

Behavior of bioactive compounds and antioxidant activity of mango (Azucar cultivar) juice during storage at 4 °C

Comportamiento de compuestos bioactivos y actividad antioxidante del jugo de mango (variedad Azúcar) durante el almacenamiento a 4 °C

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ABSTRACT

Keywords:

Antioxidant
Ascorbic acid
cv. Azucar
Mangiferin
Mango juice
Polyphenols

Mango (*Mangifera indica* L.) is one of the fruits that have shown antioxidant activity and high nutritional value. It was evaluated the effect of storage time and temperature on polyphenol content, ascorbic acid and antioxidant activity of mango (cv. Azucar) juice stored up to 64 days at 4 °C. Total polyphenol content was measured by Folin-Ciocalteu method, mangiferin and ascorbic acid were measured by HPLC (High-Performance Liquid Chromatography) and antioxidant activity was measured by ORAC (Oxygen Radical Absorbance Capacity), and ABTS•+ (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) method. Total phenolic content decreased after 16 days of juice storage. Ascorbic acid values did not show significant differences until 48 days of storage, and mangiferin content was very similar throughout storage time. The antioxidant activity measured by ORAC method was similar until the end of the storage; however, ABTS value decreased after 32 days of juice storage. In conclusion, storage up to 32 days of mango juice at 4 °C did not alter its antioxidant activity and ascorbic acid content.

RESUMEN

Palabras clave:

Antioxidante
Ácido ascórbico
var. Azúcar
Mangiferina
Jugo de mango
Polifenoles

El mango (*Mangifera indica* L.) es una de las frutas que ha mostrado actividad antioxidante y un valor nutricional alto. Se evaluó el efecto del tiempo de almacenamiento y la temperatura, en el contenido fenólico total, niveles de ácido ascórbico y actividad antioxidante, de un jugo de mango (variedad Azúcar) almacenado a 4 °C durante 64 días. El contenido fenólico total fue medido por el método de Folin-Ciocalteu, los niveles de mangiferina y ácido ascórbico fueron medidos mediante HPLC (cromatografía líquida de alta eficacia) y la actividad antioxidante fue medida mediante ORAC (capacidad de absorbancia del radical oxígeno) y ABTS•+ (ácido 2,2'-azinobis- (3-etilbenzotiazolin-6-sulfónico). El contenido fenólico total disminuyó después de 16 días de almacenamiento del jugo. Los niveles de ácido ascórbico no mostraron cambios significativos hasta el día 48 de almacenamiento, y el contenido de mangiferina mostró valores similares durante el tiempo de almacenamiento. La actividad antioxidante medida por el método de ORAC no mostró variaciones significativas durante el tiempo de almacenamiento, en contraste se observó una disminución de los valores de ABTS después del día 32 de almacenamiento. En conclusión, el almacenamiento del jugo de mango (variedad Azúcar) durante 32 días a 4 °C no da lugar a cambios en su actividad antioxidante o contenido de ácido ascórbico.

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High consumption of fruit and vegetables has been associated with protection against chronic diseases such as cancer, cardiovascular and neurodegenerative diseases (Slavin and Lloyd, 2012; Zhang *et al.*, 2014). These beneficial effects of fruits and vegetables have been attributed partly to the antioxidant activity of their phytochemical constituents (Wojdyło *et al.*, 2014).

Mango (*Mangifera indica* L.) is a popular tropical fruit due to its taste, aroma, and flavor; it is cultivated in more than 100 tropical and subtropical countries (Yang *et al.*, 2013). There are thousands of mangoes cultivars worldwide (Liu *et al.*, 2013) and the most important mango Colombian cultivars in terms of production are the cultivars Hilacha, Tommy, Keitt, and Azucar; being the latter currently import into the United States (US) and Europe on a large scale (Krenek *et al.*, 2014).

Mango has high nutritional value and is an important source of dietary antioxidants such as carotenoids, ascorbic acid and phenolic compounds (Manthey and Perkins, 2009). The phenolic compounds include flavonoids, phenolic acids, xanthenes and gallotannins (Kim *et al.*, 2010), although this composition changes between different mango cultivars (Kim *et al.*, 2010; Manthey and Perkins, 2009).

Among the compounds mentioned above, special interest has been focused on ascorbic acid and mangiferin. The ascorbic acid also known as vitamin C is an important micronutrient in the human diet involved in the antioxidant defense, protecting proteins and lipid membranes from oxidative damage caused by Reactive Oxygen Species (ROS) due to its reducing capacity, and it is also known as neuroprotective agent (Du *et al.*, 2012).

Mangiferin is a C-glucosyl xanthone which can be found in several plants such as *Mangifera indica* (Negi *et al.*, 2013); it is predominantly found in peel and stem bark of mango fruit. Mangiferin contribute to prevent fenton reaction and lipid peroxidation because its catechol motive can forms a stable complex with Fe³⁺ (Benard and Chi, 2015). Mangiferin also has a potent capacity to neutralize ROS, such as peroxy radical, hydroxyl radical, hydrogen peroxide, superoxide anion, and its activity is similar to ascorbic acid (Benard and Chi, 2015).

Considering that mango is a seasonal fruit with a short shelf life, its processing is important for consumers to ensure a longer life (Alikhani, 2014). Therefore, mango fruit is processed into several products such as puree, nectar, and juice (Appiah *et al.*, 2011); these products should be kept by consumers between 0 to 4 °C to prevent their spoilage (Kaddumukasa *et al.*, 2017); however, there are few studies about changes in its bioactive compounds and antioxidant activity during storage time at that temperature.

Currently, consumers prefer foods which may have a positive impact on their health. Therefore, it is important to consider the effect of juice storage time on bioactive compounds and antioxidant activity because this may affect consumers acceptance (Beh *et al.*, 2012). Thus, it was evaluated the changes of ascorbic acid, total phenols and antioxidant activity of mango (cv. Azucar) juice stored up to 64 days at 4 °C; besides, we investigated if mango juice had mangiferin, considering it has been proposed as one of the most important antioxidants of mango (Matkowski *et al.*, 2013).

MATERIALS AND METHODS

Raw material

Mature mango (cv. Azucar) grown in Colombia's Caribbean coast was purchased from a local market in Medellín, Colombia (June 2015). Mango got ripened after storage at 25 °C, and ripeness degree was determined according to the following criteria: peel color, firmness, and flavor based on Colombian Technical Norm (NTC *by its initials in Spanish*) 5139 (Zapata *et al.*, 2017). Ripe mango was immersed in a solution of sodium hypochlorite (100 ppm), washed with water, peeled and cut into small pieces which were used immediately to prepare the juice.

Juice preparation

The juice was prepared as described in a previous study with some modifications (Zapata *et al.*, 2017). The juice was prepared by mixing mango pieces and water (1:4) in a blender for several minutes; the total soluble solids of the juice were measured at 20 °C using a refractometer. Xanthan gum (0.07%) was added to the juice as stabilizer. Finally, juice was pasteurized at 85 °C for 10 min, sweetened with 2 g L⁻¹ of sucralose, and packaged into low-density polyethylene bag and immediately stored at 4 °C until use.

Determination of total phenolic content

The total phenolic content was determined according to a modified Folin–Ciocalteu described by Prior *et al.* (2005). The juice (10 µL) was mixed with 125 µL of Folin–Ciocalteu reagent and 400 µL of sodium carbonate solution (7.1% w/v); the resulting solution was brought up to a final volume of 1000 µL. The mixture was incubated at room temperature for 30 min in the dark. The absorbance was measured at 760 nm against a blank. Standard solution of gallic acid was used to perform calibration curves. The results were expressed as mg of Gallic Acid Equivalents, mg GAE L⁻¹.

Determination of ascorbic acid

Ascorbic acid was quantified using a Shimadzu Prominence (LC-20AD) HPLC system equipped with autosampler (SIL-20A/HT) and PDA detector (SPD 6AUV) in juice stored up to 64 days at 4 °C. Juice was filtered through cellulose membrane filter (0.45 µm), and then gradient separations were done using Lichrospher (Merck) RP C18 column (5 µm, 250x4 mm) at 35 °C; gradually, 20 µL of juice was injected using autosampler. The mobile phase was 0.1% formic acid in water run at 0.8 mL min⁻¹ under isocratic conditions. Ascorbic acid was quantified at 245 nm using a calibration curve, and the results were expressed as mg ascorbic acid L⁻¹ (Shakya and Navarre, 2006).

Determination of mangiferin

Shimadzu Prominence (LC-20AD) HPLC system described previously was used for determination of mangiferin in juice stored up to 64 days at 4 °C. The flow rate was 0.6 mL min⁻¹ and the injection volume was 10 µL. Analysis was carried out with Lichrospher RP C18 column (5 µm, 250x4 mm) using a gradient elution of 0-1 min 5% solvent B (5% (v/v) acetic acid in water with acetonitrile 50:50), 2-10 min 5-25% solvent B, 10-40 min 25-55% solvent B, 40-45 min 55-90% solvent B, 45-50 min 90-55% solvent B, 50-55 min 55-5% solvent B, 55-60 min 5% solvent B, and solvent A was 2% (v/v) acetic acid in water. Column temperature was 30 °C and mangiferin was determined at 258 nm (Luo *et al.*, 2012). The determination was done only one time.

Antioxidant activity determined by ORAC (Oxygen Radical Absorbance Capacity) assay

ORAC assay was performed as described by Ou *et al.* (2001) with some modifications, in mango juice stored up to 64 days at 4 °C. The working solution was prepared by

mixing 21 µL of 10 µM fluorescein solution, 2899 µL of 75 mM phosphate buffer (pH 7.4), 50 µL of 600 mM AAPH (2,2'-Azobis(2-amidinopropane) dihydrochloride) and 30 µL of juice. Fluorescence was measured on a Perkin Elmer LS45 spectrofluorometer with a thermostatted multicell. The results were expressed as µM Trolox® L⁻¹, according to the following equation:

$$ORAC = \frac{(AUC - AUC^{\circ})}{(AUC_{Trolox} - AUC^{\circ})} f[Trolox]$$

Where:

AUC: is the area under the curve of the sample.

AUC[°]: is the area under the curve for the control

*AUC*_{Trolox}: is the area under the curve for Trolox

f: is the dilution factor for juice = 24.

Antioxidant activity determined by ABTS•+ assay

10 µL of juice was added gradually to 990 µL of diluted ABTS•+ (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) in phosphate buffer (pH 7.4), and the resulting solution was incubated at room temperature for 30 min in the dark. The absorbance was measured at 734 nm against a blank. Trolox standard solution was used to perform the calibration curves, and the results were expressed as µM Trolox L⁻¹ (Re *et al.*, 1999).

Kinetic model

It was performed an adjustment model to determine the kinetic behavior of antioxidant activity and mango components during storage and to estimate the rate of decrease of mango components during the storage.

Statistics

Assays were conducted by triplicate and data were reported as the mean±standard deviation (SD). The differences between groups were estimated by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. A *P*-value less than 0.05 (*P*<0.05) was considered statistically significant. The results were analyzed using GraphPad Prism software version 6.0. A regression model was found to estimate the kinetic behavior of antioxidant activity and mango components throughout time.

RESULTS AND DISCUSSION

Juice

The juice fruit content was 18%, and its total soluble solids were 8 °Brix.

Total phenolic content

The values of total phenols showed a reduction during juice storage time (Figure 1). The highest values of total phenolic content were observed up to 16 days of juice storage corresponding to 126.5 ± 7.9 and 123.9 ± 4.5 mg GAE L⁻¹, respectively and they did not show significant

differences between them. After 32 days of storage, juice showed a decrease in its phenolic content, and it was significantly different in comparison to values observed at the beginning of the storage. The lowest total phenols value was observed at the end of the juice storage, corresponding to 96.23 ± 3.25 mg GAE L⁻¹.

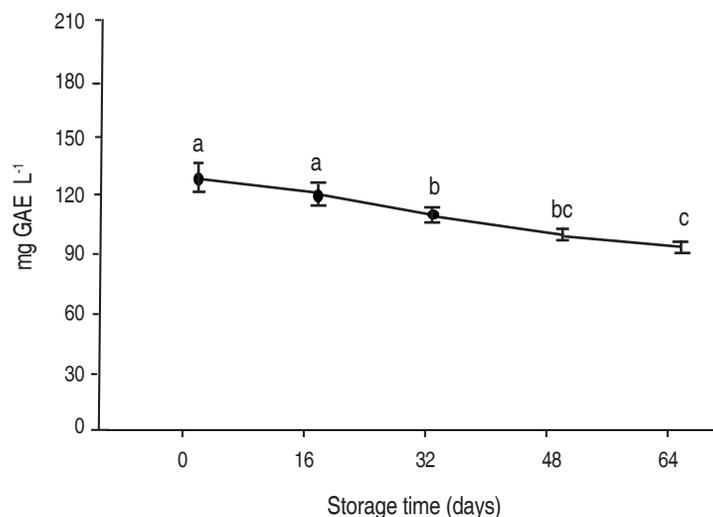


Figure 1. Effect of storage time and temperature on the total phenolic content of mango juice. Values are the means \pm standard deviation of three replicates. The same small letters indicate not significant statistically difference ($P > 0.05$). Tukey's post-hoc test. GAE: Gallic Acid Equivalents.

These results agree with previous studies which have observed a decrease phenolic compounds in juices after 20 days at 4 °C; this could be explained by polyphenol oxidase activity which reduces phenolic compounds (Mizobutsi *et al.*, 2010).

Total phenolic content values up to 48 days found in the present study were higher than those reported by Beh *et al.* (2012) which found a total phenolic content of 9.26 mg GAE L⁻¹ in fresh juice prepared with mango from Malaysia. While, total phenolic content value was similar to the value reported by Abdullakassim *et al.* (2007), corresponding to 100 mg GAE L⁻¹ in fresh juice prepared with mango from Thailand. However, some authors have reported higher phenolic content in juice prepared with mango from Iran, Malaysia and Algeria corresponding to 567.2, 804.00 and 413.5 mg GAE L⁻¹, respectively (Mahdavi *et al.*, 2010; Saci *et al.*, 2015; Wern *et al.*, 2016).

Ascorbic acid content

Another important component of mango is the ascorbic acid which is known as a potent antioxidant. Therefore, their levels in juice were determined. Ascorbic acid in the juice was quite stable during most of the evaluated days, and its content was within a range of 9.30 to 16.35 mg ascorbic acid L⁻¹ (Figure 2). Ascorbic acid values did not show significant differences between different storage time compared to day 1, except for juice stored for 64 days, which showed the lowest ascorbic acid value corresponding to 9.3 ± 0.14 mg ascorbic acid L⁻¹. Ascorbic acid decrease at the end of the storage could be attributed to an ascorbic acid degradation induced by light, oxygen and ascorbate oxidase (Castro *et al.*, 2016; di Venere *et al.*, 2011). Moreover, ascorbic acid values showed by mango (cv. Azucar) juice were lower than those reported by Mahdavi *et al.* (2010) for a fresh juice formulated with Mango from Tabriz-Iran, corresponding to 146.5 mg L⁻¹.

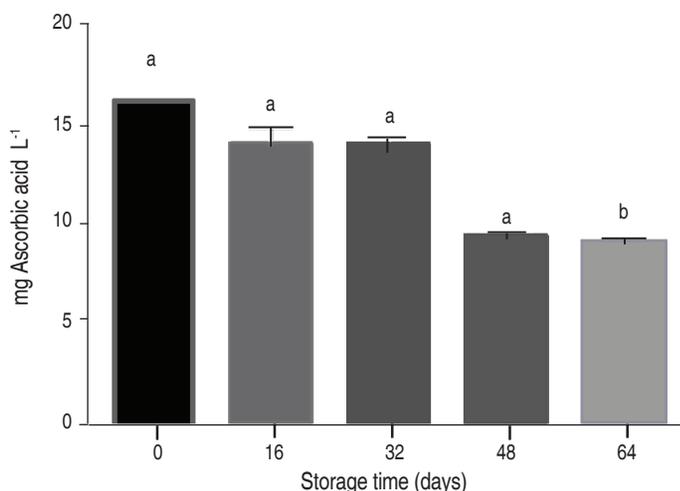


Figure 2. Ascorbic content of mango juice stored up to 64 days at 4 °C.

The variation of ascorbic acid and phenolic content depends on several factors such as cultivar, harvest date, ripeness, processing techniques and location of mango fruit (Manthey and Perkins, 2009).

Mangiferin content

Mangiferin content in mango (cv. Azucar) juice was evaluated by HPLC-DAD. At the beginning of the storage, the juice showed the highest mangiferin levels corresponding to 11.50 mg L⁻¹ (Figure 3A) detected at 258 nm with a retention time of 4.3 min. After 64 days of storage, the juice showed the lowest mangiferin levels (8.33 mg L⁻¹). Mangiferin levels were 9.21, 8.64 and 8.54 mg L⁻¹ after 16, 32 and 48 days of storage, respectively (data not shown). Mangiferin standard showed a retention time of 4.3 min (Figure 3B).

Mangiferin content was similar over the storage time and this is an important finding considering it has shown a strong antioxidant activity in vitro (Matkowski *et al.*, 2013). Mangiferin is also able to reduce induced oxidative stress in rats' brain (Márquez *et al.*, 2012).

Antioxidant activity

It was determined the antioxidant activity in a mango juice stored up to 64 days by two different methods ORAC and ABTS•+. ORAC values were similar from the beginning to the end of juice storage, and they did

not show significant differences between them. The highest and the lowest ORAC values were observed at the beginning and the end of the storage, corresponding to 2563.16±129.36 and 1933.16±104.35 μmol Trolox L⁻¹, respectively (Table 1).

The scavenging activity of mango juice against ABTS radical was similar until 32 days of storage compared to the juice stored for 1 day at 4 °C, and it did not show significant differences between them (Table 1). After 16 days of juice storage, it was observed the highest ABTS•+ value, corresponding to 656.57±12.35 μmol Trolox L⁻¹ and the lowest value was shown after 64 days of storage, corresponding to 533.60±14.69 μmol Trolox L⁻¹. Antioxidant activity measured by ABTS•+ assay decreased after 32 days of juice storage and showed significant differences compared to ABTS•+ values shown by juice stored during 1 day at 4 °C.

Antioxidant activity has been associated with phenolic content (Wojdyto *et al.*, 2014). However, other components of mango such as ascorbic acid and carotenoids are also known as potent antioxidants (Fiedor and Burda, 2014; Manthey and Perkins, 2009). Therefore, the antioxidant activity observed in this study cannot be only attributed to its phenolic compounds, but also the activity of the several antioxidants present in the juice and the synergistic activity between them.

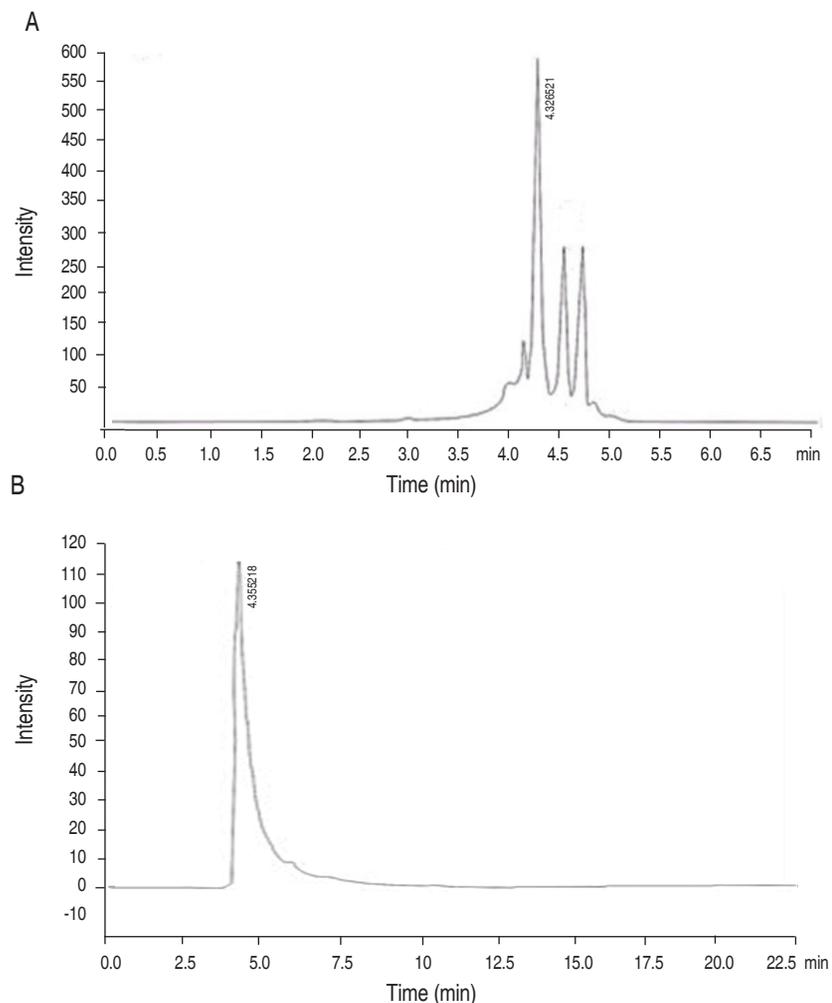


Figure 3. Identification of mangiferin by HPLC. A. Mango (cv. Azúcar) juice; B. Mangiferin standard.

Table 1. Antioxidant activity of the mango juice. The same small letters indicate not significant statistically difference ($P>0.05$). Tukey's post-hoc test.

Juice storage time (days)	ABTS ($\mu\text{mol Trolox L}^{-1}$)	ORAC ($\mu\text{mol Trolox L}^{-1}$)
1	632.71 \pm 19.05 ^a	2563.16 \pm 129.36 ^a
16	656.57 \pm 12.35 ^a	2551.96 \pm 120.18 ^a
32	632.37 \pm 18.42 ^a	2335.43 \pm 124.76 ^a
48	591.96 \pm 6.59 ^b	2275.18 \pm 128.03 ^a
64	533.60 \pm 14.69 ^b	1933.16 \pm 104.35 ^a

Kinetic model

Table 2 presents the kinetic behavior of mangiferin, polyphenols, ascorbic acid and antioxidant activity of

mango juice stored at 4 °C. There was a relationship between time and mangiferin, ascorbic acid and antioxidant activity values. Mangiferin showed more susceptibility to

degradation over time with respect to total phenols and ascorbic acid in the juice. Regarding to antioxidant activity, ABTS values decreased faster than ORAC values indicating that molecules with capacity to neutralize free radicals

through hydrogen atom transfer (ORAC mechanism) were more stable during storage at 4 °C, compared to molecules which reduces free radicals through electron transfer (ABTS mechanism) (Schaich *et al.*, 2015).

Table 2. Kinetic modeling parameters of mango juice stored at 4 °C.

Regression variables	Regression equation	Model type	R ²
Mangiferin vs. time	$mg\ mangiferin\ L^{-1} = 11.454 - 0.733 \ln(days)$	Logarithmic	0.9931
Polyphenols vs. time	$mg\ GAE\ L^{-1} = 128.448 - 0.518 * days$	Linear	0.9657
ABTS vs. time	$\mu mol\ Trolox\ L^{-1} = 651.938 - 0.0273 * days^2$	Quadratic	0.9334
Ascorbic acid vs. time	$mg\ Asc.\ acid\ L^{-1} = 16.5318 - 0.1184 * days$	Linear	0.9008
ORAC vs. time	$\mu mol\ Trolox\ L^{-1} = 2645.9 - 9.755 * days$	Linear	0.8996

CONCLUSIONS

This study found that storage time and temperature influence on composition and antioxidant activity of mango (cv. Azucar) juice. Although total phenolic content decreased after 16 days at 4 °C, antioxidant activity measured by two methods remained stable until 32 days of storage; also, ascorbic acid and mangiferin content were very similar until near the end of the storage time. Considering that mango juice has antioxidant activity and it is a good source of antioxidants, it could potentially help to prevent oxidative stress in vivo.

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