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KINETIC ANALYSIS OF THE STABILITY OF ANTIOXIDANTS IN BLACKBERRY (*Rubus fruticosus* L.) LIQUOR

ANÁLISIS CINÉTICO DE LA ESTABILIDAD DE LOS ANTIOXIDANTES EN LICOR DE ZARZAMORA (*Rubus fruticosus* L.)

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Abstract

The kinetic analysis of the stability of monomeric anthocyanins, ascorbic acid and antioxidant capacity of blackberry (*Rubus fruticosus* L.) liquor was performed as well as physicochemical evaluation of fresh fruit and liquor. °Brix, pH, titrable acidity, density and moisture content were evaluated for fresh fruit and results were similar to reported ones. Liquor was characterized during 180 days of aging in term of its physicochemical properties. Contents of monomeric anthocyanins (ACyMon) and ascorbic acid in backberry liquor agreed with reported data but kinetics of their change during aging has not yet been published. A first order kinetics described (R^2 >90%) decrements of anthocyanins, ascorbic acid and antioxidant capacity. ACyMon, polymeric colour, total phenols, antioxidant capacity and ascorbic acid correlated well (R >0.7) with each other. Rate constants and half-life time for anthocyanins showed that they were more prone to degradation than ascorbic acid and antioxidant capacity which decreased slower than anthocyanins and ascorbic acid, having a half-life time of 630.13 days. It was noteworthy that the relative rate of loss of ascorbic acid and total anthocyanins in relation to antioxidant capacity are both higher than 1 (4.54 and 5.09 respectively), due to the existence of other reactive species such as phenols that contributed to antioxidant capacity.

Keywords: blackberry liquor, antioxidants, kinetic analysis.

Resumen

Se realizó el análisis cinético de la estabilidad de antocianinas monoméricas (AcyMon), ácido ascórbico y capacidad antioxidante del licor de zarzamora al igual que la evaluación fisicoquímica de la fruta fresca y del licor. Se determinaron, °Brix, pH, acidez titulable, densidad y contenido de humedad para la fruta fresca y los valores encontrados fueron similares a los reportados. El licor se caracterizó durante 180 días de añejamiento en términos de sus características fisicoquímicas. Los contenidos de ACyMon y de ácido ascórbico coincidieron con los reportados, pero la cinética de sus cambios durante el añejamiento no ha sido reportada. Cinéticas de primer orden describieron adecuadamente (R^2 >0.90) los decrementos de antocianinas, ácido ascórbico y capacidad antioxidante. ACyMon, color polimérico, fenoles totales y actividad antioxidante correlacionaron adecuadamente entre ellos (R >0.7). El tiempo de vida media de las antocianinas mostró que son las especies más susceptibles a la degradación, seguidas por el ácido ascórbico. La actividad antioxidante, sin embargo, disminuyó más lentamente que las antocianinas y el ácido ascórbico, con un tiempo de vida media de 630.13 días. Es notorio que las velocidades relativas de pérdida tanto del ácido ascórbico como de las antocianinas con respecto a la capacidad antioxidante fueron ambas mayores que 1 (4.54 y 5.09 respectivamente), lo que puede deberse a la existencia de otras especies reactivas como fenoles que contribuyeron a la actividad antioxidante.

Palabras clave: licor de zarzamora, antioxidantes, análisis cinético.

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1 Introduction

Blackberry is a fruit having a high concentration of a number of phenolic compounds with marked antioxidant capacity. Works by Rice-Evans *et al*., (1997) demonstrated that antioxidant capacity of such phenolic compounds is 5 to 7 times higher than for ascorbic acid. Moreover, according to Wang *et al* (1997), the antioxidant capacity of ascorbic acid is similar to that reported for Trolox reagent.

Processing of blackberry fruit aims to retard natural degradation processes so preserving different compounds including antioxidants. Degradation and polymerization of anthocyanins during processing may provide sensorial stability to the product by stabilizing its colour, flavour and odour. Often, maceration and aging as in the case of wines and liquors gives place to the generation of other phenolic compounds that contribute to the antioxidant capacity. It has been proved that a number of fruit extracts containing phenolic compounds have higher antioxidant capacity than the same in pure form which have a synergic effect when found in such extracts. Protecting effect of ascorbic acid and α-tocopherol on lipoproteins is increased in the presence of catechin, epicatechin and caffeic acid (Gómez-Sampedro and Zapata-Montoya, 2016; Cerón-Montes, 2015; Liao and Yin, 2000; Vinson *et al*., 2001).

There are works dealing with products elaborated with berries such as jam (Zafrilla *et al*., 2001; Wicklund *et al*., 2005; Howard *et al*., 2010; Holzwarth *et al*., 2012), liquors (Heinonen *et al*., 1998; Montoya-Gómez *et al*., 2005; Leyva 2009; Galego *et al*., 2013; Sokól-Letowska *et al*., 2014) and juices (García Alonso *et al*., 2002; Lee *et al*., 2005; De Beer *et al*., 2014) in which the antioxidant capacity and stability have been investigated including stability during storage. As blackberry is concerned, most studies deal with identification of its main anthocyanins and phenolic compounds as well as with its antioxidant capacity (Céspedes *et al*., 2000; Sellapan *et al*., 2002; Acosta-Montoya *et al*., 2010). Results have been compared with other red fruits such as raspberry and cranberry (Pantelidis *et al*., 2007; Koca and Karedeniz, 2009). However, no studies dealing with kinetics of antioxidant degradation in blackberry liquor have been found.

Chemical reaction kinetics deals with the rate and order of a given chemical transition which are parameters that characterize the reaction in terms of velocity and on the dependence of rate and concentration of molecules involved in the reaction (Levenspiel, 1972) in such a way that for a zero-order reaction respect to reactant A, the rate of reaction is constant and independent of the concentration of A. For a first order reaction, the rate depends linearly to the concentration of A. Higher reaction orders are found when rate depends to the square and sometimes third and higher powers (including non-integers) of the concentration of reactant (Levenspiel, 1972). Reaction orders of 0 and 1 are often found in food processing and storage (Gómez-Sampedro *et al*., 2016). The typical rate equation is given by Equation (1).

$$\ln \frac{[A]}{[A]_0} = -kt \tag{1}$$

In which $[A]$ is the concentration of A at a certain time t, $[A]_o$ is the initial concentration of A, k is the reaction rate constant and t is the time of reaction.

Also, for a given reaction, the half life time of reactant A is given by Equation 2. This parameter characterizes a given chemical kinetics by stating the time at which a certain reactant modifies its concentration by one half.

$$t_{1/2} = \frac{0.693}{k} \tag{2}$$

The vast majority of reactions occurring in foods are 0 or 1st order (Labuza, 1984); especially oxidation reactions leading to colour loss have been regarded as 1st order reactions. Many reactions occurring in processed foods have not as yet been characterized in terms of reaction constant and order of same and consequently, it would be very useful knowing the reaction rate and order of key antioxidants of fruits leading to a better processing conditions and shelf life control of products. The aim of this work was to characterize the reaction kinetics for the loss of main antioxidants and antioxidant capacity of blackberry liquor.

2 Materials and methods

In this work, evaluation of physicochemical parameters of blackberry fruit and liquor are reported as well as a kinetic descriptions of anthocyanins, ascorbic acid and antioxidant capacity during aging of liquor.

2.1 Materials

Maltodextrin-standardized food grade papain with an activity between 90-110 UTyr (Enmex, S.A. de C.V., Tlalnepantla, Mexico) was used for the study. All reagents used in this investigation were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

2.2 Blackberry fruits

Blackberry mature fruits (*Rubus fructicosus*) were bought in the province of Zumpahuacán, Estado de México, crop season April 2013. Fruits were sanitized by washing them with tap water, disinfected with a 250 ppm sodium hypochlorite solution, rinsed with tap water and frozen at -2°C until further use.

2.3 Preparation of liquor

Blackberry liquor was prepared according to Montoya *et al*., (2005), by using commercial sucrose, ethylic alcohol and blackberry fruits previously thawed as described next.

An alcoholic fruit extract was prepared by adding ethylic alcohol to a 1000-mL bottle previously washed with a 70% v/v alcohol solution and properly rinsed with tap water. 500 g of mashed fruit were added to the bottle, until a 1000 mL mark (approximately 1:1 fruit : alcohol ratio) and bottles were sealed by using parafilm. Such mixture was stored at 25°C in a shelf under dark conditions and during this time the bottle was turned upside down every day for one month. After this time, the contents were filtered through a cloth and through Watman paper number 40 to eliminate suspended particles. On the other hand, a 35 ° Bx syrup was prepared by adding commercial sucrose (approximately 500 g per each litre of water). The liquor was prepared by mixing extract and syrup in a 1:1 proportion in an amber bottle, sealed and aged at 25°C under dark conditions during 180 days.

2.4 Physicochemical analyses

2.3.1 Determination of pH

pH of fruit and liquor was evaluated by using a pH meter HACH Sens ion 1. Model 51700-23, China. The electrode used was a Hanna instruments part code: HI 1332B. Determinations were carried out at the beginning of the experiment for fresh fruit and at time intervals during 180 aging days.

2.3.2 Titratable acidity

Titrable acidity of fruit and liquor was determined according to the NMX-FF-011-1982 (1982) and expressed in g equivalents of citric acid/100 g of fruit. For liquor, it was expressed in g equivalents of citric acid/100 mL of liquor. Determinations were carried out at time intervals during 180 aging days.

2.3.4 Density and °Brix of liquor

Density was obtained by weight difference by using a 10-mL pycnometer at 20°C AOAC Official Method 930.17 (2007). Determinations were carried out at time intervals during 180 aging days. For °Brix determination by measurement of the refraction index with an Atago digital refractometer.

2.3.5 Moisture content

Moisture content was determined in an oven by weight difference, according to AOAC method (2007). Determinations were carried out at time intervals during 180 aging days.

2.3.6 Alcohol content

Alcohol content was evaluated according to NOM-142-SSA1-1995. 50 mL of liquor were distilled and product brought to 50 mL with distilled water. A Gay Lussac meter was used.

2.5 Determination of monomeric anthocyanins (ACyMon)

A fruit extract was prepared by using a 80:20 solvent metanol:HCL (0.01N) (Holzwrath *et al*., 2011). After extraction, sample was sonicated during 10 min and stirred for 1 h and filtered through Watman paper number 40 and freeze-stored until further use.

ACyMon were determined by using the differential pH method as reported by Giusti and Wrolstad (2001) and expressed as cyanidin 3-glucoside. The fruit extract or liquor was diluted by using a pH 1 buffer (KCl 0.025 M) and a pH 4.5 buffer (CH_3COONa 0.4M). Maximum absorbance at 510 nm and 700 nm were obtained and the following equations were then used:

$$A_{bs} = (A_{510nm} - A_{700nm})_{pH1.0} - (A_{510nm} - A_{700nm})_{pH4.5} \quad (3)$$

$$ACyMon\left(\frac{mg}{L}\right) = \left(\frac{A_{bs} \times MW \times DF \times 1000}{\varepsilon \times 1}\right) \quad (4)$$

In which A_{bs} is the absorbance of the diluted simple, MW is the molecular weight of cyanidin 3-glucoside (449.2), ε = 26,900 cm^{-1} M^{-1} in pH 1 buffer and DF is the dilution factor. Results were expressed as cyanidin 3-glucoside. Determinations were carried out at time intervals during 180 aging days.

2.6 Determination of polymeric colour

Polymeric colour (PC) which is an index of the degradation of monomeric anthocyanins was evaluated according to Giusti and Wrolstad (2001). Liquor was diluted with water up to 2.8 mL and added with 0.2 mL of 20% sodium metabisulfite. Colour density was evaluated by mixing 0.2 mL of water to 2.8 mL of sample and left to rest for 20 min. Absorbance were measured at 420, 510 (λ_{max}), and 700 nm (Equation 5)

Polymeric colour, PC, (sample treated with sodium metabisulfite) and control sample, DC were obtained by the following equation:

$$\frac{PC}{DC} = [(A_{420nm} - A_{700nm}) + (A_{510nm} - A_{700nm})] \times FD \quad (5)$$

In which FD is the dilution factor.

The % of polymeric colour is (Equation 6):

$$\%CP = \frac{PC}{DC} \times 100\% \quad (6)$$

2.7 Determination of total phenols

Total phenols were evaluated by using the colorimetric technique reported by Singleton and Rossi (1965). 200 μL of extract or liquor were treated with 200 μL of Folin Ciocalteau reagent during 6 min and then, the reaction was stopped by adding sodium bicarbonate (2%) and leaving for 30 min. Absorbance at 765 nm was measured and results expressed in gallic acid equivalents (GAE). Determinations were carried out at time intervals during 180 aging days.

2.8 Determination of antioxidant capacity

Antioxidant capacity was determined by the DPPH method for liquor and fruit extracts (Chen *et al*., 1999). The DPPH (2,2- diphenyl-2-picryl-hidrazil) radical has an unpaired electron and a blue-violet colour which changes to pale yellow when reacts with an antioxidant substance. Colour change is measured spectrophotometrically at 517 nm (Domínguez-Hernández, 2016). The results of the assay were expressed as millimolar Trolox per g of dry weight in terms of Trolox equivalent antioxidant capacity (TEAC) as well as IC_{50}. Determinations were carried out during 180 aging days.

2.9 Determination of ascorbic acid

Ascorbic acid was determined according to AOAC method (2000) using the 2,6-Dichlorophenolindophenol (0.01%) method. Results were expressed in mg ascorbic acid / 100 g of fruit. Determinations were carried out during 180 aging days.

2.10 Kinetic analysis

During 180 days of aging, ascorbic acid, ACy Mon and antioxidant capacity were analyzed by means of a first order equation (Equation 1). Rate constant were obtained from the slope of the fitted line and $t_{1/2}$ were also evaluated by means of Equation 2 to characterize obtained kinetics.

2.11 Statistical analysis

All tests were carried out by triplicate. One way RM ANOVA were carried out by using the software SigmaStat 3.5 (Systat Software Inc., USA). A Student-Newman-Keuls test was used for comparison of means. The level of significance for each test was 2α = 0.05.

3 Results and discussion

3.1 Physicochemical analysis

3.1.1 Characterization of fresh fruit

Blackberry fruit (raw material) was characterized in terms of °Bx, pH, titrable acidity, density and moisture content. Results are presented in Table 1 as well as those reported by Valencia *et al*., (2013) which were similar to the results obtained in this work.

3.1.2 Characterization of liquor

Liquor was prepared and characterized at time intervals during 180 days of aging in term of its physicochemical properties. Results are shown in Table 2. It was possible to observe that alcohol content and titrable acidity decreased while °Brix increased. As expected, the higher the °Bx, the higher

Table 1. Blackberry fruit characterization in terms of °Brix, pH, titrable acidity and moisture content, and compared to the results reported by Valencia *et al*. (2013).

	°Brix*	pH*	Titrable acidity* (% citric acid)	Density* (g/ml)	Moisture content* (%)
Results	11± 0.2	3.59±0.02	0.79±0.91	1.1607±0.0007	83.6± 2.4
Valencia *et al*. (2013)	10.5	3.4	0.93	-	82.98

*Mean and its corresponding standard deviation

Table 2. Physicochemical properties of blackberry liquor during 180 aging days.

Aging time (Days)	°Brix*	pH*	Titrable acidity* (g CA/100 mL)	Density* (g/mL)	Alcohol* (%)
0	30 ±0.1	4.05 ± 0.01	0.2048 ±0.001	1.0709 ± 0.0001	58 ± 1.0
15	30 ± 0.1	4.08 ±0.01	0.2048 ± 0.001	1.0715 ±0.0001	52.72 ±1.0
30	30.5±0.1	4.11 ±0.01	0.1856 ± 0.001	1.0724 ±0.0001	47.44 ±1.0
45	30.5±0.1	4.11 ±0.01	0.1792 ± 0.001	1.0724 ±0.0001	42.16 ±1.0
60	30.5±0.1	4.13 ±0.01	0.1729 ± 0.001	1.0725 ±0.0001	36.88 ±1.0
75	31 ± 0.1	4.14 ±0.01	0.1728 ± 0.001	1.0728 ±0.0001	31.6 ±1.0
90	31 ± 0.1	4.15 ±0.01	0.1728 ± 0.001	1.0738 ±0.0001	26.32 ±1.0
105	31 ± 0.1	4.15 ±0.01	0.1697 ± 0.001	1.0740 ±0.0001	21 ±1.0
120	31.5±0.1	4.15 ±0.01	0.1665 ± 0.001	1.0755 ±0.0001	19.5 ±1.0
135	31.5±0.1	4.21 ±0.01	0.1664 ± 0.001	1.0764 ±0.0001	17.4 ±1.0
150	31.5±0.1	4.24 ±0.01	0.1536 ± 0.001	1.0801 ±0.0001	17 ±1.0
165	32 ±0.1	4.26 ±0.01	0.128 ± 0.001	1.1586 ±0.0001	17±1.0
180	32 ±0.1	4.28 ±0.01	0.0896 ± 0.001	1.1692 ±0.0001	17 ±1.0

*Mean and its corresponding standard deviation

the density of liquor, until reaching a value of 1.1692 ± 0.0001 at 180 days of aging. Also, decreasing alcohol content and titrable acidity (ascorbic acid) suggested that esterification reactions took place. It has been reported that aging of distilled beverages such as rum (Espinoza-Velázquez *et al*., 2016; González, *et al*., 2006). Also, acidity gave place to protonation of acetaldehyde which induced formation of ethylic bonds so increasing pH of sample (Romero-Casales, 2008).

3.2 Antioxidant compounds and antioxidant capacity

3.2.1 Fresh fruit

In Table 3, values for contents of monomeric anthocyanins, ascorbic acid and total phenols are shown, as well as antioxidant capacity of fresh blackberry. It is noteworthy that, experimental values (117.71 ± 1.7 mg C-3G/100g fruit) were very similar to those reported by Pantelidis *et al*., (2007) who found a value of 116.59 mg C-3G/100g fruit). Content of ascorbic acid was 18.62 ± 0.54 mg equivalents of ascorbic acid (mg EAA)/100 g of fruit which is slightly higher to value reported by Panteledis *et al* (2007) who reported 14.3-17.5 mg EAA/100 g of fruit. Antioxidant capacity (AOC) of fresh fruit resulted 2367.32 ± 36.6 μmol ET/100g of fruit) which falls within the range of those results by Sellapan *et al*., (2002) who reported 1804-2035 μmol ET/100g of fruit. Total phenols found for strawberry fruit were 319.25 ± 5.9 mg gallic acid equivalents (mg GAE)/100g of fruit. This value was similar to that by Koca *et al*., (2009) who reported 173-326 mg GAE/100g of fruit.

3.2.2 Liquor

In Table 4, contents of monomeric anthocyanins, polymeric colour, total phenols, antioxidant capacity and ascorbic acid during aging of blackberry liquor are shown and in Table 5, Pearson correlations for ACyMon, polymeric colour, total phenols, antioxidant capacity and ascorbic acid showed they correlated well (R >0.7) with each other. There was a positive association between antioxidant capacity and content of anthocyanins and with ascorbic acid.

Table 3. Monomeric anthocyanins, ascorbic acid, total phenols and antioxidant capacity for the fresh fruit.

	Monomeric anthocyanins* (mg C-3-G/100g WB)	Ascorbic Acid* (mg EAA/100g)	Total Phenols* (EAG/100g)	Antioxidant Capacity* (μmol ET/100g)
Fresh Fruit	117.71±1.7	18.62±0.54	319.25±5.9	2367.32±36.6

*Mean and its corresponding standard deviation

Table 4. Content of monomeric anthocyanins, total phenols, antioxidant activity and ascorbic acid, in blackberry liquor during aging.

Aging time (Days)	Monomeric Anthocyanins* (mg c-3-g/100 mL)	Polymeric Colour (%)	Total Phenols* (mg EAG/ 100 mL)	Antioxidant Activity* (μmol ET/100 mL)	IC50 (mL/ 100 mL DPPH)	Ascorbic Acid* (mg/ 100 mL)
0	14.15 ± 0.14	32.00 ± 0.83	43.70 ±0.30	338.69 ±2.7	0.6190± 0.02	16.46±0.46
15	12.20 ± 0.35	33.86 ± 0.78	46.19 ±0.95	332.35 ±1.10	0.6335± 0.03	15.46±0.75
30	11.62 ± 0.25	34.23 ± 0.59	46.75 ±1.11	326.32 ±4.63	0.6449± 0.02	15.31± 0.25
45	11.48 ± 0.17	34.55 ± 0.52	47.41 ±0.36	318.13 ±3.07	0.6772± 0.02	14.42±0.67
60	10.04 ± 0.14	34.74 ± 0.66	47.93 ±0.87	308.17 ±8.53	0.6846± 0.01	13.96 ±0.56
75	9.01 ± 0.09	36.76 ± 0.88	49.05 ±0.44	302.88 ±5.98	0.7395± 0.02	13.82± 0.82
90	9.20 ± 0.21	36.91 ± 1.26	50.70 ±0.88	301.55 ±3.33	0.7628± 0.02	10.75±0.82
105	8.96 ± 0.16	37.62 ± 0.17	53.51 ±0.42	299.70 ±13.97	0.7689± 0.01	10.49±0.77
120	8.15 ± 0.11	39.27 ± 0.67	54.47 ±0.55	296.39 ±8.51	0.7934± 0.02	9.84±0.7
135	6.51 ± 0.10	40.00 ± 2.63	52.53 ±1.12	295.72 ±5.42	0.7955±0.01	8.82±0.57
150	6.07 ± 0.2	42.10 ± 0.56	51.97 ±0.68	283.59 ±7.41	0.8210±0.02	8.07±0.32
165	5.09 ± 0.06	44.03 ± 1.35	50.94 ±0.28	283.06 ±5.42	0.8432±0.01	7.32±0.15
180	4.94 ± 0.07	44.87 ± 0.33	50.85 ±1.06	274.48 ±9.93	0.8876± 0.01	7.12±0.2

mg c-3-g/100mL: mg equivalents of cyanidcin 3 glucósid/L
mg EAG/ 100mL: mg equivalents of gallic acid/100 mL
μmol ET/100 mL: micro mol of Trolox Equivalents /100mL
*Mean and its corresponding standard deviation

Anthocyanins are pigments that greatly contribute to antioxidant capacity in fruits (Espinosa-Velazquez *et al*, 2016; Snyder *et al*., 2012). It has been shown that anthocyanins effectively trap oxygen reactive species and have antioxidant capacity against hydrogen peroxide (H_2O_2), peroxide (ROO·), superoxide (ROO·) and hydroxide (OH·) radicals as well as over singlet oxygen ($^1O_2$) (Garzón, 2008; Wang and Lin, 2000) and this is the reason why, when decreasing concentration of these species during aging, the antioxidant capacity also decreased.

Polymeric colour (PC) during aging of liquor inversely correlated with contents of monomeric anthocyanins. This was possible due to degradation of monomeric anthocyanins and increment of the polymerization of anthocyanins. Colour stability of aged wines is given by polymerized pigments. It has been shown that ascorbic acid has anti-radical capacity so having important contribution to antioxidant capacity of liquor (Patras *et al*., 2009, Atala *et al*., 2009) in such a way that decrement during aging contributed to the lowering of the antioxidant capacity.

3.3 Kinetic analysis

It is important to characterizing the reaction kinetics for the loss of main antioxidants of blackberry liquor. Contents of anthocyanins, ascorbic acid and antioxidant capacity decreased with aging time as shown in Figure 1, while total phenols increased during the first 120 days then decreased and stabilized as shown in Table 4. A 60% loss of anthocyanins was found during 180 days of aging time. Such degradation possibly took place due to the action of H_2O_2 and by polymerization reactions as described by Fennema and Tannebaum (1993). Ascorbic acid decreased by 57% which might be due to low pH values which promoted oxidation by enzymatic browning. Also, it is important to note that decrement of ascorbic acid was associated to a decrement of monomeric anthocyanins (Hernández-Carrillo *et al*, 2015; García-Viguera and Bridle, 1999). Antioxidant capacity as evaluated by means of the DPPH method, decreased by 19%, such decrement was probably due to presence of anthocyanins and other antioxidants such as cinamic acid derived compounds, flavonoids as well as by ascorbic acid loss (Céspedes *et al*., 2010). Total phenols decreased by 15% during the first 120 days and then decreased by 6% and stabilized until the end of the aging time. Increment of phenols was due to the synthesis of new polymers induced by the conversion of acetaldehyde to piranthocyanins which are more stable as described in wine aging (Cheynier *et al*., 2005). Presence of sucrose might be responsible of the increment of flavonoids as reported in raspberry

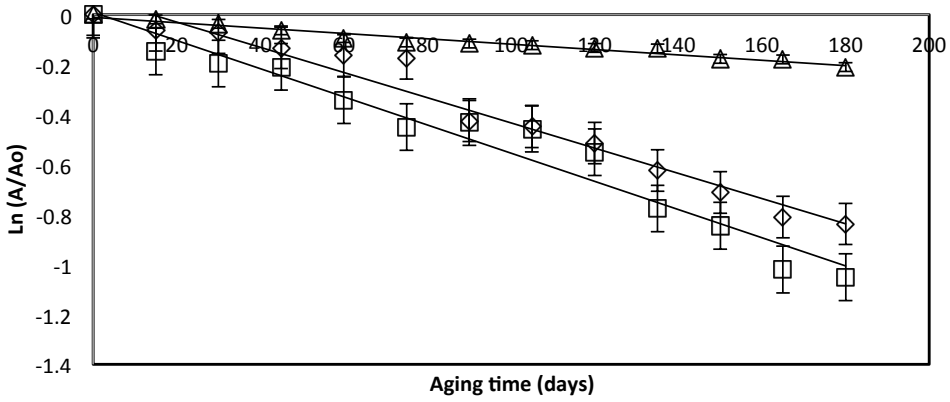

Fig. 1. Kinetics of anthocyanins (□), ascorbic acid (⋄) and antioxidant activity (△) on the blackberry liquor during aging time (180 days). Lines represent first order fitting according to Equation 1.

Table 5. Pearson correlations for monomeric anthocyanins, polymeric colour (%), total phenols, antioxidant activity ET, antioxidant activity DPPH and ascorbic acid.

	Monomeric Anthocyanins	Polymeric % Colour	Total Phenols	Antioxidant Activity (ET)	Antioxidant Activity DPPH	Ascorbic Acid
Monomeric anthocyanin	1.000					
Polymeric colour %	-0.973	1.000				
Total Phenols	-0.779	0.713	1.000			
Antioxidant Activity ET	0.976	-0.947	-0.804	1.000		
Antioxidant Activity DPPH	-0.968	0.969	0.814	-0.980	1.000	
Ascorbic Acid	0.959	-0.960	-0.831	0.948	-0.975	1.000

Table 6. Values of rate constant (k) and half-life time for monomeric anthocyanins, antioxidant activity and ascorbic acid for blackberry liquor.

Component	$k \times 10^{-3}$ (day^{-1})	$t_{1/2}$ (days)
Monomeric anthocyanins	5.6	123.77
Antioxidant capacity	1.1	630.13
Ascorbic acid	5.0	138.62

liquor (Sokol-Letowska *et al*., 2014).

A first order kinetics of the type of Equation 1, correctly described (R^2 >90%) decrements of anthocyanin, ascorbic acid and antioxidant capacity. Chemical compounds react and convert to other species and the rate constant indicates the velocity of change of concentration and the half life time of each chemical species is an indicator of the time that a given compound reduces its concentration by a factor of 0.5 and is a standardized parameter that indicates the time at which each compound is degraded (Pérez-Alonso *et al*., 2015; Steinfeld, 1989). Rate constant were higher for anthocyanins, followed by ascorbic acid and antioxidant capacity while half-life time was higher for antioxidant capacity, followed by ascorbic acid and anthocyanins as shown in Table 6.

The ratio of rate constants showed the relative speed of decay of each analysed parameter in Figure 1. It is noteworthy that the relative rate of loss of ascorbic acid and total anthocyanins in relation to antioxidant capacity are both higher than 1 (4.54 and 5.09 respectively), which indicated that both, ascorbic acid and total anthocyanins decreased their concentration faster than the rate at which antioxidant capacity diminished which was due to the existence of other reactive species such as phenols that contributed to antioxidant capacity of the liquor.

Conclusions

Physicochemical analysis of fresh blackberry and liquor showed similar results to reported ones. Liquor

kinetics performed during 180 days provided useful information for control of aging blackberry liquor and a first order kinetics described decrements of anthocyanin, ascorbic acid and antioxidant capacity. Also, the half-life time for anthocyanins indicated that they were the most prone to degradation. However, antioxidant capacity decreased slower than anthocyanins and ascorbic acid, having a half-life time of 630.13 days. It was noteworthy that the relative rate of loss of ascorbic acid and total anthocyanins in relation to antioxidant capacity was 4.54 and 5.09 respectively, indicating that ascorbic acid and total anthocyanins decreased their concentration faster than the rate at which antioxidant capacity declined.

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Nomenclature

A K	reactant A reaction rate constant
t	time of reaction
$t_{1/2}$	half time of reactant
Abs	absorbance of the diluted simple
A510nm	maximum absorbance at 510 nm
A700nm	maximum absorbance at 700 nm
A420nm	maximum absorbance at 420 nm
PC	polymeric colour
DC	control sample
FD	dilution factor

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