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Nutritional, antinutritional and phytochemical status of okra leaves (*Abelmoschus esculentus*) subjected to different processes

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The aim of this study was to analyze the nutritional quality and antinutritional factors of okra leaves subjected to different process. Proximate composition, calcium, magnesium, potassium were determined, as well as lectin, tannin, saponin and total phenolic compounds. The okra leaves showed a predominance of carbohydrates, fibers and proteins that were not significantly affected by process, being also considered source of calcium, magnesium and potassium. Extracts obtained from bleached, cooked, lyophilized leaves and from 30% fraction, buffer showed the presence of lectin. The tannin contents found were 3.39% in lyophilized leaves, 0.45% in fresh leaves, 0.44% in bleached leaves and 0.27% in cooked leaves. The presence of saponin was not detected. The extract showed content of phenolic compounds of 19.27 mg of GA/g. The okra leaves can be included in human diet as a nutritionally suitable food.

Key words: *Abelmoschus esculentus*, antinutritional factors, minerals, nutritional quality.

INTRODUCTION

The world population growth has led to an increase in nutritional deficiencies and diseases related to the lack of essential nutrients in human diet, particularly affecting vulnerable populations. One of the world's greatest challenges is to secure sufficient and healthy food for all, and to do so in an environmentally sustainable manner.

In order to reduce these conditions, the attention has been increasingly focused on exploring non-conventional food sources that provide nutritional and pharmaceutical benefits, highlighting dark-green leafy vegetables, good sources of minerals and vitamins (Raju et al., 2007; Burchi et al., 2011). Vegetables have gained prominence

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because they are sources of bioactive compounds, and are important sources of ingredients for use as functional foods (Nithiyanantham et al., 2012). The use of okra leaves (*Abelmoschus esculentus*) as vegetable popularly known as okra, belonging to the family Malvaceae, widely distributed in Africa, Asia and America is among the possible alternatives (Doreddula et al., 2014). World production of okra as fresh vegetable is estimated at 6 million t/year (Sergius and Esther, 2014); are commonly used both as food as salad fresh or cooked and for curative purposes, showing low calories, a good source of edible fiber,contains important bioactive compounds such as carotene, folic acid, thiamine, riboflavin, niacin, vitamin C, oxalic acid and amino acids (Roy et al., 2014). Besides, the nutritional properties mentioned above, some authors have reported a variety of functional activities attributed to this fruit such as anti-diabetic, anti-hyperlipemic, anti-inflammatory, anti-fungal and antioxidant (Khomsug et al., 2010; Doreddula et al., 2014). The antioxidant activity of the fruit is due to its content of phenolic compounds, which are effective antioxidants and can be used in the prevention of degenerative processes such as cancer, cardiovascular diseases and diabetes (Doreddula et al., 2014).

Moreover, vegetal sources may contain substances harmful for human health, affecting the bioavailability of nutrients. Among these substances, lectins, tannins and saponins stand out. However, to use plant leaves as an alternative source of nutrients, it is necessary to study them for the presence of antinutritional factors and how to inactivate them for subsequent safe consumption. Studies on the nutritional characteristics and phytochemical compounds of okra leaves are scarce. In this sense, in order to introduce the market a new plant product, a study on the nutritional quality and presence of phytochemical compounds in okra leaves *(Abelmoschus esculentus)* subjected to different treatments was carried out, aiming its use for human consumption.

MATERIALS AND METHODS

Samples

Okra leaves (*A. esculentus*) were grown in the municipality of Sapé, state of Paraiba, Brazil. A total of 6 kg of okra leaves were rinsed thoroughly in running water and distilled water and dried at room temperature. Samples of fresh leaves were separated for the following thermal treatments: blanching for 2 min in boiling water and cooking for 20 min in boiling water. Cooked, bleached or fresh leaves were dried at 25°C and stored. Another portion of the leaves were separated for lyophilization at temperature of -36°C and pressure of 300 mmHg, and ground in a Willey-type electric mill, obtaining fine flour.

Proximate composition and minerals

Moisture, ash, proteins and lipid determinations were performed according to AOAC (2006). The magnesium (Mg), potassium (K) and calcium (Ca) concentrations were determined by atomic absorption spectroscopy from the ash solution (AOAC, 2006). All analyzes were performed in triplicate.

Phytochemical analysis

Fresh, bleached, cooked and lyophilized leaf samples were crushed and submitted to extraction in Tris-HCl 0.1 M pH 7.4 with 0.15 M NaCl under stirring for 3 h at 25°C. The suspension obtained was centrifuged at 5000 rpm at 4°C for 20 min and the precipitate was discarded. The supernatant, called total extract, was filtered and submitted to hemagglutinating activity assay. The filtrate of the lyophilized sample was submitted to precipitation with ammonium sulfate $(NH_4)_2SO_4$ using the saturation range: 30%. After saturation with ammonium sulfate, the solution was left to rest for 8 h and centrifuged at 5000 rpm at 4°C for 20 min. The fraction obtained was dialyzed and lyophilized, and then submitted to hemagglutination activity, in which human erythrocytes types A, B and O were used, provided by the Blood Center of Paraíba and rabbit erythrocytes, obtained from the Experimental Laboratory of the Department of Molecular Biology, UFPB. The hemagglutinating activity of the different treatments was determined by double serial dilutions and the presence of HA were macroscopically determined overnight. The lectin specificity was determined by inhibition with sugar, and was macroscopically viewed (Soares et al., 2012). Lectin of fraction 30% (2 mg/mL) was initially diluted in 50 ml of 0.1 M Tris-HCl pH 7.4; 0.15 M NaCl buffer, separate into two equal aliquots and submitted to pH from 2.0 to 13.0. Then, the mixtures were incubated in 37°C for 30 min and the hemagglutinating activity was performed with rabbit erythrocyte at 3% by double-serial dilution. The resistance of lectin from okra leaves (*A. esculentus)* was assessed against trypsin-like proteolytic enzyme. The effect of the β-mercaptoethanol reducing agent on the hemagglutinating activity and the denaturing agent urea was assessed (Soares et al., 2012). To assess the effect of temperature on the lectin present in 30% fraction of okra leaves (*A. esculentus*), 2 mg/mL was used. The solution was aliquoted and submitted to heating in a thermal cycler with temperatures ranging from 40 to 100°C. Every 10 min corresponding to a variation of 10°C, an aliquot of 100 µL was collected and the hemagglutinating activity was determined as previously described. The sample was tested in duplicate and in serial dilution.

Tannin levels were determined in bleached, cooked and lyophilized okra leaves (*A. esculentus*) according to procedure recommended by F to D method (Bubba et al., 2009). Saponin levels were determined using an agar solution buffered with 25 mM bibasic phosphate pH 7.4. About 5 mL of agar solution were added to 0.5 ml of rabbit erythrocytes at 3%, homogenized and placed in a Petri dish to solidify. After solidification, 20 and 40 µL of samples were added in wells. The result was observed within 30 min and overnight by the presence of a hemolytic halo. As positive control, the fraction of *Luffa operculata* seeds presenting hemolytic halo was used.

The total phenolic compounds were determined using the Folin-Ciocalteau reagent with gallic acid as stock solution and the reading was performed in UV / VIS at 765 nm (Vallverdú-Queralt et al., 2011).

Statistical analyses

The results were submitted to statistical tests, using the Assistat software version 7.6 beta. In order to meet the methodological assumptions of parametric tests and obtain consistent results, the sample homogeneity and the Kolmogorov - Smirnov (KS) normal distribution tests were applied. Analysis of variance (ANOVA) for multiple comparisons was also applied where $p \leq 0.05$ was set to indicate statistical significance with the Tukey's t test .

Table 1. Proximate composition and Levels of minerals of okra leaves (*Abelmoschus esculentus)*, wet basis.

Parameters evaluated	Lyophilized leaf	Fresh leaf	Bleached leaf	Cooked leaf
Moisture (%)	9.13±0.19[c]	74.83±1.17[b]	73.31±0.38[b]	81.53±0.11[a]
Lipids (%)	2.00 ±0[a]	0.93±0.18[b]	1.00±0.16[b]	1.07±0.18[b]
Protein (%)	23.62±0.23[a]	6.10±0.61[b]	6.22±0.23[b]	6.06±0.39[b]
Ashes (%)	15.39±0.08[a]	2.75±0.44[b]	2.84±0.84[b]	2.49±0.40[b]
Calcium (mg/100 g)	691±0.22[a]	382.50±0.71[b]	357±0.43[c]	366.50±0.42[d]
Magnesium (mg/100 g)	438±0.64[a]	232.50±0.31[b]	237.50±0.18[c]	138.50±0.66[d]
Potassium(mg/100 g)	670.50±0.55[a]	167.50±0.68[b]	110.5±0.86[c]	63±0.91[d]

Means followed by different letters between columns indicate significant differences with an error probability of p ≤ 5%, according to the Tukey's t test.

RESULTS AND DISCUSSION

Proximate composition and minerals

The proximate composition of lyophilized, fresh, bleached and cooked okra leaves are shown in Table 1. The cooking process was able to significantly increase the moisture content by 6% compared to the fresh leaf due to the swelling of plant cells. There was no significant difference in the protein, lipids and ash contents between fresh, bleached and cooked leaves. The moisture content in the fresh leaf (74.83%) found in this study corroborates value found in literature (Effiongh et al., 2009), of 78.83%, assessing fresh okra leaves. In fresh leaves of *Petroselinum crispum*, *Anethum graveolens*, *Lactuca sativa* and *Brassica oleracea*, Caunii et al. (2010) found levels higher than those found in this work; 87.71, 85.95, 94.91 and 92.52 %, respectively. The leaves of okra had lower lipid content and similar ash content compared to okra seeds, which showed lipid content 28 to 31% and ash 3.42% (Adelakun et al., 2012), indicating that the chemical composition varies according to the examined part of the plant. Mensah et al. (2008) found low proteins in fresh leaves *Amaranthus cruentus* (4.6%). Caunii et al. (2010) analyzed fresh *L. sativa* and found values of 1.62% protein. Singh et al. (2001) found higher protein values in coriander and spinach leaves; 22.2 and 26.5%, respectively. Effiong et al. (2009) studied fresh okra leaves and found values twice greater than those found in this study, 13.75%; variations in macronutrients within the same species are a reflection of soil, climate and irrigation conditions of vegetables during cultivation. The calcium, magnesium and potassium contents were also higher in lyophilized leaves due to the concentration of nutrients found in dehydrated products, while the cooking process reduced these levels due to the solubilization of minerals in the cooking water, and it is important to use the cooking water for other culinary preparations. The calcium and magnesium contents in fresh, bleached, cooked and lyophilized okra leaves account for more than 15% of the recommended daily intake (RDA) for these minerals, which are 800 and 300 mg, respectively, being then considered food sources of these minerals (IOM, 2000).

The seeds of okra have a lower content of calcium, magnesium and potassium (Adelakun et al., 2012) in comparison to the leaves of okra. The magnesium contents were higher when compared to other leafy vegetables such as coriander, spinach, amaranth and carrot leaves, which values are 3.7 ± 0.04, 10.2 ± 0.05, 3.1 ±0.04 and 1.8 ± 0.01 mg/100 g, respectively (Singh et al., 2001). Caunii et al. (2010) found for lettuce, calcium, potassium and magnesium contents of 36, 45 and 6 mg/100 g, respectively. The calcium and magnesium contents in fresh leaves are very close to those found in a similar study (Effiongh et al., 2009), of 321.00 ± 0.88 for calcium, 180.00 ± 2.30 for magnesium; however, for potassium, there is value well above that found in this study and in other studies with leaves, of 1128.1 ± 2.19 mg/100 g.

Phytochemical analysis

The lectin present in the extracts of bleached, cooked, lyophilized leaves and in the fraction 30% has specificity for sugars present in the membrane of rabbit erythrocytes, not recognizing membrane carbohydrates present in ABO human blood system. The analyses showed that the lectin present in extracts was inhibited by mucin (625 mM). The presence of lectin was not detected in fresh leaves due to the high mucilage content in which this protein is complexed, because the lectins are proteins that are capable of specifically and reversibly binding to carbohydrates in their mono- or oligosaccharide forms, thereby agglutinating cells and precipitating the oligosaccharides and glycoproteins (Soares et al., 2012), however, with application of the thermal treatment, it was observed that, lyophilized okra leaves had a higher specific hemagglutination activity, of 11.14 UH / mgP, followed by fraction 30%, with 4.77 UH / mgP, bleached leaves of 2.57 UH / mgP and cooked leaves of 2.10 UH /

mgP. Similar results obtained by Seena et al. (2006) and Leite et al. (2009) in cooking causes the reduction of specific hemaglutination activity in plants. Lectin of fraction 30% of okra leaves was inactivated at 100°C in a time of 30 min. As for the stability against different pH, the lectin found in okra leaves was stable in acidic pH (1 to 6), with maximum activity at pH 7 and inactivation at pH 12 and by the action of agents such as beta mercaptoethanol in concentration of 20 mM and by denaturing agent urea. Similar results for this study were reported by Cheung et al. (2010), that evaluated the lectin from *Musa acuminata* remains functional within the pH range from 1 to 13 and rated by Yao et al. (2010), that evaluated the lectin from *Setcreasea purpurea* as capable of promoting clustering at extreme pH values, with 100% hemagglutination within pH range from 5 to 9. Lectins from *Hypnea cervicornis*, *Craniela australiensis* and *Aspergillus nidulans* are specific to mucin glycoprotein (Shing et al., 2011). Leite et al. (2009) studied the presence of lectin in fresh, bleached and cooked *Amaranthus* leaves and detected the presence of lectin only in fresh leaves. The presence of saponins in fresh, bleached, cooked and lyophilized leaves was not detected because there was no hemolysis of erythrocytes. Ee and Yates (2013) in experiments with wattle *Acacia saligna* seeds observed the presence of saponin in raw seeds (3.0 $g.100^{-1}$) and after cooking for 10 min (1.06 $g.100^{-1}$) and that heat processing is usually applied to the seeds before consumption to eliminate antinutritional factors, such as protease inhibitors, lectins, alkaloids, saponins and oxalates, which can interfere with the digestion and absorption of nutrients.

The analysis of the presence of tannin in fresh okra leaves found values of 0.44 ± 0.02% tannic acid equivalent and there was no significant difference when leaves were bleached (0.45 ± 0.05%); however, the cooking process reduced the tannin content (0.27 ± 0.06%) to acceptable levels. Astringent and antinutritional functions are assigned to tannins; the latter, if ingested in large amounts. The analysis of the presence of tannin in fresh okra leaves found values of 0.44 ± 0.02% tannic acid equivalent, lower than that reported in literature, of 1.2% in fresh okra leaves (Singh et al., 2001). Leite et al. (2009) investigating this component in *Amaranthus* leaves observed significant increase from fresh (1.07%) to bleached leaves (2.79 %). Ferreira et al. (2008) found higher concentrations of tannins and saponins in *Moringa oleifera* leaves compared to those determined in the present study. The content of total phenolics in *A. esculentus* extract was 19.27 ± 0.9 mg GAE/g. The content of total phenolics in *A. esculentu* was lower than that found in other leaves; *T. brasiliensis* (38.53 ± 0.63 GAE/g)), *C. macrophyllum* (66.14 ± 3.56 GAE/g) (Sousa et al., 2007); *B. crassifolia* (35.93 mg GAE/g), *I. edulis* (24.50 mg GAE/g) (Pompeu et al., 2012), but higher than values found in fresh okra leaves (less than 0.15 mg GAE/g), seeds (2.13 µg/g) and root (0.55 µg/g) (Leiote et al., 2009), in *Cosmos caudatus* (18.83 mg GAE/g), *Centella asiatica* (7.79 mg GAE/g) and *Oenanthe javanica* leaves (7.41 mg GAE/g) (Huda-Faujan et al., 2009). For some derivatives of phenolic acids, antioxidant activity has been reported, except for those of high molecular weight, such as tannins.

Conclusion

The heat treatment showed the absence of saponins, inactivation of lectins, reduced levels of tannins and maintained the protein, lipids and minerals similar to fresh leaves. The lyophilization was also an appropriate processing for conservation of nutrients in okra leaves. Fresh, lyophilized and heat-treated leaves of okra showed a source of calcium and magnesium, and furthermore antioxidant activity can be included in the human diet as a nutritionally adequate food.

Conflict of interests

The authors did not declare any conflict of interest.

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