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Antioxidant activity of *Vaccinium oxycoccus* in commercial, natural and extract juices

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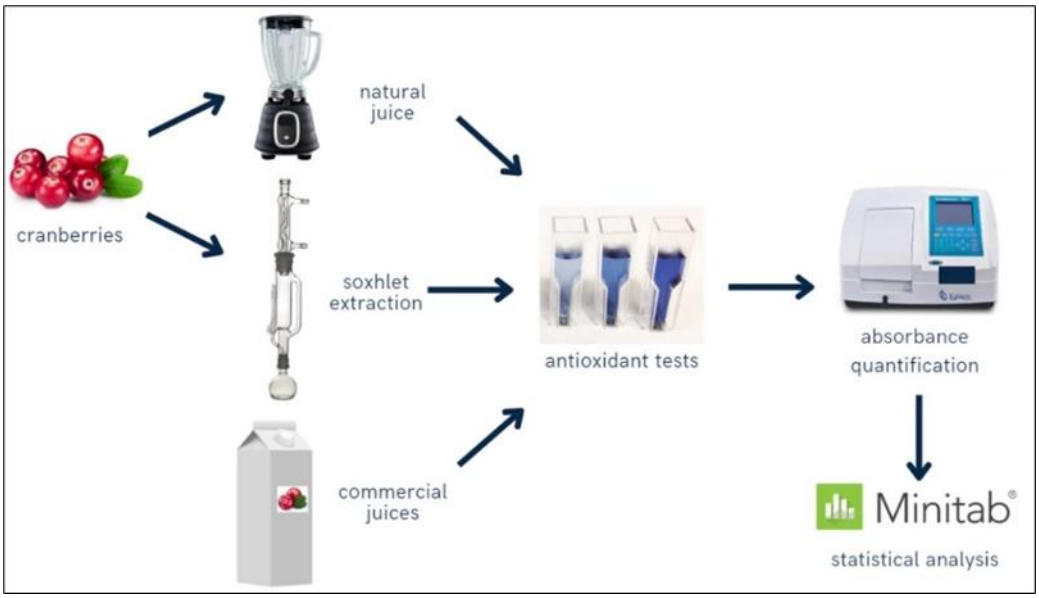
Abstract

Cranberries are one of the fruits with the highest concentration of antioxidant compounds; being these, which are attributed a great nutraceutical potential, however, their content and antioxidant capacity depend on many factors, so when comparing the antioxidant activity in commercial juices from three different brands, a natural juice and two fresh cranberry extracts obtained by Soxhlet (methanol and water), the effect of this can be analyzed. The evaluation was carried out by DPPH, FRAP, total phenolic content, total flavonoid content, and anthocyanin content. The MeOH extract presented the highest values in all the methods. Although it was proven that cranberries do indeed contain a high content of antioxidant compounds, the method by which a juice is prepared is not effective enough to extract most of these; this indicates that the activity presented by commercial juices is not necessarily due to the compounds provided by the fruits.

Keywords: Antioxidant; Cranberry; Commercial juice; Radical scavenging; Nutraceutical

Graphical abstract



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1. Introduction

In recent years, the consumption of vegetables and fruits rich in antioxidants has become popular and may have a beneficial correlation with our health. Within the variety of plant foods consumed by humans, wild berries such as cranberries have a high concentration of antioxidant compounds (mainly polyphenols, flavonoids, and anthocyanins), vitamin C and potassium (figure 1), with a nutritional value that is quite attractive for health, nutrition, and the food industry (1,2). For all this, cranberries are the protagonists of commercial products such as commercial juice boxes. These juices are marketed and promoted highlighting their supposedly high antioxidant content, which raises the question of whether this activity is due to cranberries or the additives that are used industrially. Antioxidants are chemical compounds that fight free radicals, highly reactive species responsible for oxidative stress in our cells (3,4).

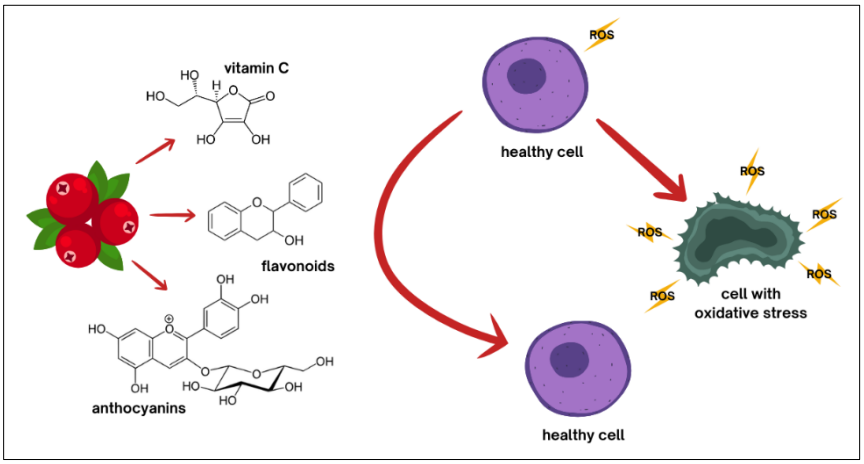

Figure 1 Antioxidant contents in cranberries reported, and relation for oxidative stress (1,3)

Oxidative stress is an imbalance between free radicals and antioxidants in favor of the former, causing the oxidation of biomolecules (proteins, fatty acids, and/or nucleic acids), leading to a disruption of redox signaling. (5,6), promoting aging, different types of cancer, cardiovascular diseases, neurodegenerative diseases, and there is even an inverse relationship with depression (7–11). The neuroprotective activity by these compounds is giving for the donation of electrons or hydrogen to inhibit free radicals, preventing damage to neuronal cells caused by oxidative stress; additionally, some antioxidants molecules, as certain flavonoids, are involved in signaling pathways related to cellular protection (12).

2. Material and methods

2.1. Commercial and natural juice vs extracts

Commercial juices from three different economic strata were selected (costly, intermedium and economic). Preparation of natural juice: fresh ripe cranberries were liquefied with drinking water in a 1:2 ratio (mass/mass). They were subsequently filtered, labeled, and stored for testing. Two differential Soxhlet extractions were carried out, the first with methanol and the second with water, for 3 hours each (13,14).

2.2. Equipment

In test adapted to the 96-well microplate, the Acurris smartreader 96-T microplate reader was used. For the rest of the tests the measurements were performed whit the UV-Vis spectrophotometer moder VE-5100UV.

2.3. Calibration curve for each technique

For each of the required techniques, a calibration curve was made which allowed us to determine the concentration of each equivalent according to the absorbance, as described below, for:

- Total phenolic content; standard: gallic acid 0.02-0.30 mg/mL.
- Total flavonoid content; standard: quercetin 0.1-1.0 mg/mL.
- FRAP; standard: quercetin 0.01-0.10 mg/mL.

- DPPH; standard: gallic acid 0.02-0.10 mg/mL.
- Total phenolic content

Folin Ciocalteu (FC) reagent methodology (15,16). 150.0 µL of sample, 2250.0 µL of distilled water, 150.0 µL of FC reagent, and 450.0 µL of Na_2CO_3 (20% mass/volume) were placed in an assay tube. For this part positive control (gallic acid) and a blank were run. The assay tube was incubated for 2 hours in darkness and subsequently the UV-VIS spectrophotometer at 760 nm.

2.4. Total flavonoid content

Total flavonoids by the methodology (17) with modifications. 4.0 mL of distilled water were added to a test tube, 1.0 mL of the sample (from this step, when adding any substance to the tube it was homogenized with a vortex), 0.3 mL of $NaNO_2$ (1.0 M), 0.3 mL $AlCl_3$ (10% mass/volume), 2 mL of NaOH (1M), and made up to 10.0 mL with distilled water; It was incubated for 40 minutes in the dark. 3 mL of the contents of the tube were transferred to a cell and the absorbance was measured at 510 nm with UV-VIS spectrophotometer.

2.5. Anthocyanin content

The determination of monomeric anthocyanins was carried out with a differential pH test. (18,19), 300.0 µL of each sample in cells into two groups, subsequently a sodium acetate buffer (pH 4.5) was added to one of the cell blocks and a potassium chloride buffer (pH 1.0) to the other block. They waited 15 minutes, and the readings were taken at two wavelengths (515 and 700 nm) in UV-VIS spectrophotometers. Subsequently, the absorbance was calculated following the equation:

$$A = (A_{515\,nm} - A_{700\,nm})_{pH:1.0} - (A_{515\,nm} - A_{700\,nm})_{pH:4.5}$$

Afterwards, the concentration of monomeric anthocyanins (MW: 449.2 g/mol, cyanidin-3-glucoside) in the original sample was calculated using the following equation, with dilution factor (DF) at 0.1, and molar extinction coefficient (ε) 26 900 L/mol cm, The results are expressed in mg/L of cyanidin-3-glucoside (C3G):

$$Monomeric\ anthocyanin\left(\frac{mg}{L}\right) = \frac{(A * MW * DF * 1000)}{\varepsilon \times 1}$$

2.6. DPPH

The DPPH scavenging test is carried out using the methodology (20) adapted to 96-well plate. 25 µL of the sample and 200 µL of a DPPH solution (initial absorbance 0.750) were placed in each well, for reference use 25 µL of the methanol and 200 µL of a DPPH solution. It was incubated for 40 minutes in the dark and the absorbance was measured with microplate reader with a 492 nm filter. The percentage of inhibition was determined by:

$$\%\ DPPH\ scavenging = \frac{Abs_{Rerence} - Abs_{Sample}}{Abs_{Reference}} x\ 100$$

2.7. FRAP

It was carried out using the methodology of (21). 60 mL of reagent, 5 mL of TPTZ (10 mM) was mixed with 5 mL of $FeCl_3$ (20 mM) in 50 mL of acetate buffer pH= 3.6; incubated at 37 °C for 7 minutes with shaking. 200.0 µL of sample and 1800.0 µL of reagent were added to each cell and incubated in the dark for 40 minutes. The absorbance was read in UV-VIS spectrophotometer at 593 nm.

2.8. Statistical data analysis

The analysis of normality and relationship of the data was carried out using Minitab 18.0 software using 95% confidence (p<0.05), using the Kruskal-Wallis test due to the nature of the data.

3. Results and discussion

The antioxidant substance content of the juices, 3 tests were addressed, determination of total phenols, total flavonoids, and monomeric anthocyanins (Table 1). It can be highlighted that in general the methanolic extract is the one with the highest concentration of phenols (4.036 ± 0.538 g GAE/L), flavonoids (0.817 ± 0.007 g QE/L), and anthocyanins (0.277 ± 0.033 g C3G/L). Which is explained by the Soxhlet method of obtaining, which is one of the conventional methods,

widely used to obtain extracts, due to its low cost, simple process and good yields if we compare it with some other techniques such as maceration (22,23) and the solvent used, due to the solubility of these compounds. Literature report cranberries contain many phenolic compounds, mainly those derived from the phenylpropanoid pathway, such as flavonols, anthocyanins, phenolic acids, proanthocyanidins, etc. (24).

Table 1 Results obtained from total phenols, flavonoids, and anthocyanins. Kruskal-Wallis test ($p<0.05$)

Sample	Total phenolics (g GAE/L)	Total flavonoids (g QE/L)	Total anthocyanidins (C3G g/L)
Fresh Natural juice	1.757 ± 0.269^{C}	0.265 ± 0.060^{B}	0.009 ± 0.003^{D}
Economic stratum commercial juice	2.926 ± 0.218^{B}	0.103 ± 0.015^{D}	0.007 ± 0.001^{D}
Intermedium stratum commercial juice	2.102 ± 0.189^{C}	0.184 ± 0.039^{C}	0.020 ± 0.001^{C}
Costly stratum commercial juice	2.935 ± 0.284^{B}	0.242 ± 0.034^{B}	0.027 ± 0.001^{C}
Fresh fruit methanol extract	4.036 ± 0.538^{A}	0.817 ± 0.007^{A}	0.277 ± 0.033^{A}
Fresh fruit water extract	1.212 ± 0.221^{D}	0.116 ± 0.004^{D}	0.041 ± 0.010^{B}
Positive control	0.1 mg/ml GA	0.1 mg/ml Quercetin	------

Therefore, considering what was expressed by commercial juices, similar results would be expected when comparing them with natural juice. What is not the case, since the one with the lowest number of total phenols is the natural juice, similar results were obtained in flavonoids and monomeric anthocyanins, this gives us a reason to question the origin of the antioxidant activity present in commercial juices. As reported by the manufacturers of the 3 commercial juices, in addition to cranberry, other added compounds are citric acid, malic acid, ascorbic acid, tannic acid, anthocyanins, sodium citrate, acesulfame potassium, among some other preservatives. All these compounds, in addition to their function as preservatives to extend the shelf life of products, function with an increase in antioxidant activity. (25,26), which explains the results obtained. Therefore, although the cranberry is providing the content of compounds beneficial to our health, this is overshadowed by the addition of external agent's characteristic of all these processed products. providing the content of compounds beneficial to our health, this is overshadowed by the addition of external agent's characteristic of all these processed products.

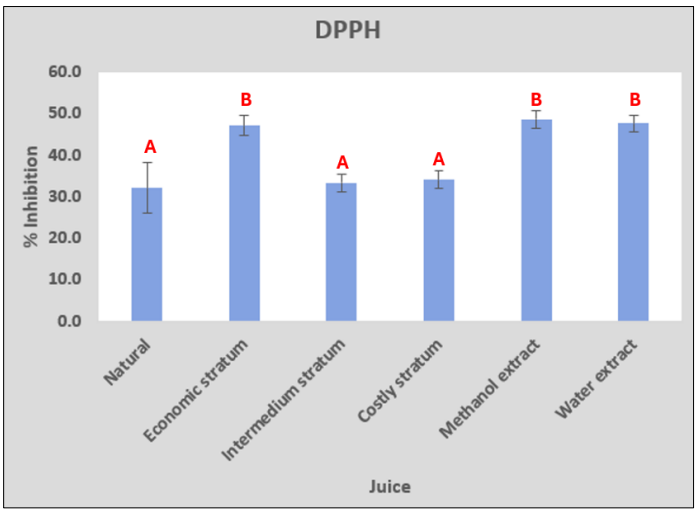

Figure 2 Determination of the antioxidant potential, with respect to the % scavenging of the DPPH free radical, of commercial juices, natural juice, and fresh fruit extracts

In the DPPH free radical scavenging test, two groups were obtained between which there is no statistically significant difference. In group one, the one with the best results, are the economic stratum juice and the two extracts (methanolic and aqueous). On this part, it is important to highlight that the two commercial juices (expensive and intermediate) and the natural one present similar result; so, despite their higher phenolic content (compounds with proven antioxidant activity), the inhibition of free radicals was not influenced. In addition to that, the fact that the inhibition is the same as

in natural cranberry juice tells us about two possible issues, the first is that the activity per se of the cranberry is being lost due to the industrial process and what we see is only of the additives or that there is no positive synergy between cranberry and the additives, in terms of antioxidant activity

Finally, the last aspect evaluated was the FRAP test, which determines the ability of a sample to resist oxidative stress through the cationic pathway (from Fe(III) to Fe(II)). In this part, the trend of the methanolic extract is maintained as the sample that stands out the most. On the other hand, those that obtain the worst results are the aqueous extract and the natural juice, which again questions the result obtained in commercial juices, since they present a better result than the natural juice associated with additives such as citric acid, malic acid, tannic acid and anthocyanidins, normally used. as stabilizers and/or preservatives.

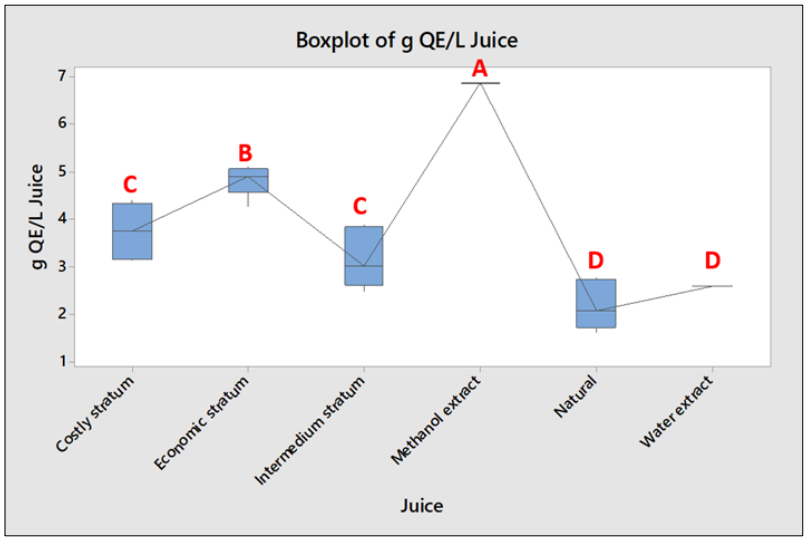

Figure 3 FRAP test by commercial, natural and extract blueberry juice. Kruskal-Wallis Test ($p<0.05$)

As established above, the price is not necessarily an indicator of quality in terms of the antioxidant activity of the commercial juice, however, in terms of antioxidant content, the commercial juices slightly dominate over the natural ones, this given the additives used as preservatives.

4. Conclusion

Cranberries have remarkable differences in their antioxidant capacity according to the extraction methods used. The results obtained show that the natural juice and aqueous extract of cranberries have a significantly lower antioxidant capacity compared to commercial juices and methanolic extract. This is reflected in the antioxidant capacity tests, such as FRAP (Ferric Reducing Antioxidant Power) and DPPH (2,2-diphenyl-1-picrylhydrazyl), where both commercial juices and the methanolic extract showed a higher capacity for hydrogen atom transfer. These findings suggest that cranberries contain a significant amount of antioxidant compounds that are not completely extractable water.

Compliance with ethical standards

Disclosure of conflict of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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