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ORIGINAL ARTICLE

Assessment of Bioactive Compounds, Physicochemical Composition, and In Vitro Antioxidant Activity of Eggplant Flour

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Abstract

Background: The eggplant (Solanum melongena) is a fruit of world consumption. Its processing in the form of flour is a way to avoid losses and to take advantage of its nutritional characteristics.

Objective: This study assessed the physicochemical composition (moisture, proteins, lipids, crude fiber, carbohydrates, minerals, niacin, saponins, titratable acidity, dietary fiber, and total phenols) of eggplant flour prepared from the whole fruit dehydrated in an oven.

Methods: In vitro antioxidant activity was assessed using the following methods: 2.2-diphenyl-1-picrylhydrazyl radical (DPPH); Ferric Reducing/Antioxidant Power (FRAP); and analysis of polyphenols using HPLC (chlorogenic acid, caffeic acid, ferulic acid, and rutin).

Results: It was possible to observe: 23.09% carbohydrates; 13.34% proteins; 1.85% lipids; 39.19% total fibers; 1,540 mg/100 g total soluble phenolic compounds; 840 mg/100 g saponins; minerals (potassium, magnesium, copper, iron, zinc, manganese); and niacin. In vitro antioxidant activity was observed through DPPH (455.6 mg ascorbic acid/100 g) and FRAP (486.8 mg ascorbic acid/100 g). The HPLC method determined the presence of ascorbic acid, tyrosine, and phenolic acids (chlorogenic acid, caffeic acid, and ferulic acid).

Conclusion: The eggplant flour had great fiber content in addition to good content of phenolic compounds and saponins with important antioxidant capacity observed through in vitro assays. As a result, eggplant flour is a good addition to the diet of the population, since it can bring potential health benefits. (Int J Cardiovasc Sci. 2017;30(3):235-242)

Keywords: Solanum Melongena; Antioxidants; Diet, and Nutrition; Phenolic Compounds; Hyperlipidemias.

Introduction

The eggplant (*Solanum melongena*, L.) is a fruit consumed worldwide and commonly grown in subtropical and tropical regions. It originated in India and was introduced in Brazil by the Portuguese in the 16th century.[1] It is currently grown by small-scale producers in practically all the Brazilian territory; however, eggplant production suffers heavy losses during the harvest period due to oversupply.[2]

The eggplant is a good source of minerals and vitamins. In addition to being rich in fiber and having low lipid content, it contains a variety of phytochemicals, such as polyphenols, which provide important health benefits.[3] One way to avoid losses and capitalize on the nutritional characteristics of eggplant is processing it into flour. Eggplant flour (EF) is a highly desirable food ingredient to enrich the diet; however, there are few data on its chemical composition.[4]

Studies have shown that phenolic compounds of eggplant have the potential to reduce intestinal glucose

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absorption and provide cellular antioxidant protection, preventing oxidation and diabetes complications,[5] especially those phenolic compounds present in eggplant peel.[6] In addition, the eggplant peel is rich in anthocyanins and has therapeutic potential for the treatment of hyperlipidemia and prevention of atherogenic cardiovascular diseases by inhibiting lipid peroxidation.[7] Gonçalves et al.[8] conducted a literature review comprising 25 articles on the species *S. melongena* and concluded that this species has a mitigation effect on dyslipidemia, particularly hypercholesterolemia, when used in the form of juice made from the fruit with peel.

Cooking methods tend to reduce the content of polyphenols in fruits and vegetables, for example by simply removing their peel, since the higher concentrations of these substances are often present in the external parts.[9] Even though several studies have addressed the eggplant in its different forms (*in natura*, juices, teas, extracts), studies that present a broad analysis of EF produced from the whole fruit dehydrated in an oven are scarce. The authors consider that this process is more accessible to the general public than the freeze-drying process.

The goal of the present study was to assess the physicochemical composition of EF prepared from the whole fruit dehydrated in an oven, characterizing qualitatively and quantitatively phenolic compounds, niacin, and saponins, in addition to assessing in vitro antioxidant activity using 2.2-diphenyl-1-picrylhydrazyl radical (DPPH) and Ferric Reducing / Antioxidant Power (FRAP) assays.

Material and Method

Standards and reagents

High-Performance Liquid Chromatography (HPLC) reagents and standard chlorogenic acid 3878, caffeic acid 0625, ferulic acid 12.870, and rutin 5143 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The samples were analyzed using a Varian HPLC system, with a tertiary gradient pump, variable-wavelength UV/VIS absorbance detector, and an automatic sampler with a refrigerated compartment (Varian Canada Inc., Mississauga, ON, Canada). The Folin-Denis reagent (Fluka) was purchased from Sigma-Aldrich Brazil LTDA.

Preparation of samples

The samples of EF used for analysis were purchased in stores of Rio de Janeiro, Brazil. According to the manufacturer, the eggplants produced in the State of Rio de Janeiro had originally been sanitized, sliced, and dehydrated in an oven at a temperature between 62°C and 70°C for approximately ten hours. Subsequently, they were placed in containers, sealed until complete cooling and the next day they were ground and packaged immediately. The final result was a dark beige thick powder. Samples from different lots were homogenized and the analyses were performed in triplicate.

Physicochemical Analysis

Moisture content was determined by weight loss in an oven set at 105°C until constant weight.[10] The determination of fixed mineral residue (FMR) was based on the burning of organic matter in a muffle furnace at 550ºC until the ashes were white or slightly grey. The ashes allowed the analysis of specific minerals since they were composed of macro-and micronutrients-and trace elements.[10]

Proteins were determined through Kjeldahl digestion process, by which organic matter was decomposed by sulfuric acid and nitrogen was transformed into ammonia.[10]

Lipids were determined through continuous diethyl ether extraction in a Soxhlet apparatus, followed by the removal of the ether through evaporation.[10]

The analysis of crude fiber content was conducted in an industrial digester (Marconi MA-444/CI) subjecting the samples to acid digestion with 1.25% sulfuric acid solution, followed by alkaline digestion with 1.25% sodium hydroxide.[10]

Total carbohydrates were estimated by difference, subtracting the values obtained for moisture, proteins, lipids, ashes, and fibers from 100.

Total titratable acidity (TTA) was determined by titration with 0.1 N NaOH, using alcoholic solution of phenolphthalein to determine color change.[10]

Total dietary fiber was determined using the method proposed by Prosky et al.,[11] in addition to soluble and insoluble dietary fiber.[12,13]

The determination of minerals (K, Ca, Na, Mg, Cu, Fe, Mn, and Zn) was performed through atomic absorption spectrophotometry, using an Analytik Jena spectrophotometer (Model ContrAA® 700).

Niacin concentrations were determined using the method proposed by Horwitz et al.[14]

Total phenols were determined using Folin-Ciocalteu reagent.[15]

Saponins content was determined using the double-solvent extraction gravimetric method described by Harborne.[16,17]

In vitro antioxidant activity

Sample extraction[18]

The EF was weighted (500 mg) and placed in 15-mL plastic tubes to which 10 mL of 80% methanol were added. The mixture was vigorously shaken using a vortex mixer for two minutes. The samples were then centrifuged at 5000 rpm for 15 minutes and the supernatant was filtered using Whatman filter paper (0.45 μm). The waste was re-extracted two more times with additional 10 mL of 80% methanol. The three extracts were combined to carry out the DPPH and FRAP assays and the analysis of phenolic acids through HPLC assay.

Scavenging method of DPPH free radical[19]

Ferric reducing/antioxidant power[20]

Analysis of phenolic acids and flavonoids through HPLC

The samples were analyzed using a Gemini-NX reverse-phase (RP)-HPLC column (100 x 4.5 mm) (Phenomenex, CA, USA), using 1 mL/min solvent flow rate, and detection was at 215 nm. Gradient elution was carried out with a mixture of the following solvents: Solvent A: 0.05% trifluoroacetic acid (TFA) in 10% aqueous acetonitrile (ACN); and solvent B: 0.05% TFA in 60% aqueous ACN (v/v), starting with 100% solvent A and reaching 40% solvent A and 60% solvent B in 30 minutes.

Phenolic acids and flavonoids were identified through comparisons with the retention time (RT) and UV spectra of authentic standards analyzed under identical analytical conditions. Ascorbic acid, tyrosine, chlorogenic acid, caffeic acid, ferulic acid, and rutin were analyzed. The same standards were used for calibration curves to quantify these substances in the EF. The results are presented as mean and standard deviation.

Statistical analysis

Data are expressed as mean ± standard deviation for continuous variables. The linear regression analysis was performed to estimate ascorbic acid, tyrosine and phenolic acids in EF, after analysis of assumptions for the use of the regression analysis. The statistical analysis was performed using the Statistical Package for Social Sciences, version 21 (SPSS Inc., Chicago, IL, USA).

Results

The results of the centesimal composition are shown in Table 1 and the results of minerals, niacin, soluble phenolic compounds, and saponins are shown in Table 2.

In vitro antioxidant activity

Activity of DPPH free radical elimination

The increase in DPPH radical scavenging in a dose-dependent manner due to the elimination of the methanolic extract of EF is 455.6 ± 3.27 ascorbic acid equivalent (mg). The results of the determination of antioxidant activity performed using the FRAP 486.8 ± 86.8 ascorbic acid equivalent (mg).

The following compounds were detected through HPLC assay: ascorbic acid (RT = 1.06 min); tyrosine (RT = 1.68 min); chlorogenic acid (RT = 7.36 min); caffeic acid (RT = 8.57 min); and ferulic acid (RT = 9.24 min) (Figure 1). Calibration information and estimation of phenolic compounds and antioxidants present in EF are shown in Table 3.

Discussion

The Brazilian Government Health Authority has shown a growing interest in the development and promotion of strategies for the prevention of cardiovascular diseases, mainly for a better control of the known risk factors, such as smoking, obesity, sedentary lifestyle, hyperglycemia, hypertension, and hypercholesterolemia.[21] Thus, the search for healthy and low-cost alternatives that can contribute to the health of the population becomes imperative.

A study conducted by Perez and Germani[4] showed that EF had high fiber content (44%). A similar result was obtained by Possetti and Dutra (2011),[22] who found 45% fibers. In addition to low content of lipids (1.99%),

Table 1 – Centesimal composition of eggplant flour

Centesimal composition (g)	Mean ± standard deviation
Kcal	162.37 ± 11.65
Total carbohydrates[1]	23.09 ± 0.50
Proteins	13.34 ± 0.50
Lipids	1.85 ± 0.03
Total dietary fiber	39.19 ± 0.08
Soluble dietary fiber	10.36 ± 0.17
Insoluble dietary fiber	28.83 ± 0.10
Ashes	4.70 ± 0.04
Moisture	11.89 ± 0.34

[1] *Calculated by difference.*

Table 2 – Minerals, niacin, phenolic compounds, and saponins present in eggplant flour

mg/100 g	Mean ± standard deviation
Potassium	2396.0 ± 83.8
Magnesium	158.1 ± 1.1
Sodium	68.1 ± 1.4
Copper	1.0 ± 0.03
Iron	2.9 ± 0.06
Manganese	2.5 ± 0.03
Calcium	130.9 ± 2.2
Zinc	2.1 ± 0.01
Niacin	1.49 ± 0.08
Total soluble phenolic compounds[1]	1540.0 ± 0.1
Saponins	840 ± 0.89

[1] *Results are expressed in terms of gallic acid equivalent (mg).*

there were 6.2% ashes, 25.54% carbohydrates, and 8% proteins. Even though fibers do not supply nutrients to the body, they are essential in the diet. They promote a number of health benefits in view of which the use of EF is considered a potential food ingredient.

In the present study we found 39.2% total fibers, 1.85% lipids, 4.7% ashes, 23.1% carbohydrates, and 13.34% proteins, in addition to a good mineral content, highlighting manganese, zinc, and copper. It is observed that, although there are small variations between the different studies, the EF features around 40% fibers and low lipid content. When compared with wheat flour, it was observed that EF featured higher levels of protein, ashes, total dietary fiber, and total sugars, whereas carbohydrate content was lower. In this way, the high dietary fiber content, the high water absorption capacity,

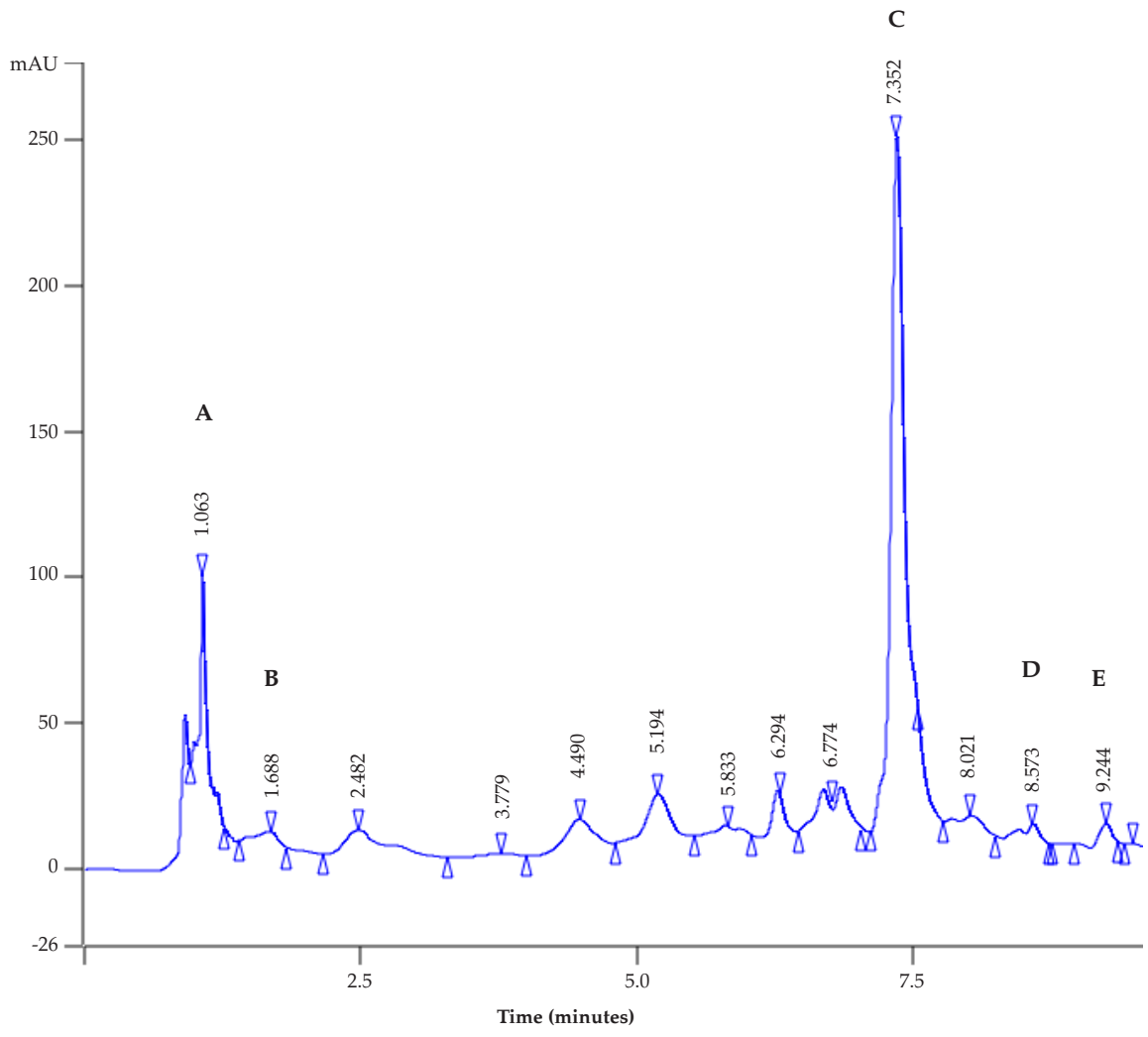

Figure 1 – *Phenolic compounds and antioxidants extracted from the eggplant flour: [a] ascorbic acid; [b] tyrosine; [c] chlorogenic acid; [d] caffeic acid; and [e] ferulic acid.*

Table 3 – Calibration information and estimation of ascorbic acid, tyrosine, and phenolic acids in eggplant flour

Polyphenols	Linear range (µg/mL)	Calibration curves	r^2	Mean ± standard deviation (mg/g)
Ascorbic acid	10-100	y = 2668.4 x – 35546	0.9921	1.73 ± 0.20
Tyrosine	2-100	y = 1069.4 x – 1243.6	0.9972	1.21 ± 0.06
Chlorogenic acid	2-100	y = 6236.2 x + 12574	0.9979	1.73 ± 0.69
Caffeic acid	2-100	y = 10415 x – 25130	0.9985	0.19 ± 0.02
Ferulic acid	2-100	y = 6667.9 x + 21757	0.9969	0.04 ± 0.02
Rutin	2-100	y = 1659.1 x + 4048.9	0.9964	1.73 ± 0.20

and the ease of grinding make EF a good alternative to be mixed with wheat flour. This mixed flour can be used in the preparation of bakery products (cookies, breads, cakes, and pasta), expanding the number of products to supplement the daily fiber intake.[4]

An analysis performed with the eggplant pulp showed a concentration of 62.5 mg gallic acid equivalent/100 g in the fresh pulp.[23] This value is lower than that found in the present study with EF prepared from the whole fruit, which was 1,540 mg gallic acid equivalent/100 g in the flour. This is an expected result, since eggplant skin is rich in polyphenols and 100 g EF represent 1,000 g of the fruit. Another study showed greater presence of total phenols in extracts prepared from the eggplant peel (2,200 μg/g) than in extracts made from the pulp (390 μg/g).[24]

The results of a study assessing the centesimal composition of 20% eggplant extract showed that nutrient concentrations found in the present study were much larger than those found by those authors: 0.118% proteins; 0.052% ashes; 0.0366% lipids; 0.038% insoluble fibers; 0.025% soluble fibers; and 256.66 mg/dL total polyphenols. Even though the amount of polyphenols was significant, they were not able to protect mice against the oxidation of LDL-cholesterol, probably due to the presence of biogenic amines, especially histamine. These results do not support the popular use of *S. melongena* extract as hypocholesterolemic agent. On the other hand, Guimarães et al.[25] assessed *S. melongena* powder and found 15.09% protein, 1.42% lipids, 13.89% fibers, 0.22% calcium, and 0.31% phosphorus, and qualitative tests assessing composition showed positive results for the presence of polyphenols and saponins. These results are similar to those found in the present study regarding proteins and lipids (13.34 and 1.85%, respectively); however, far below with respect to fiber (39.19%, i.e., fiber concentration in EF was 2.8 times greater) and calcium concentrations.

A study on extracts prepared from the pulp and the peel of eggplant showed a greater presence of soluble phenolic compounds in the peel than in the pulp, and the ability to inhibit DPPH radical was greater in all the extracts prepared from the pulp of eggplant than in those prepared from the peel. These results suggest that greater phenolic content allows moderate antioxidant activity linked to free radical scavenging potential.[5] The present study showed similarity between the results of DPPH and FRAP, thus revealing the antioxidant capacity of EF, possibly due to the important presence of phenolic compounds.

A comparison performed between raw, grilled for 4 to 5 minutes on both sides, and boiled in water for 10 minutes over moderate heat 100 ºC and freeze dried after the process, showed the following concentrations of total polyphenols/100 g dry matter: raw = 910 mg; grilled = 1,803 mg; and cooked = 1,991 mg. The chlorogenic acid concentrations were as follows: 154 mg; 549 mg; and 439 mg, respectively. The concentrations of caffeic acid were: 12.8 mg; 23.3 mg; and 33.9 mg, i.e., the grilling and boiling processes increased polyphenols concentrations. The authors also observed higher free radical scavenger capacity in boiled and grilled samples.[26] The amount of total polyphenols was much greater in the present study, i.e., 1,540 mg/100 g EF, probably due to the longest period during which the eggplants were exposed to heat at a constant temperature of approximately 70 ºC.

It is important to highlight that the polyphenols commonly present in the human diet are not necessarily the most active within the body. This fact is due to low bioavailability, since they are little absorbed by the intestine, highly metabolized, or eliminated quickly. Furthermore, the metabolites found in blood and specific organs, which result from a liver or digestive activity, may differ from ingested substances in terms of biological activity.[9] Variations in phenolic content concentrations between several studies occur due to different growing conditions.[27]

A study assessed the hypolipidemic effect of three capsules containing 360 mg dry extract of *S. melongena* L. (eggplant) or 360 mg placebo/day in 28 women with dyslipidemias under nutritional control during 90 days. The authors concluded that the dry extract of *S. melongena* L. had a modest effect on lipid profile, with no hepatotoxic effect or adverse reactions; however, it should be noted that the clinical response found in the study did not reach the values established by the III Brazilian Guidelines on Dyslipidemias and Atherosclerosis.[28]

Freeze-dried eggplants exhibited the following phenolic acids determined through HPLC assay: caffeic; p-coumaric; ferulic; gallic; protocatechuic; and p-hydroxybenzoic. The amount of hydroxycinnamates (caffeic, ferulic, p-coumaric, and chlorogenic acids) was from 9 g/g to 12 ug/g fresh matter.[29] In the present study, the EF had the following hydroxycinnamates: caffeic, ferulic, and chlorogenic acids, totaling 1,96 mg/g flour.

The major limitation of the present study is a lack of comparison between different forms of EF preparation, to analyse how it would change the composition of EF.

However, it is worth mentioning that the EF was produced by hand, thus making the procedure easier, without the need of a freeze dryer, or difficult-to-access equipment. An electric oven is sufficient, allowing low-cost access to nutrients and phenolic compounds present in this flour.

The present study demonstrates that EF is a good addition to the diet of the general population, since it is a source of fiber, minerals, phenolic compounds, saponins, ascorbic acid, tyrosine, and phenolic acids, in addition to exhibiting an important antioxidant activity observed by means of in vitro assays. However, it is observed that although several studies have assessed the eggplant in different ways, the studies on EF are limited to inserting it mixed with other flours to prepare cakes and cookies. No studies were found that had actually assessed the effect of EF on humans in order to know if it would have some hypolipidemic effect or could reduce oxidative stress. Clinical trials are needed to answer these questions.

Conclusion

The EF analyzed exhibited high fiber content and low lipid content. Also, it had good mineral content, highlighting manganese, zinc, and copper, in addition to phenolic compounds and saponins with important in vitro antioxidant capacity. In view of the results, the EF is a good addition to the diet of the population, since it enriches the diet and brings health benefits.

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Author contributions

Conception and design of the research: Scorsatto M, Silva AJR, Rosa G, Oliveira GMM. Acquisition of data: Scorsatto M, Pimentel AC, Sabally K. Analysis and interpretation of the data: Scorsatto M, Pimentel AC, Sabally K. Statistical analysis: Scorsatto M, Silva AJR, Sabally K. Obtaining financing: Rosa G, Oliveira GMM. Writing of the manuscript: Scorsatto M. Critical revision of the manuscript for intellectual content: Scorsatto M, Silva AJR, Rosa G, Oliveira GMM. Supervision/as the major investigador: Scorsatto M, Rosa G, Oliveira GMM.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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