http://dx.doi.org/10.1590/0100-2945-211/14

DIETARY FIBRE CONTENT, PHENOLIC COMPOUNDS AND ANTIOXIDANT ACTIVITY IN SOURSOPS (*Annona muricata* L.)[1]

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ABSTRACT - Healthy eating is associated with the consumption of fruits, which are notable for their beneficial effects on human health. The aim of this study was to evaluate the proximate composition, composition of fibers and components with antioxidant activity in soursops varieties Crioula, Lisa and Morada of physiological maturity (PM) and mature (M). The protein, lipid and moisture contents did not differ between soursop varieties, but the ash contents were higher in the Morada-PM (0.56%±0.03) and the Morada-M (0.82%±0.10) varieties. The Crioula-M variety showed higher levels of total dietary fibre (5.76%±0.12). The Lisa-M variety showed higher levels of insoluble dietary fibre (4.46%±0.00). The Lisa-M variety also showed a higher level of phenolic compounds (284.25 mg gallic acid/100 g of soursop pulp), differing significantly ($p < 0.05$) from the Crioula-PM soursop (154.40 mg of gallic acid/100 g of soursop pulp). Under the DPPH• system, the soursops that showed highest antioxidant activity were the Crioula-M (EC50 of 156.40 g.g $DPPH^{-1}$) and the Crioula-PM (EC50 of 162.41 g.g $DPPH^{-1}$), which differed significantly from the Morada soursops. The results suggest that the consumption of soursops is useful for increasing concentrations of bioactive compounds and dietary fibre.
Index terms: tropical fruit, dietary fibre, phenolics, antioxidant activity.

CONTEÚDO DE FIBRA ALIMENTAR, COMPOSTOS FENOLICOS E ATIVIDADE ANTIOXIDANTE EM GRAVIOLA (*Annona muricata* L.)

RESUMO - Alimentação saudável e equilibrada está associada ao consumo de frutas, visto que, frequentemente, apresentam elementos com efeitos benéficos à saúde humana. O objetivo deste estudo foi avaliar a composição centesimal, composição de fibras e componentes com atividade antioxidante em soursops variedades Crioula, Lisa e Morada em estádio de maturação fisiológica (PM) e madura (M).. Os conteúdos de proteínas, lipídios e umidade não diferiram entre as variedades de graviola, mas os teores de cinza foram maiores nas variedades Morada-PM (0,56% ± 0,03) e Morada-M (0,82% ± 0,10). A variedade Crioula-M apresentou níveis mais altos de fibra dietética total (5,76% ± 0,12). A variedade Lisa-M apresentou níveis mais elevados de fibra alimentar insolúvel (4,46% ± 0,00) e também maior nível de compostos fenólicos (284,25 mg ácido gálico/100 g polpa de graviola), diferindo significativamente ($p < 0,05$) da graviola Crioula-PM (154,40 mg ácido gálico/100 g polpa de graviola). Em sistema DPPH•, as graviolas que mostraram atividade antioxidante mais elevada foram Crioula-M (EC_{50} de 156,40 g.g $DPPH^{-1}$) e o Crioula-PM (EC_{50} de 162,41 g.g $DPPH^{-1}$), que diferiram significativamente das graviolas Morada. Os resultados sugerem que o consumo de graviola é útil para aumentar as concentrações de compostos bioativos e fibra dietética.
Termos para Indexação: fruta tropical, fibras alimentares, compostos fenólicos, atividade antioxidante.

[1](Trabalho 211-14). Recebido em: 21-07-2014. Aceito para publicação em: 27-02-2015.
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INTRODUCTION

The soursop (*Annona muricata* L.) is considered a good source of natural antioxidants, and all parts of the fruit are used in traditional folk medicine (BASKAR et al., 2007). The antioxidant activity of plant extracts (NUNES et al., 2012) and the mutagenic and antimutagenic properties of fruit, including frozen, have been investigated, and the data suggest that freezing fruits helps prevent biological damage (SPADA et al., 2008). The fruit contains carbohydrates, proteins, folic acid, calcium, phosphorus, iron, vitamin C, large amounts of vitamins B1 and B2 and fibre (DEMBITSKY et al., 2011).

In recent years, the term "functional food" has been attributed to foods containing some components that evoke specific physiological responses in addition to their nutritional functions and sensory acceptance (ZERAIK et al., 2010). The inclusion of fruits and their products in dietary recommendations requires that these foods provide appreciable amounts of vitamins, minerals and fibre. According to Nicklas et al. (2011), dietary fibre provides significant health benefits for adults, including reduction of the risk of coronary heart disease and type-2 diabetes and maintenance of a healthy body weight. It can be argued, therefore, that a diet rich in vegetables is associated with a longer life expectancy (MELO et al., 2008).

Evidence shows that the protective effects of some foods are associated with their antioxidant contents, such as vitamin C, vitamin E, carotenoids and phenolic compounds, which are the most abundant antioxidants in most plants (ZERAIK et al., 2010, Rocha et al., 2011). Antioxidants in food show the ability to modulate complex mechanisms involved in maintaining a healthy physiology, reducing the early onset of diseases related to degradation due to oxidative stress. These foods include mushrooms, mangos, tomatoes, lemons, oranges, orange kinkans (kumquats), tea, garlic, guavas, papayas, açai and soursops (ARUOMA et al., 2012).

The pulp of the soursop (*Annona muricata* L.) is consumed *in natura* or used in the preparation of foods, drinks and preserves, playing an important role in the nutrition and economics of its producing region (NUNES et al. 2012). Given this context, the current study aimed to characterise the Crioula, Lisa and Morada soursop varieties in terms of their dietary fibre contents and other components with antioxidant activity.

MATERIALS AND METHODS

The Crioula, Lisa and Morada Soursop (*Annona muricata* L.) varieties in natura were obtained from the Instituto Agronômico de Pernambuco (IPA) at different stages of maturation [Physiological maturity (PM); Maturity (M)]. Soursops were harvested using the disc collection method proposed by Livera (1992), and the following physical characteristics were observed:

PM: firm texture; matte, light green rind; resistant spines, prominent and slightly apart.

M: soft texture; bright, light green rind that is slightly yellowish; brittle spines, not very prominent and more distant.

Physicochemical characterisation

The soursops were analysed by AOAC methods (AOAC, 2006) with respect to the following physical and chemical parameters: moisture, ash, protein, lipid, total dietary fiber and insoluble dietary fiber content. Moisture (g/100 g) was determined by drying at 105 °C to a constant weight. Ash (g/100 g) was created by heating in an oven at 600 °C for 6 h. Protein (g/100 g) was analyzed according to the Kjeldahl method. Lipids (g/100) were calculated by weight loss after a 6-cycle extraction with petroleum ether in a Soxhlet apparatus. Total dietary fiber and insoluble dietary fiber were determined and expressed as g/100 g. Soluble dietary fiber was calculated by subtracting the insoluble dietary fiber proportion from the total dietary fiber. Total carbohydrate was calculated by difference, rather than analyzed directly. This is referred to as total carbohydrate by difference and is calculated by the following formula: 100 − (weight in grams [protein + fat + water + ash] in 100 g of food). It should be clear that carbohydrate estimated in this way includes fiber. Moreover, the available carbohydrate was also calculated by difference; the amount of dietary fiber is analyzed and subtracted from total carbohydrate, thus: 100 − (weight in grams [protein + fat + water + ash + dietary fiber] in 100 g of food).

Quantification of phenolic compounds

Hydroacetone and hydromethanolic extracts were obtained with a sequential extraction procedure using 80% acetone and 80% methanol. In this system, 20 g of the pulp was continuously stirred at a temperature of 24 °C±2 °C for 20 minutes with acetone (80%), and then the sample was centrifuged at 4000 rpm. The supernatant was collected and the precipitate was resuspended in the same solvent and subjected to the same process described above for another two periods of 20 minutes, totalling 60 minutes of extraction. At the end of the extraction period, the supernatants were combined and concentrated under reduced pressure at 40 °C, and the final measured volume was 50 mL. The precipitate was reused for extraction with methanol (80%) under the conditions described above. The extracts obtained were stored in covered containers and kept in a freezer (-18°C) until analysis.

The assessment of the phenolic content of the extracts was performed using the spectrophotometric method with the Folin-Ciocalteau reagent (Merck), as described by Wettasinghe and Shahidi (1999). Total phenolic content was determined by interpolating the absorbance of the samples against a calibration curve constructed with gallic acid (20-240 µg/mL), and the results are expressed in mg of gallic acid equivalent per 100 g of soursop pulp.

Measurement of Antioxidant Activity

The hydroacetone and hydromethanolic extracts were combined and subjected to analysis of the radical scavenging ability of 1,1-diphenyl-2-picrylhydrazyl (DPPH•) using the method described by Brand-Williams et al. (1995) and modified by Sánchez-Moreno et al. (1998). The combined extracts with different concentrations of phenolic compounds were added to the solution of DPPH• in methanol (0.1 M), reaching a final concentration of 7 g/L, 13 g/L and 21 g/L of soursop pulp. The absorbance at 515 nm was monitored in a spectrophotometer (Shimadzu UV-1650PC) until the reaction plateaued. The concentration of DPPH• remaining in the reaction medium was calculated from the standard curve for DPPH•, and the percentage of the remaining DPPH• ($DPPH_{REM}$%) concentration of each extract was calculated using the following expression: $\%DPPH\bullet_{REM} = [DPPH\bullet]_T / [DPPH\bullet]_{T/0}$, where, $DPPH_T$ is the concentration of the DPPH radical at the time the reaction reached a plateau, and $DPPH_{T/0}$ is the initial concentration of DPPH (reaction time zero).

Then, the extract concentration required to reduce the initial concentration of DPPH• (EC_{50}) by 50% was calculated using the graph of the concentration of the sample (g fruit. DPPH g^{-1}) versus $DPPH_{REM}$%, and the result was expressed in g of fruit per g DPPH•. The kinetic behaviours of the extracts were classified according to the time needed to reach a steady-state EC_{50} value (TEC_{50}), defined as rapid (TEC_{50} <5 minutes), intermediate (TEC_{50} = 5-30 minutes) and slow (TEC_{50} > 30 minutes). The (AE) was calculated considering the EC_{50} value and the time it took to reach the EC_{50} value (TEC_{50}) and was classified as low (AE <1), medium (AE> 1 and ≤ 5), high (AE > 5 to ≤ 10) or very high (AE> 10) according to the value obtained from the following expression (SÁNCHEZ-MORENO et al., 1998): $AE = 1 / EC_{50} \times TEC_{50}$.

Statistical analysis

The data were statistically analysed using analysis of variance (ANOVA) and the Kruskal-Wallis test, and a significance level of 5% was adopted. Bioestat ® 1.0 was used for the analyses.

RESULTS AND DISCUSSION

The most relevant macronutrients in the soursop fruit (*Anonna muricata* L.) are carbohydrates, including dietary fibre (Table 1), which collaborate with rheological properties of food (NWOKOCHA; WILLIAMS, 2009). The results of the proximate composition analyses of the Crioula, Lisa and Morada soursop varieties show that the varieties in the maturation stage were not significantly different ($p < 0.05$) in terms of protein, lipid and moisture content (Table 1). Concerning ash content, it was observed that the Morada variety in both maturity stages was higher than in the other varieties ($p < 0.05$).

The results of the chemical composition analysis of soursops are similar to those described by Sousa et al. (2011) for soursop pulp, whose composition is 83.16% moisture, 0.48% ash, 1.09% protein, 2.28% fat and 12.99% carbohydrate. Similar values were also described by Pareek et al. (2011), who found the composition of soursops to be 81% moisture, 1% protein, 0.6% fat, 17.25% carbohydrate and 0.6% ash. These variations, although very small, can be explained by differences in the maturation stages and varieties of soursops analysed in each study, as well as to environmental conditions related to the production of fruit.

A significant difference in total dietary fibre content was observed between the Lisa variety at physiological maturity (Lisa-PM), which had the lowest total dietary fibre content ($p < 0.05$), and the mature Crioula-M soursop, which had the highest concentration of total fibre. Regarding insoluble fibre, only the Lisa soursop variety showed differences between the two maturation stages, the percentage of insoluble fibre was higher in the ripe fruit. All of the varieties were similar ($p < 0.05$) in soluble fibre composition (Table 1), among mature soursops or between soursops in the physiological maturity.

The total dietary fibre found in soursops in the current study was equivalent to that found by Guerra et al. (2004) and Enweani et al. (2004):

4.31% and 4.33%, respectively. However, the data collected by Lima (2011) show that the soursop contains 1.9% fibre. Salgado et al. (1999) argue that variations in the fibre content of fruits can be attributed to various factors, such as cultivar-analysed sample representativeness, growing conditions, and analytical methods, among others.

Traditionally, dietary fibre is defined as the portions of plant foods that are resistant to digestion by enzymes. The fibre has been classified as soluble and fermentable in the colon and insoluble fibre, which has a thickening action but may be fermented to a limited extent in the colon. It is worth noting that most prebiotics are non-digestible carbohydrates that are fermented in the colon (ANDERSON et al., 2009). Inulins and fructooligosaccharides act selectively to stimulate the growth of bifidobacteria, which are considered beneficial for human health (WAITZBERG et al., 2012).

This information about fruit is examined against other relevant effects of each type of fibre. Insoluble fibre is not slimy: it is weak and slowly fermentable in the colon, acting mainly in the large intestine and contributing to increased faecal volume due to water retention, reducing intestinal transit time, the delay of glucose absorption and the hydrolysis of starch. Insoluble fibre has a high water retention capacity and has the property of forming gels in aqueous solutions. Once in the stomach and small intestine, soluble fibre increases the viscosity of the bolus, decreasing the activity of some digestive enzymes and directly influencing the rate of digestion and the absorption of nutrients. This influence is directly linked to the moderation of postprandial glucose and the insulin response, lowering cholesterol and regulating appetite (MIRA et al., 2009).

Soursops are also considered an important source of functional compounds such as phenolic compounds. The varieties of soursops studied showed no significant differences ($p < 0.05$) in total phenolic compounds between the different stages of maturation, but the Lisa-M variety showed a significantly ($p < 0.05$) higher content of phenolic compounds (284.25 mg of gallic acid/100 g of soursop pulp), than the Crioula-PM variety (154.40 mg of gallic acid/100 g of soursop pulp), as shown in Table 2. Similarly, Souza et al. (2012), found that soursop pulp had 281.0 mg gallic acid/100 g, although a lower value was found by Kuskoski and Asuero (2006), who observed 84.3 mg of gallic acid/100 g of soursop pulp.

Tiburski et al. (2011) claimed that phenolic acids and flavonoids, while not essential to human survival, may provide protection against chronic diseases with long-term consumption because they are associated with antioxidant effects. However, Melo et al. (2008) stated that the antioxidant capacity of a fruit cannot be explained solely based on its total phenolic content. Therefore, the antioxidant capacity of soursops was evaluated and expressed using the EC_{50} value, which represents the extract concentration necessary to reduce the initial concentration of DPPH• by 50%, and further parameters were observed as well: time to reach the steady state EC_{50} (TEC_{50}) and antiradical efficiency (AE), the latter of which is a parameter that combines both factors.

The EC_{50} values of TEC_{50} and the EA of the Crioula, Lisa and Morada soursops are shown in Table 2. The samples of the Crioula soursop in the two ripening stages showed the lowest EC_{50} values, differing significantly ($p < 0.05$) from the Morada variety. Villaño et al. (2007) stated that the EC_{50} data are inversely related to the antioxidant activity of a compound, expressing the amount of antioxidants necessary to decrease the concentration of radicals by 50%; therefore, the smaller the $EC_{50,}$ the higher the activity of the antioxidant compounds.

As for the time needed to reach EC_{50} (TEC_{50}) and antiradical efficiency (AE), only the Morada-PM soursop showed kinetic behaviour above the 5-minute intermediate reaction speed, showing DPPH•, while the other samples showed rapid speeds. Different results were found by Melo et al. (2008) and Souza et al. (2012), who found in intermediate antioxidant activity in soursops. All of the varieties of soursops studied, regardless of maturation, showed low efficiency (AE <1), with no significant differences ($p < 0.05$) between samples (Table 2).

The results of this study suggest that the consumption of soursops can be useful for increasing the concentrations of biologically active substances in the body. Several studies have shown that soursops are easy to produce and commercialize and have excellent sensory acceptance, a beyond-adequate nutritional profile and claims to functional and therapeutic benefits.

TABLE 1- Mean values for the physicochemical composition of soursops (*Annona muricata* L., Crioula, Lisa and Morada varieties) in Physiological Maturation (PM) and Maturation (M)* stadium.

	Crioula-PM	Crioula-M	Lisa-PM	Lisa-M	Morada-PM	Morada-M
Moisture (%)	79.70±1.10a	80.48±0.44a	80.66±0.55a	81.14±0.33a	79.34±0.34a	80.57±0.66a
Ash (%)	0.07±0.01a	0.13±0.01a	0.07±0.01a	0.16±0.01a	0.56±0.03b	0.82±0.10b
Protein (%)	1.02±0.02a	1.05±0.03a	1.09±0.06a	1.10±0.02a	1.04±0.03a	1.03±0.02a
Lipid (%)	0.00±0.00a	0.01±0.01a	0.00±0.00a	0.01±0.01a	0.00±0.00a	0.01±0.00a
Total dietary fibre (%)	4.51±0.64ab	5.76±0.12a	3.72±0.22b	5.69±0.08ab	4.40±0.18ab	5.43±0.11ab
Total carbohydrate (%)**	14.70±1.57a	12.57±0.39ac	14.47±0.30a	11.91±0.32bc	14.66±0.58a	12.14±0.67bc
Available carbohydrate (%)**	10.19±2.14ab	6.81±0.37ac	10.74±0.09b	6.22±0.31c	10.25±0.75abd	6.71±0.56acd
Insoluble dietary fibre (%)	3.55±0.61ab	3.92±0.03ab	2.78±0.00a	4.46±0.00b	3.46±0.00ab	3.65±0.06ab
Soluble dietary fibre (%)	0.96±0.08a	1.84±0.11b	0.94±0.22a	1.23±0.08b	0.94±0.18a	1.77±0.06b

[a,b,c,d] The means within a line followed by different superscripts are significantly different at p <0.05 by Kruskal-Wallis test.
* The mean of three replicates.
** Calculated by difference

TABLE 2- Mean values for the phenolic compounds and antioxidant activity in soursops (*Annona muricata* L., varieties Crioula, Lisa and Morada) in Physiological Maturity (PM) and Maturity (M)* stadium.

	Crioula-PM	Crioula-M	Lisa-PM	Lisa-M	Morada-PM	Morada-M
Phenolic Compounds (mg gallic acid/100g)	154.40 a	188.55ab	284.25ab	358.92 b	217.10ab	264.65ab
EC_{50} (g fruit. DPPH g^{-1})	162.41 a	156.40 a	184.44ab	179.25ab	219.06 b	212.08 b
T_{EC50} (min)	4.40ab	3.70ab	3.25ab	2.83 a	6.71 b	4.70ab
Kinetic Classification	Rapid	Rapid	Rapid	Rapid	Intermediate	Rapid
Antiradical Efficiency	0.003 a	0.002 a	0.002 a	0.002 a	0.001 a	0.001 a
Classification Antiradical	Low	Low	Low	Low	Low	Low

[a,b] The means within a line followed by different superscripts are significantly different at p < 0.05 by Kruskal-Wallis test.
* The mean of five replicates.

CONCLUSIONS

The soursops analysed in this study had high moisture contents and high concentrations of carbohydrates. The soursops showed no significant differences between varieties or maturation stages in dietary fibre content. The Lisa variety had a higher concentration of phenolic compounds, but the Crioula variety showed the greatest potential for antioxidant activity, as it had lowest EC_{50} value. The bioactive compounds and dietary fibre present in soursops, alone or combined, can offer protection and improved quality of life by reducing the development of morbidity resulting from oxidative processes.

ACKNOWLEDGEMENTS

The authors wish to thank the CNPq for its financial support and the Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) for the scholarships it provided.

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