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FOOD TECHNOLOGY

Effects of thermal processing on nutritional composition and toxicity of jackfruit seeds

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ABSTRACT: This study evaluated the impacts of thermal processing on the chemical composition of jackfruit seeds and their toxicity. The ash content of the seeds subjected to roasting was higher (3.21%) compared to the seeds in the other treatments. However, the contents of moisture (5.29%) and protein (10.49%) were lower. The protein content showed a significant difference ($P \leq 0.05$) among the studied groups (10.49%). The lipid content was lower in the seeds subjected to cooking (1.13%), while the carbohydrate content showed a significant difference ($P \leq 0.05$) among the groups studied (54.11%). Raw seeds had the highest fiber content (25.20%). Regarding toxicity, only the cooked jackfruit seed flour showed cytotoxic potential. However, none of the samples caused irritation or vascular disorders in fertilized eggs. These results suggested that heat treatment can ensure the safety of seeds against cytotoxicity; although, it reduces the content of some macronutrients.
Key words: food composition, cytotoxicity, thermal treatment, Artocarpus heterophyllus, residue utilization.

Efeitos do processamento térmico na composição nutricional e toxicidade de sementes de jaca

RESUMO: Este estudo teve como objetivo avaliar os impactos do processamento térmico na composição química das sementes de jaca sua toxicidade. O teor de cinzas das sementes submetida à torra foi maior (3,21%) em comparação com as sementes nos outros tratamentos. No entanto, os teores de umidade (5,29%) e proteínas (10,49%) foram menores. O teor de proteínas apresentou diferença significativa ($P \leq 0.05$) entre os grupos estudados (10,49%). O teor de lipídeos foi menor nas sementes submetidas ao cozimento (1,13%), enquanto o teor de carboidratos apresentou diferença significativa ($P \leq 0.05$) entre os grupos estudados (54,11%). As sementes brutas apresentaram o maior teor de fibras (25,20%). Em relação à toxicidade, apenas a farinha de sementes de jaca cozidas apresentou potencial citotóxico, embora nenhuma das amostras tenha causado irritação ou distúrbios vasculares em ovos fecundados. Esses resultados sugerem que o tratamento térmico pode garantir a segurança das sementes frente à citotoxicidade, embora reduza o teor de alguns macronutrientes.
Palavras-chave: composição de alimentos, citotoxicidade, tratamento térmico, artocarpus heterophyllus, utilização de resíduos.

INTRODUCTION

The jackfruit tree (*Artocarpus heterophyllus* L.) is a member of the Moraceae family and is native to Southeast Asian countries. However, it is widely found in tropical countries like Brazil, due to favorable conditions such as light, soil type, relative humidity, and temperature (MADRUGA et al., 2014).

The fruit of the jackfruit tree, known as jackfruit, its renowned as the largest edible fruit globally, weighing between 4.5 and 50 kilograms and measuring approximately 90 cm in length (GOSWAMI & CHACRABATI, 2016). In Brazil, jackfruit exists in two distinct cultivar forms, distinguished by the texture of their flesh: "soft" jackfruit, which has a tender flesh and perianth, and "hard" jackfruit, with firmer flesh and perianth (SWAMI & KALSE, 2018).

Jackfruit pulp can be consumed fresh or used in the preparation of jellies, juices, and jams. It is abundant in carbohydrates, protein, fiber, vitamins, and minerals, making it a valuable source of bioactive compounds and antioxidants in the diet. Conversely, the seeds of jackfruit account for 8 to 15% of the fruit's total weight and can be consumed after roasting on grills, toasting in ovens, or boiling in water. They are utilized in both sweet and savory dishes (WAGHMARE et al., 2019). Despite their significant industrial and commercial potential, these seeds are often

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discarded as waste, leading to environmental pollution and wastage of fruit parts with high nutritional value (ZHANG et al., 2018).

Despite being natural and highly nutritious, jackfruit seeds contain compounds originating from the secondary metabolism of plants, which are responsible for producing defense factors in plants. Some toxins, as well as anti-nutritional factors, have been found in the seeds of various edible fruits, including cherry, plum, peach, mango (GARCIA-RODRIGUEZ et al., 2009), mangaba, cagaita, jatobá, araticum and tucumã (FONSECA et al., 2013), and rambutan (KUMORO et al., 2020). The heat treatment employed during the cooking of some seeds is considered the most effective process for reducing these substances. It helps inactivate potential toxic compounds, improves the technological and functional properties of the seeds, and enables their use in various culinary preparations (BENEVIDES et al., 2011).

Therefore, by exploring the comprehensive utilization of jackfruit and its potential application in the food industry, this study evaluated the impact of thermal processing on the nutritional value of jackfruit seeds. The objective is to assess their suitability for human nutrition.

MATERIALS AND METHODS

The jackfruit seeds used in this study were obtained from twenty fruits sourced from a commercial plantation located in the municipality of São José de Mipibu, in the state of Rio Grande do Norte, Brazil. All subsequent procedures were conducted at the Laboratory of Food Experimentation and Analysis of the Nutrition Department at the Federal University of Pernambuco.

Preparation of dried jackfruit seed flour (DJSF)

The jackfruit seeds were washed in running water and dried in an electric convection oven without steam (WICTORY ® WCV-535) at 60 °C for 24 hours. Subsequently, they were ground using a high-speed food grinder (BERMAR ® Model BM-35) and further processed in an analytical mill (IKA ® A11 Basic) until particles with a size of 0.84 mm (No. 20 mesh) were obtained. The resulting flour was then stored in polyethylene bags and kept refrigerated until the time of analysis.

Preparation of cooked jackfruit seed flour (CJSF)

After being washed with running water, a portion of the jackfruit seeds was cooked in an open pan at 100 °C for 45 minutes using a 1:5 ratio of seeds to water. Following the cooking process, the seeds were spread out on trays and placed in a forced-air circulation oven at 40 °C for 24 hours to dry. Once dried, they were ground and packed following the same conditions mentioned in the previous topic.

Preparation of roasted jackfruit seed flour (RJSF)

Washed jackfruit seeds were roasted in an electric convection oven without steam (PRACTICA ® Model Miniconv Classic Stainless SV) at 160 °C for 45 minutes. Subsequently, they were ground and stored under refrigeration, following the same steps as mentioned in the previous topics.

Obtaining the extracts

Following the methodology described by CAETANO et al. (2011), three aqueous extracts were prepared from each jackfruit seed flour sample. Initially, 5g of the dry residue was mixed with 30 mL of water and subjected to constant agitation using a magnetic stirrer for 20 minutes. The resulting residue was then subjected to two additional extraction cycles using the same process, resulting in a total extraction time of 60 minutes. The solutions obtained from the three cycles were combined, and the final volume was adjusted to 100 mL. All extracts were stored in amber containers and refrigerated until the cytotoxicity and irritant potential analysis were performed.

Chemical composition

Analysis of moisture, ash, protein, lipids, carbohydrates, and total dietary fiber were determined using standard procedures (AOAC, 2016). The tests were carried out with three repetitions for each jackfruit seed flour.

Cytotoxic evaluation

The cytotoxic activity of the extracts was evaluated using the 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT method, as described by ALLEY et al. (1988). The HCT116 (human colorectal carcinoma) and HL-60 (human promyelocytic leukemia) cell lines were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% antibiotic solution (penicillin and streptomycin). The cells were maintained in a humidified incubator at 37 °C with 5% CO2. For the cytotoxicity assay, HCT116 (at a concentration of 10^5 cells/mL) and HL-60 (at a concentration of $3x10^5$ cells/mL) were plated in 96-well plates and incubated for 24 h. Then, 10µL of the prepared extracts were added to the wells at final concentration of 100µg/mL and 50 µg/mL.

Doxorubicin, a drug with known cytotoxic properties, was used as the standard at a concentration of 10µg/mL. After 72h of re-incubation, 25µL of MTT solution (5mg/mL) was added to each well, and the plates were incubated for an additional 3 hours. The culture medium with MTT were then aspirated, and 100µL of dimethyl sulfoxide (DMSO) was added to each well. The absorbance was measured at a wavelength of 560nm using a microplate reader. The experiments were performed in four repetitions.

Irritation potential

The irritation potential of the aqueous extracts obtained from the jackfruit seed flours was assessed using the fecundated chicken egg chorioallantoic membrane test (HET CAM) following the methodology proposed by FREIRE et al. (2015). The extracts were tested at different concentrations, and both positive and negative control substances were used. Sodium laurel sulfate at 1% concentration served as the positive control, while saline solution at 0.9% concentration served as the negative control. After applying the substances, the test subjects were observed for 5 minutes to detect any irritant effects such as bleeding, coagulation, or lysis. The irritation potential was quantified using a specific equation, and the results were classified based on the following parameters: non-irritant (0-0.9), slightly irritant (1-4.7), moderately irritant (5-8.9), and severely irritant (9-21). Each substance was applied five times to ensure reliability of the results.

$$IP = (301 - bleeding) \times 5/300 + (301 - vasoconstriction) \times 7/300 + (301 - coagulation) \times 9/300$$

Statistical analysis

The obtained results were subjected to descriptive statistical analysis, including the calculation of mean and standard deviation. This analysis was performed using Excel ® 2016 (Microsoft ®). For data with a normal distribution, the analysis of variance (ANOVA one-way) was conducted, followed by Duncan's test to compare the means obtained. The significance level was set at 5%.

RESULTS AND DISCUSSION

Chemical composition

The data regarding the chemical composition of raw jackfruit seeds and those subjected to different types of thermal processing are expressed in table 1, as percentages per 100 g of product on a dry basis.

The moisture contents of the three flours analyzed ranged between 5.29% and 7.63%, with RJSF having the lowest value and CJSF having the highest value. These results were higher than those found by EKE-EJIOFOR et al. (2014), who reported 6.60% and 4.44% moisture content for cooked and roasted seeds, respectively. According to OLANIPEKUN et al. (2015), the longer the exposure time of the sample to dry heat (180 °C), the lower the moisture values, thus supporting the fact that roasting as a pre-treatment reduces free water levels more effectively than prior cooking. The roasting process contributes to a decrease in moisture content, which in turn enables an extended shelf life for the product. There was a significant difference ($P \leq 0{,}05$) among the samples for each applied method.

In the ash analysis, the three flours in the present study showed values ranging from 1.62% to 3.25%, with the RJSF having the highest value and the CJSF having the lowest. This latter value may be attributed to leaching of minerals due to water action (LIU et al., 2019). According to OLANIPEKUN (2015), the intestinal absorption and bioavailability of minerals in jackfruit seeds are increased when they are subjected to roasting. The results obtained in the dried and roasted seed flours were higher than those reported by EKE-EJIOFOR et al. (2014), who

Table 1 - Physicochemical characterization of jackfruit seed flour (%/100g dry sample) submitted to different heat treatments.

	Moisture	Ash	Protein	Lipid	Carbohydrate	Fiber
DJSF	6.46 ± 0.05^{b}	2.62 ± 0.07^{b}	11.28 ± 0.03^{a}	1.54 ± 0.25^{a}	54.11 ± 0.22^{b}	25.20 ± 0.001^{a}
CJSF	7.63 ± 0.03^{a}	1.62 ± 0.02^{c}	10.62 ± 0.02^{b}	1.13 ± 0.08b	57.07 ± 0.05^{a}	21.90 ± 0.001^{b}
RJSF	5.29 ± 0.06^{c}	3.21 ± 0.03^{a}	10.49 ± 0.11^{c}	1.47 ± 0.01^{a}	53.19 ± 0.12^{c}	25.10 ± 0.001^{a}

Results expressed as means of three repetitions ± standard deviation.
Averages followed by the same vertical letters do not differ significantly by Duncan test at 5% significance level.
DJSF – dried jackfruit seed flour.
CJSF – cooked jackfruit seed flour.

obtained values close to 2.5% for jackfruit seeds subjected to the two heat treatments in question. There was a significant difference ($P \leq 0.05$) among the samples for each applied method.

The protein content of the studied jackfruit seed flours ranged from 10.49% to 11.28%, with DJSF having the highest content and RJSF exhibiting the lowest value. Higher values were reported by JUÁREZ-BARRIENTOS et al. (2017), (13.86% and 13.18%) for cooked and roasted seeds, respectively. The variation in protein content can be attributed to the varietal differences among the fruits (AKMEEMANA et al., 2022). It is worth mentioning that moist heat application can lead to protein reduction through solubilization and leaching processes (EYOH, 2020). Conversely, dry heat can denature and degrade certain proteins. Additionally, the roasting process can decrease the amino acid profile of proteins in jackfruit seeds, with lysine, arginine, cysteine, and histidine being particularly affected (ZUWARIAH et al., 2018). The protein content in this study showed a significant difference ($P \leq 0.05$) among the samples for each applied method.

The lipid content of jackfruit seed flour varied between 1.13% (CJSF) and 1.54% (DJSF). There was a significant difference ($P \leq 0.05$) in lipid content among the samples for each applied method. The results were higher than those reported by EKE-EJIOFOR et al. (2014): 0.77%, 0.66%, and 0.27%, for raw, cooked, and roasted jackfruit seeds, respectively. Conversely, the present study reported lower lipid content than JUÁREZ-BARRIENTOS et al. (2017): 6.56% and 5.69% for cooked and roasted jackfruit seeds, respectively. GAOL et al., (2019), remind that thermal processing reduces lipid content in jackfruit seeds. The authors suggest that hot water cooking releases unsaturated fatty acids through inactivation of lipase by heat, reducing fat content via leaching. On the other hand, dry heat cooking damages lipid molecules, causing their breakdown into volatile fatty acids released into the air, reducing lipid content in roasted seeds.

The carbohydrate content of the flours ranged from 53.19% to 57.07%, with CJSF having the highest value and RJSF showing the lowest. EKE-EJIOFOR et al. (2014) reported values higher than those obtained in this study (72.05% and 72.16%, for cooked and roasted seeds, respectively). The results of the present study revealed a significant difference ($P \leq 0.05$) in carbohydrate content among the samples for each method applied. The decrease in carbohydrate content observed in the roasted samples can be attributed to non-enzymatic browning reactions that occur during the roasting process (TALABI, 2016). Conversely, the increase in carbohydrate content in the cooked seeds is due to the hydrolysis of starch present in the seeds, resulting in total reducing sugars (SINGH et al., 2020).

The total dietary fiber values ranged from 21.9% to 25.20%, with cooked jackfruit seed flour having the lowest value and dried jackfruit seed flour exhibiting the highest. There was a significant difference ($P \leq 0.05$) among the samples for each method applied. Similar satisfactory results were observed in the studies conducted by EKE-EJIOFOR et al. (2014) (35%-47%) and JUÁREZ- BARRIENTOS et al. (2017) (37.2%-39%). These studies evaluated the dietary contents in dried, cooked and roasted jackfruit seeds, and concluded that both fruits' seeds are rich in soluble and insoluble fiber. The heat treatment employed had an influence on their fiber values.

Cytotoxic evaluation

The results presented in table 2 suggested that the extracts used in this study did not exhibit significant cytotoxic activity at the tested concentrations, considering the evaluated incubation period of 3 hours.

However, it is worth noting that artocarpine, a lectin found in jackfruit seeds, has been reported to possess cytotoxic activity, particularly against tumor cells (CHAN et al., 2018). The precise mechanisms underlying the cytotoxic activity of artocarpine are not fully understood, but it is believed that this lectin can induce cell apoptosis through the activation of specific enzymes (BURCI et al., 2018).

BURCI et al. (2018) conducted a study analyzing the cytotoxicity of various extracts derived from jackfruit seeds and concluded that they were active against breast (T47D), colon (HT-29), and melanoma (B16F10) tumor cells, attributing this action to the presence of artocarpine in the seeds. LEE et al. (2018) conducted an animal study using artocarpine isolated from jackfruit seeds to induce apoptosis in three osteosarcoma cell lines (U2OS, MG63, and HOS) over a period of 18 days. The results showed that artocarpine was able to reduce tumor volume by 40% in the studied animals.

Regarding table 2, only the extract from cooked jackfruit seeds demonstrated a % inhibition different from zero, indicating that this extract was able to inhibit 22.57% of cellular activity. According to MURAMOTO (2017), artocarpine is highly heat-stable and is only degraded at temperatures above 150 °C, such as during the process of roasting.

Table 2 - Cytotoxicity assays conducted on the aqueous fraction obtained from heat-treated jackfruit seeds against two different cell lines.

--------------------------------% of inhibition--------------------------------				
Samples	HL-60	SE	HCT-116	SE
DJSF - 100µg/mL	0.00	0.00	0.00	0.00
CJSF - 100µg/mL	0.00	0.00	22.57	0.00
RJSF - 100µg/mL	0.00	0.00	0.00	0.00
Distilled water	0.00	0.00	8.89	3.95
Doxorubicin - 10µg/mL	77.60	2.61	66.29	1.24

SE – standard error.
HL-60 - Human promyelocytic leukemia cells.
HCT-116 - Human colorectal carcinoma cells.
DJSF – dried jackfruit seed flour.
CJSF – cooked jackfruit seed flour.
RJSF – roasted jackfruit seed flour.

Irritation potential

The irritation potential test was conducted to evaluate the effects of treated jackfruit seed extracts on the vascularization of fertilized chicken eggs. No vascular changes, such as hemorrhage, vasoconstriction, or coagulation, were observed in the chorioallantoic membrane of the studied eggs.

Similar findings were reported by OKTAVIA et al. (2017) when using methanolic extract of jackfruit seeds. These consistent results suggested that the phenolic compounds present in Artocarpus species are responsible for inhibiting angiogenesis in the analyzed cases. According to SWAMI & KALSE (2018), angiogenesis is a process involved in tumor growth, but its activity can be inhibited by the combined action of flavonoids, saponins, and tannins. These secondary metabolites are present in jackfruit seeds and are known to be partially heat resistant at temperatures around 130 °C (CHAABAN et al., 2017).

CONCLUSION

The macronutrient contents of the jackfruit seeds undergo changes when subjected to heat compared to seeds that have not undergone thermal processing. However, these changes in carbohydrate, fiber, and protein contents open possibilities for the use of jackfruit seed flour in culinary applications and as an ingredient in product formulations. The results of this study suggested that heat processing makes jackfruit seeds safe for consumption. However, it is crucial to acknowledge the importance of conducting further investigations into alternative extraction methods (such as methanolic, alcoholic, etc.) and their potential influence on the cytotoxic effects of the seeds, as well as its mechanism of action. This is particularly relevant as the current study solely utilized an aqueous solution of the extract. Despite the alterations in macronutrient values caused by heat, jackfruit seeds still possess a good nutritional composition, which adds value to this by-product and increases the potential for its comprehensive utilization.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript.

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