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Original Paper

Nutritional and Physicochemical Properties and Safe Consumption of Jackfruit Seeds *(Artocarpus heterophyllus Lam.)*

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***Abstract*— Fruit and vegetable byproducts, such as peels, rinds, and seeds, have been the focus of functional food research in recent years. Byproducts of jackfruit, such as seeds, are functional foods/ingredients. However, studies on the nutritional and physicochemical properties of jackfruit seeds and their safe consumption are limited. Thus, this study aimed to determine the nutritional and physicochemical properties of raw, roasted, and boiled jackfruit seeds as well as how these seeds can be safely consumed. Nutrient composition, total dietary fiber composition, fermentability, resistant starch content, antinutrients, heavy metals, and microbial load of jackfruit seeds were determined using AOAC standard methods. Jackfruit seeds are a good source of protein (9.9–10.2 g/100 g), ash (3.3–3.8 g/100 g), carbohydrates (21.45–82.15 g/100 g), dietary fiber (12.11–13.83 g/100 g), resistant starch (19.9–25.6 g/100 g), and amylose (20.61–23.03 g/100 g). Phytic acid, tannic acid, and heavy metal contents as well as microbial load of raw and thermally processed jackfruit seeds were within acceptable limits; however, the microbial load in raw seeds was above the acceptable limit. The starchy structure of processed jackfruit seed expands its granules and exhibits an increased surface area, thereby leading to better digestion. In conclusion, processed jackfruit seeds can serve as a potential functional food or ingredient. Consumers and food industry professionals should be aware of the beneficial effects of jackfruit byproducts.**

Keywords*— *jackfruit seeds, functional food, dietary fiber, resistant starch, amylose, starch structure

I. INTRODUCTION

Jackfruit *(Artocarpus heterophyllus Lam.)* is considered a poor man's fruit because of its low cost and high production and is also called a "miracle food" due its high nutritional value. Although jackfruit seeds are edible and may replace some major staple food items [1], they are underutilized. Jackfruit seeds have significant nutritional benefits and constitute approximately 10%–15% of the fruit's weight [2]. In some Asian countries, jackfruit seeds are considered snacks, similar to nuts; they are cooked and consumed either boiled or roasted with salt for approximately 25 minutes [3]. These seeds can also be consumed as a dessert by adding sugar during the boiling process [4]. Jackfruit seeds can also be collected from ripe fruit, dried in sunlight, and stored for future use [5].

Jackfruit seeds are considered perishable in nature, and they are often discarded as waste due to challenges faced during their processing and storage. However, when seeds are stored under a cool and moist temperature, they can last for approximately 1 month. Roasting the seeds can also extend their shelf life; furthermore, the roasted seeds can be grinded into powder to further increase their value [5,64]. Jackfruit seed powder can be used as a flour substitute and incorporated in different types of flour in baked products [6,7]. Despite these uses, jackfruit seeds remain underutilized because they are often removed or discarded from the fruit [8]. Jackfruit seeds are underrecognized among consumers and food producers [8]. Recent studies on jackfruit seeds have focused on characterizing the physicochemical properties of starch from cooked jackfruit seeds and highlighting the use of jackfruit seeds as a flour substitute. In the present study, we aimed to determine the nutritional and physicochemical properties of raw, roasted, and boiled jackfruit seeds and their safety for human consumption. The results of this study will enhance our understanding of the nutritional and health benefits of jackfruit seeds.

II. MATERIALS AND METHODS

A. Materials

Fresh and mature jackfruits *(tinumbaga)* were purchased from a local dry market in Indang and Tagaytay City in Cavite. This local market was chosen because of the abundant supply of jackfruit in the area. All jackfruits *(Artocarpus heterophyllus Lam.)* purchased were submitted to the Bureau of Plant Industry for species authentication.

B. Methods

1) Preparation of jackfruit seeds

The fresh mature jackfruit was cut manually using a knife, and seeds were separated from the pulp and rinds. The soft white jackfruit seed covering was removed, and the collected seeds were washed with water. After washing, the collected

seeds were divided into three different groups according to preparation: raw, roasted, and boiled. During the roasting process, seeds were placed in a pan without touching and baked in an oven at 198°C for 30 minutes. The boiled seeds were immersed in 1 inch of water and boiled at 100°C for approximately 35 minutes or until fork tender. After the roasting and boiling process, jackfruit's outer white skin was peeled and removed. Similar to raw jackfruit seed, which served as the control, all samples were freeze-dried before analysis.

2) Chemical analysis

All chemicals and reagents used were of analytical grades (AR). The nutritional and physicochemical properties of jackfruit seeds as well as their safety were analyzed following the Association of Official Analytical Chemists (AOAC) methods. Amylose, dietary fiber contents and fermentability were performed in duplicate and triplicate for all other parameters.

3) Nutritional properties

a) Moisture content

Moisture content was determined using the gravimetric method–AOAC 925.09 [9]. Approximately 10 g of the prepared jackfruit seed samples were placed in dried and weighed petri dishes and heated for 5–6 hours at 100°C in an air-drying oven. Dried samples were placed in a desiccator, cooled, and weighed. Moisture content was calculated using the formula (1):

$$\% \text{ Moisture} = \frac{\text{weight of moisture}}{\text{weight of sample}} \times 100 \quad (1)$$

b) Ash content

The ash content of the jackfruit seeds was determined using the gravimetric method–AOAC 923.03 [9]. A porcelain crucible containing 2 g of the samples was ignited and heated inside a muffle furnace at 550°C for 8 hours. Weight was recorded upon cooling to room temperature and was computed using (2):

$$\% \text{ Ash} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100 \quad (2)$$

c) Protein content

The protein content of the jackfruit seeds was determined using the Kjeldahl method–AOAC 945.18 [9]. One gram of film sample was placed in separate digestion tubes, and 15 mL of concentrated H_2SO_4 was added. The solution was digested using the Buchi digester block until it was clear. Then, it was cooled and distilled in a Buchi Nitrogen Analyzer. A 32% NaOH and 2% H_3BO_3 were added to the clear solution to liberate ammonia gas. Nitrogen content was measured by titrating ammonia with 0.1 N Standard HCl. Nitrogen and protein content was computed using (3):

$$\% \text{ protein} = \% \text{ nitrogen} \times \text{protein factor} \quad (3)$$

Wherein:

$$\% \text{ nitrogen} = \frac{\text{ml std HCl} \times \text{N HCl} \times 1.4007}{\text{weight of sample}} \times 100$$

and Protein factor is 6.25

d) Total fat content

The fat content of the sample was determined using the acid hydrolysis method–AOAC 945.38 [9]. Four grams of the samples were weighed into a 25-mL digestion tube containing 1 g of *Celite*. The fat content of the samples was extracted using Soxtec System HT6 and hydrolyzed using Soxtec System 1047. Ether was then evaporated from the extracted fat. After evaporation, the extract was dried at 105°C for 2 hours using a drying oven. Then, the extract was cooled to room temperature and weighed. Fat content was computed using (4):

$$\% \text{ Fat} = \frac{\text{weight of cup before extraction - weight of cup after extraction}}{\text{weight of sample}} \times 100 \quad (4)$$

e) Total carbohydrate content

The carbohydrate content of the jackfruit seed sample was determined using difference computation [9]. This was computed using (5):

$$\% \text{ Carbohydrates} = 100 - (\%\text{ash} + \%\text{moisture} + \%\text{fat} + \%\text{protein}) \quad (5)$$

f) Dietary fiber analysis

Total dietary fiber, soluble dietary fiber, and insoluble dietary fiber contents were determined using AOAC 991.43 [9]. Duplicate samples of jackfruit seeds, weighing 1 g each, were mixed with 40 mL of MES-TRIS buffered pH 6.0 at 24°C. It was then added and mixed thoroughly with 100 µl of alpha-amylase solution. The sample solutions were covered with aluminum foil and were placed in a water bath for 35 minutes at 95°C–100°C. After heating, the samples were cooled to 60°C. The beaker walls were scraped with a spatula and rinsed with 10 mL water. The samples were then added to a 100-µl protease solution and incubated for 30 minutes at 60°C. Then, 5 mL of 0.561 hydrochloric acid solution and 200 µl of amyloglucosidase solution were added to the samples, followed by 30 minutes of incubation at 60°C. The resultant mixtures were used for insoluble and soluble dietary fiber analyses.

For insoluble dietary fiber content estimation, mixtures were filtered through a crucible containing fritted glass disk and *Celite* in duplicate. The residues were washed with water, 95% ethanol, and acetone; dried; and weighed. Then, one of the duplicate residues was analyzed using the Kjeldahl method for indigestible protein content, whereas the other residue was incinerated in a muffle at 550°C until the ash content was obtained. Insoluble dietary fiber was computed using (6):

$$\text{IDF}, \% = \frac{\text{WR - P - A - B}}{\text{weight test portion}} \times 100 \quad (6)$$

Wherein:

WR = average weights for duplicate test portion determinations; P = protein weight in the first sample residues;

A = weight of ash in the second sample residues; B = blank weight (mg); weight test portion = average of two test portion weights taken.

For soluble dietary fiber content estimation, mixtures obtained were filtered through a crucible containing fritted glass disk and *Celite* in duplicate. The filtrate was added into four volumes of 95% ethanol and preheated to 60°C. The precipitate was filtrated through a crucible containing fritted glass disk and *Celite.* The residue was washed with 15 mL of 78% ethanol, 95% ethanol, and acetone. The crucible containing the residue was dried, cooled, and weighed to calculate the residue weight. The Kjeldahl method was used to analyze one of the duplicates for indigestible protein content, whereas the other was incinerated in a muffle at 550°C until the ash content was obtained. Soluble dietary fiber was calculated using (7):

$$\text{SDF, \%} = \frac{\text{WR - A - B}}{\text{weight test portion}} \times 100 \qquad (7)$$

Wherein:
WR = average weight for duplicate test portion determinations; P = protein weight in the first sample residues; A = weight of ash in the second sample residues; B = blank weight (mg); weight test portion = average of two test portion weights taken. The total dietary fiber was calculated as the sum of the insoluble dietary fiber and insoluble dietary fiber using (8):

$$\text{TDF} = \text{Soluble dietary fiber} + \text{Insoluble dietary fiber} \qquad (8)$$

g) Dietary fiber fermentability

The human inoculum was prepared from a healthy donor (not taking antibiotics). The donor's diet was not controlled before collection. Feces were collected immediately after defecation. Polyethylene plastic containers previously flushed with CO_2 served as containers to maintain anaerobic conditions. Collected samples were placed in ice before transport to the laboratory. The inoculum was prepared 1 hour after the collection of feces. Pooled feces were diluted six-time weight/volume with sterilized 0.9% (w/v) NaCl solution and immediately homogenized using a normal blender for 1 minute. The medium was filtered using double layer cheesecloth and stored in the presence of CO_2 at 39°C [10]. The previously prepared freeze-dried sample, 1 g of each, was homogenized using a laboratory stomacher to simulate mastication in the mouth for 1 min with the addition of 15 mL salivary fluid (mixture of 8 g NaCl, 2.38 g Na_2HPO_4, 0.19 g KH_2PO_4, and 100 mg mucin in 1 L of distilled water). The mush was adjusted to pH 6.75 to obtain 200U/L enzyme activity using α-amylase (E.C 3.2.1.1). To simulate gastric digestion, pH was adjusted using 5 M HCl to pH 1.2 and successively adding 15 mL gastric fluid (mixture of 0.03 M NaCl with 300 U/L of pepsin adjusted to pH 1.2). Gastric digestion lasts up to 120 min at 37°C, followed by addition of 0.1 M $NaCO_3$ to adjust pH to 6.00. To simulate digestion in the small intestine, 15 mL of pancreatic fluid (a mixture of 0.05 g of pancreatin with 0.3 g bile extract in 35 mL 0.1 M $NaCO_3$) was added. The final mixture was neutralized to pH 7.00 using 1 M NaOH and then 5 mL of 120 mmol/L NaCl and 5 mL of 120 mmol/L KCl were added. The mixture was subjected to *in vitro* digestion for 120 min at 37°C in the dark to simulate the action in the large intestine. Dialysates (dilution in the dialysis membrane) and a nondialyzed fraction (residue) were separated and placed in polyethylene vials. Reagent blanks were run as controls in triplicate to check contamination. A method proposed by Trinidad *et al.* [11] was used for the colonic digestion of the nondialyzed fraction (residue). A portion of 1 gram of freeze-dried sample was placed in a fermentation flask, followed by addition of 40 mL media (20 mL $NaHCO_3$ buffer solution [0.04 M $NaH_2PO_4.H_2O$ + 0.5 M KH_2PO_4]), 0.1 mL resazurin solution (0.1%), and 2 mL reducing solution (1.25 g cysteine-HCl, 50 pcs KOH pellets, and 1.25 g Na_2S in 100 mL distilled water) in each flask. Fermentation was conducted by placing the bottles in a water bath at 37°C for 1 hour. Approximately 10 mL of fecal inoculum was added into the mixture and incubated at 37°C for 24 hours. The reagent blank was run to correct contamination. Merthiolate solution (1 mL [0.6 g/100 mL]) was added to deactivate microorganisms in each flask. The mixture was sonicated, and 5 ml aliquot from the supernatant was obtained and subjected to short-chain fatty acid analysis using high-performance liquid chromatography.

h) Amylose and amylopectin content

An assay kit was used to determine amylose content based on the principle of the concanavalin-A binding method [12]. A 25-mg sample was accurately weighed, placed in a screw cap tube, and completely dispersed by heating in 1 mL dimethylsulfoxide for 15 minutes with stirring in a vortex mixer. The tube was stored and allowed to cool for 5 minutes. Then, 2 mL of 95% ethanol was added to the tube and continuously stirred in a vortex mixer. After adding another 4 mL of ethanol, the tube was capped and inverted for mixing. The starch was allowed to form overnight and then centrifuged at 2000g for 5 minutes. The supernatant was discarded, and the tubes were drained on tissue paper for 10 minutes to drain all ethanol. To the recovered starch pellet, 2 mL of DMSO was added. The tube was placed in a boiling water bath for 15 minutes and mixed occasionally to ensure no lump formation. Immediately after heating, 4 mL of Concanavalin A solvent (30 mL of a 600mM, pH 6.4 sodium acetate buffer diluted to 100mL with 24 distilled water) was added, mixed thoroughly, and transferred in a 25-mL volumetric flask. The contents were diluted with the volume of concanavalin A solvent, this mixture is solution A.

The resulting solution was analyzed within 2 hours. To analyze the amylose content, 1 mL of the solution was transferred to a 2-mL microfuge tube. Next, 0.5 mL of Con A was added to the tube, and then the tube was capped and gently mixed via repeated inversion. The tube was allowed to stand for 1 hour at room temperature and centrifuged at 14,000g for 10 minutes in a microcentrifuge at room temperature. Approximately 1 mL of the supernatant was transferred to a 15-mL centrifuge tube and then 3 mL of sodium acetate buffer was added, mixed, and heated in a boiling water bath for 5 minutes to denature the Con A. The tube was then heated to 40°C,

equilibrated for 5 minutes, and treated with 0.1 mL amyloglucosidase/α-amylase enzyme mixture. The tube was incubated at 40°C for 20 minutes and centrifuged at 2,000g for 5 minutes. In an aliquot of the supernatant, the amylose was enzymatically hydrolyzed to D-glucose, which was analyzed using a glucose oxidase/peroxidase reagent. In a separate aliquot of the acetate/salt solution, the total starch was hydrolyzed to D-glucose and measured colorimetrically using glucose oxidase/peroxidase. The concentration of amylose in the starch sample was estimated as the ratio of GOPOD absorbance at 510 nm of the supernatant of the Con A precipitated sample to that of the total starch sample. The absorbance was measured at 510 nm against a reagent blank. The amylose content was measured using (9):

$$Amylose, \% = \frac{Absorbance\ (Con\ A\ supernatant)}{weight\ test\ portAbsorbance\ (Total\ starch\ aliqout)ion} \times \frac{6.15}{9.2} \times \frac{100}{1}$$

$$Amylose, \% = \frac{Absorbance\ (Con\ A\ supernatant)}{weight\ test\ portAbsorbance\ (Total\ starch\ aliqout)ion} \times 66.8 \quad (9)$$

The amylopectin content of the jackfruit seed samples was computed using (10):

$$\% \text{ Amylopectin} = 100\% - \% \text{ Amylose} \quad (10)$$

i) Resistant starch content

Resistant starch analysis of the jackfruit seeds was performed using the Megazyme method of AOAC 2002.2 [14]. First, 100 mg of jackfruit seed samples were weighed and placed into individual corning screw cap culture tubes with a size of 16 × 125 mm. Then, 4 mL of pancreatic α-amylase (10 mg/mL) containing AMG (3 U/mL) was added in each sample. The tubes were mixed in a vortex mixer and incubated for 16 hours at 37°C under continuous shaker conditions. Then the samples were removed from the water bath, and the contents were treated with 4.0 mL of ethanol (99% v/v). This was accompanied by vigorous stirring on a vortex mixer; after mixing the samples. The samples were centrifuged at 1,500 g for 10 minutes, and 2 mL of 50% v/v ethanol was added to the pellet, where resistant starch is recovered as pellet with vigorous stirring on a mixer. The pellets were resuspended in 6 mL of 50% v/v ethanol, then undergone centrifugation for 10 minutes. Carefully decant the supernatants and invert the tubes to drain excess liquid.

To measure the resistant starch content of the sample, add a magnetic stirrer bar and 2 mL KOH to each tube and resuspend the pellets by stirring for approximately 20 minutes in an ice/water bath, then 8 mL of 1.2 M sodium acetate buffer with a pH of 3.8 and 0.1 mL of AMG (3300 U/mL) were added to the sample while stirring. The samples were then placed in a water bath for 30 minutes at 50°C and were incubated on a vortex mixer. The samples were diluted to a final volume of 100 mL with distilled water. Then, 0.1 mL aliquots of the samples were transferred into glass test tubes, and 3 mL of GOPOD reagent was added to the mixture. Finally, the samples were incubated for 20 minutes at 50°C and absorbance was measured at 510 nm against the reagent blank.

For samples that contained >10% resistant starches, the following formula was used for estimating resistant starch content (11):

$$\Delta E \times F \times \frac{100}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180} = \Delta E \times \frac{F}{W} \times 90 \quad (11)$$

4) Physicochemical properties

a) Water activity

The water activity was measured using the method of Rayaprolu *et al.* [15]. First, the homogenized freeze-dried jackfruit seed sample was placed in a container with a cover layer at the bottom, and then the samples were placed into the chamber to accurately measure the water activity of the jackfruit seed samples.

b) Color

The color of the jackfruit seeds was analyzed with a Chroma Meter (CR-300; Minolta, Tokyo, Japan) using the Hunter system, which identifies color using three attributes: *L** or lightness (white = 100, black = 0), *a* (red = positive, green = negative) and *b* (yellow = positive, blue = negative). The color difference (ΔE), measures the distance in color space between two colors [16] and was determined by comparison to a standard white tile with colorimeter values of *L** = 94.5, *a* = −1.0, and *b* = 0.2 using (12):

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (12)$$

c) Texture

The samples were analyzed using a TA1-Texture Analyzer [15] with the following conditions: probe of 2 mm cylinder, speed of 2 mm/minute, 0005-kilogram force mm for the trigger, and a sample compression of 10%. The sample was sheared, compressed, and extruded through the bottom openings. Since the blades were set further apart than on the 10-blade version, a reduction was observed in the force of bulk shearing or compression on samples with many particles or foods with nonuniform texture. The accessory operates from ambient temperatures up to 100°C and is fitted with a spill container, which can also be used with the 10-blade version.

d) Starch granule

A micrograph of starch granules of the seed was captured using the Hitachi TM 3000 tabletop digital scanning electron microscope. The images were obtained at a magnification of 1000 and 2000 times. The freeze-dried jackfruit seed samples were placed in an aluminum holder and sealed with gold film using double-sided tapes to identify each sample [17].

5) Safety assessment

The safety of jackfruit seeds was determined by analyzing the antinutrients, heavy metals, and microbiological properties of raw and processed seeds.

a) Phytic acid

Five-gram samples in duplicates were incinerated at 600ºC for 4 hours. Residues were dissolved in hydrochloric acid and drops of concentrated nitric acid. The mixture was diluted to 50 mL with deionized water and filtered. Then, 0.5 mL of the aliquot solution was placed in a 50-mL volumetric flask. Then, 5.0 mL acetate buffer, 0.50 mL 1% ascorbic acid, and 5.0 mL 1% ammonium molybdate were added to the flask and diluted to 50.0 mL with deionized water. The solution was left to stand for 1 hour before reading in a UV–Vis Spectrophotometer at 660 nm [18].

b) Tannic acid

The tannic acid content of the samples was determined following the method of Abiola *et al.* [18]. One gram of samples was weighed in a 50.0-mL volumetric flask. Then, 23.8% ethanol solution and 10.0 mL of 1.5% metaphosphoric acid were added. The mixture was vigorously mixed for 1 minute, diluted with 70% ethanol, and filtered (#42 Whatman filter paper). Then, 2 mL of the filtrates were transferred to a 25.0-mL volumetric flask containing 10.0 mL deionized water, 5.0 mL Folins–Denis reagent, and 5 mL of 0.1 N anhydrous sodium bicarbonate solution. The mixture was mixed vigorously and left to stand for 90 minutes before reading in a UV–VIS Spectrophotometer (Shimadzu-UV-VIS) at 720 nm.

c) Heavy metals

The heavy metal content of the samples was determined using the Hicsonmez method [19]. First, 1 gram of samples were weighed and added in a 100-mL volumetric flask containing 5 mL 35% H_2O_2 and 3 mL 65% HNO_3. This mixture was allowed to stand for overnight. Then the flask with the sample was heated until the solution was clear and then cooled. Then, 3 mL of 65% HNO_3 and 9 mL of 37% HCl were added, and the solution was heated until a small volume of the samples was left. Next, deionized water was added to achieve a volume of 100 mL before reading. Then, heavy metal concentrations were analyzed using inductively coupled plasma-optic emission spectrometry (ICP-OES).

d) Aerobic count

The pour plate method was used to estimate the concentration of microorganisms in the jackfruit seed samples. First, the seed sample of 20 g was soaked by placing it in 180 mL of water for approximately 60 minutes, then the sample dilution of 10-fold up to 10^6 was prepared. After the preparation, the samples were placed on the nutrient agar and mannitol salt agar plates for analysis, where the samples were incubated for 1–3 days at 28°C [20]. The average colony count was computed following the method proposed by Borgis *et al.* [21] using (13):

$$\text{Colony Forming Unit/g} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Weight of sample}} \quad (13)$$

e) Total coliform count

The pour plate method was used to estimate the concentration of microorganisms in the jackfruit seed samples. First, the seed sample of 20 g was soaked in 180 mL of water for approximately 60 minutes. A sample dilution of 10-fold up to 10^6 was prepared following the method proposed by Braide *et al.* [22]. After the preparation, a lauryl tryptase broth was used to grow and detect coliform organisms in samples in Durham invert tubes. Then, the tubes were inoculated with 1 mL of each sample and incubated at 35°C for 24 hours. The presence of gas indicated that the samples were positive for coliforms, whereas the absence of gas indicated that the samples were negative for coliforms [23].

f) Yeast and mold count

The pour plate method was used to estimate the concentration of microorganisms in jackfruit seed samples. Approximately, 20 g of the seed sample was soaked in 180 mL of water for about 60 minutes, a sample dilution of 10-fold up to 10^6 was prepared. After the preparation, the spread plate technique was used in a potato dextrose agar for the analysis, where the samples were incubated for 1 day for yeast detection and for 4 days for mold detection [22].

The average colony count was computed following the method proposed by Borgis *et al.* [21] using (14):

$$\text{CFU/g} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Weight of sample}} \quad (14)$$

g) Escherichia coli count

The seed sample of 20 g was soaked in 180 mL of water for approximately 60 minutes, a sample dilution of 10-fold up to 10^6 was prepared. After the preparation, the sample was placed on the nutrient agar and mannitol salt agar plates and swirl plates for a uniform distribution of samples. The samples were incubated and observed for 1 day at 37°C [22].

6) Raw, roasted, and boiled jackfruit seeds as a functional ingredient

Jackfruit seed samples used in food products were raw, roasted, boiled and freeze-dried. The jackfruit seeds were added to all-purpose flour at varying concentrations (10%, 30%, and 50%) as functional food ingredients. Fortification with 10%, 30%, and 50% of different types of flour is commonly used when developing bakery products.

7) Data analysis

All experiments were conducted in duplicates or triplicates, and results are expressed as mean ± SD. All data were analyzed using analysis of variance and Duncan's multiple range test to determine significant differences between samples and treatments at a 95% level of significance ($P < 0.05$) using the SAS program.

III. Results and Discussion

A. Nutritional properties

1) Moisture content

Moisture accounts for the water content of the seed samples and their total solid content. It is also an index of flour storability. Reduced moisture content implies better shelf life and stability. Moisture contents of samples generally depend

upon the drying process duration. Results of proximate analysis in Table 1 showed that the moisture content was 7.96 g/100 g in raw jackfruit seeds, 6.50 g/100 g in roasted seeds, and 3.26 g/100 g in boiled seeds. Raw jackfruit seeds had a higher moisture content than thermally processed seeds as they did not undergo thermal processing because the seeds' granules still maintain its structural integrity. In roasted seeds, dry heat results in the quick withdrawal of water because of the high temperature; therefore, it has low moisture content [24]. In boiled seeds, starch gelatinization occurs due to moist heat from the boiling water, which results in a much larger granule because of the disruption of cell membranes in the seeds [25]. Boiled seeds are softer and more porous and hence have reduced moisture content. Low moisture content affects the physical properties of seeds and increases the quality and shelf life of foods [26].

2) Ash content

Ash content serves as an indicator of the mineral content of food samples. As shown in Table 1, the ash content was 3.76 g/100 g in raw jackfruit seeds, 3.54 g/100 g in roasted seeds, and 3.26 g/100 g in boiled seeds. A similar result was reported by Onyeike and Oguike in a previous study [27]. Ash content was highest in raw as seeds did not undergo thermal processing, whereas the ash content in roasted and boiled seeds was decreased due to the presence of some inorganic compounds that might have been released during thermal processing [28,29].

3) Protein content

The boiling process did not significantly affect the protein content of jackfruit seeds, as shown in Table 1. The protein content was 9.89 g/100 g in raw jackfruit seeds, 10.19 g/100 g in boiled seeds, and 9.47 g/100 g in roasted seeds. This decrease in the protein content of roasted seeds might be due to the loss of nitrogenous volatile compounds [30], as a result of the high temperature during the cooking process. On the other hand, a very slight increase in protein content was observed in the boiled seeds, which might be due to the proteolytic enzyme that releases the inherent proteins to their amino acids and peptides constituent to form a more protein aggregate [31]. This was also observed in a study conducted by Tian *et al*. [32] on boiled peanuts, where degradation of polypeptides released free amino acids and peptides, and the released peptides formed more complex aggregates, thereby increasing the protein content of boiled peanuts.

4) Total fat content

The total fat content of raw jackfruit seeds was 56.94 g/100 g, which decreased to 22.50 g/100 g upon roasting. The marked decrease in fat content during roasting might be due to the leaching of fats during dry heat processing with high temperatures [27]. However, this study also showed that the boiling process resulted in a tremendous decrease in the fat content (1.14 g/100 g) of jackfruit seeds. With the presence of water during boiling, it can be assumed that almost all fat content leached out in the cooking water.

5) Total carbohydrate content

The carbohydrate content was 21.45 g/100 g in raw jackfruit seeds, 57.99 g/100 g in roasted seeds, and 82.15 g/100 g in boiled seeds. Similar results were reported by Kumar *et al*. [33], where the carbohydrate content of roasted and boiled jackfruit seed flour was higher (72.16% and 72.05%, respectively) than that of raw flour (28.01%). The carbohydrate content of jackfruit seeds was computed by difference computation. An increase in carbohydrate content was related to the decrease in other nutrient compositions, such as moisture, ash, protein, and fat contents, as an effect of thermal processing [30, 34].

TABLE I. NUTRIENT COMPOSITION OF RAW, ROASTED, AND BOILED JACKFRUIT SEEDS

Parameters (g/100g)	**Raw**	**Roasted**	**Boiled**
Moisture	7.96 ± 0.17^{a}	6.50 ± 0.33^{b}	3.26 ± 0.07^{c}
Ash	3.76 ± 0.09^{a}	3.54 ± 0.08^{b}	3.26 ± 0.07^{c}
Crude protein	9.89 ± 0.13^{b}	9.47 ± 0.12^{c}	10.19 ± 0.13^{a}
Total fat	56.94 ± 1.54^{a}	22.50 ± 1.12^{b}	1.14 ± 0.04^{c}
Carbohydrates	21.45 ± 1.07^{c}	57.99 ± 2.90^{b}	82.15 ± 4.11^{a}
Note: Values are presented as means (±) standard deviation (n = 2); superscript letters (a, b, and c) indicate mean values that were significantly different between samples ($P < 0.05$).			

6) Dietary fiber

The dietary fiber composition of raw, roasted, and boiled jackfruit seeds is shown in Table 2. The results of this study showed that raw jackfruit seeds had a total dietary fiber content of 13.93 g/100 g, insoluble dietary fiber content of 13.83 g/100 g, and soluble dietary fiber content of >0.10g/100g. This finding revealed that the type of fiber in jackfruit seeds was mostly insoluble, which is similar to the findings of Kumari *et al.* [35]. The same results were observed in roasted and boiled jackfruit seeds. The TDF in roasted and boiled seeds was approximately 12.73 g/100 g and 12.21 g/100 g, respectively. The IDF was 12.63 g/100 g for roasted and 12.11 g/100 g for boiled seeds. Hence, the dietary fiber composition was not affected by heat processing in this study. Several studies have demonstrated that total dietary fiber is not affected by thermal heating [36]. Some effects due to processing included rearrangement or redistribution of soluble and insoluble dietary fiber; however, it was not observed in this study because dietary fiber in jackfruit seeds was mostly insoluble. Foods with a TDF of ≥10 g are considered high-fiber foods, especially those with large amounts of insoluble dietary fiber; these foods are considered beneficial for gut health. Insoluble dietary fiber speeds up digestion and adds bulk to stool. In addition, insoluble fiber keeps the bowels moving and prevents constipation. Moreover, certain insoluble fibers are fermentable

by the bacteria in colons, hence preventing the risk of colon cancer [37].

TABLE II. DIETARY FIBER IN RAW, ROASTED, AND BOILED JACKFRUIT SEEDS

Parameters (g/100g)	Raw	Roasted	Boiled
Total dietary fiber	13.93 ± 0.19^{a}	12.73 ± 0.18^{b}	12.21 ± 0.17^{c}
Soluble dietary fiber	<0.10%	<0.10%	<0.10%
Insoluble dietary fiber	13.83 ± 0.19^{a}	12.63 ± 0.18^{b}	12.11 ± 0.17^{c}
Note: Values are presented as means (±) standard deviation (n = 2); superscript letters (a, b, and c) indicate mean values that were significantly different between samples ($P < 0.05$).			

7) Dietary fiber fermentability

Fermentation occurs in all dietary fibers, but the degree of fermentation varies widely. Concerning intestinal physiology, dietary fiber should not be considered from a single perspective but rather as a term that covers various moieties with varying physicochemical properties [38]. Soluble fibers dissolve in water, whereas insoluble fibers do not. Viscous fibers thicken in water, forming very viscous solutions or viscoelastic gels. Fermentable fibers are readily metabolized by the gut microbiota (i.e., bacteria that normally colonize the large intestine). Fermentation of fiber results in the formation of short chain fatty acids (acetate, propionate, and butyrate) and gases. In this study, as shown in Table 3, it was proven that jackfruit seed produces more propionate than acetate and butyrate because of its insoluble dietary fiber. This result conforms with the study results of Opyd *et al.* [39], who reported that high propionate content is related to high insoluble dietary fiber in apple seed and pomace. The production of butyrate content might be similar to the study of Ge *et al.* [40], due to the fermentability of resistant starch in sorghum.

TABLE III. *IN VITRO* FERMENTABILITY OF JACKFRUIT SEEDS– SHORT-CHAIN FATTY ACID PRODUCTION

Parameters (ppm)	Roasted	Boiled
Acetate	609 ± 38	609 ± 25
Butyrate	1089 ± 227	1089 ± 197
Propionate	6219 ± 146	6219 ± 143
Note: Values are presented as means (±) standard deviation. ppm: parts per million		

8) Amylose and amylopectin contents

The amylose and amylopectin contents of raw, roasted, and boiled jackfruit seeds are presented in Table 4. The amylose content was 23.03 g/100 g in raw jackfruit seeds, 20.76 g/100 g in roasted seeds, and 20.61 g/100 g in boiled seeds. The experiment results indicated significant changes in the amylose content of the jackfruit seeds during boiling and roasting. This may be due to gelatinization and retrogradation during and after boiling and roasting of the jackfruit seeds. In addition, significant changes were observed in the amylose content due to the structural makeup of the jackfruit seeds, wherein the starch granules of the jackfruit seeds swell completely. The amylose content decreased due to complete solubilization and leaching out of amylose from the starch granule during thermal processing [41].

TABLE IV. STARCH CONTENT IN RAW, ROASTED, AND BOILED JACKFRUIT SEEDS

Parameters (g/100g)	Raw	Roasted	Boiled
Amylose	23.03 ± 0.57^{a}	20.76 ± 0.53^{b}	20.61 ± 0.52^{b}
Amylopectin	76.97 ± 0.57^{b}	79.24 ± 0.53^{a}	79.39 ± 0.52^{a}
Note: Values are presented as means (±) standard deviation in (n = 2) for amylose and difference computation for amylopectin. Superscript letters (a and b) indicate mean values that were significantly different between samples ($P < 0.05$).			

9) Resistant starch

Thermal processing methods can increase or decrease the resistant starch content depending on the type of food. This study showed that the resistant starch content of raw jackfruit seeds was 19.88 g/100 g, which increased to 24.42 g/100 g and 25.56 g/100 g when boiled and roasted, respectively (Table 5). High temperature treatment, such as broiling and roasting, increases the resistant starch content of jackfruit seeds. The dry, high-temperature cooking method is more effective than moist cooking method in resistant starch formation. This is because starch gels containing high moisture can cause crystallization of amylose, thereby generating resistant starch between glass transition temperature and melting temperature [42]. This was also observed in low temperature/long baking periods. The temperature of bread may reach around 100°C and stay for a long period, which can cause propagation and crystallinity, leading to the generation of more resistant starch in bread.

Also, in this study, boiling increased resistant starch content in jackfruit seeds. However, some studies have reported that boiling decreases resistant starch content in boiled chickpeas and other grains and seeds [43]. This decrease might

be due to the catabolism of amylose inhibitors that increase during boiling. In the present study, the increase in resistant starch in boiled jackfruit seeds can be explained by the principle of starch retrogradation that arises after boiling and gelatinization [44].

The recommended intake of resistant starch was estimated to be 5–6 grams per meal, which can be supplied liberally by the RS found in jackfruit seeds. The application of resistant starch in foods as a functional ingredient in bakery products, cereals, beverages, and yogurt can improve the appearance, taste, and texture of food while providing positive health benefits to humans, such as improvement in colon health and reduction of chronic diseases [45].

TABLE V. STARCH CONTENT IN RAW, ROASTED, AND BOILED JACKFRUIT SEEDS

Parameters (g/100g)	**Raw**	**Roasted**	**Boiled**
Resistant starch	19.88 ± 0.00^{c}	25.56 ± 0.00^{a}	24.42 ± 0.00^{b}
Note: Values are presented as means (±) standard deviation (n = 2) for resistant starch. Superscript letters (a, b, and c) indicate mean values that were significantly different between samples ($P < 0.05$).			

B. Nutritional properties

1) Water activity

The water activity (aw) of a food is the ratio of the vapor pressure of the food itself when in a completely undisturbed balance with the surrounding air media to the vapor pressure of distilled water under identical conditions. A significant change in the aw of raw, roasted, and boiled jackfruit seeds was observed, as presented in Table 6. The water content was 0.419 ± 0.006 for raw jackfruit seeds, 0.301 ± 0.005 for roasted seeds, and 0.124 ± 0.005 for boiled seeds. The aw scale extends from 0 (bone dry) to 1.0 (pure water), but most foods have aw in the range of 0.2 for very dry foods to 0.99 for fresh, moist foods. Aw is usually measured as equilibrium relative humidity (ERH) [41].

TABLE VI. WATER ACTIVITY IN RAW, ROASTED, AND BOILED JACKFRUIT SEEDS

Parameters (g/100g)	**Raw**	**Roasted**	**Boiled**
Water activity	0.419 ± 0.006^{a}	0.301 ± 0.005^{b}	0.124 ± 0.005^{c}
Note: Values are presented as means (±) standard deviation (n = 2) for resistant starch. Superscript letters (a, b, and c) indicate mean values that were significantly different between samples ($P < 0.05$).			

2) Color

Based on the results, a comparable significant difference in the color content of raw, roasted, and boiled jackfruit seeds was observed, as presented in Table 7. The color L* in jackfruit seed refers to the lightness value, where a decreasing effect in lightness was observed in boiled (84.09 ± 0.12) < roasted (85.07 ± 0.25) < raw (87.84 ± 0.23). Similar trends were observed with a*, which refers to greenness to redness: boiled (0.94 ± 0.07) < roasted (1.28 ± 0.10) < raw (1.36 ± 0.07). While b* in jackfruit seed, referring to blueness to yellowness, had an increasing effect when boiled (13.57 ± 0.20) > roasted (11.04 ± 0.23) > raw (9.59 ± 0.007) [16]. The thermal process is related to the change in color; it increases the *a and b* values while decreasing the L* value. Thermal processing affects the color of the seeds and results in the development of brown color in the seeds.

TABLE VII. COLOR CONTENT IN RAW, ROASTED, AND BOILED JACKFRUIT SEEDS

Parameters	**Raw**	**Roasted**	**Boiled**
L*	87.84 ± 0.23^{a}	85.07 ± 0.25^{b}	84.09 ± 0.12^{c}
a*	1.36 ± 0.07^{a}	1.28 ± 0.10^{a}	0.94 ± 0.07^{b}
b*	9.59 ± 0.07^{c}	11.04 ± 0.23^{b}	13.57 ± 0.20^{a}
Note: Values are presented as means (±) standard deviation. Superscript letters (a, b, and c) indicate mean values that were significantly different between samples ($P < 0.05$).			

3) Texture

Texture is considered a primary parameter that affects chemical changes in food products [46,65]. In freeze drying, removing water through sublimation leaves a highly porous structure [46], which produces the softness of seeds, especially in thermally processed seeds [16]. The texture of whole raw, roasted, and boiled jackfruit seeds is presented in Table 8. The results revealed that there were no significant differences between raw and thermally processed jackfruit seeds in terms of their textural properties, including cohesiveness, springiness, gumminess, and chewiness. However, in a study by Nwosisi *et al.* [47], roasting highly affected the textural properties of jackfruit seeds, possibly due to the temperature and duration of roasting. However, significant differences were observed in hardness 1 and 2, stiffness, fracture force, adhesive force, and adhesiveness. Raw jackfruit seeds have the highest value in hardness, stiffness, fracture forces, and adhesive forces compared with thermally processed seeds. These results were similar to those reported by Erkan *et al.* [48]. Hardness 1 values were 2.595 kgf–0.365 kgf and hardness 2 values were 2.278 ± 0.847–0.169 kgf, which indicated that the raw jackfruit seeds had a harder seed structure, whereas the boiled seeds had the

softest structure. Stiffness values ranged from 2.354 kgf.mm to 0.461 kgf.mm; raw seeds had the highest value (2.354 kgf.mm), indicating a lack of flexibility. Boiled seeds were the most flexible, with a value of 0.461 ± 0.113.

The fracture force value ranged from 0.024 kgf to 0.073 kgf, indicating the brittleness of the seed. The raw seeds had the highest fracture force value, whereas the boiled seeds had the lowest fracture force value. On the other hand, the adhesive force value ranged from 0.044 ± 0.075 to 0.008 ± 0.005, indicating that raw seeds have a much intact structure, and roasted seeds had the lowest value indicating that the structure was affected by heating.

The adhesiveness of the seeds ranged from 0.005 kgf.mm to 0.001 kgf.mm, which proved that the seed structure was affected by thermal heating. The low adhesiveness value of the roasted and boiled seeds indicates that low adhesiveness is related to a higher starch gelatinization process in the seeds. Textural profile analysis is an important indicator of food products. As shown in this study and other studies, thermally processed seeds are softer than raw seeds [49]. Cohesiveness, springiness, and low adhesiveness are related to thermally processed seeds due to starch gelatinization of seeds [50], whereas hardness, stiffness, and high adhesiveness are related to a harder texture of seeds.

TABLE VIII. TEXTURE ANALYSIS IN RAW, ROASTED, AND BOILED JACKFRUIT SEEDS

Parameters (g/100g)	**Raw**	**Roasted**	**Boiled**
Sample height (mm)	15.220 ± 1.832[a]	13.670 ± 1.827[a]	14.065 ± 1.352[a]
Hardness1 (kgf)	2.595 ± 0.835[a]	0.929 ± 0.383[b]	0.365 ± 0.116[c]
Hardness2 (kgf)	2.278 ± 0.847[a]	0.489 ± 0.250[b]	0.169 ± 0.068[c]
Area1 (kgf.mm)	1.003 ± 0.661[a]	0.446±0.305[a]	0.370 ± 0.093[a]
Area2 (kgf.mm)	0.043 ± 0.059[a]	0.067 ± 0.054[a]	0.018 ± 0.018[a]
Cohesiveness	0.018 ± 0.021[a]	0.204 ± 0.149[a]	0.054 ± 0.058[a]
Springiness (mm)	0.667 ± 0.331[a]	1.076 ± 0.203[a]	0.723 ± 0.353[a]
Springiness Index	0.371 ± 0.174[a]	0.622 ± 0.083[a]	0.562 ± 0.295[a]
Gumminess (kgf)	0.054 ± 0.071[a]	0.127 ± 0.093[a]	0.019 ± 0.022[a]
Chewiness (kgf.mm)	0.050 ± 0.070[a]	0.152 ± 0.133[a]	0.020 ± 0.027[a]
Fracture force (kgf)	0.073 ± 0.036[a]	0.027 ± 0.010[b]	0.024 ± 0.006[b]
Adhesive force (kgf)	0.044 ± 0.075[a]	0.005 ± 0.007[b]	0.008 ± 0.005[b]
Adhesiveness (kgf.mm)	0.005 ± 0.015[a]	0.001 ± 0.005[c]	0.002 ± 0.005[b]
Stiffness (kgf.mm)	2.354 ± 0.540[a]	0.967 ± 0.437[b]	0.461 ± 0.113[c]
Note: Values are presented as means (±) standard deviation (n = 2); superscript letters (a, b, and c) indicate mean values that were significantly different between samples (P < 0.05).			

4) Starch structure

The results of scanning electron microscopy of raw, roasted, and boiled jackfruit seeds are presented in Fig. 1. The thermal process affects the physical characteristics of starch, such as starch gelatinization and retrogradation, as well as the structure of the seeds [51]. The shape of the seeds was round and bell granular, similar to that reported in a study by Azeez *et al.* [24]. The size of the seed granules under 1000 and 2000 magnification differed from each other, with raw seeds having a smaller size granule due to an intact seed granule than processed seeds having a larger size granule due to the separation of cotyledon cell compartment inside the seeds [24,51]. The granules of the boiled and roasted seeds were expanded, which increased their surface area and porosity [52].

The rough surface of the granules indicates the presence of insoluble fractions from the plant's cell [53], which correlates to the insoluble dietary fiber of the seeds. Regarding the structure, thermally processed seeds retain their structure even with the larger size because of their resistance to heat due to high starch content [54].

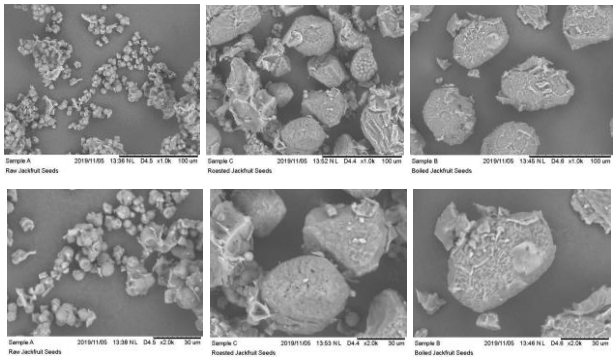

Fig. 1. SEM of raw, roasted, and boiled jackfruit seeds at 1000x and 2000x magnification.

C. Safety assessment

1) Antinutrients

The phytic acid content was 202.94 mg/100 g in raw jackfruit seeds, 251.45 mg/100 g for boiled seeds, and 232.77 mg/100 g for roasted seeds (Table 9). The phytic acid content was increased during thermal processing, and this result was

consistent with that of a study by Mbah *et al.* [55] in leguminous seeds. According to Torre *et al.* [56], the phytic acid content increases during the boiling process as phytic acid binds to starch through hydrogen bonding in the phosphate group, resulting in the formation of a complex structure [55]. As shown in Table 9, the tannic acid content was 10.80 ± 0.34 in raw jackfruit seeds, 9.11 mg/100 g in boiled seeds, and 11.96 mg/100 g in roasted seeds. The decrease in tannic acid during boiling was due to the leaching of tannic acid in the cooking water, as it is easily dissolved in water or alcohol to form colloidal solutions [18]. The high tannic acid content (11.96 mg/100 g) was due to the binding of tannin to protein, starches, and minerals [55]. According to Amadi *et al.* [57], jackfruit seeds contain higher phytic and tannic acid contents than jackfruit leaves and pulp but within acceptable levels. In the present study, the phytic and tannic acid contents of raw, roasted, and boiled jackfruit seeds were within the acceptable levels (250 mg/100 g and 20 g/100 g, respectively) [58]. In the boiled seeds, the phytic acid content slightly exceeded the acceptable levels.

TABLE IX. ANTINUTRIENTS IN RAW, ROASTED, AND BOILED JAKCFRUIT SEEDS

Parameters	Raw	Roasted	Boiled
Phytic acid (mg Phosphorus/100g sample)	202.94 ± 5.08^{c}	232.77 ± 3.68^{b}	251.45 ± 3.38^{a}
Tannic acid (mg tannic acid/100g sample)	10.80 ± 0.34^{b}	11.96 ± 0.33^{a}	9.11 ± 0.32^{c}
Note: Values are presented as means (±) standard deviation (n = 3); superscript letters (a, b, and c) indicated mean values that were significantly different between samples (P < 0.05).			

2) Heavy metal content

Consumption of foods contaminated with heavy metals may lead to toxicity and cause several disorders, such as organ malfunctions and chronic diseases [59]. The heavy metal contents of raw, roasted, and boiled jackfruit seeds, as shown in Table 15, were within the maximum permissible level set by the World Health Organization, European Food Safety Authority, and US Food and Drug Administration [60]. The variety of the seed as well as the location where the plant is grown influence the heavy metal content.

TABLE X. HEAVY METAL ANALYSIS IN RAW, ROASTED, AND BOILED JAKCFRUIT SEEDS

Parameters (g/100g)	Raw	Roasted	Boiled	WHO/EFSA and USFDA
Cadmium	ND (<0.05)	ND (<0.05)	ND (<0.05)	0.1
Lead	ND (<0.04)	ND (<0.04)	ND (<0.04)	0.1
Mercury	ND (<0.07)	ND (<0.07)	ND (<0.07)	0.5
Arsenic	ND (<0.07)	ND (<0.07)	ND (<0.07)	0.1
Note: ppm: parts per million, ND: not detected				

3) Microbiological properties

The results of the present study revealed that raw jackfruit seeds exceeded the maximum acceptable levels of counts for aerobes (2.2×10^6 est.), coliforms (1.4×10^6 est.), and yeast and molds (1.0×10^4). These same results were reported by Noah and Ogunfowote [61]. The roasted and boiled jackfruit seeds had microbiological counts within acceptable levels, thereby making them safe for consumption. Some microorganisms are destroyed or inactivated during high-temperature cooking.

TABLE XI. MICROBIOLOGICAL PROPERTIES IN RAW, ROASTED, AND BOILED JAKCFRUIT SEEDS

Parameters (g/100 g)	Raw	Roasted	Boiled	Center for Food Safety
Aerobic plate count, CFU/g	2.2×10^6 est.	4.1×10^3	5.0×10^3	$<10^3$
Coliforms, CFU/g	1.4×10^6 est.	2.2×10^2	5.4×10^2	$<10^3$
Yeast and Molds, CFU/g	1.0×10^4	1.8×10^3	1.3×10^3	$<10^3$
Escherichia coli, MPN/g	<1.8	<1.8	<1.8	<3
Note: <1.8 means the sample is negative for *Escherichia coli*				

IV. CONCLUSIONS

Thermal processing may contribute to the nutritional and physicochemical properties of jackfruit seeds and its safe consumption. The nutritional composition of jackfruit seeds favors thermal processing in terms of high source of nutrient composition, total and insoluble dietary fiber, and resistant starch. The physicochemical properties of jackfruit seeds, including water activity, color, and texture, were all improved due to thermal processing. Safety assessment of jackfruit seeds indicated that the levels of antinutrients, heavy metals, and microbes were within acceptable levels, indicating that the seeds

had undergone thermal processing. Jackfruit seeds are recommended to be eaten roasted and boiled. These seeds can also be used for fortification or as a complete or partial substitute to various food products, such as extruded and baked products like bread and pastries. Jackfruit seeds can also serve as an alternative source of cocoa because of their similar flavor and aroma to cacao beans [62]. Furthermore, jackfruit seeds are considered gluten-free food [63]; hence, they are recommended for use within gluten-free products.

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CONFLICT OF INTEREST

All authors declare no conflicts of interest in this paper.

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