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Article

Total polyphenol content and antioxidant activity of commercial Noni (*Morinda citrifolia* L.) juice and its components

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The plant *Morinda citrifolia* L. (noni) has been the focus of many recent studies due to its potential effects on treatment and prevention of several diseases. However, there are few *in vivo* and *in vitro* studies concerning its composition and antioxidant capacity. The aim of the present study was to determine the total polyphenol content (TPC) and antioxidant capacity of a juice commercialized as noni juice, but containing grape, blueberry and noni fruits. Commercial noni juice was compared against its separate constituents of blueberry and grape juice. Folin-Ciocalteu and DPPH• methods were used to determine the concentration of total polyphenol content and antioxidant activity, respectively. Commercial noni juice presented higher values of TPC (91.90 mg of gallic acid/100 mL juice) and antioxidant activity (5.85 mmol/L) compared to its 5% diluted constituents. Concentrated blueberry juice presented higher TPC and antioxidant activity than the other juices analyzed. Considering that the blueberry and grape juices account for only 10% in the composition of commercial noni juice, it can be inferred that these two components contribute significantly to the antioxidant activity. Therefore, additional studies are necessary in order to elucidate the contribution of the noni juice as an antioxidant.

Uniterms: Noni juice/antioxidant activity. *Morinda citrifolia* L. Total polyphenols/determination. Antioxidants.

A planta *Morinda citrifolia* L. tem sido objeto de muitas pesquisas decorrente de seus efeitos benéficos no tratamento e prevenção de muitas doenças. No entanto, são escassos os estudos *in vivo* e *in vitro* sobre os compostos presentes e sua capacidade de atuar como antioxidante. Objetivou-se com este trabalho determinar o índice de polifenóis totais (IPT) e a capacidade antioxidante do suco de noni comercial, constituído de uva, mirtilo e a fruta do noni. O suco de noni comercial foi comparado com seus constituintes (mirtilo e suco de uva) separadamente. Os métodos Folin-Ciocalteu e DPPH• foram utilizados para determinar a concentração de polifenóis totais e capacidade antioxidante, respectivamente. O suco de noni apresentou valores superiores de IPT (91,90 mg ácido gálico/100 mL de suco) e atividade antioxidante (5,85 mmol/L) quando comparado aos seus constituintes diluídos a 5%. O suco de mirtilo, na sua forma concentrada, apresentou IPT e atividade antioxidante superior aos demais sucos. Considerando que os sucos de mirtilo e de uva representam apenas 10% na composição do suco de noni, pode-se inferir que estes dois constituintes contribuem de forma significativa para a atividade antioxidante. Assim, estudos adicionais são necessários para elucidar a contribuição do suco de noni como sequestrador de radicais livres.

Unitermos: Suco de noni/atividade antioxidante. *Morinda citrifolia* L. Polifenóis totais/determinação. Antioxidantes.

INTRODUCTION

Popular medicine has been using natural and herbal products for centuries in all cultures of the world. Scientific community and health professionals have shown an increasing interest in research investigating the therapeutic benefits related to these products. Among the widely used medicinal plants, is *Morinda citrifolia* L. (noni), which was discovered 2000 years ago by ancient Polynesians (Wang *et al.*, 2002; Zin, Hamid, Osman, 2002).

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Recently, this plant has been the focus of several studies due to its nutraceutical properties (Chan-Blanco *et al.*, 2006; Dixon *et al.*, 1999; Zin *et al.*, 2006). The beneficial health effects observed might result from specific compounds extracted from the roots, leafs, peel and fruit such as nitric oxide, alkaloids and sterols with antioxidant potential (Chan-Blanco *et al.*, 2006).

Studies on antioxidants have reported the use of isolated nutrients in the treatment and prevention of diseases. However, a large variety of compounds that can act synergistically in protecting cells and tissues are found in foods (Bianchi, Antunes, 1999).

Phenolic compounds such as flavonoids are the most active and common antioxidants present in fruit and vegetables (Nijveldt *et al.*, 2001). These compounds have an antioxidant function which result from a combination of chelating properties and scavenging of free radicals as well as inhibition of oxidases and other enzymes (Alonso *et al.*, 2004; Trueba, 2003).

According to Wang *et al.* (2002) juice of noni has been consumed as a medicinal alternative for many diseases such as arthritis, diabetes, high arterial pressure, headaches, AIDS, cancer, peptic ulcer, and others.

Given wide use of this fruit in an array of products in different countries, *in vitro* and *in vivo* studies elucidating its therapeutic and nutritional properties have become important.

Commercial noni juice is traditionally made by fermentation of noni fruits in sealed containers for 2 months at ambient temperature (Nelson, 2006). Fresh noni juice is made by direct squeezing of noni fruits (Nelson, 2006). Some noni juice is made by boiling of noni fruits for hours. Since noni is considered a fruit with a bitter taste, addition of sweet tasting components allow changes in organoleptic aspects, favoring the commercialization of noni juice.

Considering the increase in commercialization of this product and the lack of scientific studies regarding the composition of noni juice and its beneficial implications in treatment of diseases, the aim of the present study was to determine the total polyphenols content (TPC), and antioxidant capacity of commercial noni juice and its components.

MATERIAL AND METHODS

Samples

Samples of noni juice were acquired through a distributer from the southern region of Brazil. Because this juice comprises 5% blueberry juice and grape juice, these two products were also acquired in order to perform the present study. Blueberry juice was purchased at a store of natural products in the United States and grape juice from local markets.

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid 97%) (Sigma-Aldrich Chemical Co®) was used as the reference antioxidant and DPPH• (2,2-diphenyl-1-picrylhydrazyl) (Sigma-Aldrich Chemical Co®) was used as a free radical and both were used to determine the antioxidant activity. Total polyphenol content was determined using the Folin-Ciocalteu (Merck KGaA®) reagent.

Spectrophotometric readings were determined using a Shimadzu 1601® UV spectrophotometer. All analyses were carried out in the Food Science Laboratory in Nutrition of the University of Vale of Itajaí (UNIVALI), with the collaboration of the Center for Chemical and Pharmaceutical Research, UNIVALI - Itajaí (SC).

Total Polyphenol Content (TPC)

This method consists of determining the total polyphenol content through oxidation of phenolic compounds using a mix of phosphotungstic and phosphomolybdic acids in base medium, producing blue acids of tungsten and molybdenum. Absorbance of these acids was then read at 765 nm (Folin, Ciocalteu, 1927; Moyer *et al.*, 2002). Results were expressed in mg gallic acid/100 mL noni juice. Each assay was performed in triplicate.

Antioxidant Activity - DPPH• Method

This method is based on decreased absorbance of DPPH• radical read at 515 nm, due to the action of antioxidants. A 0.1 mL volume of the sample was added to 3.9 mL of DPPH• dissolved in methanolic solution and carefully homogenized. Free radical was freshly prepared before each assay while protected from light (Villaño *et al.*, 2007). Absorbance was read at 515 nm at different time intervals until the reaction reached stability. DPPH• concentration was determined by a calibration curve obtained by linear regression (Van Den Berg *et al.*, 1999).

Results were expressed inTEAC (Trolox-equivalent antioxidant capacity, mmol/L of sample). In order to determine the TEAC value, two dilutions were carried out in duplicate, considering four absorbance values for calculation. Data were interpolated on a Trolox calibration curve (absorbance variation *versus* Trolox concentration) and samples were suitably diluted in order to keep their values within the calibration curve. To determine the TEAC value of each assayed solution, the Trolox concentration was multiplied by dilution factor (Van Den Berg *et al.*, 1999).

Stability of phenolic compounds from noni juice during storage under refrigeration

Considering the indication of daily consumption of 30 – 60 mL of noni juice, the pack was maintained under refrigeration (4 °C) after opening, in order to evaluate possible changes in polyphenol composition during storage. Over a period spanning four weeks, the time taken to use about 1 L of noni juice, a sample of 30 mL was collected every week in order to determine the total polyphenol content, according to Folin, Ciocalteu (1927).

Statistical analysis

Results were expressed as mean ± standard deviation. Statistical analyses were carried out using Statistica software version 5.0 by applying ANOVA. Significant differences among samples were evaluated by Tukey's test at a significant level of 0.05.

RESULTS AND DISCUSSION

Total Polyphenol Content

Polyphenolic compounds have been implicated in antioxidant activity of fruits, vegetables and derived beverages (Trueba, 2003). Table I shows total polyphenol content (means±standard deviation) of noni, blueberry and grape juices.

Data contained in Table I shows that commercial noni juice presented higher values of TPC compared to blueberry and grape juice diluted at 5%. Considering that noni juice constitutes a blend of 5% of blueberry and grape juice, these two components represent 31.65 mg of total polyphenols. It can be inferred that noni juice corresponds to 65.56% of total polyphenols against 27.90% and 6.54% which represent the 5% blueberry and grape juice, respectively. Because noni is considered a fruit with a bitter taste, addition of sweet tasting components allow changes in organoleptic aspects, favoring the commercialization of noni juice.

Studies on the chemical composition of noni have reported that phenolic compounds are predominant in the juice, acting as free radicals scavengers and serving to prevent several diseases (Chan-Blanco *et al.*, 2006; Dixon, Mcmillen; Etkin, 1999).

Yang et al (2007) evaluated the polyphenol content and antioxidant activity of noni juice during storage at different temperature. At time zero, fresh noni juice presented polyphenol content of the 210 mg gallic acid/100 ml - a value higher than that found in the present study. According to the authors, the processing of the noni juice and storage influenced the polyphenol content and antioxidant activity. This could explain the lower results found in the present study.

Su and Silva (2006) evaluated polyphenol content in different kinds of blueberry products (juice, vinegar and wine) and reported that the highest content was found in processed juice from pulp, presenting 29.2 mg EGA/100 mL juice. In the present study, lower values were found (25.64 mg GA/100 mL juice). However, it should be noted that this occurred in 5 % diluted juice, presenting higher values than the study of Su and Silva (2006) when in its concentrated form.

Furthermore, Malacrida and Motta (2005), evaluating grape juice, reported higher values of total polyphenols (143 mg GA/100 mL juice) in original grape juice compared to reconstituted grape juice (105 mg GA/100 mL juice). According to the authors, high temperatures during flavor recovery, evaporation, type of extraction and reactions occurring during juice storage can influence total polyphenol content. Grape juice 5 % diluted used in the present study presented TPC of 6.01 mg GA/100 mL juice, a value similar to that found by Malacrida and Mottha (2005).

Table 1 shows the total polyphenol content of concentrated juices. It is evident that blueberry juice presented TPC five times higher than noni juice and four times higher than grape juice. However, no significant differences were observed (p>0.05) between grape and noni juices. Blueberry juice presented statistically significant (p<0.001) values when compared with grape and noni juices.

TABLE I - Means and standard deviation of Total Polyphenol Content of Noni, Blueberry and Grape Juices

Sample	**TPC*** (mg GA /100 mL juice)[‡]	**TPC**[†] (mg GA /100 mL juice)[‡]
Noni Juice	91.90 ± 0.52 [a]	91.90 ± 0.52 [a]
Blueberry Juice	25.64 ± 0.83 [b]	512.00 [b]
Grape Juice	6.01 ± 0.85 [c]	120.00[a]

* TPC: Total Polyphenol Content of commercial noni juice (5 % blueberry; grape juice) [†] TPC: Total Polyphenol Content of commercial noni juice (5% blueberry; grape juice) and its components in its concentrated form. Different letters in the same column indicate significant difference (P>0.05) [‡]Results expressed in mg gallic acid/100 mL juice.

TABLE II - Means and standard deviations of antioxidant activity of noni, blueberry and grape juices

Sample	**DPPH•** * (mmol/L juice) ‡	**DPPH•** † (mmol/L juice) ‡
Noni juice	5.85 ± 2.24 [a]	5.85 ± 2.24 [a]
Blueberry juice	1.72 ± 0.11 [b]	34.40 [b]
Grape juice	0.37 ± 0.02 [c]	7.40 [a]

* **DPPH•**: 2,2-diphenyl-1-picrylhydrazyl. Antioxidant activity of commercial noni juice and of 5% diluted grape and blueberry juices.† **DPPH•**: Antioxidant activity of commercial juice noni, blueberry and grape concentrated juices. Different letters in the same column indicate significant difference (P>0.05). ‡ Results expressed in mMol/L juice.

Antioxidant activity

With the increasing interest in function and diversity of antioxidants, some methods have been developed in order to determine this activity in food, beverages and biological samples (Dávalos, Bartolomé, Gómez-Cordovés, 2005).

Among chemical methods applied to determine the antioxidant activity of a compound, DPPH• (2,2-diphenyl-1-picrylhydrazyl) is one of the most used methods because it is practical, fast and stable (Espin *et al.*, 2000).

Mean values and standard deviations of antioxidant activity of noni, blueberry and grape juices are presented in Table II.

Noni juice (5.85 mmol/L) presented a higher TEAC value than 5 % diluted blueberry (1.72 mmol/L) and grape (0.37 mmol/L) juices. Regarding results of antioxidant activity of noni juice and its components, it was observed that 64.27 % of antioxidant activity could be represented by noni juice, and 29.40 % and 6.32 by 5 % diluted blueberry and grape juices, respectively.

Concerning antioxidant activity of concentrated blueberry juice, it can be inferred that this presented antioxidant activity 6 times higher than commercial noni juice and 4.5 times higher than grape juice. On the other hand, no significant differences were observed ($p>0.05$) between grape and commercial noni juices. Blueberry juice presented statistically significant ($p<0.001$) values compared to grape and noni juices.

Kuskoski *et al.* (2005, 2006) reported that samples rich in anthocyanins present the highest antioxidant activity. It is important to bear in mind that this correlation does not depend solely on concentration and antioxidant quality but also on its interaction with other compounds and the method used to determine the antioxidant activity.

Blueberry is considered a fruit rich in anthocyanins and antioxidants since it presents particularly high polyphenol content in peel and pulp which confer a protective function to cell wall (Kaur, Kapoor, 2001).

Frankel *et al.* (1998) and Vinson *et al.* (1999) reported that in grape juice, the antioxidant activity was similar to that found in red wines. Grape juice contains a higher content of glycosylated phenolic compounds than red wine which, according to Hollman *et al.* (1995), are more easily absorbed than the respective aglicons. On the other hand, the presence of ethanol in wine increases the absorption of phenolics since it prevents polyphenol precipitation in the digestive tract.

Nevertheless, consumption of grape juice as a source of phenolic compounds can present advantages over wine, in that the lack of ethanol allows juice to be consumed by the majority of people, children included, as well as by those with diseases such as hepatitis (Romero-Pérez *et al.*, 1999).

CONCLUSION

Commercial noni juice and its components can be considered good sources of polyphenols and antioxidants.

Grape and blueberry concentrated juices presented higher TPC and antioxidant activities than commercial noni juice, contributing significantly to the capacity of free radical scavenging, besides representing a lower cost option.

These results suggest that consumption of commercial noni juice, as well as its components, can have potential beneficial effects on heath. However, additional studies *in vitro* and *in vivo* are necessary in order to elucidate and identify other compounds that justify the use of noni juice as a free radical scavenger compared to its components.

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