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Characterization, Thermal and Morphological Analysis of Blanched Pretreated Winged Bean Flour

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Abstract: Legumes are a nutritionally dense food source that is widely consumed throughout the world. Winged bean seeds are one of the constituents of nuts. Apart from their use as a culinary item, winged bean seeds can be utilized as a raw material to manufacture compostable coatings and films that help to extend the shelf life of food. Biodegradable coatings and films are intended to have physical and chemical qualities that enable them to perform these purposes. This study aimed to determine the chemical composition, morphology, thermal and infrared spectral of pretreated and raw winged bean flour. Proximate analysis revealed how pretreatment decreases winged bean flour's carbohydrate, protein, fat, and ash content. Throughout this period, the water content increased. Thermogravimetric analysis indicates two primary decomposition processes of pretreated winged bean flour showed prominent peaks at 271ºC and 404.2ºC with mass loss of 42.09 % and 32.95 %, respectively. Morphology analysis revealed that the average diameter of granules is the range value of 5-15 µm for pretreated flour. In accordance with the results of morphological analysis, the shape of the flour particles is uneven and tends to aggregate. On the other hand, infrared spectral exhibit polysaccharide in pretreated flour is expressed as 1,4 glycosidic linkages, amide I and amide II at bands 1161 cm^{-1}, 1647 cm^{-1}, and 1541 cm^{-1}, respectively.

Keywords: Pretreated flour, winged bean flour, thermal, morphological

1. Introduction

The winged bean *(Psophocarpus tetragonolobus*) is a perennial herbaceous legume that may reach a height of 3 to 4 meters [1]. As a leguminous plant, practically every portion of the winged bean can indeed be utilized. Fruit, leaves, petals, tubers, and pods contain large levels of protein and oil [2,3]. The ripe, dried seed of the winged bean is the most nutritious portion. Their exceptional nutritional quality is primarily due to their high protein content (30-42 %) and high amino acid composition [3]. In addition, winged bean protein isolate has a high lysine content and other essential amino acids [4]. Based on the solubility properties, winged bean protein is more soluble in a wide pH range than soy protein. It has foam properties that are more stable and easier to digest [5].

Research on the nutritional value of winged beans has mainly focused upon the mature seed, which contains high protein and oil. A study of nutritive value of animals has been performed on using seed as a diet replacement for rats and chicken. In human studies, winged bean seed has been used in food preparation to make bread, tempeh, miso, tofu, and soy sauce [1]. The oil content of winged bean seeds reaches 15-20.4 % [6]. The oil content consists of 30-40 % saturated fatty acids and 60-70 % with a composition of 10-20 % is behenic acid.

In contrast, the composition of unsaturated fatty acids is 60-70 %, with most of which consists of oleic and linolenic acids [7]. Oil extracted from winged bean seeds have some advantages compared to soybean oil. This is

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related to high oxidative stability, solid oil content, and good thermal conductivity. It makes the possibility to use the winged bean oil as a good cooking oil [8]. Winged bean seeds extract has medicinal benefits. The content of bioactive peptides in winged bean extract has angiotensin-converting enzyme inhibitor activity, antimicrobial property, and antioxidant activity [9].

Numerous studies have been conducted to prepare winged bean seeds and improve their material properties for human use. Blanching and soaking winged bean seeds improved the water content of the flour and reduced its protein solubility. However, blanching for a brief period (1 minute) renders the lipoxygenase enzyme inactive [10]. Protease inhibitors can be eliminated from winged bean seeds by heating them at 120 ^{0}C for 45 minutes in an autoclave. This promotes the protein digestibility of winged bean seeds. The prolonged heating duration in the autoclave reduces urease activity, leading to a drop in trypsin inhibitor; it also lowers protein solubility in 0.2 % potassium hydroxide [11]. Direct feeding of winged bean seeds to Japanese quail can lead to mortality. Meanwhile, winged beans that have been autoclaved and supplemented with 0.5 % methionine perform similarly to soybeans when used as Japanese quail food [12].

Furthermore, no study has been conducted to compare the winged bean flour's physicochemical, morphology, and thermal properties with different treatments. This work aims to characterize and analyze the raw and pretreated winged bean flour's physicochemical, thermal, and morphological properties. It is expected to provide helpful information that can offer some probability of applying winged bean flour in food industries.

2. Materials and Methods

2.1 Materials

Dry matures winged bean (*Psophocarpus tetragonolobus*) seeds were acquired from Ambarawa Central Java, Indonesia. The dried seeds were cleaned thoroughly, and immature seeds were removed. All reagents used were Merck chemicals.

2.2 Processing Seeds to Obtain Flour

The winged bean seeds were soaked in tap water with a ratio of seed to water 1:2 at room temperature for 12 hours, then divided into two parts corresponding with variable pretreated and raw seeds. The first part was then pretreated with boiled for 30 minutes to inactivate enzymes. The boiled seeds and the raw dehulled manually. Dehulled seeds were dried at temperature 50ºC for 24 hours using the cabinet airflow dryer and ground in a laboratory blender. The grounded seeds were passed through a sieve 100 mesh size screen to obtain winged bean flour. The flour was dried at temperature 50ºC for 24 hours and stored in an airtight plastic container. The second part was treated the same as the first part but without being boiled in water [3].

2.3 Proximate Analysis

Analyses of moisture content, carbohydrate, ash, lipids, and proteins (The Kjeldahl method by a factor of 5.46 x %N) were performed in accordance with the Association of Official Analytical Chemists-AOAC (1997) methodology [13]. The entire experiment was conducted in triplicate.

2.4 Thermal Analysis of the Winged Bean Flour

Thermogravimetric analysis of winged bean flour was performed using a thermal analyzer (Perkin Elmer, Pyris Diamond) and air as the carrier gas (200 ml/min flow rate). The sample and reference material (alumina powder) were weighed at 3,3042 mg and 5,3042 mg, respectively. The sample is heated in an alumina pan at a rate of 10ºC/min from 25 to 580ºC [14].

2.5 Morphological Analysis of the Winged Bean Flour

To investigate morphology of winged bean flour, scanning electron microscopy (SEM) was utilized. Previously, samples were dried in an oven at 105ºC for 48 hours. SEM was utilized to obtain electron micrographs (JEOL, JSM-6100). Under identical optical conditions, a micrometric scale was projected [15].

2.6 Fourier Transform Infrared Spectroscopy Analysis

Using a Fourier transform infrared (FTIR) spectrophotometer (Shimadzu) and KBr pellets (Merck), the infrared (I.R.) spectra of winged bean flour was determined. 2 mg of sample and 150 mg of KBr were crushed, dried, and pressed into a small disk at room temperature. KBr does not absorb infrared photons between 4000-400 cm^{-1} in wavelength. Consequently, the complete spectrum of the material was obtained [16].

3. Results and Discussion

3.1 Proximate Analysis

The total carbohydrate of raw winged bean seed was calculated by difference was 33.65 %. The content of carbohydrate is a little different with other varieties of winged bean, 33.0 % [17], 34.7 % [18], 33.3 % and 28-31.6 % [19]. The level of carbohydrate of winged bean flour compares in the middle with other seed flour. The carbohydrate content of fruit seed flour ranges from 15% in peach to 78% in Suriname cherry [20, 21]. Some bean flour has a greater carbohydrate content than winged bean flour, pea flour (72% d.b.), lentil flour (69% d.b.), and chickpea flour (67% d.b.) [22]. After 30 minutes of boiling, the total carbohydrates in winged bean flour decreased to 32.81 %. This is most probably since the composition of other components contained in flour changes as a result of process factors. Carbohydrates are valued differently because their value is dependent on the variable variables that impact the value of the other components [23]. Soaking, peeling, fermenting, and cooking beans significantly increase their nutritional content. The impact of soaking, stripping, and heating on the content of oligosaccharides is additive. Almost 50 % of the raffinose and above half of the sucrose and stachyose are lost throughout the process [24].

Table 1 - Proximate analysis of winged bean flour

	Composition, %	
	Raw	**Pretreated**
Carbohydrate (d.b)	33.65 ±0.02	32.81±0.12
Protein (d.b)	36.59±0.07	34.97 ±0.14
Lipid (d.b)	16.10±0.16	16.02±0.08
Moisture content (d.b)	9.42±0.05	12.65±0.02
Ashes (d.b)	4.24±0.04	3.55±0.17

Protein concentration of winged bean flour in this experiment found in the range of protein contains winged bean with other varieties, and it is between 29.8-37.4 % [19]. The protein of fruit seed reported had a value between 29-32 % [21]. A comparison between fruit seed protein and winged bean flour protein showed that both contain almost the same protein. Moreover, the protein of winged bean with other varieties could be higher than fruit's seed. Some legume flour has a protein content lower than winged bean flour, lentil (26.34 g/100 g d.b.), chickpea (24.41 g/100 g d.b.), and pea (22.25 g/100 g d.b.) [22]. The result of the proximate analysis of winged bean flour is shown in Table 1. As shown in Table 1, pretreatment lowered the protein content of flour from 36.59 to 34.97 %. This is due to the presence of proteins that leach and dissolve into the water, as was the case with the legume Senna occidentalis [25].

The lipid content of the winged bean seed is in the range of lipid content other seed for about 13.11 %. Proximate analysis result of some fruit (cherry, jackfruit, orange, melon, peach, and Suriname cherry) found the range of oil content in those fruits was between 3-39 % [21]. The citrus fruit seed contains between 28-35 % of the oil. Oil content in an orange seed range from 24 % to 41%, depending on the cultivar [26]. In turn, the highest oil content of citrus fruit seed is 54.2 % [27]. The oil content of winged bean seed was between 16.4-21.3 % on a dry basis) [28], lipid content in the winged bean seed was 18 % [29]. Another cultivar of winged bean seed was reported to have lipid content between 14.8-17.9 % [30]. The fat content of some legume flour was reported lower than winged bean flour, chickpea (5.57 g/100 g dry basis), pea (1.83 g/100 g dry basis), and lentil (1.25 g/100 g dry basis) [22]. The fat content of winged bean flour prepared with and without heating somehow does not vary substantially (Table 1). Winged bean flour that has not been treated has 16.10 % lipids, whereas those that have been cooked have 16.02 % lipids. This corresponds to the findings obtained with five beans [31]. Seeds typically reserve energy employing glycids or lipids, depending on the soil type, environment, and germination. Generally, a grain with a high lipid content during the latency phase has a low starch content. The fatty then will convert to glycids, which will provide energy for the exothermic germination process [21].

The ash content of winged bean seed was found to be 3.04 %. It was lower than was reported before in between 3.6-4.9 % [17, 18, 6]. The ash content of winged bean seed was similar to the ash content of fruit seed, ranging between 2.4 % to 3.9 % [21]. When unprocessed and processed winged bean flours are compared, the ash content is observed to reduce (Table 1). The ash percentage of unprocessed winged bean flour is 4.24 %, whereas that of heated winged bean flour is 3.55 %. Cooking significantly decreases the ash content. The findings obtained are similar to those obtained with five beans. This decline may result from endospermic compounds comprising micro and macro components leaching into the water [31].

The moisture content of pretreated winged bean flour has increased. Probably due to the fact that the flour's absorption is more than the heating process during blanching. The morphological features of pretreated flour influence its absorption properties. The SEM analysis revealed that the pretreated flour had a tendency to clump due to the larger granule size.

3.2 Thermal Property

The pretreated winged bean flour shows two new decomposition steps after the stability (Fig. 1). A decomposition attributed to decomposition and oxidation of organic matter (amylose and amylopectin) occurs in consecutive reactions and with mass loss of 42.09 % and 32.95 %, respectively. The final residue was 16.77 %. Meanwhile, with unprocessed winged bean flour, a significant mass decrease of 76.18 % happened at one stage, yielding a 15.96 % residue. The residue content is higher than other kinds of flour, soya flour is 6.26 %, and the complex cornmeal is 0.65 %. The water loss in the first step is in range value 7.8 % - 13.1 % [14].

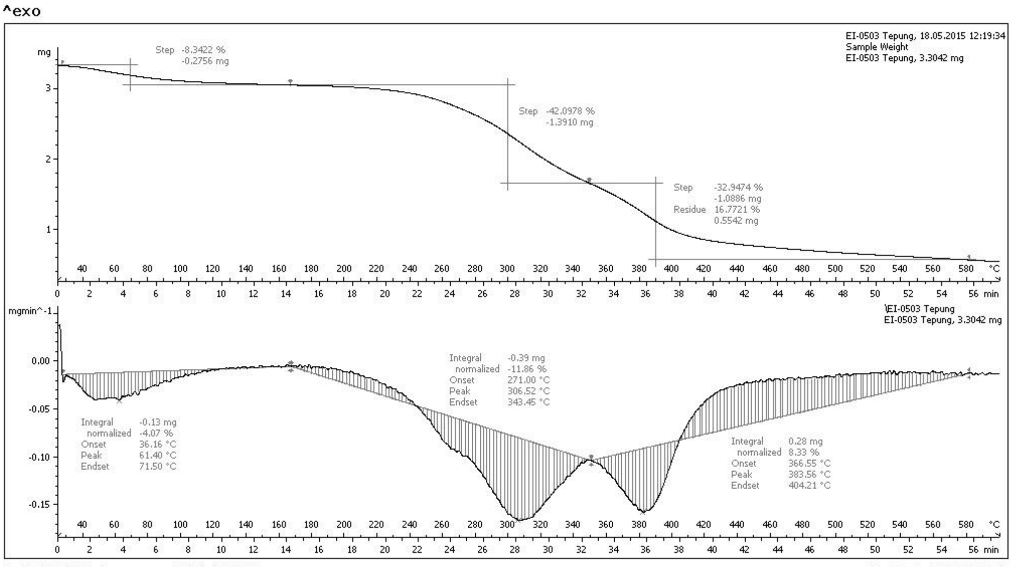


Fig. 1 - TG and DTG of pretreated winged bean flour at flowrate 200 ml/minute, rate 10ºC/min

The thermogravimetry (TG) and differential thermal analysis (DTA) of the raw winged bean flour are shown in Fig. 2. The curve demonstrates that the thermal decomposition process proceeds in three stages: stage 1 (T≤61.8ºC), where mass loss (8.342 %) is exacerbated by evaporation of volatile materials, particularly water; stage 2 (61.8 ºC≤T≤306.52ºC), where mass loss is resulted by the principal decomposition of starch; and stage 3 (T≥306.52ºC) where carbon accumulation occurs. The principal decomposition process is initiated by rapid dehydration and decomposition of the glucose ring's hydroxyl group into water molecules. Chain cleavage occurs at this step when the C-C-H, C-O, and C-C bonds are broken [32]. The breakdown of the resultant gas consists mainly of H_2O, CO, CO_2, and chemicals such as CH_4 and C_2H_4. Between 271ºC and 404.2ºC, the sharp peak on the D.T.G. curve shows the potential of a single reaction mechanism or the union of numerous reaction mechanisms [33,34].

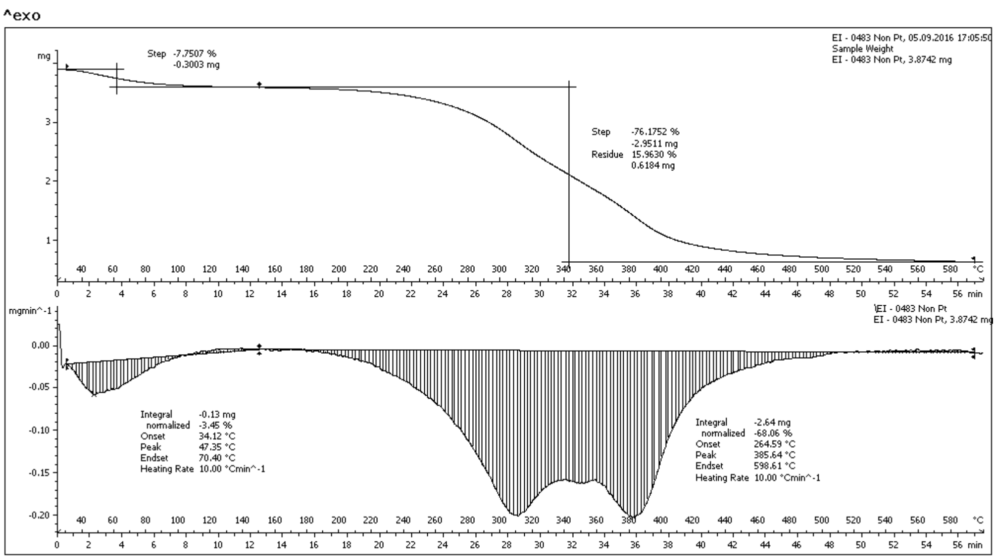
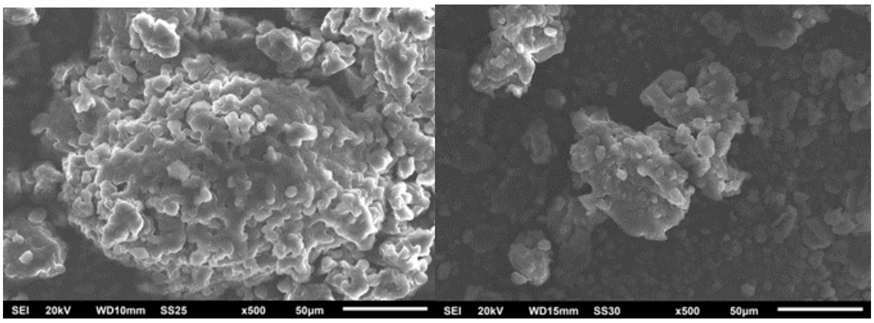


Fig. 2 - TG and DTG of raw winged bean flour at flowrate 200 ml/minute, rate 10ºC/min

3.3 Morphological Analysis

Scanning electron microscopy was used to investigate the granule morphology of winged bean flour. The morphology of treated and untreated winged bean flour is shown in Fig. 3. The results showed that the treated winged bean flour granules were irregular in structure with low sphericity and some granules forming aggregates. This presence could be mainly associated with protein aggregates and fiber components [35]. Meanwhile, untreated winged bean flour did not establish significant aggregates in the granules. This is because the flour is unheated, which reduces protein clumping. When exposed to high temperatures, protein molecules have a tendency to aggregate. While contrasted to the SEM results for locust bean flour, the presence of starch granules varying in size from small to large with round, oval, irregular, or cuboidal forms indicates the existence of fissures or dents for small or large granules. This morphology means a high protein, fat, and fiber content [36].



(a) (b)

Fig. 3 - SEM micrograph of; (a) pretreated magnified 500 X, and (b) raw winged bean flour magnified 500X

Size majority of granules was found in the range 5-15 µm, but small ones with a diameter less than 5 µm contributed only less than 10 % of the lot. The average of winged bean flour is below the granule size average of Jackfruit seed (32 to 47 µm), and most native legume flours were reported [15].

3.4 FTIR Analysis

The infrared absorption spectroscopy analysis result confirmed the presence of starch and protein in winged bean flour is shown in Fig. 4 and Fig. 5. The major functional groups in winged bean flour displayed absorbance peaks within a frequency band 400-4000 cm^{-1}. The band corresponding to the C-H stretching of the starch appears at 2854.74 cm^{-1} for pretreated and raw winged bean flour. The stretching of the O-H bonds for pretreated and raw appears at 3282.95 cm^{-1} and 3294.53 cm^{-1}, respectively, shifted from 3400 cm^{-1} which it should be. The infrared spectrum of winged bean flour reveals typical saccharide bands between 1091 and 1161 cm^{-1}, as either a result of C-C vibrations, C-O stretching, and C-H bond deformations [16].

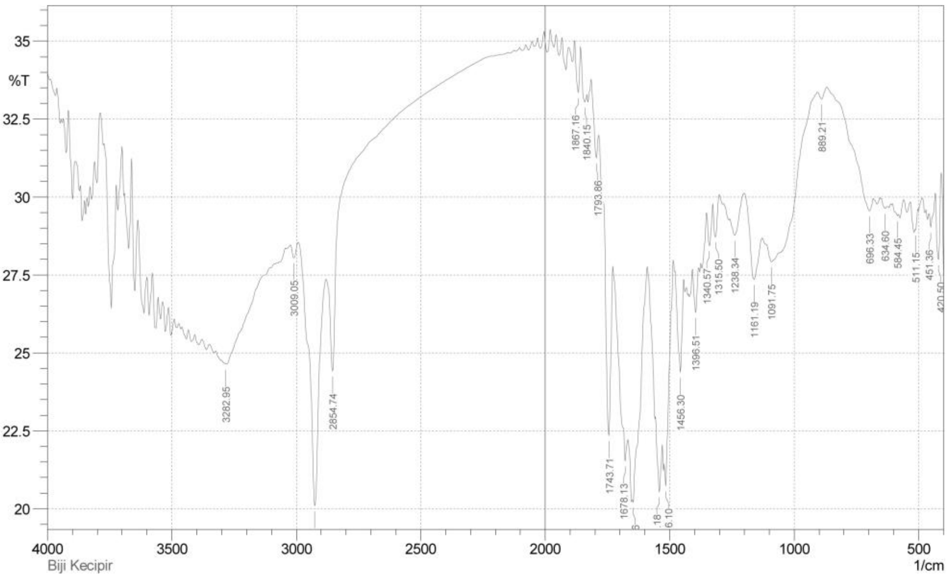


Fig. 4 - FTIR result of pretreated winged bean flour

The characteristic polysaccharides with 1→4 glycosidic bonds exhibit absorption bands in the spectral range 1175-1140 cm^{-1}, a spectroscopic manifestation of glycosidic bond formation; this property is used to distinguish the various structural transformations of polysaccharides in the presence of glycosidic bonds [37]. 1→4 glycosidic linkages are visible in the spectral absorption bands 1161 cm^{-1} for pretreated and 1163.11 cm^{-1} for raw winged bean flour in Fig. 4 and 5.

The amide I and II bands are most prominent at 1650 cm^{-1} and 1540 cm^{-1}, respectively [38]. The infrared spectra for winged bean flour show characteristic protein bands, amide I and amide II, which are shifted from 1650 cm^{-1} and 1540 cm^{-1} to 1647 cm^{-1} and 1541 cm^{-1} for treated winged bean flour, respectively. Meanwhile, raw winged bean flour shifted to 1658.84 cm^{-1} and 1545.03 cm^{-1}, respectively. Peak shifts are driven by enhanced oscillator power and molar concentration [39].

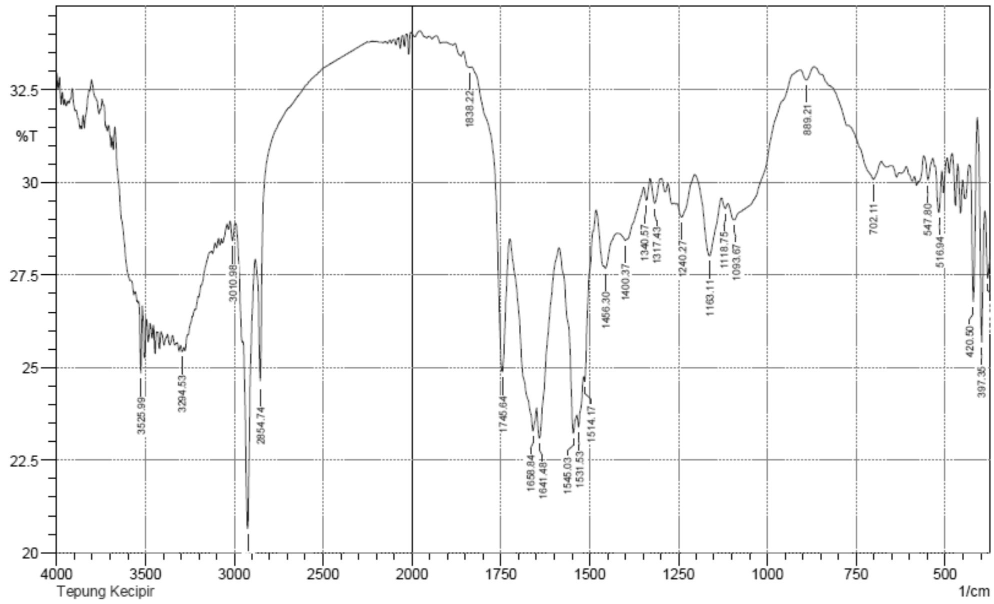


Fig. 5 - FTIR result of raw winged bean flour

4. Conclusion

The winged bean flour contains high protein and carbohydrates. Analysis of infrared spectral result showed the polysaccharide, glycosidic linkage, protein in the formamide I, and amide at specific bands. The morphological analysis showed that granules of winged bean seed flour form aggregate due to the protein content. Furthermore, the diameter of the granules is under the average size diameter of most native legumes. The thermal analysis results in the water content lost in the first step are the range value of the other same type of flour. The analysis raises the possibility of using this product in the industry, either as an essential raw material or as a component for another blend. The vast application possibilities are the bakery, pastry, animal diet, fermented food, and packaging as a component in the biodegradable composite.

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