Food and Nutrition Sciences, 2014, 5, 488-494
Published Online March 2014 in SciRes. http://www.scirp.org/journal/fns
http://dx.doi.org/10.4236/fns.2014.56058

Scientific Research

Effect of Light on Stability of Anthocyanins in Ethanolic Extracts of *Rubus fruticosus*

Elizabeth Contreras-Lopez, Araceli Castañeda-Ovando*, Luis Guillermo González-Olivares, Javier Añorve-Morga, Judith Jaimez-Ordaz

Universidad Autonoma del Estado de Hidalgo, Academic Area of Chemistry, Pachuca, Mexico
Email: *ovandoa@uaeh.edu.mx

Received 23 July 2013; revised 23 August 2013; accepted 31 August 2013

Abstract

Blackberry (*Rubus fructicosus*) is one of the fruit with the highest concentration of anthocyanins; however, its use is limited for making jams, jellies and liqueur, and recently, fruit concentrates in combination with pomegranate, blueberry and grape. One of the main problems with these pigments is their poor stability in solution, mainly in beverages as liqueur and juices, which depends on factors such as chemical structure, pH, temperature, light, water activity, and presence of oxygen. The effect of light on total monomeric anthocyanins content as well as the degradation rates and browning of two blackberry ethanolic extracts is to establish the conditions for storage of liqueur without adding artificial food coloring. The initial content of anthocyanins on extract without storage was 106 mg·l^{-1}. The study assessed the light irradiation effect on the anthocyanins of blackberry ethanolic extract. The anthocyanins degradation followed the second order reaction kinetics with respect to illuminance of the light source. The $t_{1/2}$ value at high illuminance (3968.30 lx) was 28.20 hours.

Keywords

Blackberry; Light; Anthocyanins Stability; Ethanolic Extracts

1. Introduction

Color and appearance are perhaps the most important quality attributes of food due to the ability and facility that the human has to perceive them, and are the first evaluated by the consumer. Colorants, which could be natural or synthetic, are the chemical substances responsible for creating these properties [1]. Anthocyanins are a group

*Corresponding author.

How to cite this paper: Contreras-Lopez, E., *et al.* (2014) Effect of Light on Stability of Anthocyanins in Ethanolic Extracts of *Rubus fruticosus*. *Food and Nutrition Sciences*, **5**, 488-494. http://dx.doi.org/10.4236/fns.2014.56058

of naturally occurring phenolic compounds, defined as phenolic flavonoids that have the chemical structure C_6-C_3-C_6 [2]. These polyphenolic substances are glycosides of polyhydroxy- and polymethoxy-derivates of 2-phenylbenzopyrylium or flavilium salts. As flavonoids, the structure of aglycon (**Table 1**) consists of two benzene rings (A and B) combined by the mediation of the oxygen-containing pyrane ring (C) [3]. **Table 1** shows the most common aglycons with different substitution patterns of hydroxylation and methoxylation which produce an orange-red (pelargonidin) to blue-red (delphinidin) color at about pH 1.

Although anthocyanins present a large variety of colors making them a viable utility in food, their stability is greatly affected by factors such as pH, temperature, oxygen, ascorbic acid, some nucleophile used as food additives (SO_2, NO_2^-, H_2O_2) and light [2]. Higher stability of anthocyanins, which occurs in acid conditions, has been investigated, but their stability and color are affected by the substituents into the aglycon [4]. These compounds could be in four structural forms in both aqueous solutions and foods (depending on the pH): the quinoidal-base (blue), the red flavilium cation and the pseudo-carbinol base and the chalcone which are colorless [5]-[7]. A limitation that has restricted the use of anthocyanins as colorants in food systems is that their intensity is reduced and a blue shade increases in neutral pH where the hemicetal and the quinoidal structures are formed. They are less stable and may rapidly degrade [2]. Temperature also affects the stability of anthocyanins which are being destroyed during the processing and storage of food. Color degradation was observed when the temperature increases [8].

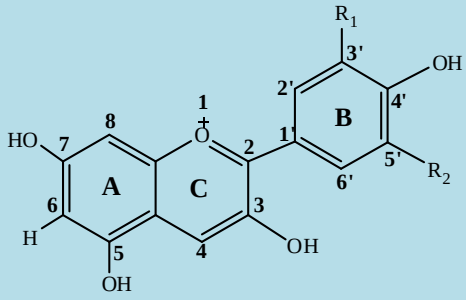
On the other hand, the unsaturated structure of anthocyanins makes them susceptible to molecular oxygen attacks. For many years it has been known that when grape is bottled hot, the degradation of color from purple to dull brown is reduced by the complete filling of the bottles. These effects have also been investigated in other juices containing anthocyanins [9]. Recent researches have shown that ascorbic acid and anthocyanins disappear simultaneously from the fruit juice, suggesting some direct interactions between the two molecules. Some authors have reported the effect of light on the degradation rates of anthocyanins. The aim of this work was to evaluate the effect of the presence of light and the aging time in the content of monomeric anthocyanins from blackberry alcoholic extract used for manufacturing artisanal liquor.

2. Materials and Methods

2.1. Samples

Fresh fruit harvested in Zacualtipan, Hidalgo (Mexico) and stored ethanolic extract of blackberry were provided

Table 1. Structural identification of most common anthocyanidins or aglycons [4]. Me: methyl group.



General anthocyanidins structure

Name	Abbreviation	Substitution pattern		Color (pH 1)	$\lambda_{vis\text{-}max}$ (nm)
		R_1	R_2		
Cyanidin	Cy	OH	H	orange-red	510
Delphinidin	Dp	OH	OH	blue-red	522
Malvidin	Mv	OMe	OMe	blue-red	520
Pelargonidin	Pg	H	H	orange	505
Peonidin	Pn	OMe	H	orange-red	532
Petunidin	Pt	OMe	OH	blue-red	546

by a local micro-industry which manufactures artisanal blackberry liqueurs.

2.2. Reagents

All the reagents used were analytical grade. Solutions were prepared with deionized water (Milli-Q from Millipore; MA, USA). The determination of monomeric anthocyanins was done using two buffer systems: potassium chloride buffer, pH 1 (0.25 mol·l^{-1}), and acetate buffer solution, pH 4.5 (0.4 mol·l^{-1}). The rates of degradation and browning were determined with sulfite solution (0.6 mol·l^{-1}) from potassium metabisulfite ($K_2S_2O_5$).

2.3. Preparation of Blackberry Extracts

Two kinds of extracts were used: fresh and stored extracts. The stored extract was prepared by manufacturers and was stored during five years at room temperature in transparent PET bottles.

Fresh blackberries were provided by the micro-industry. Fresh extract was obtained mixing 500 g of blackberries with 600 ml of ethanol. The resulting mixture was macerated in a dark place at room temperature for four months. After that, ethanolic extract was obtained using a mechanical press and stored in a dark place for a month and finally it was decanted after sedimentation. This extract (extract without storage, EWS) and the stored extract (SE) were used to evaluate the effect of light on the content of monomeric anthocyanins as well as degradation and browning rates.

2.4. Kinetics of Anthocyanins Degradation

The effect of light on the degradation of anthocyanins was determined. Aliquots of 20 ml of EWS and SE were put in screw-cap test tubes. The tubes were irradiated with three light sources (992.06, 2380.95, and 3968.30 lx) for five days in a chamber (**Figure 1**) while two samples were stored in a dark place at room temperature. At intervals of 24 hours, both the rate of degradation and the rate of browning were measured. Analyses were performed in triplicate.

The light sources selected cover the light intensity range in which real alcoholic extracts are stored in the manufacturing micro-industry until they are used for preparing liquors.

2.5. Determination of Monomeric Anthocyanins

Total extract monomeric anthocyanins content was determined using the pH differential method [10] in two buffer systems (pH 1.0 and pH 4.5). The concentrated samples were diluted with the corresponding buffer and the absorbance was measured at 510 and 700 nm. Analyses were performed in triplicate.

Total monomeric anthocyanins were calculated as cyanindin-3-glucoside [11] [12], which is the most com-

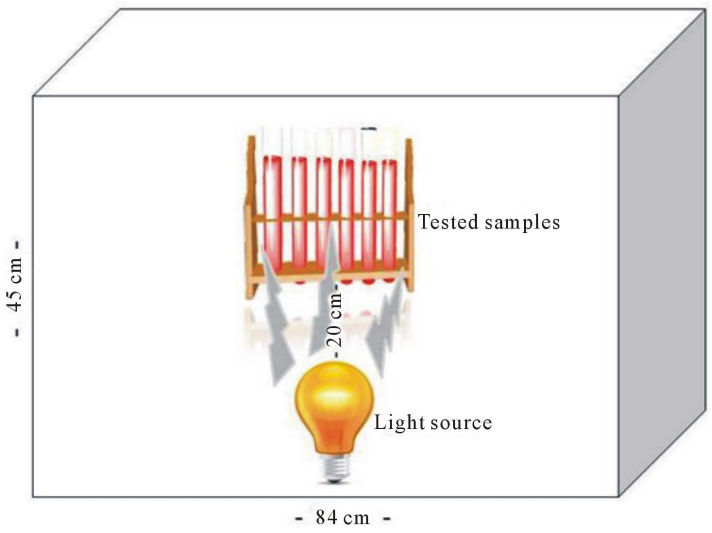


Figure 1. Chamber shows light source and distance to the tested sample.

mon anthocyanic compound (Equations (1) and (2)). When suspended solids or colloids exist in the sample, the absorbance at 700 nm is a correction. The absorbance at 510 nm was measured because this wavelength was the maximum absorbance of the extracts.

$$\text{Monomeric anthocyanins } (\text{mg}\cdot\text{L}^{-1}) = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l} \quad (1)$$

where:

$$A = \left(A_{510\text{ nm}} - A_{700\text{ nm}}\right)_{\text{pH}_1} - \left(A_{510\text{ nm}} - A_{700\text{ nm}}\right)_{\text{pH}_{4.5}} \quad (2)$$

MW (molecular weight) = 449.2 gmol^{-1} for cyanidin-3-glucoside; DF = dilution factor; ε 510 nm= 26,900 $\text{l}\cdot\text{cm}^{-1}\cdot\text{mol}^{-1}$ for Cyanidin-3-glucoside (pH = 1); l = path length in cm.

2.6. Determination of Degradation and Browning Rates

Two aliquots of 500 µl of EWS and SE were diluted to 10 ml with distilled water for the control solution, and for the sample solution 1 ml of HSO_3^- was added and diluted with distilled water to 10 ml. Degradation and browning rates were determined at 420, 515 and 700 nm and were calculated according to Equations (3) to (5) for both solutions. During the analysis the target was distilled water [11].

Color density was calculated with a control solution according to the Equation (3). A measure of polymeric color of the sample solution was obtained by applying the same procedure as used in determining color density. Polymeric anthocyanins are resistant to bisulfite bleaching.

$$\text{Color density} = \left(A_{420\text{ nm}} - A_{700\text{ nm}}\right) + \left(A_{510\text{ nm}} - A_{700\text{ nm}}\right) \times DF \quad (3)$$

The percent of polymeric color was calculated according the Equation (4).

$$\%\ \text{Polymeric color} = \frac{\text{polymeric color}}{\text{color density}} \times 100 \quad (4)$$

The *browning index* was calculated according to Equation (5). Results of the analysis of the sample solution were used.

$$\text{Browning index} = A_{420\text{ nm}} \times DF \quad (5)$$

3. Results and Discussion

3.1. Kinetic Modeling for the Extract without Storage

The initial content of total monomeric anthocyanins of EWS was 106 $\text{mg}\cdot\text{l}^{-1}$ which was considered to calculate the spectral data to cyanidin-3-glucoside. The effect of light on the content of monomeric anthocyanins was plotted as a function of time (**Figure 2**). The order of reaction for the degradation of anthocyanin by action of light was calculated by graphical method [13]. **Figure 2** shows various graphs for each of the reaction orders: order zero, **Figure 2(a)**, first order, **Figure 2(b)** and second order, **Figure 2(c)**. **Figure 2(a)** shows that monomeric anthocyanins concentration decreased 76% in one week when the extract was irradiated with a light source of 3968.30 lux. The decrease in the concentration of monomeric anthocyanins, which give the red color to fruit drinks, is a loss of food quality [8]. The monomeric anthocyanins concentration decreased 29% (P = 0.05) in extract stored at darkness. These results showed that this storage method is efficient to preserve the quality of blackberry extract. Increasing the intensity of the light source causes the gradual loss of monomeric anthocyanins (**Figure 2(a)**).

The linear correlation coefficients for each reaction order were calculated (**Table 2**). It is clear from **Figure 2** and **Table 2** that the degradation of blackberry anthocyanins from ethanolic extract followed second order reaction kinetics with respect to the illuminance of the light source (greater values for R^2). This model has also been reported by Albarici & Pessoa [14] for degradation anthocyanins from nonpasteurized acai pulp due to temperature effects. In that study, the concentration of anthocyanins decreased exponentially over time, showing half-life ($t_{1/2}$) = 10.14 hours.

Considering a second-order kinetic, the rate constant (k) and $t_{1/2}$ for anthocyanins from EWS were calculated

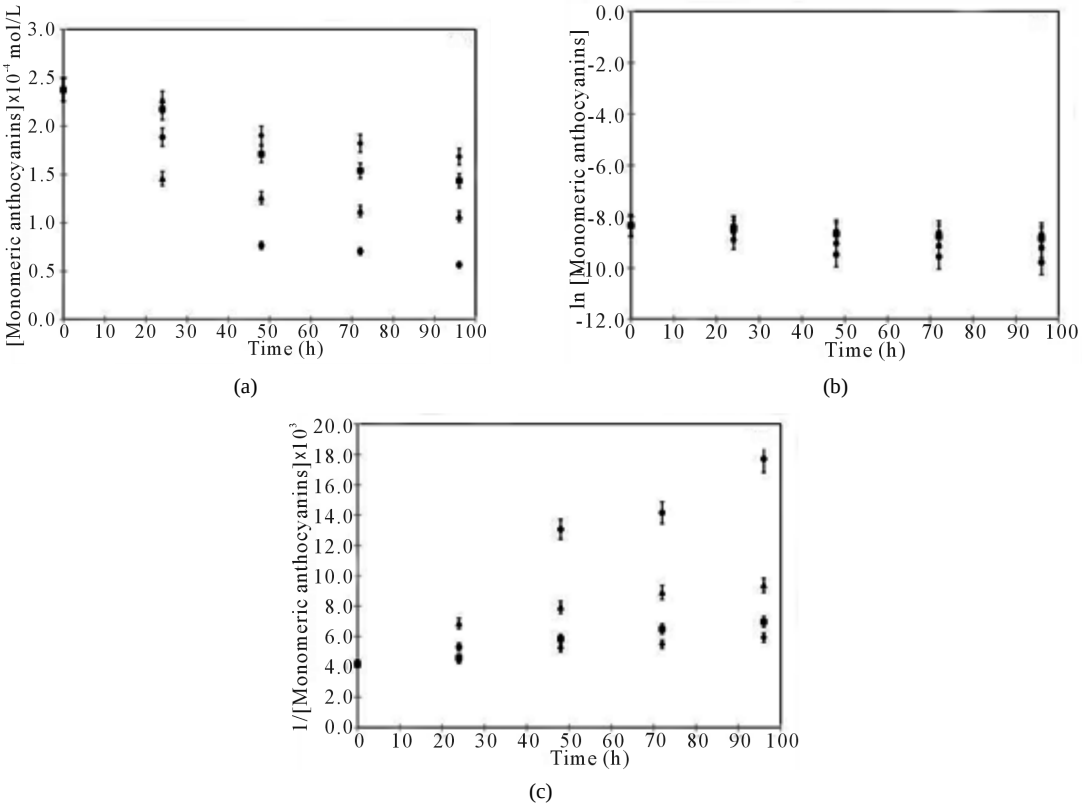


Figure 2. Anthocyanins degradation rate in the presence of light. (a) zero-order, (b) first-order, and (c) second-rder. Legends: ◆ 100 µlx, ■ 992.06 lx, ▲ 2380.95 lx, and ● 3968.30 lx.

Table 2. Linear correlation coefficients (R^2) for graphical representations of each reaction orders.

Illuminance condition	Reaction order		
	Zero	First	Second
100 µlx	0.9533	0.9618	0.9678
992.06 lx	0.9453	0.9594	0.9697
2380.95 lx	0.7601	0.8359	0.9056
3968.30 lx	0.8632	0.9008	0.9333

(Table 3). According to the results, it was observed that the color degradation of anthocyanins was greater when the extract was exposed to radiation sources of high illuminance (3968.30 lx) and $t_{1/2}$ was longer in darkness storage (100 µlx). The $t_{1/2}$ value at high illuminance (3968.30 lx) was 28.20 hours; which suggest that anthocyanins from blackberry are most susceptible to high temperature that high illuminance, since Wang and Xu [8] has reported lower $t_{1/2}$ values for anthocyanins thermal degradation (16.7, 8.8, 4.7 and 2.9 hours at 60˚C, 70˚C, 80˚C and 90˚C, respectively).

On the fifth day of our kinetic study the rates of degradation and browning were measured. There was no significant change (P = 0.05) between experimental conditions (storage at dark conditions and under light irradiation). The color density was set from 4.39 to 6.96, the polymeric color from 3.71 to 5.21 and the rate of browning from 2.56 to 4.32. At the end, the juice that was irradiated with 3968.30 lx showed the greatest loss of color (Figure 3).

3.2. Stored Extract

The initial concentration of monomeric anthocyanins in stored blackberry extract was 1.67 $mg \cdot l^{-1}$. The kinetic

Table 3. Rate constant and half-life following second-order reaction kinetics.

Illuminance condition	k ($mol^{-1} \cdot l \cdot h^{-1}$)	$t_{1/2}$ (h)
100 µlx	18.75	224.52
992.06 lx	30.91	136.19
2380.95 lx	51.55	81.66
3968.30 lx	149.28	28.20

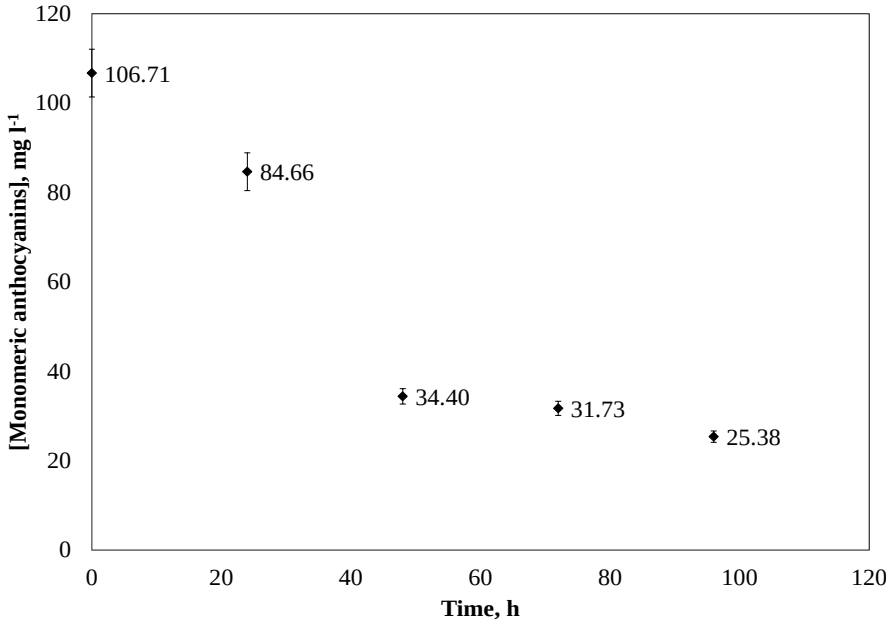


Figure 3. Anthocyanins degradation for fresh juice irradiated with 3968.30 lx.

parameters were not calculated due to the small variation observed in the concentration of monomeric anthocyanins.

These results showed no significant difference between the initial concentration of monomeric anthocyanins and the concentration at the end of the study; which would indicate that anthocyanins were totally degraded. This could be attributed to a deficient handling of the blackberry extracts by manufacturers (too long storage time at room temperature in transparent containers). According to the results obtained, blackberry extract should be stored in darkness at all stages of production (from extraction to packaging) and the packaging must be in amber bottles.

4. Conclusion

The results from the present study provide detailed information regarding the changes in the kinetic stability of blackberry anthocyanins from alcoholic extracts under light exposure and storage at darkness. Degradation of blackberry anthocyanins followed the second order reaction kinetics with respect to illuminance of the light source. Knowledge of the chemistry of the anthocyanins and factors affecting their stability can be used to minimize degradation by the appropriate selection of processes. In this way, the study of the effect of light on anthocyanins provides information needed for the packaging of products rich in these substances such as liquor or juice concentrates, avoiding the addition of artificial colors to mask the degradation of anthocyanins as natural components.

Acknowledgements

The authors acknowledged SNI (CONACyT) for the stipend received. ACO gratefully thanks PROMEP for fi-

nancial support.

References

[1] Shibamoto, T. and Bjeldanes L.F. (2009) Introduction to Food Toxicology Food Science and Technology. Academic Press, California.

[2] Castañeda-Ovando, A., Pacheco-Hernández, M., Paez-Hernández, M., Rodríguez, J. and Galán-Vidal, C. (2009) Chemical Studies of Anthocyanins: A Review. *Food Chemistry*, **113**, 859-871.

[3] Konczak, I. and Zhang, W. (2004) Anthocyanins—More Than Nature's Colours. *Journal of Biomedicine and Biotechnology*, **5**, 239-240. http://dx.doi.org/10.1155/S1110724304407013

[4] Giusti, M.M. and Wrolstad, R.E. (2003) Acylated Anthocyanins from Edible Sources and Their Applications in Food Systems: A Review. *Biochemical Engineering Journal*, **14**, 217-225. http://dx.doi.org/10.1016/S1369-703X(02)00221-8

[5] da Costa, C.T., Nelson, B.C., Margolis, S.A. and Horton, D. (1998) Separation of Blackcurrant Anthocyanins by Capillary Zone Electrophoresis. *Journal of Chromatography A*, **799**, 321-327. http://dx.doi.org/10.1016/S0021-9673(97)01043-1

[6] Kennedy, J.A. and Waterhouse, A.L. (2000) Analysis of Pigmented High Molecular-Mass Grape Phenolics Using Ion-Pair, Normal-Phase High Performance Liquid Chromatography. *Journal of Chromatography A*, **866**, 25-34. http://dx.doi.org/10.1016/S0021-9673(99)01038-9

[7] Fleschhut, J., Kratzer, F., Rechkemmer, G. and Kulling, S.E. (2006) Stability and Biotransformation of Various Dietary Anthocyanins *in Vitro*. *European Journal of Nutrition*, **45**, 7-18. http://dx.doi.org/10.1007/s00394-005-0557-8

[8] Wang, W.-D. and Xu, S.-Y. (2007) Degradation Kinetics of Anthocyanins in Blackberry Juice and Concentrate. *Journal of Food Engineering*, **82**, 271-275. http://dx.doi.org/10.1016/j.jfoodeng.2007.01.018

[9] Barreiro, M.J. and Sandoval, B.A.J. (2006) Operaciones de Conservación de Alimentos por bajas Temperaturas. Equinoccio, Caracas.

[10] Wrolstad, R.E., Durst, R.W. and Lee, J. (2005) Tracking Color and Pigment Changes in Anthocyanin Products. *Trends in Food Science and Technology*, **16**, 423-428. http://dx.doi.org/10.1016/j.tifs.2005.03.019

[11] Giusti, M.M. and Wrolstad, R.E. (2001) Anthocyanins. Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy. In: Wrolstad, R., Ed., *Current Protocols in Food Analytical Chemistry*, John Wiley & Sons, Inc., New York, F1.2.1-F1.2.13.

[12] Lee, J., Durst, R.W. and Wrolstad, R.E. (2005) Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study. *Journal of AOAC International*, **88**, 1269-1278.

[13] Levine, I.N. (2003) Fisicoquímica. Mc Graw Hill, México.

[14] Albarici, T.R. and Pessoa, J.D.C. (2012) Effects of Heat Treatment and Storage Temperature on the Use of Açaí Drink by Nutraceutical and Beverage Industries. *Ciência e Tecnologia de Alimentos*, **32**, 9-14. http://dx.doi.org/10.1590/S0101-20612012005000026