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Detection of Amylose and Evaluation of Structure and Morphology of Isolated Starch from Indigenous Rice Variety (Begun Bichi, India)

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ABSTRACT

The presence of amylose and amylopectin in rice or any other cereals is an important parameter to determine the quality of rice or cereals. Due to emerging interest on indigenous rice varieties in recent time, we have chosen one indigenous rice variety of India (Begun Bichi) and determined the amylose and amylopectin content by standard Juliano's method. The amylose content for the prepared solution is 12.37% and the stock solution is 14.93%. The amylopectin content is 87.62% and 85.06% for the prepared and stock solution respectively. The increment of amylose in stock solution from the prepared solution was also found and this result was corroborated with the previous literature. We have also isolated the pure starch from this rice via alkali extraction methods. The morphology of the isolated starch was characterized by scanning electron microscopy (SEM) and powder XRD (p-XRD). The microstructure of this rice starch granules appears mainly as polyhedral (5.06 mm in size). The different p-XRD peaks observed at 15°, 16.5°, 18.5°, 22.5°, and 26° indicates A-type crystallinity. Based on the data collected from various experimental results, the Begun Bichi rice starch can be used both in the food as well as the pharmaceutical industry.

Key words: Indigenous rice, Amylose content, Amylopectin content, Polyhedral, Stock solution

The global market for industrial starches is expanding and current demand is being met by potatoes, corn, wheat, tapioca, and rice [1]. Starch is an attractive raw material for edible packaging due to its low cost, renewable nature, and biodegradability [2]. Starch products are identified not only for food purposes but also in the textile, detergent, paper, and adhesive industries [3]. The structure and composition of starch molecules (mainly amylose and amylopectin) may be affected by the physical, chemical, and functional properties of rice flour starch during food processing [4]. Recent trends in industry focus on new applications of starch that include chelating, antifreeze, drying aid, lipidicide, flavor carrier, herb coating, and fermentation [5-6]. Rice starch has the smallest particle size among other commercial starches; the whitest powder colour and a neutral state which altogether makes it an important ingredient in different industries [7]. Gelatinized rice starch is very smooth and creamy, and does not have any flavour, hence its preferable use in producing baby powder and pharmaceutical filling agent [7-8]. Rice starches have also been used in laundry for sizing good fabrics and skin cosmetics due to their small granule size and higher solubility in water [3]. Rice water may contain a little amount of zinc and selenium which have been found to show antimicrobial effects [9], and these anti-aging components have also been used as raw materials for the application of skincare [10]. In particular, rice water is also found to be useful for the treatment of diarrhoea, cholera or gastroenteritis [11]. Indigenous rice is a good source of phytochemicals and these components are most likely involved in the reduction of degenerative human diseases due to their anti-oxidative and free radical scavenging properties [12].

Starch is made up of polysaccharides, the main ingredient of rice which is more than 80% of all ingredients that might play a significant job in our diet and aids in determining the blood glucose levels [13]. Starch naturally occurs as separate molecules called granules [14-15]. The starch granule's size and shape are influenced by the origin from which the starch is being isolated. The climatic

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conditions and agricultural practices affect the shape of the starch granules [15]. The shapes and sizes of these granules may be spherical, ellipsoids, polygon, platelets, and irregular tubules [16]. Grain size can be determined by different techniques such as microscopy (optical microscopy, scanning electron microscopy, SEM), screening, electrical resistance, laser light scattering, and Field-flow fractionation [15]. Scanning electron microscopy (SEM) has been frequently used to determine the granule size. It provides a more detailed perspective on particle surface properties and grain formation [17]. The structure of rice granules is varying in size and shape in different cultivars. The scanning electron microscopy images showed that the starch grains of all types of rice are mainly polyhedral, elliptical, irregular and angular in shape. Rice starch grains are the smallest grains found in cereals, ranging in size from 2 to 10μm for single type [14, 18-19] and 80 μm for the compound type [16]. Moreover, the rice starch granules provide a texture-like perception because they are small in size and have low glycemic index value [20]. It is recommended as a fat replacement in food industries in the form of small granules made from rice starch [21-22]. Rice starch is increasingly being used as a fat substitute because its water-binding properties can mimic oil-sensitive properties such as slippery mouthfuls and tenderness [23-24].

Rice cultivation is facing various problems like high soil acidity, the solubility of toxic elements, low fertility, high salinity, shallow pyrite layers, thick and raw peat, and floods and drought [25-26]. Indigenous rice varieties are more adaptive to the land conditions [25], which require low inputs of fertilizers and pesticides. They are also recognized for their tolerance to iron toxicity [26]. Traditional landraces rice varieties have higher nutritional quality than that of the conventional and high yielding rice varieties, and perhaps contain more effective bioactive compounds [27-28]. Rice of intermediate amylose content is favoured by the major world rice market [29]. Since the indigenous rice varieties are less affected by chemical exposure therefore it will be useful if we use the collected starch from it. Additionally, the natural phytochemicals in such rice grains (Begun Bichi and Kalizira rice) attract immense attention for the prevention of cardiovascular diseases and cancer [30]. Herein we have taken Begun Bichi of Indian variety as indigenous rice for this particular study. The amylose and amylopectin contents of this rice variety have been determined. The pure starch from this rice variety has been isolated by known methods and the size of the starch granules was determined by scanning electron microscopy (SEM). The crystalline or amorphous nature of the starch granules were also determined by powdered crystal X-ray diffraction technique (p-XRD).

MATERIALS AND METHODS

Sample collection

For this study, the Begun Bichi (India), an indigenous rice variety was collected from Raiganj, Uttar Dinajpur, West Bengal, India. The rice grains were cleaned well and dried, ground in a mixture grinder, and stored properly in an air tight plastic container at room temperature before the experiment.

Sample storage

The collected rice variety was cleaned and all the impurities such as stones, broken husks, and parts of silage were removed. The rice samples were sun-dried under shadow circumstances for three days and the samples were stored in plastic baskets in house conditions.

Flour preparation

Mortar, pestle, and blender were used for sample preparation. The collected indigenous rice sample is de-husked using a small wooden mortar and pestle. After de-husking the rice grains were ground using a grinder at medium speed. The mortar, pestle, and blender were cleaned carefully to avoid the mixing with other varieties. Rice flour was sieved with 100 mesh sieve and stored in air-tight containers until it was analysed.

Chemical and reagents

The following chemicals and reagents were used for the determination of amylose content. Standard potato amylose was purchased from Sigma-Aldrich (India). Glacial acetic acid and acetone was purchased from Spectrochem. Iodine flakes, potassium iodide, NaOH, and NaCl were purchased from Merck. The solvents and reagents were used without any further purification.

Instrumentation details

The absorbance of different solutions was measured by a UV-VIS spectrophotometer, Lasany International, India (Model LI-2700). Digital pH meter (Systronics, India), laboratory centrifuge (REMI, R-8C, India), magnetic stirrer (REMI 2MLH, India), and blender (LG, India) were purchased from different companies as mentioned in the parenthesis. The powder X–ray diffraction studies (PXRD) were done by PANalyticalX' Pert Pro diffractometer using CuK α-radiation. Scanning Electron Microscopy (SEM) and Electron–Dispersive X–ray Spectroscopy (EDS) were performed using JEOL JSM–IT 100 electron microscope.

Determination of amylose content

Initially, 40 mg of pure potato amylase was weighed into a beaker. 1mL of 95% ethanol and 9 mL of 1N NaOH were added to it and mixed well. The mixture was then heated for 15 minutes on a boiling water bath. The solution was cooled to room temperature and the contents transferred into a 100 mL volumetric flask while distilled water was added to make up the volume. According to the Juliano's method [31], 1, 2, 3, 4, and 5mL of the prepared solution was transferred into 100mL volumetric flasks. After that 0.2, 0.4, 0.6, 0.8, and 1 mL of 1N acetic acid solution were added respectively for preparing the standard solutions. 2 mL of 0.2% iodine solution was added to all the flasks and shaken well. Finally, the prepared solutions were kept in a dark place for 20 minutes. The absorbance of the solutions was measured at 620 nm using UV-Vis double beam spectrophotometer. The standard curve was drawn against the absorbance and concentration of amylose. For the blank test, 5 mL of 0.09N NaOH solution was added to a 100 mL volumetric flask. Then 1 mL of 1N acetic acid and 2 mL of 0.2% iodine solution was added and kept in the dark for 20 minutes and the absorbance measured at 620 nm. To determine the amylase and amylopectin content in the sample, 100 mg of rice flour was weighed into a beaker and the same procedure was carried out. 5 mL of the starch solution was pipetted in a 100 mL volumetric flask. 1 mL of 1N acetic and 2 mL of 0.2% iodine solution was added then the volume was made up to the mark with distilled water. The mixture was shaken well, and kept in the dark for 20 minutes. Finally, absorbance was measured at 620 nm using a UV-VIS double beam spectrophotometer (LI-2700). The amylose percentage of the sample was determined as described by Juliano [31].

Amylopectin content was calculated using the following equation [32-33].

Amylopectin (%) = (100 – Amylose %)

Starch isolation

We used the alkali extraction method Reddy and Bhotmange, 2013 [34], with some modification for starch isolation from rice powder. 100 g of rice powder was dispersed in 1500 mL of distilled water and the pH adjusted to 10 with 1N NaOH. The solution was then kept for an hour with moderate stirring. After completion of stirring, the solution was centrifuged at 5000 rpm for 30 minutes. The residue was extracted with 1L of 2% NaCl and kept for 24 hours at 4°C. The solution was again centrifuged at 5000 rpm for 30 minutes. The residue obtained was extracted with 300 mL of 0.1N NaOH twice and the solution kept for 48 hours at 4°C. The solution was again centrifuged at 5000 rpm for 30 minutes and the supernatant liquid was discarded when a white starch layer was obtained. The residue (starch layer) was re-suspended with 80% aqueous ethanol and blended for 1 min using a magnetic stirrer. The solution was further heated in a water bath for 1hr at 80°C. It was cooled to room temperature and allowed to settle for 4 h at 4°C. The supernatant liquid was discarded again and the residue was dehydrated by rotary evaporator and the pure starch powder was collected.

RESULTS AND DISCUSSION

Waxy rice flour is used in the food industry as a thickening agent in white sauces, gravies, and desserts. Baby food, beer, noodles, and breakfast cereals mainly use rice flour with low-amylose (12%-20%) and medium-amylose (20%-25%) content. Low-amylose rice has higher water absorption and pollination ability than high-amylose rice. To compare the others cereals as resistant starch (RS), rice starch has multifaceted health benefits ranging from reduced risk of colon cancer [35], restoring or healing the lining of the large intestine, to positive improvements on insulin sensitivity as well as in the immune system [36]. Dietary consumption of RS was shown to increase the body's ability to kill the malignant (damaged) cells by up to 30% [37]. Reduced insulin sensitivity is considered as a biomarker for metabolic syndrome, e.g., the combination of risk factors leading to increased risk of heart diseases, obesity, cancer, and other major health diseases [38]. Low-amylose type rice flour is suitable for industrial application than high-amylose rice flour [39]. In the present study, the size of Begun Bichi was determined and the rice starch granules was found to be small while the value of amylose content was also found to be low. Therefore, the Begun Bichi rice is a good choice for industrial applications.

Amylose and amylopectin

The amylose content of Begun Bichi rice variety is shown in (Table 1) which has been determined according to Juliano's method [31]. The calibration curve was obtained by plotting the absorbance against the pure potato amylose content (mg/mL). The linear regression equation for the calibration curve was y = 2.2204x + 0.0073 (R^2 = 0.9994). With the help of this equation the amylose content was determined. The amylose content of Begun Bichi is 12.37% and 14.93% for the prepared solution and the stock solution. The values indicate that the amylose content of this rice variety increases in stock solutions as compared to the prepared solutions. The increase of amylose content from prepared to the stock solution is 2.56%. According to Sowbhagya and Bhattacharya [40], there is an increase in the amylose content of the extracts upon storage up to 20 days if left in cold or at room temperature. The total increase is 1–2% in the first few days of storage. The Begun Bichi rice variety contains low amylose and is famous for its use in special dishes and is served on special occasions. The amylopectin content for the prepared and stock solution was 87.62% and 85.06% as derived from the standard curve (Fig 1-2). Rice with higher amylopectin content is more delicious than higher amylose content [41]. Amylopectin permits greater access to digestive enzymes than amylose [42]. Since this Begun Bichi rice is high in amylopectin, it therefore gives more energy, is delicious and easy to digest.

Table 1 Amylose and amylopectin content of Begun Bichi rice determined from the standard curve

Variety	Solution	Abs 620nm	Concentration (mg/L)	Amylose (%)	Amylopectin (%)
Begun Bichi	Prepare	0.282	0.123732	12.3732	87.6268
	Stock	0.339	0.149389	14.9389	85.0611

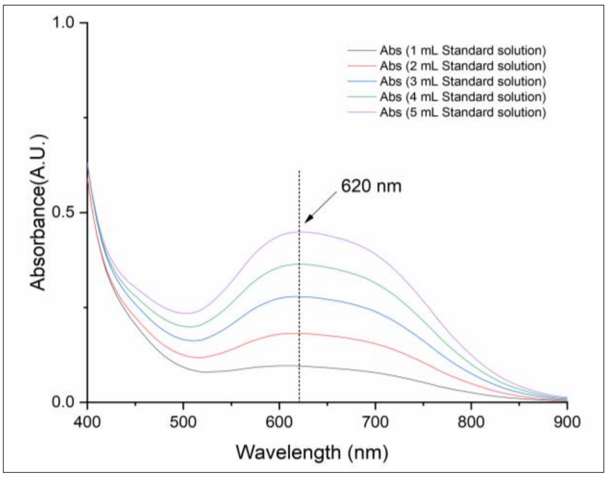


Fig 1 Absorbance of various standard potato amylose solutions

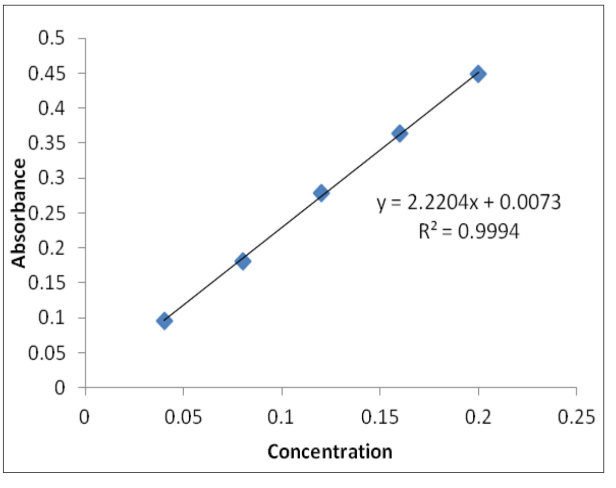


Fig 2 Standard curve for potato amylose

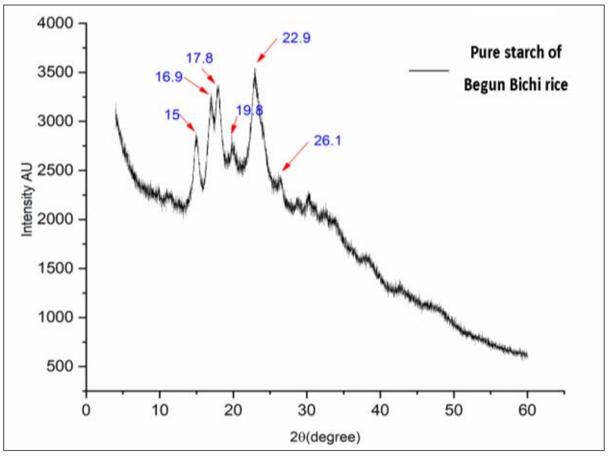


Fig 3 Powder X-ray diffraction (p-XRD) analysis of Begun Bichi rice

Powder X-ray diffraction (p-XRD) analysis

X-ray diffraction (p-XRD) is an analytical tool used for the identification of crystalline material and to investigate the molecular structure of a crystal. Various XRD patterns are displayed by native starch granules depending upon their botanical origin. Native starch is classified into A, B, and C types based on these patterns [43]. The two principal crystalline patterns of native starches based on the X-ray diffraction patterns have been classified as A or B. The A-type starches are founds in cereals, while B-type starches are found mainly in tubers and high-amylose starches. The X-ray diffraction pattern helps to determine the structure of starch grain crystal. In the A-type starch grain crystal, X-ray diffraction patterns at 2θ shows the first peak around 15°, the second peak near 18°, and the third main reflection around 23° [44]. For the B-type starch, peaks could be found at about 5.5º and 17.2° in 2θ scale [45]. As is evident from (Fig 3), the p-XRD pattern of rice starch for Begun Bichi shows typical A-type crystallinity with 2θ values at around 15°, 16.5°, 18.5°, 22.5°, and 26° which corroborated with existing literature.

Microscopic study

The morphological properties of rice starch granules were measured by Scanning Electron Microscope (SEM). The SEM images at different resolution of Begun Bichi starch granules are shown in (Fig 4). The starch granule surfaces were observed to be crystalline when viewed under SEM at an altered magnification range (X1400 to X2700). The large, medium and small granules of starch consists of a mixed population in the range of 5µm to 10µm. Granule size may also be expressed as the average length of the major and minor axes, mean maximum diameter, mean granule volume or mean surface area [15]. In this study, the average size of the granule is expressed as the average length of the major and minor axis equivalent diameter. The average size of Begun Bichi starch granules is 5.06 µm. Begun Bichi starch granules have an intact structure having a crystalline surface with pores on some parts of the granules. Images were taken at different magnification rates to observe the close structure of the granules and pores from the surface. Scanning electron micrographs of starch granules of Begun Bichi variety showed irregularly shaped crystalline granules predominant in the starch matrix.

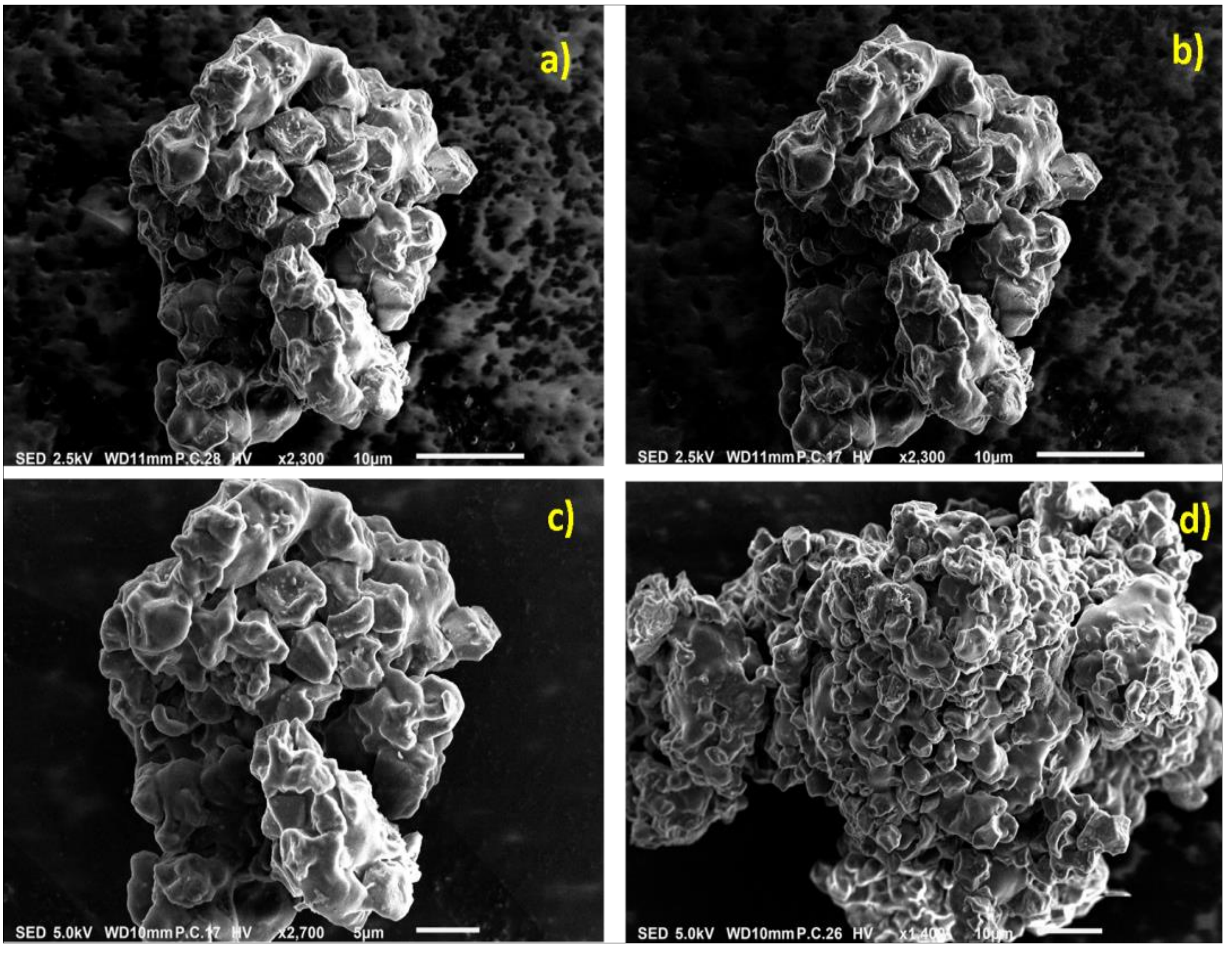
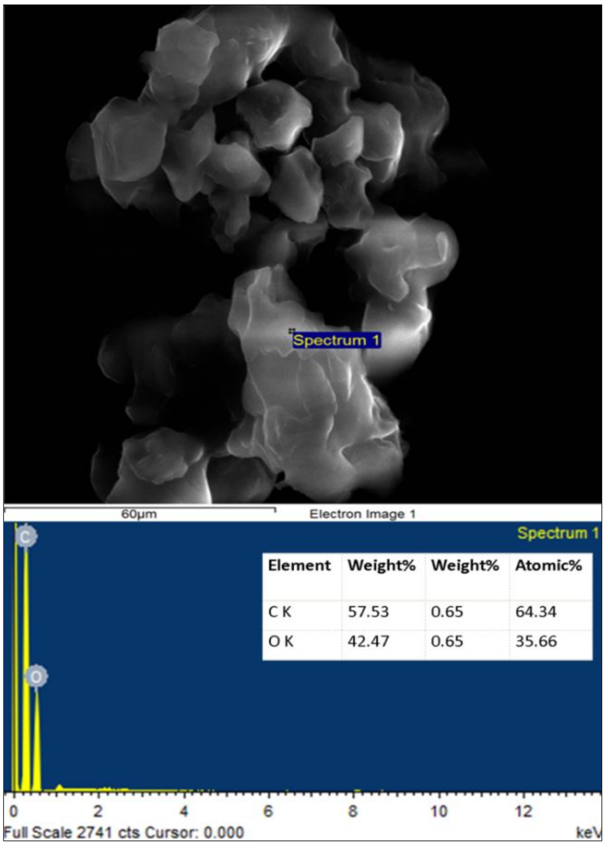


Fig 4 SEM images at different resolution of Begun Bichi rice starch



Element	Weight%	Weight%	Atomic%
C K	57.53	0.65	64.34
O K	42.47	0.65	35.66

Fig 5 SEM-EDS images of Begun Bichi rice starch

Energy dispersive X-ray scattering analysis (SEM-EDS) of the starch confirmed the presence of C (64.34%) and O (35.66%) elements only (Fig 5). It is thus apparent that the presence of only C and O elements indicate pure starch was isolated during starch the isolation procedure from Begun Bichi rice.

SEM images from studies have revealed that the starch grains from all types of rice are mainly polyhedral [14, 18]. Some variation was observed in different rice cultivars of different literature. Ashwar *et al*. [46], reported that some Indian rice varieties are polyhedral, ranging from 4.02 to 5.2 mm in size. Sodhi and Singh [47], reported that the size of the average granules of rice grown in India range from 2.4 to 5.4 mm. On comparison, we find that the granules of Begun Bichi are small in size. The size of Sri Lankan rice varieties BW 267/3 is 5.52 µm and BW 367 is 5.34 µm. These rice varieties are used in the pharmaceutical industry because of its very small particle size [48]. In corroboration with the findings of Piyumini *et al*. [48], we can safely use the Begun Bichi rice starch in the pharmaceutical industry after modifications.

Comparison of morphology of various starch granules with starch of Begun Bichi rice

Oats starch contains the lowest amount of carbohydrates and Sorghum starch contains the highest amount of carbohydrates (Table 2). According to Soulaka and Morrison 1985 [49], the starch granules type is determined by its size; when the size of a granule is greater than 9.9 µm then it's an A-type granule, and when the size of a granule is less than 9.9 µm then it's a B-type granule. Depending upon the cereal's source, starch granules are of different sizes and shapes. Barley, Millet, Rye, and Wheat starches have two granule populations differing in size.

Table 2Morphology and composition of various native starch granules

Starch/Cereal	Shape	Crystal Type	Size (µm)	Amylose (%)	Starch (%)	Reference
Barley	Lenticular (A-type), spherical (B-type)	A and B Both Type	2–5, 15–25	22-27	62.7	[16,58]
Maize (waxy and normal)	Spherical/polyhedral	A type	2–30	23-32	65	[16, 54]
Millet	Polyhedral	A type	4–12, 2.5-24	0-28.4	62.9-73	[16, 55, 59]
Oat	Polyhedral	A type	3–10 (single) 80 (compound)	21-33	56.2	[16, 56]
Rye	Lenticular (A-type), Spherical (B-type)	A and B Both Type	10–40, 5–10	11-30	60.3	[16]
Sorghum	Spherical	A type	5–20	20-30	83.45	[16, 53]
Sago	Oval	C type	20–40	23-29	83.9	[16, 52]
Wheat	Lenticular (A-type), Spherical (B-type)	A and B Both Type	15–35, 2–10	1-37.5	59.4	[16, 58, 60]
Indigenous rice variety	Polyhedral	A Type	3–8 (single) 150 (compound)	6.86-26.2	73.7	[14, 16, 61]
High yielding varieties (HYV) rice	Polyhedral	A Type	1.5-6.1 µm	22.8-30.7	70-73.7	[62]
Begun Bichi (Ind)	Polyhedral	A Type	5.06 µm	12.3-14.9	74	This work

Small granules are of sizes between 2-12 µm (Table 2) and spherical in shape while large granules have sizes ranging between 10-40 µm and lenticular in shape [16, 50]. The size of other cereal granules is 5 µm and most of these cereals are spherical [16, 47, 51]. Only the Sago starch size is about 20-40 µm and is oval in shape [16, 47]. Rice and oat starch are found as single and compound type granules (the average size is 3–10 µm for single granules and up to 150 µm for compound granules). The shape of the rice and oat starch granules is polyhedral [16, 51]. As has been already mentioned, the starch granules are classified as A, B, and C-type according to the X-ray diffraction pattern [44, 45, and 52]. The shape, size and amylose content of various types of starch molecules are presented in (Table 2). According to the literature, the Sorghum [53], corn [54], millet [55], and oat starches [56] are A-type, sago starches are C type [52, 57] while barley [58], rye [16] and wheat [58] starches are both A and B types with crystalline pattern. As is presented in (Table 2) the shape of barley, wheat, and rye starches are lenticular and spherical, maize starches are spherical and polyhedral, millet and oat starches are polyhedral, sorghum starch is spherical, and sago starch is oval. The granule size of Begun Bichi starch is 5.06 µm and the shape is polyhedral. As per PXRD data and the granular size of Begun Bichi rice it is evident that it possesses A- type crystals.

CONCLUSION

In this particular study, we have determined the amylose content and isolated the pure starch of indigenous rice Begun Bichi (Ind). The amylose content of prepared solution and the stock solutions were determined by Juliano's method. Similarly, the amylopectin contents were also determined. The increment in amylose content from prepared to stock solution can be explained on the basis of adsorption of more iodine over starch surface. The significance of the study stands out among others in the method of starch isolation. The characterization of isolated starch by scanning electron microscopy revealed that pure starch was isolated. The study will help us and other researchers to carry out further similar studies on other indigenous rice varieties.

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Conflict of Interest

There is no conflict of interests to declare.

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