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Isolation and Characterization of Starch from Kodo Millet (*Paspalum scrobiculatum*)

RENU GARHWAL, MANISHA MALIK*, MITHUN KUMAR AND B. S. KHATKAR

Department of Food Technology, Guru Jambheshwar University of Science and Technology, Hisar-125 001 (Haryana), India

**(e-mail : malikmanisha252@gmail.com; Mobile : 9992268557)*

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ABSTRACT

In the present study, kodo millet grains were investigated for proximate composition and morphological properties. Isolated starch from kodo millet was investigated for functional, thermal (DSC) and textural (Texture analyzer) properties. Protein content of kodo millet was comparable to finger and fonio millets but lower than pearl and foxtail millets. Starch yield was significantly higher (65%) with some non-starch compounds. Values of water binding capacity, swelling power, viscosity and dispersibility were moderate but fat absorption capacity and solubility percentage were low as compared to pearl millet starch. Kodo starch had high range of gelatinization temperature in comparison with other millets such as finger, pearl and foxtail. Textural properties of kodo millet starch were also superior to other starches such as wheat and cassava. Additionally, this starch had moderate springiness, cohesiveness and hardness as compared to the above-mentioned starches. There is a scope of studying molecular characterization, pasting and structural properties and relationship among fractions of kodo millet starch.

Key words : Kodo, starch, swelling, thermal, textural

INTRODUCTION

Kodo millet (*Paspalum scorbiculatum* L.) is referred diversely as *Kodo* in Bengal, *Harka* in Punjab, *Kodra* in Gujarat, *Koduain* in Odisa and *Varagu* in Tamil Nadu. Predominantly, it is cultivated in countries like India, Pakistan, Thailand, Indonesia, Vietnam, Philippines and West Africa. Additionally, it is a noteworthy nourishment grain for the general population living in deccan level district of India and in few areas of Maharashtra, Odisha, West Bengal, Himalayas, UP and Rajasthan. Kodo millet comprises around 8% protein in which glutenin is the major protein (Deshpande *et al.,* 2015). Kodo millet is also rich in fiber, carbohydrate, fat, mineral, iron and energy with proportions of 9%, 66.6 g, 1.4%, 2.6%, 25.86-39.60 ppm and 353 Kcal/100 g, respectively (Chandel *et al.*, 2014). Phosphorus in kodo is less as compared to other millets. This millet also has good DPPH scavenging capability. Range of gelatinization temperature of kodo millet flour was found to be 76.6-90°C. This shows that kodo millet is less resistant to gelatinization and hence can be used in baking. In India, kodo grains are ground to flour for making puddings. Being gluten free, its use as gluten free ingredient is increasing day by day (Deshpande *et al.*, 2015). Several authors reported its use in making novel as well as traditional foods such as *dosa, idli, pongal, chapatti, idiyappam, puttu, biscuit, boli, cutlet, soup, bread, ladoo cookies* (Padma and Rajendren, 2013; Chakraborty and Kotwaliwale, 2016). Pasta made from mixture of kodo and wheat was found acceptable (Devi *et al.*, 2014). Combination of kodo millet flour, refined wheat flour and soy flour in proportion of 50 : 40 : 10 was used to make cold extruded vermicelli and pasta (Ranganna *et al.*, 2014). Purified starches are used for encapsulation of food ingredients (Li, 2014). These are also used to impart desirable functional roles and modify food consistency and texture (Nagarnaik *et al.*, 2015). Kodo starch is composed of two sub-units, amylose and amylopectin. Sometimes slight change in the segment structure takes place according to the method of fractionation. Structure of amylose is straight with few branches attached with the direct spine while amylopectin is branched type. Amylose content was found to be 19.6% (Zhu, 2014). Literature available on kodo millet starch is limited and it requires to be explored. This paper aims at the extraction and characterization of kodo millet starch.

MATERIALS AND METHODS

Kodo millet grains were procured from local market and stored in deep freezer at -18°C. Proximate composition and morphological properties of these grains were studied followed by the extraction of starch using two methods. Extracted starch from best suitable extraction method was then characterized for its proximate composition, functional, thermal and textural properties.

Moisture, ash, crude fat and crude protein were estimated using the methods as described by AOAC (1995). Dimensions of kodo millet grains such as length, width, and thickness in mm were determined using a Digital Vernier Caliper, 0-150 mm (Ramashia *et al.*, 2018). Thousand kernel weight was calculated by using the method described by Ramashia *et al.* (2018). Geometric mean diameter (Dg), Sphericity and Frontal area were calculated as per equations described by Ramashia *et al.* (2018). Surface area of the millet grain was calculated by using the equation described by Nadvornikova *et al.* (2018).

In first extraction method, grains were first steeped in 0.2 M acetate buffer of pH 6.5 containing 0.01 M mercuric chloride for 30 h at room temperature followed by grinding and slurried in water before being sieved successively and rapidly through 80 mesh screens. Grinding, slurring and sieving were repeated until material left on the sieve was free from starch. Proteinaceous material in starch suspension was separated without causing any modification to the starch by shaking the aqueous suspension with 1/8 of its volume of toluene (Gutierrez-Osnaya *et al.*, 2020).

In second method, Kodo millet flour was prepared by milling kodo grains in milling machine (Atta Master, Winner). Flour (100 g) was soaked in 350 ml 0.5% NaOH for 1 h with intermediate stirring followed by centrifugation (TC 450 D, Eltek) at 3000 rpm for 10 min. Washing of sediment with distilled water was done followed by centrifugation. Sediment pH was adjusted to 6.5 using 1M HCl and further centrifugation was done. Upper brown layer was removed and washed again with distilled water and centrifuged at 3000 rpm for 10 min. Drying of final sediment was done at 50°C for 24 h (Jayawardana *et al.*, 2019).

Starch obtained from both methods was then packed in air tight container and stored in deep freezer at -16°C. Out of these two methods, one method was selected based on colour and yield of extracted starch. Colour was observed visually and yield was studied by calculating percentage starch obtained. Starch was then ground first using pestle mortar and then in grinder for 30 s for its characterization.

Moisture, ash, crude fat and crude protein were estimated using the methods as described by AOAC (1995). Water binding capacity (WBC) was analyzed using the method followed by Maktouf *et al.* (2016). Fat absorption capacity (FAC) was analyzed using the procedure followed by Kumari *et al.* (2015). Starch swelling and solubility patterns were estimated using the method followed by Singh *et al.* (2017). Starch solution of 10% concentration after mixing with distilled water was boiled for 15 min and then cooled. Reading was noted at 100 rpm (Reddy and Bhotmange, 2014) for viscosity (Fig. 1). Dispersibility was estimated by using the method followed by Asaam *et al.* (2018). Percentage dispersibility was expressed as :

$$\text{Dispersibility \%} = \frac{30 - \text{Volume of settled particles}}{30} \times 100$$

Wettability was estimated using the method followed by Banupriya and Vijayakumar (2016). Bulk density was measured using the procedure followed by Adeyeye *et al.* (2020). Percentage light transmittance of starch paste was measured by the method followed by Bharti *et al.* (2019) with slight modifications. Sediment volume was analyzed using the procedure described by Wu *et al.* (2014). Gelatinization

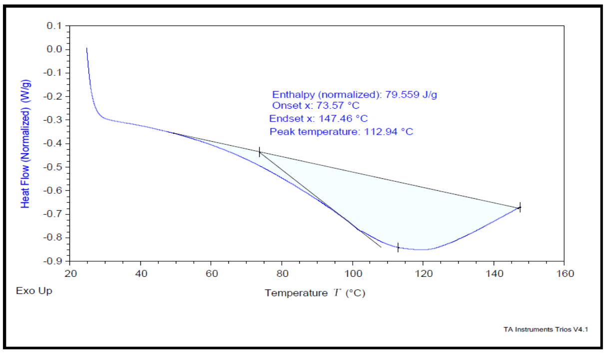

Fig. 1. Thermal properties by DSC.

characteristics of kodo millet starch were studied using Differential Scanning Calorimeter, DSC (Shaikh *et al.* (2016). Textural properties were analyzed following the procedure described by Dey *et al.*, 2016 using texture analyzer with a compression plate (P-75).

RESULTS AND DISCUSSION

Results of proximate analysis are presented in Table 1. These results are comparable to the results obtained in the previous studies. Moisture content of kodo grains was found to be 10.97% and this value was low as compared to the value estimated by Kumar *et al.* (2016) who reported it as 12.71%. The decrease in moisture value may be due to low moisture level of procured sample maintained in order to increase its shelf life and reduce its deterioration during storage and transportation. Mineral content of kodo grains was found to be 2.56%, which was comparable to the mineral value of 2.6% estimated by Saleh *et al.* (2013). Fat content was 1.98%, which was slightly higher than value of 1.4% presented by Deshpande *et al.* (2015). Protein content was found to be 8.7%, which was comparable to the earlier presented value of 8.3% (Deshpande *et al.* 2015). Differences in ash, fat and protein levels may be due to different sample variety, its cultivating conditions and analytical variations. Literature available on its composition is limited and need to be explored further.

Table 1. Proximate analysis of kodo millet grains

Parameter (%)	Mean value±SD
Moisture content	10.97±0.49
Mineral content	2.56±0.30
Fat content	1.98±0.14
Protein content	8.7±0.2

All values are mean±SD of three replicates.

Results of morphological properties of kodo grains are presented in Table 2. Dimensions of kodo millet grain including length, width and thickness were found to be 2.61, 1.94 and 1.31 mm, respectively. These values were comparable to those reported by Kumar *et al.* (2016) who observed values of 2.74, 2.23 and 1.45 mm, respectively. The range of length, width and thickness at moisture content levels from 8.19 to 12.71% was 2.61-2.74, 1.96-2.23 and 1.33-1.45 mm, respectively (Kumar *et al.*, 2016). The difference in values may be due to difference in variety and moisture content. Thousand kernel weights of the millet grains may be used to decide upon the type of fan and sieve required to separate the grains from undesirable materials. Thousand-kernel weight of kodo millet grains was found to be 2.73 g which was about half of the value of 5.74 g reported by Rao *et al.* (2020). Geometric mean diameter may be useful in attaining desired cylinder concave clearance of the thresher. Geometric mean diameter of kodo millet grains was 1.87 mm which was less than the value of 2.29 mm reported by Rao *et al.* (2020). Sphericity of kodo millet grains was found to be 0.71, which was comparable to the value of 0.76 reported by Kumar *et al.* (2016) and Rao *et al.* (2020). Frontal area and surface area of kodo millet grains were found to be 3.97 mm and 10.98 mm^2.

Table 2. Morphological properties of kodo millet grains

Parameter	Mean value±SD
Dimensions	
Length (mm)	2.61±0.01
Width (mm)	1.94±0.01
Thickness (mm)	1.31±0.01
Thousand kernel weight (g)	2.73±0.20
Geometric mean diameter (mm)	1.87
Sphericity	0.71
Frontal area (mm)	3.97
Surface area (mm^2)	10.98

All values are mean±SD of three replicates.

Colour of starch was observed visually. Based on results obtained, starch extracted by method II was considered superior to the other (Table 3). Starch extracted by this method was then used for its further analysis. Moisture content of kodo millet starch was found to be 8.25% (Table 4). Literature related to moisture content of kodo millet starch was not found. The moisture content was comparable to other millets like foxtail millet starch having value as 8.59% (Dey and Sit, 2016) and pearl millet as 7.97% (Chhabra and Kaur, 2017). Mineral content of kodo millet starch was found to be

Table 3. Colour and yield of extracted starches

Parameter	I	II
Colour	Brownish white	White
Yield (g/100 g)	38.6	65

I and II are extraction methods to extract kodo millet starch.

0.65%. Fat content of kodo millet starch was found to be 0.78%. This value was comparable to value 0.16% (Annor *et al.*, 2013). Fat content of grains was also higher than value presented by Deshpande *et al.* (2015). Protein content of kodo millet starch was found to be 0.95%. Value reported by Annor *et al.* (2013) for protein content of kodo millet starch was 0.56%. Increase in value of protein content may be due to the difference in method of protein estimation and variety. Protein content of grains was also higher than the values presented by Deshpande *et al.* (2015).

Table 4. Proximate analysis of kodo millet starch

Parameter (%)	Mean value±SD
Moisture content	8.25±0.01
Mineral content	0.65±0.28
Fat content	0.78±0.02
Protein content	0.95±0.02

All values are mean±SD of three replicates.

Water binding capacity (WBC) of kodo millet starch was found to be 0.84 g (Table 5). Nazni *et al.* (2015) reported that water absorption capacity ranged from 0.94 to 1.15 g for little millet and finger millet, respectively. The difference in values may be due to predominant availability of water binding sites in pearl millet starch as compared to kodo millet starch (Balasubramanian *et al.*, 2014). The use of starch in food products can be determined by studying water binding capacity as one of the parameters as it directly affects other functional properties of starch like viscosity, which was an important indicator of consistency and bulking of food products (Team, 2015). Fat absorption capacity of kodo millet starch was 1.24 g. This was also comparable to value presented by Sharma *et al.* (2016) for pearl millet starch who observed the fat absorption capacity of 1.31 g. Solubility and swelling power of kodo millet starch were found to be 21.6% and 7.06 g/g, respectively. Solubility per cent of kodo millet starch was higher as compared to other millets. Solubility of pearl, finger, foxtail and proso millet starches was 20.5, 7.5, 8.2 and 13.6%, respectively as presented by Zhu *et al.* (2014). On the other hand, swelling power of kodo millet starch was low as compared to all these millets, which had 14.8, 12.8, 13.8 and 14.5% swelling powers, respectively, as presented by Zhu *et al.* (2014). Viscosity and dispersibility of kodo millet starch were found to be 9.24 cps and 81.62%, respectively. Wettability of kodo millet starch was observed and it was noted that the sample started dissolving as soon as it came in contact with distilled water (in less than 1 min) and after 30 min the sample was completely hydrated and went to bottom of the beaker. This showed good wettability of kodo millet. Bulk density of kodo millet starch was found to be 0.96 g/ml, which was higher as compared to the reported value of kodo millet starch, which was 0.67 g/ml (Kumar *et al.*, 2016). The difference in values may be due to different extraction conditions and variety. Light transmittance of kodo millet starch was noted for five consecutive days with an interval of 24 h and it was observed that the values decreased day by day showing the clarity of paste as reduction from 10.74 to 8.77. In comparison to finger and little millet starch having values 5.89 and 2.28%, respectively, light transmittance value of kodo millet was higher (Nazni and Bhuvaneswari, 2016). Balasubramanian *et al.* (2014) reported similar pattern in transmittance values of pearl millet from 97.8 to 95.6%. Clarity of starch paste is one of the significant qualities of starch, which was fundamental in food products, like jams and natural product pastes, to get ideal consistency. This clarity shifted extensively with starch source, enzymatic adjustments, solutes addition and amylose/amylopectin proportion (Balasubramanian *et al.*, 2014). Sediment volume of kodo millet starch was found to be 30.1 ml/100 ml, whereas barnyard millet starch had 32 ml/100 ml sediment volume (Wu *et al.*, 2014). Sediment values of both millet starches were comparable but the

Table 5. Functional properties of kodo millet starch

Parameter	Mean value±SD
Water binding capacity (WBC) g	0.84±0.06
Fat absorption capacity (FAC) g	1.24±0.57
Starch swelling and solubility patterns	
Solubility (%)	21.6±0.38
Swelling power (g/g)	7.06±0.86
Viscosity (cps)	9.24±0.25
Dispersibility (%)	81.62±0.03
Wettability (seconds)	<1 min
Bulk density (g/ml)	0.96±0.01
Light transmittance (%)	10.74±0.9-8.77±0.92
Sediment volume (ml/100 ml)	30.1±0.15

All values are mean±SD of three replicates.

Table 6. Results of texture analysis

Compression (%)	Parameters					
	Hardness	Adhesiveness	Springiness	Cohesiveness	Gumminess	Chewiness
10	1.036	-	3.304	0.860	0.891	2.942
20	14.755	-1.835	0.893	0.915	13.497	12.051
30	82.625	-14.176	0.988	0.713	58.876	58.145

slight difference was due to difference in degree of cross-linking in starches of both millets (Balasubramanian *et al.*, 2014).

Differential scanning calorimetry of kodo millet involved various gelatinization parameters such as onset temperature (To), peak temperature (Tp), endset/conclusion temperature (Tc) and gelatinization enthalpy to be 73.57, 112.94, 147.46°C and 79.59 J/g, respectively. Values obtained were comparable to values obtained by Annor *et al.* (2014) for finger millet starch. They reported the values as 63.9, 69.2, 75.1 ^{0}C and 13.2 J/g, respectively. The difference in onset temperature might be due to different amylose value, shape and size of starch granules and internal arrangement of their fragments. The enthalpy of gelatinization of kodo millet starch is very high as compared to finger millet; this may be due to strong association of double helices that melted during gelatinization (Nazni and Bhuvaneswari, 2016).

It was observed that hardness, gumminess and chewiness increased with increase in compression percentage (Table 6). Hardness value could be attributed to chain length of amylose and amylopectin (Mehboob *et al.*, 2015). Springiness of kodo millet starch showed decrease followed by a slight increase in value with increase in compression, while cohesiveness value showed increase with increase in compression followed by a slight decrease. The change in values could be attributed to intermolecular bonding between starch molecules (Dey and Sit, 2016). Cited literature on the textural studies of its starch is limited. Therefore, its molecular characterization needs further work.

CONCLUSION AND FUTURE PERSPECTIVES

The study showed that kodo millet grains had good nutritional value with moderate protein and high mineral content. Protein in millet was comparable to finger and fonio millets but lower than pearl and foxtail millets. Based on the results of starch extraction, it was concluded that alkaline method with 65% starch yield was found optimum. Starches isolated from kodo millet contained significantly low levels of non-starch components. However, there was a scope of further improvements in quality starch yield, using some alternate methods. Values of water binding capacity, swelling power, viscosity and dispersibility were moderate but fat absorption capacity and solubility percentage were low as compared to pearl millet starch. Kodo starch had high range of gelatinization temperature in comparison with other millets such as finger, pearl and foxtail. Textural properties of kodo millet starch were also superior to other starches such as wheat and cassava starches. Additionally, this starch had moderate springiness, cohesiveness and hardness as compared to the above-mentioned starches. Molecular characterization of kodo starch and relationship among its fractions are loopholes in this area. Interactions of this starch with other food components also need researchers to be focused. Starch of kodo millet can be characterized further as there is a scope of studying its pasting and structural properties using rapid visco-analyzer and X-ray diffraction, which will be helpful in knowing kodo millet starch more deeply.

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