and Food Chemistry**, v. 51, n. 18, p. 5207-5213, 2003. PMid:12926860. http://dx.doi.org/10.1021/jf0302106

MANACH, C.; MAZUR, A.; SCALBERT, A. Polyphenols and prevention of cardiovascular diseases. **Current Opinion in Lipidology**, v. 16, n. 1, p. 77-84, 2005. PMid:15650567. http://dx.doi.org/10.1097/00041433-200502000-00013

MAZZA, G. Anthocyanins in grapes and grape products. **Critical Review of Food Science and Nutrition**, v. 35, p. 341-371, 1995. PMid:7576162. http://dx.doi.org/10.1080/10408399509527704

MAZZA, G.; MINIATI, E. **Anthocyanins in fruits, vegetables and grains**. Boca Raton: CRC Press, 1993. 363 p.

ORRIOLS, I. Tecnologia de la destilacion en los aguardients de oreijo. In: CONGRESSO INTERNATIONAL DE LA VITICULTURA,1., 1994, Atrantica, Isla de la Toja Espana. **Anais**… Atrantica,1994. p. 291-305.

OSAWA,Y. Copigmentation of anthocyanins. in: MARKAKIS, P. **Anthocyanins as food colors**. New York: Academic Press Inc., 1982. chapt. 2, p. 41-65.

PUUPPONEN-PIMIÃ, R. et al. Antimicrobial properties of phenolic compounds from berries. **Journal of applied Microbioloy**, v. 90, n. 4, p. 494-507, 2001.

SASTRE, J. A. L.; SINOVA, P. L. C.; PAUNERO, A. A. Los resíduos de la indústria del vino. In: LOPES, J. A.; DE COCA, P. L.; ASENSIO, A. **La utilización de los resíduos de la indústria vitivinícola em Castilha y Leon**. Salamanca: Verona Press, 1994. p. 15-20.

STRINGHETA, P. C. **Identificação da estrutura e estudo da estabilidade das antocianinas extraídas da inflorescência do capim gordura**. 1991. 138 f. Tese (Doutorado em Ciência de Alimentos)-Universidade Estadual de Campinas, Campinas, 1991.

VANINI, L. S. et al. Extraction and stability of anthocyanins from the Benitaka grape cultivar (*Vitis vinifera* L.) **Brazilian Journal Food Technology**, v. 12, n. 3, p. 213-219, 2009.