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Physicochemical and functional properties of breadfruit flour, commercial wheat flour and starches

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Artocarpus altilis or breadfruit is a tropical fruits as well as traditional starch rich crop and originated from Malaysia. However, this fruit is underutilized due to inadequate information regarding its physicochemical (physical and chemical) characteristics and potential use in industries. Thus, a study was carried out to produce, determine and compare the physicochemical and functional properties of breadfruit flour with commercial wheat flour and starches (corn, potato and tapioca). Breadfruit flour showed highest in ash (2.53%) and lowest in moisture content (10.94%) amongst five samples. Breadfruit has significantly higher in water and oil holding capacity than wheat flour and starches, 1.53 g/g and 1.16 g/g respectively. The swelling power was found to increase with increasing temperature for all samples. Potato starch exhibited the highest swelling power (15.02 and 27.82 g/g) at 60 and 70°C. Whereas breadfruit flour showed slight tendency to swell (5.44 g/g) at 70°C. The pasting temperature was highest (82.75°C) while breakdown viscosity was lowest (33.50 cP) for breadfruit flour. Setback values for breadfruit flour and tapioca starch were lower than other samples, 1317.50 and 1221 cP respectively, which made those samples tend to expose to high retrogradation process. Those properties for breadfruit flour has made it could be incorporated in various food products owing to the differences in their physicochemical and functional properties compared to other commercial wheat flour and starches.

Keywords: Artocarpus altilis, underutilized fruit, physicochemical properties, functional properties

INTRODUCTION

Upgrading of deserted crops to a functional and valuable food ingredients for human consumptions has captured worldwide attention. Breadfruit (*Artocarpus altilis*) is an example of deserted crops that owing a great potential values which needs to be developed (Gbadamosi and Oladeji, 2013). This fruit is believed to possessed beneficial physiological effects and a good source of carbohydrates, particularly starch. Furthermore, some literature has reported that the breadfruit is native to Malaysia and has spread through the South Pacific and Caribbean (Sikarwar et al., 2015). However, this fruit is commonly classified as one of underutilized crops in Malaysia due to lack of information on its physicochemical and functional properties plus with poor storage of the fresh fruits have made it restricted to be applied in industries (Liu et al., 2014). It has recommended that the conversion of fresh breadfruit into starch or flour would provide better shelf life and increase its versatility, indirectly its application in industries could be maximized (Ojokoh et al.,

2014).

The flour acts as a raw material for the industries of basic foods such as bread, cakes and other foods, where the wheat flour is the most commonly consumed flour. Thus, it has increased the flour demand (Amaral et al., 2016). In order to fulfill the demand, there is needs to expand the production and find other carbohydrate-rich crops to become as an alternative flour sources, such as breadfruit flour. It could be assessed from the novel and underutilized crops rather than relying on the marketed flour and starch sources like wheat, corn and tapioca, which will deficit over the years. Thus, the aim of this study is to produce the breadfruit flour, determine its physicochemical and functional properties and compare to commercial wheat flour and starches (corn, potato and tapioca).

MATERIALS AND METHODS

This study involved three (3) phases; the production of breadfruit flour, physicochemical study and functional properties of breadfruit flour in comparison to commercial wheat flour and starches (corn, potato and tapioca).

Production of breadfruit flour

Mature breadfruits were freshly picked from a tree owned by local people in Bukit Tunggal, Kuala Terengganu while the commercial wheat flour and starches were purchased from a local store in Gong Badak, Kuala Terengganu. The maturity of breadfruits were classified based on its color and fruits morphology (Anonymous, 2013). The breadfruit flour was produced from the fresh breadfruits as followed a method by Nochera and Ragone (2016) with some modifications. The fruit's skin were washed and peeled manually, then rewashed again under running tap water. The breadfruit pulps were sliced using semiautomated slicer to produce thin and uniform slices. Then the pieces were dried in electrical dryer cabinet at 50°C for 2 days (48 hours). The dried pulp were ground in a mill to produce flour and sieved through 125 µm sieve mesh (120 mesh), kept in container and stored in chiller at 4°C.

Physicochemical properties

Physicochemical properties of the samples were proximate analysis, total starch, amylose, amylopectin content, bulk density, granules morphology and pasting properties were determined in this study.

Proximate analysis

The moisture content was determined using oven drving method while the ash contents were determined by dry ashing. The nitrogen contents of samples were assessed using Kjeldahl's method. The crude protein percentage was calculated by multiplying nitrogen percent with a factor of 5.70. Also, the crude fat contents were calculated using petroleum ether as a solvent in a Soxhlet apparatus. For the determination of crude fibers content, the samples were digested with 0.13% sulphuric acid (H₂SO₄) followed by 0.313% sodium hydroxide (NaOH) solution. These methods were followed as described by American Association of Cereal Chemists (AACC, 2000). The total percentage of carbohydrate content for samples were determined by the difference method as reported by Devi et al., (2015). The energy values were determined based on a method by Olapade and Umeonuorah (2015) using Atwater's conversion factor as follows:

Energy (kcal/100g) = $(4 \times \% \text{ Crude protein}) + (9 \times \% \text{ Crude fat}) + (4 \times \% \text{ Carbohydrate})$

Total starch content, amylose and amylopectin contents

Total starch content were determined according to Zi-Ni et al. (2015) with minor modifications. The samples were treated with amyloglucosidase and the liberated glucose were measured using glucose oxidase and peroxidase (GOPOD) reagent (Robonik, India). The amylose and amylopectin content of samples were determined according to Barine and Yorte (2016) with few modifications. While amylose content was measured based on the standard curve of amylose (0-100%). The amylopectin content were calculated by the difference of total starch and the amylose contents.

Bulk density and granules morphology

Bulk density were obtained according to Olapade and Umeonuorah (2015). A 15 g sample was put into 100 mL graduated cylinder, then the cylinder was tapped forty (40) times and the results were calculated as weight per unit volume (g/cm³). The granules morphology were observed using scanning electron microscope (SEM), JEOL JSM-6360LA (Jeol Ltd, United States) followed method Turi et al., (2015) with slight modifications. The sample was evenly distributed on SEM specimen stubs with double adhesive tape and coated with a 10 nm gold layer by auto-fine coater machine, JFC-1600 (Jeol Ltd, United States). The stub with gold coated sample was then placed in the SEM chamber which was evacuated before the electron beam was turned on. A 10kV/2.05A setting was used and the aperture size being fixed at 3.

Pasting properties

The pasting properties of samples were assessed using the Rapid Visco-Analyzer, Model RVA 4500 (Perkin Elmer, Sweden), followed a method by Barber et al. (2016). Three grams (3 g) of sample was dispersed in an aluminum canister containing 25 g of distilled water. The flour-water suspension was held at 50°C for 1 minute and then heated up to 95°C and re-held for 10 minutes. Block temperature was cooled to 50°C and held for another 2 minutes. Results were expressed as viscosity in centipoise (cP) for pasting viscosity (PV), breakdown (BD), final viscosity (FV) and setback (SB), °C for pasting temperature (PT).

Functional properties

Water and oil holding capacities

The water holding capacity (WHC) and oil holding capacity (OHC) of samples were determined according to Zi-Ni et al., (2015) with several modifications. Sample (1 g, dry basis) was weight in pre-weighted 15 mL centrifuge tube and vortexed in 10 mL of distilled water or oil at ambient temperature (25 °C) for 1 minute followed by centrifugation (3610 rpm, 30 minutes). The supernatant was removed and the wet sediment was weighed. The WHC and OHC were expressed as weight of water or oil held per gram of samples.

Swelling and solubility power

Swelling and solubility power of samples were determined according to Zi-Ni et al. (2015) with slight modifications. Hundred milligram (100 mg) of flour was accurately weighed into a pre-weight 15 mL centrifuge tube, and 10 mL of distilled water was added. The tube was placed in a water bath at 60, 70, 80 and 90°C for 30 minutes until it become translucent. Then the sample's solution were centrifuged at 3610 rpm for 15 minutes and the supernatant was carefully removed. The wet sediment in the centrifuge tube was weighed (for the calculation of swelling power). For solubility analysis, an aliquot (5 mL) of the supernatant from swelling analysis was transferred into a preweighed moisture dish and dried at 110°C in an oven overnight. The moisture dish was cooled in a

desiccator and weighed for calculation of solubility.

Statistical analysis

All experiments were conducted in triplicate. All the results were expressed as mean \pm SD (standard deviation). One-way ANOVA with Duncan post test procedures of Statistical Package for Social Science, Version 24.0 (IBM SPSS Inc., Chicago, USA) was used for the analysis. The level of significance was set at p< 0.05 (95% confidence level).

RESULTS AND DISCUSSION

Physicochemical properties of breadfruit flour, commercial wheat flour and starches

Proximate analysis

Table 1 below shows the nutritional values for breadfruit flour, commercial wheat flour and starches (corn, potato and tapioca). The moisture content ranged between 10.94% and 16.10%. The highest moisture content was obtained in potato starch (16.10%) while the lowest was obtained in breadfruit flour (10.94%). Generally, the moisture content of all samples were within acceptable levels of the flours and starches (10-14%) (Abena et al., 2014). Statistical analysis showed that there was a significant difference (p<0.05) between the moisture contents obtained from all the flours and starches. The variation in moisture content may be due to the differences in the method of flour and starch processing. Devi et al., (2015) has explained the moisture content plays a vital role in determining the shelf-life of food products. High level of moisture content also is an indicator for perishability of food products. Thus, it can be assumed that the shelf life of breadfruit flour was better than wheat flour and starches for this study.

The ash content of samples were ranging from 0.11% and 2.53%. Among the flour and starches samples, breadfruit flour exhibited highest ash content (2.53%) while the lowest is tapioca starch (0.11%). The statistical analysis had shown that there was a significant difference (p<0.05) between wheat flour and starches. The ash content of breadfruit flour was higher than the cassava (1.0%) and yam flour (2.03%) but lower than *Prosopis africana* flour (4.4%) and millet flour (3.7%) (Abena et al., 2014). For corn and tapioca starch, both does not showed significant difference (p>0.05). The ash content has been clarified by Devi et al., (2015) where it reflects the presence of mineral elements in the sample. In this study, it can be suggests that the breadfruit flour could be a good source of minerals.

As regards to the crude fat content, the values ranged between 0.01% and 1.64%. Wheat flour exhibited the highest fat content, 1.64% while potato starch is the lowest value, 0.01%. However, breadfruit flour also possessed moderately high in fat content, 0.83% compared to all samples. Saeid et al., (2015) has explained that the crude fat of samples portrays its chances towards rancidity and shelf life. The lower crude fat indicates the lower chances rancidity. From fat contents, it can be observed that commercial starches are prone to have low fat content compared to flours. This is might due to the starches which have been refined form the flour to starch.

In addition, the crude protein of samples have ranged between 0.11% and 9.36%. The wheat flour possessed the highest value, followed by breadfruit flour (5.74%) among the samples. As compared to a study by Abena et al. (2014), the protein content for breadfruit flour is 4.43% which was lower from this study. Potato and tapioca starch does not show any significant difference (p>0.05) of crude protein. The differences in protein content were due to the nature properties of flour and starches and it has be explained by Birt et al., (2013) where highly refined starches have been stripped of nearly all their nutrients and fibers and just remained a lots of carbohydrates. The sample's crude fiber were ranged between 0.04% and 5.44%, where the breadfruit flour showed highest crude fiber and differed significantly (p<0.05) among all the samples. This value showed higher than vam and cassava flour where the fiber contents were 1.65% and 1.00% respectively (Devi et al., 2015). Crude fiber has brought many vital effects on human's diet where it can increase the bulk of feces, which has a laxative effect in the gut (Devi et al., 2015).

Starches samples were observed to have low crude fiber compared to breadfruit and wheat flour. This might due to the processing of starches that have been purified from the native flour. From this study, it can be seen that breadfruit possessed high in fiber which could be incorporated in foods especially, bakery goods as a source of fiber by replacing or making a composite with wheat flour. From the results, the starches have shown highest carbohydrate content ranged between 99.23% and 99.42% compared to breadfruit and wheat flour. This is because the starch has monopolized the carbohydrate composition for about 53-76% (Huang et al., 2017). Even though the breadfruit flour has lower carbohydrate content (85.46%), but then there is a previous study has pondered that breadfruit contained complex carbohydrate which could prevent onset of type II diabetes. However, the detailed scientific studies have not been clarify yet (Turi et al., 2015).

Total starch, amylose and amylopectin contents

The results for total starch, amylose and amylopectin contents are shown in Table 2. The sample's total starch are varied from 62.44% to 81.28%. Breadfruit flour showed the lowest (62.44%) and the highest is corn starch. The total starch of all samples had differed significantly (p<0.05). This was due to the flour contain other mixtures; protein, fiber, minerals and other component while the corn, potato and tapioca comprised of refined starch (Birt et al., 2013). The result for breadfruit flour in this study is in the range of total starch studied by Bakare et al. (2015) which between 53-76%. Generally, starch composed of two glucans namely amylose and amylopectin. The ratio of amylose: amylopectin were differ amongst the samples and the breadfruit has ratio of 53:47 which higher than wheat flour, 49:51. For the commercial starches, potato starch exhibited the highest amylose content with ratio of 61:39.

Madzlan et al., (2012) has explained the starches from different sources may differ in overall structure through size distribution of the granules, shape, amylose and lipid content, distribution of chain length in amylopectin and crystalline structure. Furthermore, a study by Zi-Ni et al., (2015) has enlightened that any foods that have high in amylose are tend to form more complex carbohydrate when treated with different processing methods (Eroglu and Buyuktuncer, 2017). Meanwhile, any foods that have high amylopectin can increase human insulin levels which then can prevent onset of type II diabetes (Madzlan et al., 2012).

Analysis	Breadfruit flour	Wheat flour	Corn starch	Potato starch	Tapioca starch		
Moisture (%)	10.94 ± 0.10 ^a	12.42 ± 0.09 ^{bc}	12.11 ± 0.39 ^b	16.10 ± 0.06^{d}	12.63 ± 0.03 ^c		
Dry weight basis							
Ash (%)	2.53 ± 0.02^{d}	0.76 ± 0.06 ^c	0.18 ± 0.04^{a}	0.40 ± 0.03^{b}	0.11 ± 0.07 ^a		
Crude Protein (%)	5.74 ±0.15 ^c	9.36 ± 0.14 ^d	0.33 ± 0.05^{b}	0.13 ± 0.05^{a}	0.11 ± 0.01 ^a		
Crude Fat (%)	$0.83 \pm 0.02^{\circ}$	1.64 ± 0.02^{d}	0.11 ± 0.05^{b}	0.01 ± 0.01 ^a	0.04 ± 0.02^{a}		
Crude Fibre (%)	5.44 ± 0.35^{b}	0.27 ± 0.17 ^a	0.15 ± 0.17 ^a	0.04 ± 0.07^{a}	0.30 ± 0.26^{a}		
*Carbohydrate	85.46 ± 0.35 ^a	87.98 ± 0.32 ^b	99.23 ± 0.25°	99.42 ± 0.09 ^c	99.31 ± 0.34 ^c		
by difference (%)							
Energy (kcal/100g)	372.29 ± 1.49 ^a	404.11 ± 0.69 ^c	399.21 ± 0.47 ^b	398.29 ± 0.26 ^b	398.58 ± 1.19 ^b		

Table 1. Nutritional values of breadfruit flour in comparison with wheat flour, corn starch, potato starch and tapioca starch.

Results are expressed as mean \pm standard deviation, N = 3

Mean values in the same row followed by different superscript lowercase letters ^{abcd} are significantly different (p <0.05).

*Carbohydrate by difference (%) = 100 - (% crude protein + % crude fat + % ash + % crude fibre) (Olaoye and Onilude, 2008)

Table 2; Composition and properties of breadfruit flour in comparison with wheat flour, corn starch, potato starch and tapioca starch.

Analysis	Breadfruit flour	Wheat flour	Corn starch	Potato starch	Tapioca starch
Total starch (%)	62.44 ± 0.56^{a}	64.93 ± 0.38 ^b	81.28 ± 1.51 ^e	75.77 ± 0.20 ^d	73.42 ± 0.38 ^c
Amylose (%)	33.37 ± 0.33^{a}	31.87 ± 1.33 ^a	39.64 ± 0.23 ^b	46.01 ± 1.42 ^c	40.86 ± 0.32^{b}
*Amylopectin by difference (%)	29.07 ± 0.23ª	33.07 ± 0.95 ^b	41.64 ± 1.28 ^c	29.75 ± 1.61ª	32.57 ± 0.05 ^b
Bulk density (g/cm ³)	0.47 ± 0.03^{a}	$0.67 \pm 0.02^{\circ}$	0.58 ± 0.02^{b}	0.85 ± 0.03^{d}	0.58 ± 0.01^{b}

Results are expressed as mean ± standard deviation, N = 3

Mean values in the same row followed by different superscript lowercase letters ^{abcd} are significantly different (p <0.05).

*Amylopectin by difference (%) = Total starch – Amylose (Zi-Ni et al., 2015)

Bulk density

The bulk density was varied between 0.47 to 0.85 g/cm³ where the breadfruit flour exhibiting the lowest bulk density (0.47 g/cm3). There was a significant difference (p<0.05) showed between breadfruit flour and other samples while corn and tapioca have the same values of bulk density (0.58 g/cm³). Bulk density referred to the quantity of material that can be packaged within a specified packaging space (Gbadamosi and Oladeji, 2013). The researcher also added, bulk density also depends on the combined effects of interrelated factors such as the intensity of attractive inter-particle forces, particle size, and number of contact points. So, the higher the bulk density, the greater quantity of material that can be packaged within a specified packaging space. In this study, potato starch would showed a great potential in packaging system.

Granules morphology

Figure 1 below showed the morphological properties of breadfruit flour (A, B) and potato starch (C) granules. The micrographs of breadfruit flour was observed at magnification of 1000X and 2000X using scanning electron microscope (SEM). The breadfruit granule was spherical with intended and umbilical point was at the center. The average diameter was 5-10 µm and it is in the range of diameter of breadfruit starch (4-16 µm) (Verma and Srivastav, 2017). But, the breadfruit granule is smaller than white yam starch (19.2-30.8 µm), this happens due to different types of granules properties; flour and starch (Ssonko and Muranga, 2017). Starch granule size has been reported to affect starch physicochemical properties and at the same amylose content (Ssonko and Muranga, 2017).



Figure 1; Granules micrograph of breadfruit flour (A, B) observed under the 1000X and 2000X magnifications, while potato starch (C) observed under 2400X of magnification (Preedy and Watson, 2019).

Pasting properties

The results of pasting properties of the starch samples are presented in Table 3 which included pasting temperature (PT), peak viscosity (PV), breakdown (BD), final viscosity (FV) and setback (SB) values. Breadfruit flour possessed lowest PV, 2118.5 cP compared to other samples. PV is associated with the size of granules where it is inversely proportional with PV values (Zi-Ni et al., 2015). The PT of samples ranged between 67.83-83.75°C with breadfruit flour exhibiting the highest and potato starch is the lowest of PT. Gbadamosi and Oladeji (2013) has defined the pasting temperature where it measures the minimum amount of temperature required to cook a given sample which as well as can have implications to the cost of energy used.

Besides that, Barber et al., (2016) added, the ability of starch to imbibe water and swell is primarily dependent on the pasting temperature. The higher the pasting temperature, the faster the tendency for paste to be formed. Thus from the result, breadfruit flour will form paste faster than other samples. As for BD values, breadfruit flour showed the lowest (33.50 cP) and potato starch is the highest (11340 cP) and the values were differed significantly (p<0.05) among all the flour and starches. The breakdown viscosity is a measures of the cooked starch to disintegrate (Gbadamosi and Oladeji, 2013). This implies that breadfruit flour is more stable to heat and mechanical shear than wheat flour and starches (corn, potato and tapioca).

SB values for the pasting properties refers to the rate of retrogradation of flours and starches during cooling process (Barber et al., 2016). The SB values were ranged at 1221 cP to 1887 cP and showed there is a significance difference (p<0.05) between the samples tested. Breadfruit flour is the second lowest of SB values compared than tapioca starches and this showed a good signs where those samples has higher retrogradation process during cooling and which tend to form complex carbohydrates after various cooking methods.

Analysis	Breadfruit flour	Wheat flour	Corn starch	Potato starch	Tapioca starch
PV (cP)	2118.5 ± 38.9 ^a	3018.5 ± 85.6 ^a	5459.0 ± 62.2 ^b	13051.0 ± 1253.0 ^d	8929.5 ± 9.2°
PT (°C)	82.8 ± 0.6 ^d	69.7 ± 0.6^{b}	$76.7 \pm 0.0^{\circ}$	67.8 ± 0.0^{a}	68.6 ± 0.0^{a}
FV (cP)	3402.5 ± 116.7 ^{ab}	3323.0 ± 104.7 ^a	4811.5 ± 64.4 ^c	3146.0 ± 282.8 ^a	3784.5 ± 139.3 ^b
BD (cP)	33.5 ± 9.2^{a}	1178.5 ± 26.2 ^{ab}	2534.5 ± 33.2 ^b	11340.0 ± 1462.3 ^d	6366.0 ± 124.5 ^c
SB (cP)	1317.5 ± 68.6 ^{ab}	1483.0 ± 7.1°	1887.0 ± 35.4 ^d	1435.0 ± 73.5 ^{bc}	1221.0 ± 24.0 ^a

 Table 3. Pasting properties of breadfruit flour in comparison with wheat flour, corn starch, potato starch and tapioca starch.

Results are expressed as mean ± standard deviation, N = 3; Mean values in the same row followed by different superscript lowercase letters ^{abcd} are significantly different (p <0.05).

Functional properties

Water and oil holding capacities

Water-holding capacity (WHC) measures the interaction magnitude of the samples with water molecules. Figure 2 shows the WHC and oil holding capacity (OHC) for breadfruit flour in comparison with commercial flour and starches. The WHC for all the samples were differed significantly as breadfruit flour possessed highest (1.52 g/g) while potato starch shows lowest (0.73 g/g). Other carbohydrate sources such as cassava starch (1.03 g/g), cocoyam starch (1.02 g/g) and breadfruit starch (0.71 g/g) in a study of Gbadamosi and Oladeji (2013) possessed lower WHC than breadfruit flour.

The ability of starch or flour to absorb water is desirable in food systems as it could improve yield, consistency and gives body to the wide range of foods (Gbadamosi and Oladeji, 2013). In this study, it can be said that breadfruit flour could perform well as an alternative flour to replace or combine with any commercial flour since it have highest WHC. WHC also important in baking industry because it can provides a better texture for bread and easy to be handled during processing besides decrease the loaf volume and maintaining the bread crumbs structure (Ordonio and Matsuoka, 2016). The OHC is measures the ability of starches to hold the oil. Breadfruit flour has the highest (1.16 g/g) and potato starch has lowest OHC value (0.60 g/g). The OHC values were significantly difference (p<0.05) between the samples. As a comparison to other studies, OHC of breadfruit flour was slightly higher than cassava starch (1.12 g/g) and cocoyam starch (1.02 g/g) (Gbadamosi and Oladeji, 2013).

Swelling and solubility power

Swelling power is a measure of hydration capacity or it can be said that it measures swollen starch granules with trapped water in sample (Huang et al., 2017). It is affected by amylose. amylopectin and phosphate groups in starch chains (Awokoya et al., 2016). However, Zi-Ni et al., (2015) stated that swelling power of a product was positively correlated with amylopectin content, where amylopectin can swell freely without any restriction by amylose. A study by Sikarwar et al., (2015) has added that the swelling power is related to the associative binding and strength within the starch granules. Figure 3 and 4 show the swelling power and solubility power of flours and starches. The swelling power for all samples were increased as the temperature increased.





In this study, different temperatures (60, 70, 80 and 90°C) were employed in order to know does the temperatures affect the swelling power of the samples.

Potato starch showed significant increment in swelling power after 70°C. Gbadamosi and Oladeji (2013) stated that those flour and starch which have high swelling power may serve as useful ingredients for baked goods, baby food, sauces and retorted canned foods. This is because high temperature is exerted for processing of those foods. So, from this study, breadfruit flour could be chosen as one of the functional ingredients for the foods. Solubility is measures the mobility of starch granules which facilitated enhanced dispersion of starch molecules in water (Aderinola, 2016). There is significant difference (p<0.05) observed for the solubility of all sample.



Figure 3; Effect of temperatures on swelling power of breadfruit flour in comparison with wheat flour, corn starch, potato starch and tapioca starch



Figure 4; Effect of temperatures on solubility power of breadfruit flour in comparison with wheat flour, corn starch, potato starch and tapioca starch

CONCLUSION

Breadfruit flour was studied in terms of physicochemical and functional properties in comparison with commercial flour and starches. Breadfruit flour contained high in ash and low in fat. For the functional properties, it has high in WHC and OHC, lowest breakdown viscosity and small granules morphology.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

SNMN performed the experiments, run data analysis and also wrote the draft. ZZ designed, supervised the experiments and reviewed the manuscript. NH acts as a project leader for this research grant and designed the flow of experiments.

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