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EFFECT OF VARIOUS EXTRACTION SYSTEMS ON THE ANTIOXIDANT ACTIVITY KINETIC AND COLOR OF EXTRACTS FROM PURPLE CORN

EFECTO DE VARIOS SISTEMAS DE EXTRACCIÓN SOBRE LA CINÉTICA DE ACTIVIDAD ANTIOXIDANTE Y COLOR DE EXTRACTOS DE MAÍZ MORADO

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ABSTRACT

The purpose of this study was to investigate the effect of extraction systems on the anthocyanin index, antioxidant kinetics (DPPH-radical scavenging capacity) and color of purple corn (*Zea mays*) seeds. The factors studied were: extraction system (methanol, 100%, 80%, 60%, 40, 20% v/v) using hydrochloric acid as a means of acidifying. Tristimulus colorimetry was applied to evaluate quantitatively and qualitatively the process. The estimated effect and ANOVA parameters were calculated. The anthocyanins index in different extraction systems was between 1.09 to 2.87 mg/g. The highest anthocyanin index was obtained at an extraction system of 100% methanol. To determine their radical scavenging capacity, the second-order rate constant for the oxidation of these extraction systems measured by the DPPH (1,1-diphenyl-2-picrylhydrazyl radical) method, ranged from $1.19x10^{-2}$ to $1.27x10^{-2}$ $(mg/mL)^{-1}(s^{-1})$. The greatest second-order rate constant was obtained at an extraction system of 60% methanol. Regarding the simple regressions analysis the anthocyanin index showed a better relationship with CIELAB parameters: (L^*; $r = -0.928$, $p < 0.05$), (a^*; $r = 0.982$, $p < 0.01$), (b^*; $r = 0.927$, $p < 0.05$), (C^*_{ab}; $r = 0.9793$, $p < 0.01$) and (h_{ab}; $r = -0.936$, $p < 0.05$). However, the correlation existing between color parameters and the second-order rate constant did not show a good relationship. When multiple linear regression analysis were applied considering the angular coordinates (L^*, C^*_{ab}, h_{ab}) as independent variable values, a R^2 nearing 1 was obtained for anthocyanins index. While a value of $R^2 = 0.63$ was obtained for the second-order rate constant.

Keywords: Purple corn, anthocyanins, extraction system, color, kinetic.

RESUMEN

El propósito de este estudio fue investigar el efecto de los sistemas de extracción sobre el índice de antocianinas, cinética antioxidante (capacidad de secuestro de radical-DPPH) y color de las semillas de maíz morado (*Zea mays*). Los factores estudiados fueron: sistema de extracción (metanol, 100%, 80%,

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60%, 40%, 20% v/v), usando ácido clorhídrico como medio acidificante. La colorimetría triestímulo fue aplicado para evaluar los procesos cuantitativos y cualitativos. El efecto estimado y los parámetros de análisis de varianza fueron calculados. El índice de antocianinas en los diferentes sistemas de extracción fue entre 1,09 a 2,87 mg/g. El mayor índice de antocianinas fue obtenido en un sistema de extracción de 100% de metanol. Para determinar la capacidad de secuestro del radical, constante de velocidad de segundo orden por la oxidación de estos sistemas de extracción medido por el método DPPH (1,1-difenil-2-picrilhidrazil), varío de $1.19x10^{-2}$ a $1.27x10^{-2}$ $(mg/mL)^{-1}(s^{-1})$. La mayor constante de velocidad de segundo orden fue obtenida en un sistema de extracción de 60% de metanol. En cuanto al análisis de regresión simple, el índice de antocianinas mostró una mejor relación con los parámetros CIELAB: (L^*; $r = -0,928$, $p < 0,05$), (a^*; $r = 0,982$, $p < 0,01$), (b^*; $r = 0,927$, $p < 0,05$), (C^*_{ab}; $r = 0,9793$, $p < 0,01$) and (h_{ab}; $r = -0,936$, $p < 0,05$). Sin embargo, la correlación existente entre los parámetros de color y la constante de velocidad de segundo orden no mostró una buena relación. Cuando se aplicó el análisis de regresión lineal múltiple considerando las coordenadas angulares (L^*, C^*_{ab}, h_{ab}) como variables independientes, un valor de R^2 cercano a 1 fue obtenido para el índice de antocianinas. Mientras que un valor de $R^2 = 0,63$ fue obtenido para la constante de velocidad de segundo orden.

Palabras clave: maíz morado, antocianinas, sistemas de extracción, color, cinética.

INTRODUCTION

Antioxidants are substances that when present in foods or in the body at low concentration compared with that of oxidizable substrates, significantly delays or prevents oxidation of that substrate (1). The term "oxidizable substrate" includes almost everything found in foods and in living tissues including proteins, lipids, carbohydrates and DNA. Foods manufacturers have used food-grade antioxidants to prevent quality deterioration of products and to maintain their nutritional value. Antioxidants have also been of interest to biochemists and health professionals because they may help the body protect itself against damage caused by reactive oxygen species and degenerative diseases (2).

The anthocyanins constitute a major flavonoid group that is responsible for cyanic colors ranging from salmon pink through red and violet to dark blue of most flowers, fruits, and leaves of angiosperms. They are sometimes present in other plant tissues such as roots, tubers, stems, bulbils, are also found in various gymnosperms, ferns, and some bryophytes (3). The common anthocyanidin aglycones are cyanidin (cy), delphinidin (dp), petunidin (pt), peonidin (pn), pelargonidin (pg), and malvidin (mv). They all have the basic flavylium cationic structure at low pH, and they differ from each other by having different substituents. The prevalent sugar moieties are glucose, rhamnose, xylose, galactose, arabinose, and fructose. Both mono and diglycosides are common, as well as acylated forms. The sugar moiety can be located on carbons 3, 5, 7, 3', and 5', the 3- and 5-positions being dominant (4 - 6). Anthocyanins pigments are very effective scavengers of free radicals, which have been demonstrated in vitro through such assays as free radical-scavenging capacity (6-10).

For hundreds of years, people from the Andean region have utilized native plants and crops to maintain and improve their health. The kernels of purple corn have long been used by the people of the Peruvian Andes to color foods and beverages, a practice just beginning to become popularized in the industrialized world. They also make a drink from the kernels which they call "chicha morada", which has been related by folklore to increased health benefits (11). The content of anthocyanins in purple corn (*Zea mays*), have been previously characterized and major anthocyanins were derived from: cyanidin, pelargonidin, peonidin. Also other pigments such as anthocyanin-flavanol condensed were detected in pericarp and endosperm: Catechin-(4,8)-cyanidin-3,5-diglucoside, catechin-(4,8)-cyanidin-3-malonylglucoside-5-glucoside, epicatechin-(4,8)-cyanidin-3-malonylglucoside-5-glucoside (12).

The application of colorimetric systems, based on uniform color space (CIELAB) is of great value in the quantification and characterization of the color properties of pigments and foods (13, 14). However, it is important to study in depth the relationships between color and antioxidant kinetic, which may help to understand the basic principles that influence the anthocyanins color.

The influence of extraction systems: 100, 80, 60, 40 and 20% methanol containing 1% 1N HCl on the color and kinetic analysis were studied in this paper. The color properties of extraction systems were estimated by tristimulus colorimetry.

MATERIALS AND METHODS

Sample

The purple corn (*Zea mays*) was collected from the experimental fields belonging to the Estación Experimental Agraria Baños del Inca (Cajamarca, Perú). The genetic material corresponds to an improved variety (INIA 601) basic. The purple corn (seeds) was not subjected to any form of special treatment. The purple corn underwent a reduction in size and was conducted in two parts, the first by using a mortar and pestle, the second through a Mini chopper (Moulinex Co., Berkshire, UK), with exposure time of 8 seconds. Finally, the particles were filtered through a mesh with pore diameter of 600 μm. The pulverized material is stored in hermetic plastic bags at 5 °C.

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), was obtained from Sigma-Aldrich. Hydrochloric acid, methanol HPLC, acetic acid, obtained from Merck KGaA.

Extraction of anthocyanins from purple corn

Anthocyanins from purple corn were extracted through the method described by Yang *et al.*, 2009 (15). Approximately 5 g of powdered sample was extracted with 50 mL of methanol (MeOH) at different concentrations (100, 80, 60, 40, 20%) containing 1% 1N HCl. The extraction was carried out at 40°C for one hour with constant agitation. The liquid part was placed in conical tubes and centrifuged at 3500 rpm. The supernatant was recovered and stored at 5°C before analysis.

Total anthocyanins index (TAI)

Total anthocyanins index was determined using the pH differential method described by Giusti and Wrolstad (16). To a 300 mL aliquot of extract was added 2700 mL of buffer: pH 1 (0.2M KCl and 0.2M HCl), pH 4.5 (1M CH_3COONa and 1M HCl). Absorbance was measured at 510 and 700 nm and results were calculated by means of the following formulas and expressed as cyanidin-3-*O*-glucoside, equation 1:

$$A = (A_{510}\text{-}A_{700})_{pH\ 1.0} - (A_{510}\text{-}A_{700})_{pH\ 4.5} \quad \textbf{Equation 1.}$$

Monomeric anthocyanins = (A x molecular weight x dilution factor)/(ε x l), molecular weight = 449.2 g mol^{-1}, ε = molar extinction coefficient: 26800 M^{-1} cm^{-1}, l = optical path of cuvette (cm).

Antiradical activity assay

The free radical-scavenging capacity (1,1-diphenyl-2-picrylhydrazyl) was determined through the method described by Brand-Williams *et al.*, 1995 (17). The antiradical activity conditions were: 100 μM DPPH in MeOH for different extraction systems, the reaction being developed by a ratio extract-DPPH (50:950 v/v), and the absorbances were collected for 600s to 60s intervals.

Kinetic analysis

The graphics and the experimental data were run through a Quasi-Newton algorithm implemented in the program STATISTICA 6.0 (StatSoft, Inc.) for Windows. Second-order rate constant (k_{RSC}) was calculated to determine the radical scavenging capacity (RSC) of the different extraction systems tested. The concentration decrease of DPPH radical is shown in figure 2, followed by the following equation:

$$[DPPH]_t = [DPPH]_0 e^{-kt} \quad \textbf{Equation 2.}$$

$$\ln [DPPH]_t = \ln [DPPH]_0 - kt \quad \textbf{Equation 3.}$$

where $[DPPH]_t$ is the radical concentration at any time, $[DPPH]_0$ is the radical concentration at time zero and (k) is the pseudo-first-order rate constant.

Second-order rate constant (k_{RSC}) was calculated from the slope between the constant (k) and the concentration of the different extraction systems (18). The initial estimates of $[DPPH]_t$ in time (t) was interpolated to the equation of DPPH (A_{515} = 0.01[μM DPPH] - 0.025) and the constant (k) was calculated by nonlinear regression analysis (equation 2) using the program STATISTICA 6.0 (StatSoft®, Inc.). The kinetic analysis was measured by the disappearance of DPPH at 515 nm. The experimental data were collected on a Shimadzu UV-vis 2520 spectrophotometer, in kinetics module (UVProbe).

Color measurements

Absorbance measurements were performed using a UV–Vis 2550 spectrophotometer (Shimadzu® Co. Columbia, SC, USA) with 10 mm path length semi-micro polystyrene cells. The visible spectrum was set up between (395-750 nm, $\Delta\lambda = 1$ nm) and the standard illuminant D_{65} plus the 10° standard observer were used in the calculus. CIELAB parameters L*, a*, b*, C^*_{ab} and h_{ab} were determined using an original program CromaLab® (19), following the recommendations of the Commission Internationale de L'Eclariage (20). In the CIE L* a* b* system, a* and b* are the chromaticity coordinates. Positive a* value indicates the red direction, negative a* value is the green direction, positive b* value is the yellow direction, and negative b* value is the blue direction. The chroma (C) and hue angle (h°) were calculated by the equations $C^* = (a^{*2}+b^{*2})^{1/2}$ and $h^o = (\tan^{-1}a^*/b^*)$. The color differences (ΔE^*_{ab}) between two colors in the CIELAB space are calculated as the Euclidean distance between their locations in the three dimensional space defined by L*, a* and b*. ΔE^*_{ab} is defined by the following equation $\Delta E^*_{ab} = [(\Delta L^*)^2+(\Delta a^*)^2+(\Delta b^*)^2]^{1/2}$. Color intensity (as the sum of absorbances at 420, 520 and 620 nm) (21), was also calculated.

Statistical analysis

Each experiment was performed in triplicate of three independent determinations. All statistical analyses were performed with STATISTICA 6.0 (StatSoft®, Inc.) and STATGRAPHICS Plus 5.1 (Statistical Graphics® Corp.). One-way analysis of variance (ANOVA) by Duncan's test was used to compare the means. Differences were considered to be significant at $p < 0.05$. The relationships existing between the second-order rate constants (k_{RSC}), anthocyanin index and the CIELAB color parameters were assessed by simple and multiple regression models.

RESULTS AND DISCUSSION

Color coordinates and total anthocyanins index (TAI)

To investigate color quality in a systematic way it is necessary to objectively measure color. In this context, color denotes the visual appearance of the product whereas pigments or colorants are the chemical compounds that impart the observed color (22).

Figure 1 shows the average spectral response corresponding to different extraction systems, observing an increase in the absorption spectrum with decreasing solvent polarity.

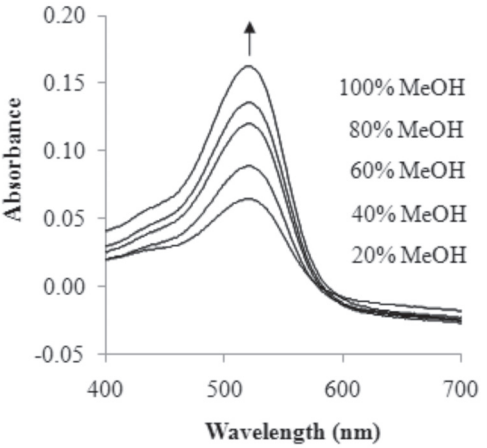

Figure 1. Change in absorption spectrum of different extraction systems.

All the extracts from different extraction systems were quantified with respect to the anthocyanins index. The effect of extraction systems are shown in table 1. The anthocyanin index increased significantly ($p < 0.05$). This suggests that the best extraction system was (100% MeOH, containing 1% 1N HCl). However, the anthocyanin index in the extraction systems is variable; these changes are mainly due to the solvent used, pH, temperature and extraction time (15, 23, 24).

Table 1. Index of anthocyanins and colour coordinates for each extraction system assayed.

Parameters	Solvent methanol (% v/v)				
	20%	40%	60%	80%	100%
TAI (mg/g)	1.09±0.11^e	1.93±0.10^d	2.51±0.17^c	2.76±0.14^b	2.87±0.14^a
CI	0.075±0.002	0.097±0.003	0.130±0.004	0.155±0.003	0.193±0.003
L*	98.17±0.14	97.64±0.25	96.71±0.11	96.15±0.04	95.23±0.15
a*	6.12±0.04	8.70±0.13	11.30±0.17	12.36±0.08	14.02±0.07
b*	2.13±0.01	2.49±0.12	2.99±0.01	3.24±0.10	3.83±0.12
C^*_{ab}	6.48±0.10	9.05±0.02	11.69±0.11	12.78±0.13	14.54±1.22
h_{ab}	19.23±0.01	15.98±0.02	14.80±0.02	14.67±0.08	15.29±0.01
ΔE^*_{ab}	2.71±0.08	2.88±0.10	1.18±0.12	1.96±0.10	

Letters (a - e) indicate significant difference at $p < 0.05$.
TAI = anthocyanins index; CI = color intensity ($A_{420}+A_{520}+A_{620}$).

Kuskoski *et al.*, 2003 (25) reports a maximum extraction for fruits (*Eugenia umbelliflora* Berg) using ethanol containing 0.1% HCl at 5°C for 48 h. Besides, it is important to note that there is a direct relationship between the extraction and anthocyanin content; this relationship is enhanced when there is an increase in the concentration of solvent. Xavier *et al.*, 2008 (23), reports that higher ethanol concentrations extracted less anthocyanin, regardless of the solvent/solid ratio used because the diffusivity of the anthocyanin in a vegetable matrix was affected by both the concentration and the type of the solvent. However, the type of matrix and solvent used plays an important role for the extraction of anthocyanins; this relationship is observed in three red grape varieties: refošk, merlot and cabernet. For refošk variety the organic solvent mixture acetone/water (1:1 v/v) was found to be the most suitable solvent. For cabernet grape, 100% ethanol and 50% acetone and for merlot grape, 70% ethyl-acetate, were found to be the most suitable solvents (26).

With respect to the color coordinates it is observed that an increase in solvent polarity produces changes, L* value (lightness) being increased, whereas scalar coordinates a* (redness) and b* (yellowness) decreased. On the other hand, the angular coordinates show different changes, chroma (C^*_{ab}) is the quantitative component of chromaticity, and hue (h_{ab}) is its qualitative expression; chromaticity is the bidimensional parameter that correlates with the visual sensation attribute colorfulness (27). The differences among the extracts were found in terms of chroma (C^*_{ab}) with values ranging from 6.48 to 14.54 CIELAB units, which indicate that the differences observed among the color characteristics of the different extraction systems were mainly quantitative. While the hue (h_{ab}) values ranged from 14° to 19°. In the L*a*b* color space, color difference can be expressed as a single numerical value, ΔE^*_{ab}, which indicates the size of the color difference but not in what way the color is different (28). In our study, the ΔE^*_{ab} color difference is greater between 20 to 60%, while between 60 to 100% is lower. The locations of the color point corresponding to the extraction system studied within the (a*b*) and (C^*_{ab}L*) plane are diagrammed in figure 2.

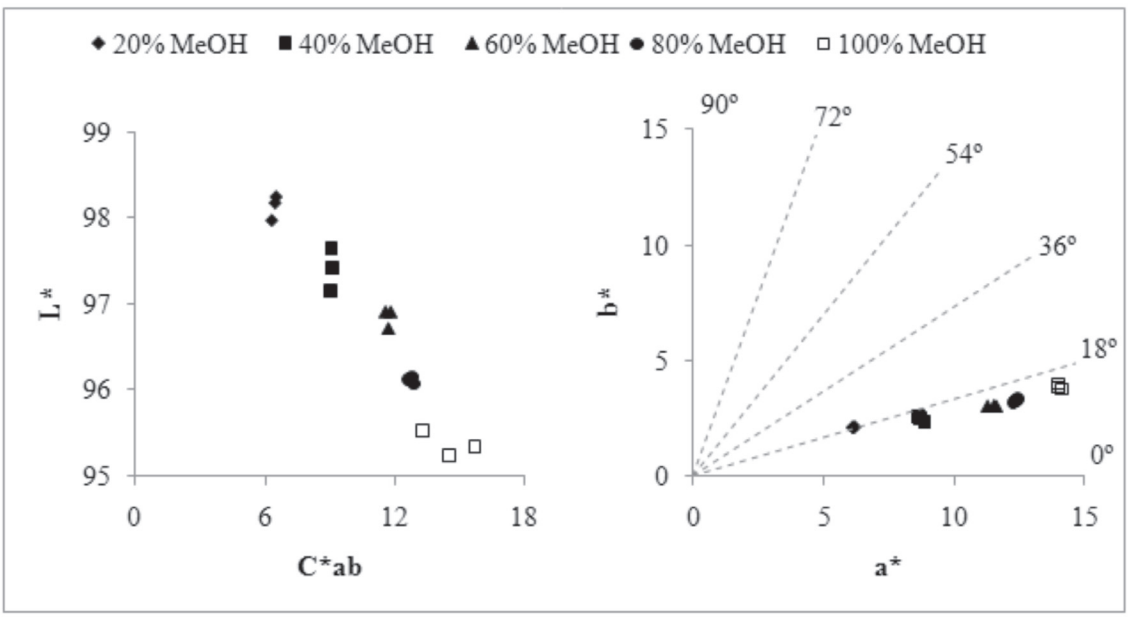

Figure 2. Distribution of different extraction systems within the (C^*_{ab}L*) and (a*b*) color plane.

Radical scavenger capacity of purple corn

In the presence of the extracts, a decrease in the absorbance at 515 nm was observed. Measurements were made for 600 s. Therefore, and taking into account the equation: $-d[DPPH]/dt = k[DPPH] = k_{RSC}[AH]_n [DPPH]$, the pseudo-first-order rate constant, k and the second-order rate constants k_{RSC} were calculated in different extraction systems (figure 3).

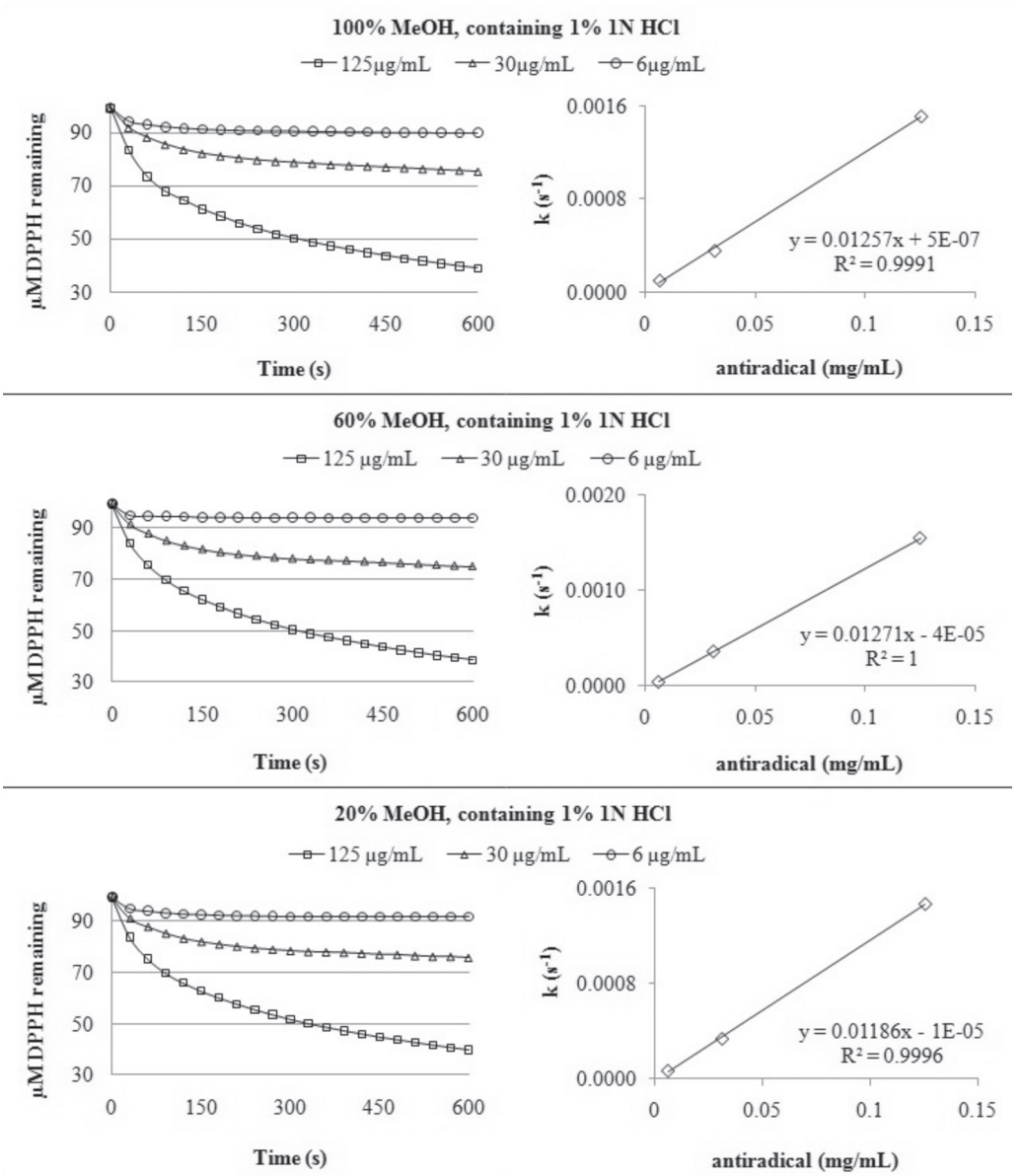

Figure 3. Spectrophotometric recordings of the disappearance of DPPH in the presence of increasing concentrations of different extraction system and pseudo-first-order rate constant (*k*) dependence on antiradical concentration.

Table 2 shows the values of rate constants obtained. Moreover, taking into account the extraction, the concentration 60% methanol has a high k_{RSC} value, followed by 80% > 100% > 40% > 20%. According to these results, the second-order rate constants (k_{RSC}) increases with decreasing methanol concentration. Nevertheless, there is a direct relationship between the concentrations of 40%, 20% and the value of the rate constants.

Table 2. Kinetic parameters obtained for different extraction systems.

Solvent (% v/v)	ARP $(mg/mL)^{-1}$	Max. Inhibition (μM DPPH)	k_{RSC} $(mg/mL)^{-1}(s^{-1})$
MeOH 20%	9.71 ± 0.14^{d}	58.37 ± 0.63^{e}	$1.19x10^{-2} \pm 0.004^{d}$
MeOH 40%	9.84 ± 0.20^{cd}	59.71 ± 0.55^{d}	$1.20x10^{-2} \pm 0.002^{d}$
MeOH 60%	11.68 ± 0.11^{a}	61.43 ± 0.48^{a}	$1.27x10^{-2} \pm 0.001^{a}$
MeOH 80%	10.20 ± 0.06^{b}	60.96 ± 0.86^{bc}	$1.25x10^{-2} \pm 0.001^{b}$
MeOH 100%	9.92 ± 0.07^{c}	60.66 ± 0.31^{cd}	$1.21x10^{-2} \pm 0.002^{c}$

Letters (a - e) indicate significant difference at $p < 0.05$.
Antiradical power = ARP.

Effect of extraction system on the color properties, anthocyanins and kinetic analysis

The correlation of the extraction systems are shown by the Pearson coefficient (29, 30) for color coordinates, anthocyanins index and the second-order rate constants (k_{RSC}) (table 3). According to these results about the second-order rate constants (k_{RSC}) there is no statistical difference with the color coordinates, and the anthocyanin index (TAI). However, there is a moderate relationship between (k_{RSC} vs TAI; r = 0.6428) and (k_{RSC} vs h_{ab}; r = -0.7495). The correlations between the anthocyanins index and color coordinates were also determined. A negative correlation was observed between (TAI vs L*; r = 0.9276, $p < 0.05$), indicating that high values of L* are associated with lower anthocyanins index. These results were corroborated by Montes *et al.*, 2005 (13) for jaboticaba fruits (L*; r = -0.85). Yang *et al.*, 2009 (15) reported similar correlations for purple corn (L*; r = -0.9263).

Table 3. Correlations between the second-order rate constants (k_{RSC}), anthocyanin index and the color parameters.

	L*	a*	b*	C^*_{ab}	h_{ab}	TAI	k_{RSC}
TAI	-0.928*	0.982**	0.927*	0.979**	-0.936*	1.000	0.643^{ns}
k_{RSC}	-0.413^{ns}	0.537^{ns}	0.391^{ns}	0.530^{ns}	-0.750^{ns}	0.643^{ns}	1.000
L*	1.000						
a*	0.981**	1.000					
b*	-0.998***	0.981**	1.000				
C^*_{ab}	-0.983**	0.999***	0.983**	1.000			
h_{ab}	0.738^{ns}	-0.855^{ns}	-0.742^{ns}	-0.849^{ns}	1.000		

^{ns}not significant; *significant at 0.05 level; **significant at 0.01 level; *** significant at 0.001 level.

A positive correlation was found between the anthocyanins index and C^*_{ab} (TAI vs C^*_{ab}; r = 0.9793, $p < 0.01$). This positive correlation indicated that high C^*_{ab} values were related to high anthocyanins index. On the other hand the correlation between anthocyanins index was high (TAI vr h_{ab}; r = -0.9358, $p < 0.05$). This indicates that high anthocyanins index originate low levels of h_{ab}. In contrast Yang *et al.*, 2009 (15) reports a correlations ratio lower (h_{ab}; r = -0 367) for the conditions of their study.

The assessment of correlations between anthocyanins index (TAI), second-order rate constants (k_{RSC}) and CIELAB color coordinates were established considering TAI, k_{RSC} and CIELAB (L*, C^*_{ab}, h_{ab}) as dependent and independent variables respectively. The multiple regression models between the angular coordinates of the CIELAB space, which allow a more intuitive interpretation of the correlations, the anthocyanins index and second-order rate constants are summarized in the table 4. The assessment of the correlations existing between the anthocyanin index, second-order rate constants and CIELAB color coordinates were established by considering the former as dependent variables and the second as the predictor.

Table 4. Partial correlation coefficients and multiple determination coefficients (R^2) among anthocyanin index, second-order rate constants and CIELAB angular color coordinates.

	L*	C^*_{ab}	h_{ab}	Intercept	R^2
TAI	-0.8217	-0.2326	-0.3217	89.4491	0.9992
k_{RSC}	0.0023	0.0010	0.0003	-0.2233	0.6303

As it can be observed, values of R^2 close to 1 show a very high correlation, which may indicate a direct relationship between the rate of anthocyanins and CIELAB parameters. By contrast, values of R^2 below 0.7 indicate a moderate correlation; thereby we can say that the radical scavenging capacity (RSC) does not depend on color, but on other factors associated with the nature of the matrix.

CONCLUSIONS

The effect of different extraction systems of purple corn on the color-antioxidant kinetic (k_{RSC}) shows a low correlation, as seen in the Pearson correlation (table 3) and linear model multiple regression (R^2 = 0.6303). However, the relationship between the angular and scalar coordinates with anthocyanins index is better. On the other hand, the radical scavenging capacity (RSC) is not necessarily due to the intensity of the color but to the presence and nature of chemical compounds that exert this activity. In relation to the parameters obtained from different extraction systems, there are statistical differences between the parameters obtained from different extractions system ($p < 0.05$), 60% methanol being the best extraction system. Tristimulus Colorimetry allows a qualitative analysis of the extracts, which is an advantage over the quantitative method based in the measurement of absorbance.

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