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Antioxidant capacity of commercial cranberry juices before and after storage at low temperature

Capacidad antioxidante de jugos de arándano antes y después de su almacenamiento a baja temperatura

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Abstract:

This study evaluated the antioxidant activity (AA) of cranberry juices available in supermarkets of San Luis Potosí, México, before and after storage at low temperature (4°C) by ABTS•, DPPH•, and FRAP methods. Twelve commercial juices were tested at the time of opening (D1) and 24 hours later (D2). The results were expressed as vitamin C equivalent antioxidant capacity (VCEAC) and percentages of free radical inhibition. The highest AA was recorded at 60 min, although it was different in the three methods. The AA decreased significantly in D2. Therefore, our study recommends the consumption of cranberry juice once the container is opened, to ensure the consumption of the antioxidant compounds associated with a beneficial effect on health.

Keywords: juices, cranberries, free radical, phenolic compounds, commercial brands.

Resumen:

Se evalúa la actividad antioxidante (AA) de jugos de arándano disponibles en los supermercados de San Luis Potosí, México, antes y después de su almacenamiento a baja temperatura (4 °C) mediante los métodos ABTS•, DPPH• y FRAP. Se analizan 12 jugos comerciales al abrirlos (D1) y 24 horas después (D2). Los resultados se expresaron como capacidad antioxidante equivalente a vitamina C (VCEAC) y porcentajes de inhibición de radicales libres. La mayor AA se registró a los 60 min, aunque fue diferente en los tres métodos. La AA disminuyó significativamente en D2. Por lo tanto, se recomienda el consumo de jugo de arándano una vez abierto el envase para garantizar el consumo de los compuestos antioxidantes asociados a un efecto benéfico para la salud.

Palabras clave: jugos, radicales libres, compuestos fenólicos, marcas comerciales.

Introduction

Fruit and vegetable juices are widely used due to their convenience and method of consumption, high concentrations of functional ingredients, and represent an appropriate medium for functional component dissolution (Wootton-Beard & Ryan, 2011). Cranberry juice is the second fruit juice of interest due to its health benefits, after citrus juices (Wootton-Beard & Ryan, 2011; Shidfar *et al*., 2012; Hyson, 2015). With various clinical studies that support its use, cranberry has established itself in the market of dietary supplements since the last decade of the twentieth century (Blumberg *et al*., 2016).

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The cranberry belongs to the *Vaccinium* genus (Ericaceae), is cosmopolitan and has 450 species (Abreu *et al*., 2014) of which the most used to produce commercial juices is *V. macrocarpon*, a shrub species native to North America (Brown & McNeil, 2006). As one of the first functional foods in America, cranberry juice has been popular for many years for treating and preventing Urinary Tract Infections (UTIs) and cardioprotective effects attributed to its phenolic compounds. Its activities have been evaluated in reproductive-age women (Bass-Ware *et al*., 2014), adults (Chen *et al*., 2013), children (Salo *et al*., 2012), and pregnant women (Heitmann *et al*., 2013). According to epidemiological studies, cranberry and cranberry juices consumption is related to a decrease in the inflammatory response associated with non-communicable diseases (cancer, diabetes, renal diseases) (Babu *et al*., 2012; Pagonas *et al*., 2012; Weh *et al*., 2016). Besides, cranberry juice is beneficial in stomach ulcer prevention (McKay *et al*., 2015) because it inhibits *Helicobacter pylori* adhesion in the gastric mucous membrane (Lin *et al*., 2011).

Cranberries are popular with consumers because of their tart taste and their health benefits (McKay *et al*., 2015). These effects are attributed to a variety of compounds with antioxidant capacities such as tannins, flavonoids (including flavonols, flavan-3-ols, anthocyanins, and proanthocyanidins), anthocyanins, procyanidins, phenolic acids (including benzoic, hydroxycinnamic, and ellagic acids), triterpenes, iridoids, and organic acids (Pappas & Schaich, 2009). The antioxidant capacity of cranberry juices in humans has been reported in numerous studies. These effects are attributed to the vitamin C increase and phenols in plasma. Also, it has been reported that cranberry juice consumption for two weeks decreased circulating oxidized LDL (Vinson *et al*., 2008).

It has been proven that fruit juices stored at room temperature have a significantly shorter shelf life than those at a lower temperature (Teleszko *et al*., 2016). There are studies for the antioxidant activity analysis of medicinal or edible plants. However, analysis of the antioxidant activity of foods consumed daily by the population is currently scarce. Packaged juices are among the most popular foods in the population. However, proper storage temperature is a requirement to maintain the packaged juices quality. The purpose of this study was to evaluate the antioxidant capacity of cranberry juices available on markets of San Luis Potosí, México, before and after storage al low temperature (4°C). The antioxidant capacity was evaluated by 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt radical cation (ABTS•), 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•), and (ferric reducing ability of plasma) FRAP methods.

1. Materials and methods

1. 1. Standard chemicals

Ammonium salt of 2,2'-azinobis- (3-ethylbenzthiazolin-6-sulphonic acid) (ABTS•) (Fluka Chemicals), potassium persulfate (Panreac), monosodium phosphate (Panreac), 2,2-diphenyl-1-picrylhydroxy (DPPH•) (Sigma), methanol (Merck), hydrochloric acid (Panreac), 2,4,6-tripyridyl-s-triazine (TPTZ) (Fluka Chemicals), ferric chloride (Panreac), sodium acetate (Panreac), and acid ascorbic (Sigma).

1. 2. Cranberry juices samples

We evaluated 12 commercial cranberry juices available in San Luis Potosí, Mexico and obtained from local retailers. The juices were named with the following acronyms: Del Valle (DV), Jumex (JX), Sonrisa (SA), Ocean Spray (OS), Hill Country (HC), Member's Mark (MM), Kirkland Signature (KS), Golden Hills (GH), Selecto (SO), Great Value (GV), Old Orchard (OD) y Healthy balance (HB).

1. 3. Antioxidant assessment

The antioxidant capacity was evaluated by ABTS•, DPPH•, and FRAP methods. Two measurements were taken, the first when the juice was opened (D1). A second measurement was taken 24 h after at 4 °C (D2) The juices were refrigerated immediately after each measure. pH readings were carried out with a Hanna HI 2211 potentiometer.

1. 4. ABTS method

To 2 ml of ABTS•+ solution, 100 *µl* of juice was added and mixed for 30 sec. The absorbance at 734 nm was measured at 5, 30, and 60 minutes (Miller *et al*., 1993).

1. 5. DPPH method

To 3 ml of DPPH• radical, 400 *µl* of juice was added. This mixture was stirred and left in the dark at room temperature; the absorbance was measured at 520 nm at 5, 30, and 60 minutes (Cheng *et al*., 2006).

1. 6. FRAP method

Nine hundred *µl* of FRAP solution preserved at 37 °C were taken, for the 120 *µl* of sample a 1:6 dilution was made, 100 *µl* of distilled water and 20 *µl* of the juice were added. The mixture was stirred and maintained at 37 °C in a thermostatic bath, proceeding to the measurement of absorption at 595 nm at 5, 30, and 60 minutes. The blank was prepared with 900 *µl* of FRAP and 120 *µl* of deionized distilled water (Benzie & Strain, 1996).

1. 7. Validation of analytical method

For the validation of the analytical method, seven calibration curves of ascorbic acid with a range of 0.4, 0.8, 1.2, and 1.6 mg/ml were made, obtaining the linear range (r2) the slope of the curve (m), and the ordered to the origin (b). The quantification of ascorbic acid was validated, a correlation coefficient greater than 0.999 was obtained. Once validated the analytical method the samples were analyzed.

The concentrations were calculated by the absorbance obtained by the ABTS•, DPPH•, and FRAP methods. The absorbances were interpolated in the respective calibration curve, using the following formula:

$$y = mx + b$$

Where:
y = A° of the problem solution
x = problem solution concentration
b = intercept
m = slope

The x was cleared in the following way: $x = (y - b)/m$. The results were expressed as Vitamin C Equivalent Antioxidant Capacity (VCEAC). The percentage of radical inhibition of the juices by method was calculated using the following formula:

$$\% \text{ Inhibition: } \frac{(A°i - A°f)}{A°i} \times 100$$

Where:
A°i = initial absorbance of the radical adjusted to the desired wavelength
A°f = final absorbance of the radical and the extract
The blank, the samples and the calibration standards were performed in independent triplicates (Basanta *et al.*, 2014).

1. 8. Statistical analysis

The results were analyzed with the Statistica Software version 10.0. The normality of the data was analyzed with a Shapiro-Wilks test. The data followed a normal distribution and analyzed by factorial ANOVA. The significant differences were obtained at the 95% confidence level.

2. Results and discussion

All juices showed antioxidant activity, the highest activity recorded at 60 minutes ($p = 0.001$). Significant differences were found between D1 and D2 ($p < 0.05$), with D1 the highest VCEAC for the three methods (figure 1), as well as a higher percentage of inhibition of free radicals (table 1). The antioxidant activity of the cranberry juices is due to the phenolic compounds, which act as reducing agents' hydrogen donors, and some have metal chelation potential (Vinson *et al.*, 2008; Côté *et al.*, 2011).

TABLE 1
Percentages of inhibition of the cranberry juices at 30 and 60 minutes

Commercial brand	ABTS• (60 min) % inhibition		DPPH• (60 min) % inhibition		FRAP (30 min) % inhibition	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
DV	82.9 ± 0.08	82.4 ± 0.30	91.1 ± 0.86	90.4 ± 0.68	**85.7 ± 0.08**	75.6 ± 0.50
GH	**90.9 ± 0.14**	85.9 ± 0.16	87.3 ± 0.22	87.1 ± 0.32	88.9 ± 0.11	87.3 ± 0.71
GV	91.1 ± 0.40	90.5 ± 0.08	87.1 ± 0.32	85.8 ± 1.64	90.6 ± 0.04	88.6 ± 0.86
HB	89.5 ± 0.59	88.9 ± 0.32	85.3 ± 1.23	84.5 ± 0.77	88.5 ± 0.40	*88.9 ± 0.41*
HC	88.3 ± 0.07	84.3 ± 0.08	96.9 ± 0.27	92.9 ± 1.57	**85.7 ± 0.04**	74.9 ± 1.60
JX	**75.2 ± 0.19**	58.1 ± 0.08	**92.8 ± 0.46**	75.4 ± 1.15	**77.5 ± 0.64**	73.8 ± 0.27
KS	**82.1 ± 0.20**	77.4 ± 0.17	95.6 ± 0.77	94.1 ± 0.19	**86.1 ± 0.07**	76.8 ± 1.87
MM	87.8 ± 0.07	86.8 ± 0.28	96.4 ± 0.22	93.9 ± 1.25	**85.4 ± 0.02**	75.8 ± 0.23
OD	90.2 ± 0.33	89.8 ± 0.07	80.7 ± 0.34	79.5 ± 3.61	***94.1 ± 0.07***	88.9 ± 0.07
OS	**83.3 ± 0.23**	77.9 ± 0.10	**96.1 ± 0.19**	92.2 ± 1.39	**85.3 ± 0.02**	75.5 ± 0.19
SA	**80.7 ± 0.32**	10.9 ± 0.04	**92.0 ± 0.66**	67.9 ± 0.63	**77.9 ± 1.29**	74.9 ± 0.15
SO	91.1 ± 0.06	90.7 ± 0.12	85.9 ± 3.04	85.2 ± 1.46	90.1 ± 0.10	87.2 ± 1.16

Source: own elaboration.
Note: All analyses were performed in triplicate. The bold values are statistically significant ($p < 0.05$). The italics values are the highest vitamin C equivalent antioxidant capacity (VCEAC). The highest percentages are shown in boldface type.

Studies on the antioxidant activities of plants and their components have typically used more than two different methods and recommended a combination of assays for scavenging electrons or radicals DPPH•, ABTS•, and FRAP, the most widely used (Moon & Shibamoto, 2009). In our study, antioxidant activity was different in the three methods in the brands evaluated. With ABTS•, the SO showed the highest VCEAC and inhibition percentage (91.1%). Juice JX was the lowest (table 1). With DPPH•, HC was the highest at (96.9%) (figure 1), and OD was the lowest (table 1). With the FRAP method, the OD showed the highest VCEAC and percentage of inhibition (94.1%) (figure 1), and JX presented the lowest (table 1).

Studies have evaluated the antioxidant capacity of cranberry fruits (Taruscio *et al*., 2004; Ehala *et al*., 2005; Skrovankova *et al*., 2005) and the effect of storage temperature on the antioxidant capacity of the fruits. The recommended storage temperature for blueberry fruit to maintain its antioxidant capacity is 2 to 4 °C (Wang & Stretch, 2001). This temperature coincides with the present study since the antioxidant activity of the juices analyzed was higher at 4 °C.

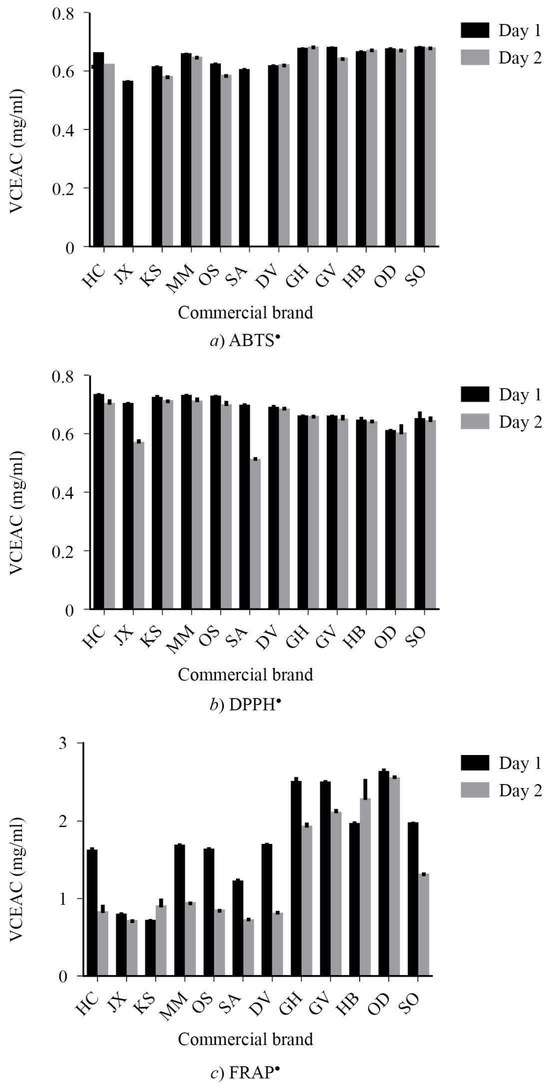

FIGURE 1
Antioxidant capacity equivalent to vitamin C (VCEAC) of the cranberry juices
Source: Own elaboration. Note: *a*) ABTS•, *b*) DPPH•, and *c*) FRAP at 30 min and 60 min days 1 y 2. All analyses were performed in triplicate.

Regarding pH measurements, all values were below 5.0 with an average of 2.76, being HC the most acidic (2.58) and GH the least (3.03) in the D1. In D2, the pH increased but remained below 5.0; GH was the most acidic on both days (table 2). The acidity of the cranberry is attributable to the polyphenols; its low pH and astringency is due to low levels of sugars but high levels of organic acids, mainly citric, malic, and quinic acids (Lu *et al*., 2017). However, polyphenols are sensitive to changes in pH because they present oxidative degradation in alkaline media, while at a pH below 5.0 they have better stability (Zeng *et al*., 2017).

TABLE 2
pH of commercial cranberry juices and additional amount of ascorbic acid according to the label on the container

Commercial brand	Day 1	Day 2 (at 4 °C)	Day 2 (room temperature)	Ascorbic acid
DV	2.71	2.95	3.12	Without adding
JX	3.01	3.12	3.27	Without adding
SA	2.80	2.87	2.93	Without adding
OS	2.60	2.98	2.98	50 mg
HC	2.58	2.76	2.71	60 mg
MM	2.70	2.72	2.83	60 mg
KS	2.66	2.69	2.73	30%
GH	**3.03***	**3.16***	3.20	60 mg
SO	2.82	3.01	2.98	60 mg
GV	2.48	2.68	2.67	Not specified
OD	2.81	2.89	2.86	2%
HB	2.92	3.14	3.15	2%

Source: own elaboration.
Note: *The highest pH value is highlighted in bold type.

One of the main findings of the study was a significant difference ($p < 0.05$) in the antioxidant activity during the two evaluations. Samples in D1 have the highest percentage of inhibition and the highest rate of VCEAC. On the other hand, for D2 after refrigeration at 4 °C by 24 h, possibly the antioxidant effect decreased since the phenolic compounds were oxidized. It has been observed that phenolic compounds are susceptible to degradation by heat, light, and dissolved oxygen (Côté *et al*., 2011; Zeng *et al*., 2017). Besides, heating, separation, and storage techniques can result in oxidation, thermal degradation, and leaching to surrounding water can increase or decreases the antioxidant activity (Wootton-Beard & Ryan, 2011). Also, the pH change modified the antioxidant capacity of the juices registered in D2. It is recommended to store the cranberry juice at 4 °C to avoid contamination with pathogens (Sewlikar & D'Souza, 2017). However, when juices are stored at a low temperature (i. e., 4 °C), the polyphenol content decreased (Tembo *et al*., 2017).

It has been suggested that juices stored at −1.5 °C possessed higher polyphenol contents, fewer color alterations, and less aroma and taste loss than at 25 °C (Gou *et al*., 2019). However, frozen juice requires thawing before consumption, during which time it may become contaminated with microorganisms. Understanding how processing and storage alter the cranberries' polyphenolic compounds, it is crucial to avoid them and maximize the health benefits of cranberry juice (White *et al*., 2011).

The compounds with antioxidant capacity present in the commercial juices of cranberry confer benefits to human health (Babu *et al*., 2012; Dang *et al*., 2012; Duffey & Sutherland, 2015). Several studies reported increases in the antioxidant capacity of plasma following consumption of cranberry juice (Liu *et al*., 2015; Liu *et al*., 2017). For these reasons, currently, cranberry juice is popular among the population and is known as a beverage that brings health benefits. Commercial juices are a source of antioxidants and may increase the consumption of fruits and vegetables to re-equate the balance between recommendations and actual intake due to their high levels of polyphenolic compounds, which have health benefits (Wootton-Beard & Ryan, 2011). It is important to note that commercial cranberry juices are high in sugars and calories. In Mexico, there is front-of-food labeling that allows consumers to identify foods with higher sugars, calories, and fat content. Therefore, commercial cranberry juice should be consumed according to the recommended portions for each age group to obtain its benefits as a functional food.

Cranberry juice is an option to increase the consumption of functional foods in a scenario where there is an increase in the incidence of diseases associated with inflammatory processes (cancer, diabetes, cardiopulmonary diseases) and a low intake of fruits and vegetables.

Our understanding of how cranberry juice has beneficial effects on human health is a challenge because of the range of product formulations and the amount of cranberry juice on the market. Especially in the Mexican population, the challenge is to register the consumption frequency and amount consumed of cranberry juicy because they are unknown. In this regard, it is crucial to carry out studies about the content of phenolic compounds, their bioavailability, antioxidant activity, and consumption of available cranberry juices to know their nutritional contribution to the Mexican diet.

Prospective

Studies on the antioxidant activity of medicinal plants and functional foods have been carried out. However, studies on commercial foods and beverages commonly consumed by the population are scarce. Cranberry juice is among the most popular due to its beneficial health effects related to its antioxidant activity. Beverage-appropriate storage and foods are essential to maintain their properties and safety. The present study found that the temperature at which cranberry juice is stored affects its antioxidant activity. Packaged juices are an alternative to improve the intake of fruits and vegetables and their bioactive compounds. However, juices must be handled and stored adequately. It is advisable to drink cranberry juices at room temperature and not to store them since this decreases their antioxidant activity. Antioxidant consumption from functional foods has been increasing due to their effect on health. The general population is looking for alternatives for their health, especially in an era of infectious diseases related to the pandemic as COVID-19, where self-care and good nutrition have become fundamental. Epidemiological studies related to the consumption of cranberry juice in Mexico are needed to know which population consumes it, as well as to disseminate information on the appropriate storage of the juices consumed by the people.

Conclusions

The compounds in cranberry juices give them antioxidant effects with great benefits for health. However, these compounds are easily degraded and significantly decrease their antioxidant activity once the container is opened. For this, we recommend consuming cranberry juice once the package is opened, along with attention to the conditions used to preserve it to guarantee the consumption of the compounds responsible for their beneficial effects on health. Fruit and vegetable juices are a rich source of antioxidants, which have health benefits. Cranberry juice is an option to increase the consumption of functional foods in a scenario where there is an increase in the incidence of diseases associated with inflammatory processes and a low intake of fruits and vegetables, especially in the Mexican population.

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