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Granules morphology, crystalline structures and thermal properties of Malaysian breadfruit (*Artocarpus altilis*) flour and commercial wheat flour

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This study was carried out to determine the granules morphology, crystalline structures and thermal properties of the breadfruit flour in comparison to commercial wheat flour. Breadfruit (*Artocarpus altilis*) flour was produced from local matured unripe breadfruit while commercial wheat flour was obtained from local store. Both flours have dissimilar granule sizes and morphology. Breadfruit flour consisted of small granules (5-10 µm), spherical in shape with intended and umbilical point at the center while commercial wheat flour has larger granules (10-15 µm), oval in shape with smooth granules surface. Both flours exhibited different types of crystalline structures determined based on X-ray diffraction patterns. Breadfruit flour gave B-type diffraction pattern at 2Ө = 14.94°, 17.03°, 22.84° and 34.30°, commercial wheat flour indicated A-type diffraction pattern at 2Ө = 15.56°, 17.66°, 18.24° and 22.99°. The value of gelatinization temperature (T_p), temperature range (T_c - T_o) and enthalpy of gelatinization (ΔH) were significantly different between breadfruit flour (77.01°C, 8.60°C, 4.83 J/g) and commercial wheat flour (64.83°C, 18.82°C, 1.36 J/g) respectively. This study revealed that the breadfruit flour exhibited different flour characteristics with commercial wheat flour which may facilitate its applications in wider industrial applications.

Keywords: *Artocarpus altilis*, granules morphology, crystalline structures, thermal properties

INTRODUCTION

Breadfruit or scientifically known as *Artocarpus altilis*, is a tropical food crop that owing large and round of fruit's morphology (Wang et al. 2011). Breadfruit belongs to the family of Moraceae and shared the same family with figs, mulberries, and jackfruit (Zerega et al.2016). Predominantly, breadfruit is known to native to Malaysia, the South Pacific and the Caribbean, plus also has been introduced into South Western Nigeria since centuries ago (Nwokocha & Williams, 2011). Breadfruit usually harvested for a source of carbohydrate particularly starch and had become a staple food for these areas (Siti Nuriah et al., 2019, Zarinah et al. 2018). There are varieties of breadfruit cultivars (*Artocarpus* spp.) that previously reported by Liu et al. (2014) such as *Meinpadahk, Meitehid* and *Rotuma*. Particularly in Malaysia, *Meinpadahk* was dominated among other cultivars. *Meinpadahk* is recognized to be a highly productive cultivar that produced fruits twice in a year, March to June and July to September as high as 269 fruits per tree annually (Alice et al., 2012). Hence, there was highly abundance of fruits during its season and prone to deteriorate after 2 to 5 days of harvesting without proper storability (Zerega et al., 2016). Processing of the fresh breadfruit into flour and starch has been reported by many studies as it could diminish those problems (Adepeju et al. 2011). There are various advantages that arise from this conversion such as better shelf life and increase its versatility, indirectly its application in industries could be maximized (Siti Nuriah et al., 2019).

To date, there is a growing tendency towards finding alternative flour and starches sources from novel and underutilized food crops, such as breadfruit rather than relying on other sources such as wheat, potato and corn flour. Consequently, it could fulfil the market demand and it is a noteworthy way to alleviate poverty and hunger worldwide (Barber et al., 2016). For instance, food-deficit countries such as South Pacific and the Caribbean have fully utilised existing starch rich crop, breadfruit for their food source (Zarinah et al., 2019). The breadfruit flour, is a less common flour type that worth to investigate and it might be able to use as substitution of other readily accessible flours such as potato, corn and wheat. Previously, breadfruit was renowned for its fat, ash, fibre and protein as reported by Alice et al. (2012). Precisely, every 100 g of breadfruit contains about 1.34 g protein, 0.31 g fat, 27.82 g carbohydrate, 1.5 g fiber, 1.23 g ash, and other nutrients such as calcium, phosphorus, iron, potassium, carotene, and vitamin B (Wang et al. 2011). Due to the fact that breadfruit flour that high in carbohydrate content (76.7%), thus has made it as a valuable source of starch as well (Huang et al. 2017).

Flour, generally comprised of a high proportion of polysaccharides namely starch (Verma & Srivastav, 2017). In order to produce new flour-based products from breadfruit, it is vital to study the flour's properties such as granules morphologies, types of crystalline structures and thermal properties. In Malaysia, Information on breadfruit flour is scarce especially in comparison to commercial flour sources, such as wheat flour. It was remarked that wheat flour has appeared as the most widely cultivated food grains among cereals for human consumption (Korma, 2016). Hence, this study was carried out to produce breadfruit flour and to determine the granules morphology, crystalline structures and thermal properties of breadfruit flour in comparison to commercial wheat flour.

MATERIALS AND METHODS

Flour samples

Breadfruit flour was produced from the processing of the fresh breadfruits as followed a method by Nochera and Ragone (2016) with some modifications. The breadfruits were collected from three (3) locations in Kampung Bukit Tunggal, near the city of Kuala Terengganu where the geographical coordinate was 5°N, 103°W. The fruits were washed and its skin was peeled manually then rewashed under running tap water. The breadfruit pulps were sliced using a semi-automated slicer to produce thin and uniform slices (thickness ~2.5mm, diameter ~ 3cm). Then the breadfruit pieces were dried in the lab dryer cabinet at 50˚C (48 hours). The dried pulp was ground using a grinder and the flour was sieved through 125 µm sieve mesh (120 Mesh), kept in airtight container and cold stored at 4°C. Commercial wheat flour was purchased from a local store in Gong Badak, Kuala Terengganu with particle size range of 180-200 µm for direct human consumption (Alimentarius, 1985). All the chemicals used in the experiments were analytical grade and purchased from Merck Milipore (Germany), Bendosen Laboratory Chemicals (Norway), Nacalai Tesque, Inc. (Japan), Tokyo Chemical Industry (Japan) and Sigma Aldrich (Germany).

Granules morphology

The granules morphology was observed using a scanning electron microscope (SEM) model JEOL JSM-6360LA (Jeol Ltd, USA) according to Nwokocha and Williams (2011) with slight modifications. The flour samples were sprinkled onto a double adhesive tape stuck on a circular aluminum stub. The aluminum stubs with flour samples on it were then coated with gold for 140 seconds in the vacuum chamber of autofine coater machine, model JFC-1600 (Jeol Ltd, USA). The stubs with gold coated samples were then placed in the SEM chamber which had been evacuated earlier before the electron beam was turned on. The SEM was operated at 10kV/2.05A and the aperture size is fixed at 3. The samples were viewed at 2000× magnifications to get the clear sample's morphology.

Crystalline structure

The crystalline structure of the flour samples was determined using a desktop X-ray diffractometer (Rigaku MiniFlex II, Japan) according to Nwokocha and Williams (2011). The samples were placed onto air sensitive sample holder of the diffractometer and scanned with a CuKa target at a voltage of 40 kV and current of 30 mA. X-ray diffraction patterns were recorded using a HyPix-400 MF (2D HPAD) detector and scanned between 6° to 40° (2θ) at the rate of 12°/minutes.

Thermal properties

Thermal properties of flour samples were determined according to method Wang et al. (2011) and Siswoyo and Morita (2010) with modifications. This analysis was done using differential scanning calorimetry, DSC (DSC Q2000, TA Instruments, USA) which operated in nitrogen gas. The range of temperature used was 20°C to 150°C at a rate of 5°/minutes under oxygen-free nitrogen flow of 50 mL/minutes. The changes of sample temperatures were measured and displayed in DSC thermograms. Flour samples (3 mg) were weighed into tarred aluminum sample pan and distilled water (6 µL) was added carefully into the sample. The pan was then sealed hermetically using a DuPont encapsulation press. The pan containing samples and water was left to be in equilibrium on a shaker (IKA® KS 130 Control, Staufen, Germany) at 200 rpm under room temperature for 24 hours. Before analysis, the DSC instrument was first calibrated using indium. Then the samples were heated. An empty aluminum pan was used as a reference. The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and gelatinization enthalpy (ΔH) were determined from DSC thermograms using the TA Universal Analysis 2000 software (V4.5A). Gelatinization enthalpy was estimated by integrating the area between the thermogram and a baseline under the peak and was expressed in terms of joules per unit weight of dry starch (J/g).

Statistical Analysis

The Statistical Package for Social Science, Version 24.0 (IBM SPSS Inc., USA) was used for the analysis. The readings of the samples were taken triplicate and all the results were expressed as mean ± standard deviation (SD). The samples means were statistically computed using Independent *t* test. The level of significance was set at $p < 0.05$ (95% confidence level).

RESULTS AND DISCUSSION

Granules morphology

Figure 1 shows the scanning electron micrograph of breadfruit flour (A) and wheat flour (B) as observed at different magnifications of 2000× and 1000× using scanning electron microscope (SEM). Based on Figure 1, the breadfruit granules were appeared spherical with indented and some having umbilical point at the center. While the average granules size for breadfruit flour were between 5 to 10 µm which is way smaller than wheat granules. In contrast to breadfruit flour, wheat flour granules has larger in size as average granules sizes were in the range of 10 to 15 µm, oval in shape with smooth granules surface (Saiter et al., 2012).

Granules size have been reported to affect the physicochemical properties of flour or starch in terms of amylose content, swelling power and water–binding capacity (Nwokocha & Williams, 2011). Besides, the variations in size and shape of the starch granules might due to their biological origins and biochemistry of the chloroplast or amyloplast as well as the plant physiological properties (Abioye et al., 2017).

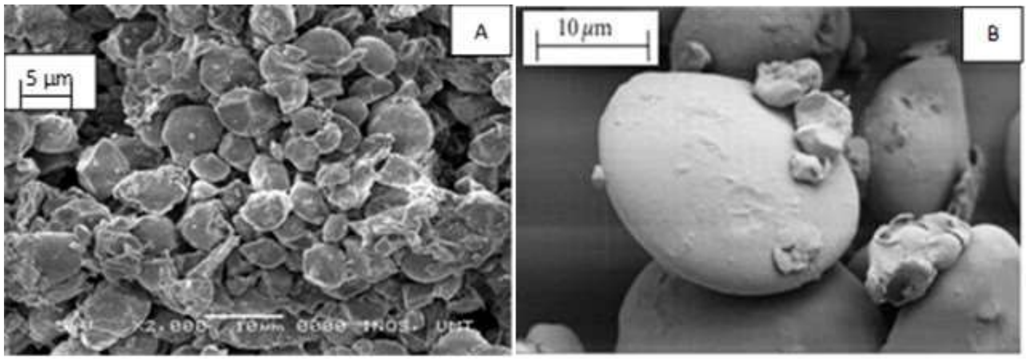

Figure 1. Granules size of (A) breadfruit flour at 2000X magnification and (B) wheat flour at 1000X magnification (Saiter et al., 2012).

Crystalline structure

The crystalline structures of the breadfruit and commercial wheat flour were determined based on X- ray diffraction patterns according to Sajilata et al. (2006) method. The X-ray diffraction patterns of these samples were presented in Figure 2. The flour, is comprised of high proportion of complex carbohydrates known as starch (Verma & Srivastav, 2017).

Based on Wang et al. (2017), the starch basically consists of alternate crystalline region and non-crystalline region (amorphous region) which made up of amylose and amylopectin polymers. In between these two regions, there is sited a microcrystalline structure. The crystalline area was deeply explained where it is structurally tight and orderly which make them hard to erode by acid and enzyme. Conversely, for the non-crystalline area, it is easily eroded by acid and enzyme. Hence, the starch characteristic of birefringence pattern and crystallinity undeniably could determine by those amorphous and crystalline regions located in the starch granules (Kumar et al., 2016).

Breadfruit flour gave strong diffraction peaks at 2Θ = 14.94°, 17.03°, 22.84° and 34.30° while commercial wheat flour gave strong diffraction peaks at 2Θ = 15.56°, 17.66°, 18.24° and 22.99°. These peaks were denoted that breadfruit flour and commercial wheat flour indicated a typical B- and A-type diffraction patterns respectively. A-type crystal commonly present in cereal whereas B-type crystal often present in tuber, fruit and stem (Kumar et al. 2016). Moreover, a previous study by Nwokocha and Williams (2011) has showed that the breadfruit starch also exhibited the same B-type crystalline structure similarly to breadfruit flour in this study. Thus, it might be said that flour and starch could have the same type of crystal structure since it comes from the same botanical origin.

In general, Wang et al. (2017) explained that the crystalline characteristics of the starch was supposed give direct impact to the application practical of the starch in both food and non-food industries. In addition, there was a small diffraction peak was observed at 2Θ = 19.33° (labeled as # in Figure 2) found in both breadfruit and commercial wheat flour. This has been attributed to the occurrence of amylose-lipid complexes. Hence, it could be said that both flours might have this amylose-lipid complexes as reported by Huang et al. (2017). In the similar report, it was reported that the results for breadfruit and white yam starches also have small diffraction patterns at 2Θ = 19.88°.

To date, there are four types of crystalline structures have been recorded which are A, B, C and V- type crystalline structures (Kumar et al., 2016). However, native starches normally display only three (3) characteristic diffraction which are A-type, B-type and C-type (Nwokocha & Williams, 2011). The A-type crystalline structure with peaks at 2Θ = 15°, 17°, 18, 23° is present in cereal starches for instance wheat and rice starches, whereas B-type, having peaks at 2Θ = 5.8°, 15°, 17°, 23° and 24° normally present in tuber, fruit and stem starches such as potato and banana starches. Whereas C- type is mixture of both A- and B-type crystal structures, and V-type crystalline structure is a complexes of amylose with compounds such as iodine, dimethyl sulfoxide (DMSO), alcohols, or fatty acids and mainly occurred in swollen granules (Kumar et al., 2016; Sajilata et al. 2006).

Generally, types of starches as aforementioned were determined by X-ray diffraction patterns whereby it largely depends on the presence of the chain lengths that making up the amylopectin lattice, the density of packing within the granules, and the presence of water (Sajilata et al. 2006). To add, amylopectin ration with short-chain fractions in the starch granules has made up ordered double helix structure was mainly attributed to the starch crystallinity which made up the sharp peaks of X- ray diffractogram (Siswoyo & Morita, 2010). Whereas the amorphous regions are believed to be formed by amylose and the branch points of amylopectin (Ma et al. 2018).

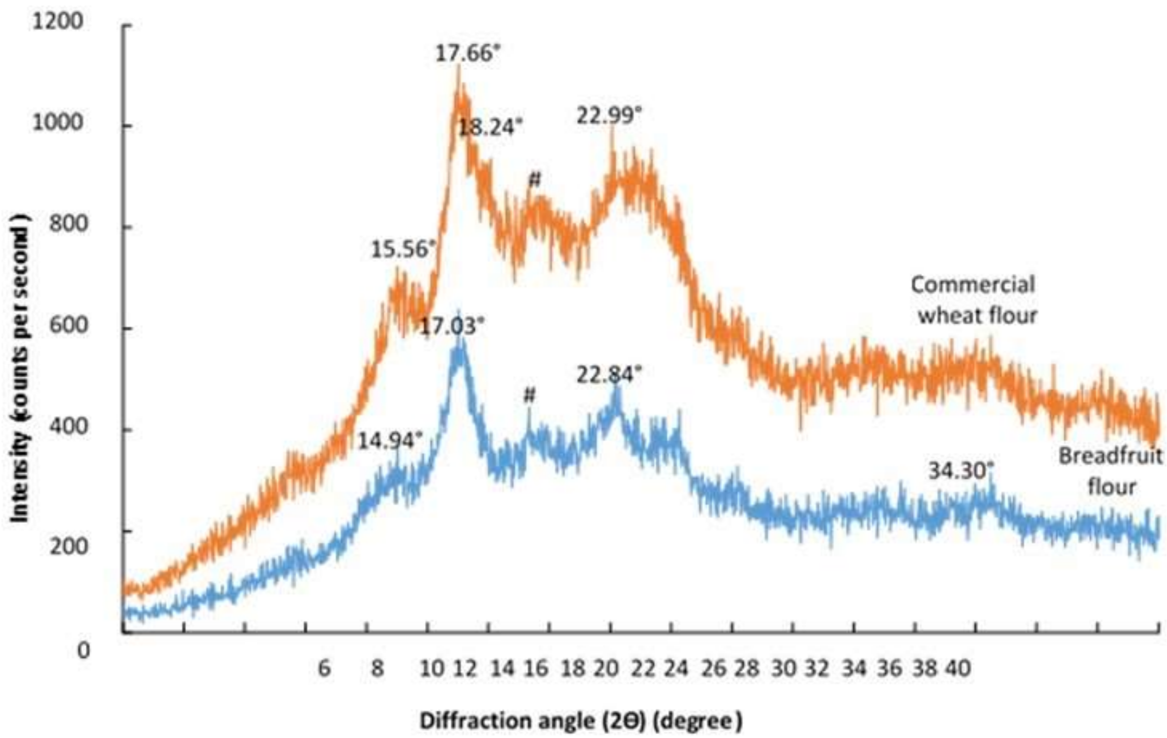

Figure 2: X-ray diffractogram of commercial wheat flour and breadfruit flour

Thermal properties

The thermal properties of breadfruit and commercial wheat flour was measured using differential scanning calorimetry (DSC) and the results were summarised in Table 1. The core of thermal transition of the flour and starch is gelatinization, where it is used to describe the molecular behavior of flour or starch related with heat and moisture content (Coral et al., 2009). The gelatinization parameters include the onset temperature (T_o), peak temperature (T_p), conclusion temperatures (T_c) and gelatinization enthalpy (ΔH). The advantage of using DSC to investigate the starch gelatinization is it permits precise control of the sample temperature while keeping the sample in a physically closed system (Ratnayake et al., 2009).

DSC enthalpies represent a net sum of all endothermic processes that take place during heating. In this study, breadfruit flour exhibited significantly higher gelatinization temperature than that of commercial wheat flour ($p < 0.05$). Gelatinization temperature defines as the peak temperature (T_p) of samples where the gelatinization begins as the starch crystals starts molten (Tribess et al., 2009). In flour, starch was predominantly as the major subset. To add, T_p is related to the length of double helix structure and it measures the quality of the starch crystalline structure (Zeng et al., 2015). The higher T_p was showed by the breadfruit flour followed by commercial wheat flour (Table 1). The difference in T_p might be caused by several factors such as amylopectin content in crystalline region and the type of granular structure such as A, B and C-type. Tribess et al. (2009) has reported that C-type X-ray diffraction pattern of banana starch that has longer amylopectin chain than other cereal starches possessed higher T_p. In this study, breadfruit flour with B-type X-ray diffraction pattern exhibited higher T_p than commercial wheat flour with A-type granular structure.

The gelatinization temperature indicates the heat stability of the crystalline structure, which depends on the granules size distribution (Zeng et al., 2015). In present study, small granules of breadfruit flour (5-10 µm) showed significantly higher gelatinization temperature than commercial wheat flour which having larger granules (10-15 µm). Thus, it might be assumed that granule size has contributed to the gelatinization of the flour. Besides, the differences in gelatinization temperature also might due to the differences in amylose content, size, form, distribution of starch granules, and also internal arrangement of starch fractions within the granules (Nimsung et al., 2007).

In this study, the breadfruit flour showed smaller temperature range (T_c - T_o, 8.60°C) than commercial wheat flour (T_c - T_o, 18.82°C). Smaller temperature range indicates that crystals present in starch have similar quality and stability compared to wider temperature range that implies most of the crystals have different stability (Zhao et al., 2018). Thus, it was indicated that breadfruit flour contained much stronger and ordered crystalline structure than commercial wheat flour, as indicated with higher T_p and ΔH than wheat flour. Breadfruit flour exhibited higher gelatinization enthalpy (ΔH) compared with the commercial wheat flour (Table 1). The ΔH defines as the required quantity of energy measured from the transition in sample (Tribess et al., 2009). It was determined by the area between the thermogram and a baseline under the peak of the DSC thermogram and it is correlated with the amount of starch in amorphous phase (Coral et al., 2009).

Table 1:Thermal properties of flour

Gelatinization	Breadfruit flour	Commercial wheat flour
Onset temperature, T_o (℃)	$75.19^a \pm 0.09$	$58.11^b \pm 1.11$
Gelatinization temperature, T_p (°C)	$77.01^a \pm 0.07$	$64.83^b \pm 0.97$
Conclusion temperature, T_c (℃)	$83.79^a \pm 0.88$	$76.93^b \pm 2.57$
Gelatinization temperature range, T_c - T_o (°C)	$8.60^a \pm 0.90$	$18.82^b \pm 2.46$
Gelatinization enthalpy, ΔH (J/g)	$4.83^a \pm 0.05$	$1.36^b \pm 0.21$

Results are expressed as mean ± standard deviation (SD), N = 3, mean values in the same row followed by different superscript lowercase letters ab are significantly different ($p < 0.05$)

Gelatinization, is an order-disorder phase transition which initially occurred in the amorphous regions due to weakened of the hydrogen bonds when heated in excess water (Siswoyo & Morita, 2010). In addition, ΔH also has been related to the degree of crystallinity value (Mamat & Hill, 2018). Flour or starches with higher crystallinity value had higher ΔH and lower swelling power. In present study, it might be assumed that the breadfruit flour showed a higher crystallinity as it requires significantly more energy input for gelatinization process than the commercial wheat flour.However, the ΔH for the breadfruit flour in this study was significantly lower than ΔH for breadfruit starch in Huang et al. (2017). The variation in ΔH of the breadfruit flour and starch were noted even though from the same botanical sources. This might be due to the presence of other components in starch such as protein and lipids that obstructed the swelling of the granules and thus increase the amount of heat required to reach the final swelling. Similar observation has been reported previously by Korma (2016). Thermal properties of the flour and starch are important indicator for determining their qualities in food products based on its granules morphology, size, molecular structure, and crystalline structure that may facilitate its applications in food and non-food industries (Lin et al. 2017).

CONCLUSION

Breadfruit flour was characterized in terms of granules morphology, crystalline structures and thermal properties in comparison with commercial wheat flour. Breadfruit flour contained smaller granules with spherical in shape and umbilical point at the center compared to commercial wheat flour. Both flours exhibited different starch crystal types, B-type and A-type respectively for breadfruit flour and commercial wheat flour based on X-ray diffraction patterns. Breadfruit flour possessed higher gelatinization and enthalpy with smaller temperature range than commercial wheat flour. This indicate the good flour qualities of breadfruit flour that could be used in wider industrial applications.

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. FTA provided an accessibility to use lab facilities at different institution.

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