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Studies on Isolation and Characterization of Starch from Pearl Millet (*Pennisetum americanum* (L.) Leeke) Grains

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ABSTRACT

The isolation, characterization and amylolytic digestion of pearl millet starch has been studied. The yield of the starch was approximately 60·2%, on a whole grain basis. The starch exhibited two-stage swelling and moderate solubility patterns in an aqueous medium. The starch contained 22·8% amylose. The gelatinization temperature range of the starch was 69·5–74·0–77·5°C. The viscoamylographic examination on starch paste (8%, w/v) showed a peak viscosity of 640 BU but it reduced considerably during holding at 93°C for 30 min. However, the viscosity of the starch paste increased abruptly (885·0 BU) during cooling (50°C) probably due to retrogradation of amylose. The extent and modes of attack by gluco-amylase and human salivary α-amylase on the native starch granules as viewed by scanning electron microscopy were investigated.

INTRODUCTION

Pearl millet (*Pennisetum americanum* (L.) Leeke) is one of the major millets cultivated under semi-arid, hot, dry conditions and found to adapt to many regions that have as low as 25–65 cm annual rainfall. It is grown extensively in India and Africa. It forms an important component in the diet of individuals in both developing and underdeveloped countries of Asia and Africa as a primary source of calorie, and contributes significantly as the often sole source of vitamins, minerals and, to some extent, protein. As in most cereals, starch is the major component (56–65%) of the pearl millet grains. There are some reports on the properties of pearl millet starch (Badi *et al.*, 1976; Beleia *et al.*, 1980), but

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detailed information on physicochemical characteristics, the extent of hydrolysis and the mode of attack by amylolytic enzymes on the native starch granules of pearl millet is lacking. This paper reports the results of a study on the physicochemical, pasting characteristics and amylolytic susceptibilities of this starch.

EXPERIMENTAL

Cereal grains

Pearl millet (BJ-104) grains were obtained in December 1987 from the University's experimental farm.

Isolation and purification of the starch

The starch was isolated and purified from pearl millet grains essentially by a wet milling method (Hoseney *et al.*, 1971), with modifications described by Wankhede *et al.* (1979).

Chemical composition of starch

Quantitative estimations of moisture, total lipid, ash and protein content of starch were performed using the standard AOAC (1960) procedures. The total sugar in the starch was determined after acid hydrolysis (4% H_2SO_4, 100°C, 8 h) using the phenol sulphuric acid method (Dubois *et al.*, 1956) with the modifications adopted by Tharanathan *et al.*, (1980). Quantitative estimation of pearl millet starch was carried out according to the procedure of Thivend *et al.* (1972) using glucoamylase to hydrolyse the starch into D-glucose. The liberated D-glucose was quantitatively estimated with glucose oxidase (Dahlquist, 1964).

Electron microscopic examination of starch granules

The native starch granules were mounted on double-sided adhesive tape attached to specimen stubs. The samples were coated with a thin layer of carbon *in vacuo* and then with about 150 Å of gold. The samples were viewed, examined and photographed in an ETEC-autoscan scanning electron microscope at an accelerating voltage of 15 kV as described elsewhere (Tharanathan *et al.*, 1980).

Gelatinization temperature range

The gelatinization temperature range was determined according to the method of Schoch and Maywald (1956).

Swelling and solubility characteristics

The swelling and solubility behaviour of starch in an aqueous system was studied by the procedure of Leach *et al.* (1959) using 5 g of starch.

Amylograph pasting characteristic of the starch

The pasting behaviour of the pearl millet starch paste (8%, w/v) was determined using a Brabender viscoamylograph as described by the American Association of Cereal Chemists (AACC, 1960).

Fractionation of the starch

The millet starch was defatted as described earlier (Wankhede *et al.*, 1977). The resultant defatted starch was dispersed in dimethyl sulphoxide and fractionated into its components, i.e. amylose and amylopectin, as described by Banks and Greenwood (1967). The amylose content was quantitatively estimated by the procedure of McCready and Hassid (1943). The calibration curve was prepared for the starch using appropriate mixtures of isolated amylose and amylopectin.

Amylolytic enzyme preparation

Freshly secreted human saliva was collected and diluted with an equal volume of 0·1 M citrate phosphate buffer (pH 6·9) and was used as human salivary amylase.

A crude glucoamylase was purchased from M/s Novo Industries, Copenhagen, Denmark. It was subjected to ammonium sulphate fractionation, followed by DEAE-cellulose and Sephadex-G-100 column chromatography as described by Wankhede *et al.* (1981).

Digestion of native and gelatinized starches of pearl millet by human salivary α-amylase and glucoamylase

The percentage hydrolysis of native and gelatinized starches by human salivary α-amylase was determined by the procedure of Tharanathan *et al.* (1980). In a typical experiment, 100 mg of starch were incubated separately with the enzyme (40 units/mg of starch) in a citrate–phosphate buffer (0·05 M, pH 6·9, 100 ml) at 37°C for specific time intervals (viz. 0·5, 1, 2, 5, 10, 20, 40, 60 and 100 min). Aliquots (5 ml) of the incubated mixtures were mixed with 10 ml of methanol. Supernatants were obtained by centrifugation at $3000 \times g$ for 10 min. The maltose in the supernatant was estimated quantitatively by the procedure of Nelson

(1944). The resultant pellet following centrifugation was washed repeatedly with distilled water and lyophilized.

The extent of hydrolysis by glucoamylase was studied by a similar method (Wankhede *et al.*, 1979). One hundred milligrammes of the native or gelatinized starches were incubated with purified glucoamylase (5 units/mg of starch) in 0·05 M sodium acetate buffer, pH 4·5 (100 ml) at 37°C. Aliquots (5 ml) were withdrawn after specific time intervals from the incubation mixtures and the control, and added to the methanol (10 ml). The reaction mixtures were centrifuged at 3000 × *g* for 15 min. The resultant hydrolysates were assayed for D-glucose quantitatively by the glucose oxidase method (Dahlquist, 1964). The pellet was dried as described previously and in both cases SEM was used to determine the mode of attack of the enzymes, (Tharanathan *et al.*, 1980).

RESULTS AND DISCUSSION

Morphological granular characteristics of the starch

The microscopic examination of pearl millet starch granules revealed that they are morphologically similar to other millet starch granules. The starch granules ranged from polygonal to round in shape with characteristic dimensions in the range 10–16 μm. Electron micrographs showed that some of the granules had deep indentations due to the pressure exerted by protein bodies. The electron photomicrograph of native starch granules at different magnifications (viz. 3200 × and 6000 ×) are presented in Fig. 1(a) and (b). Variations in shape and size of these granules may be attributed to premature biosynthesis, since the charac-

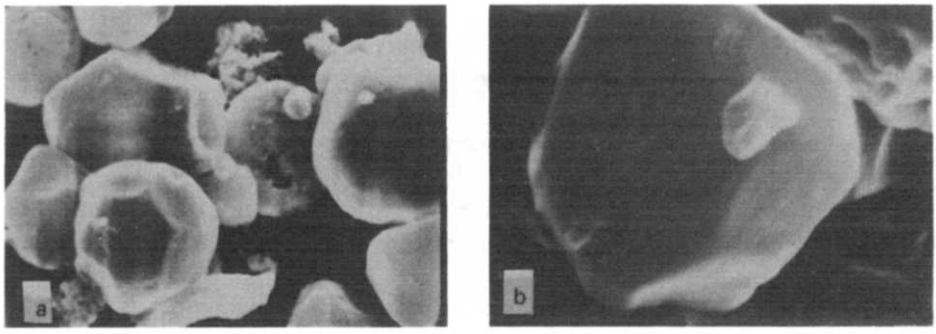

Fig. 1. Scanning electron photomicrographs (SEM) at different magnifications, of native starch granules of pearl millet; (a) 3200 × (b) 6000 × .

teristic of any starch depends entirely on the time at which they were harvested and isolated. These results are in good agreement with the results reported by Badi *et al.* (1976).

Chemical composition of the starch

The chemical composition of the starch is presented in Table 1. The results revealed that the pearl millet grains contained 65·2% starch by quantitative determination. However, the extraction of starch from the grains was only 60·18% in the present study. After repeated purification, the protein content of the starch was reduced to 0·68%. This may be due to the presence of highly hydrated pentosans and insoluble protein which entrap the starch granules in the matrix. However, Beleia *et al.* (1980) reported that the protein content of pearl millet starches ranged between 0·44 and 0·77%. The variations in protein contents of the starches may be due to isolation and purification methods adopted by different workers. In addition, the varietal differences, origin of the grain samples, stage of maturity, and storage of grains cannot be overlooked. The amylose content of the starch was approximately 22·85%. According to Badi *et al.* (1976) pearl millet starch contained 17% amylose. However, Beleia *et al.* (1980) have investigated the starches from different varieties and hybrids of pearl millet and reported that the amylose contents of pearl millet starches ranged between 20 and 22%.

TABLE 1
Chemical Composition (%) and some of the Properties of Pearl Millet Starch[a]

Yield[b]		60·18
Moisture		10·18
Ash		0·78
Protein ($N \times 6{\cdot}25$)[c]		0·68
Total lipid		0·92
Total sugar		87·25
Amylose		22·85
Amylopectin		77·15
Gelatinization temperature range (°C)[d]		69·5–74·0–77·5
Starch granule characteristics		
Granule shape	Round	Polygonal
Granule size (μm)	(10–12)	(10–15·5)

[a]Each value represents the mean of three determinations.
[b]The value represents the mean of three determinations, on whole grain basis.
[c]N is the microkjeldahl nitrogen content.
[d]The values represent initial, middle and final gelatinization temperatures.

Gelatinization temperature range

To probe into the granular structure of pearl millet starch, the gelatinization temperature range was studied. It was observed that the starch had the initial, middle and end-point gelatinization temperatures of 69·5, 74·0 and 77·5°C respectively (Table 1). These results varied slightly but fell within the ranges reported by several investigators (Beleia *et al.*, 1980; Badi *et al.*, 1976). However, it was observed that the gelatinization temperature range for the starch was considerably higher as compared to other millet starches (Wankhede *et al.*, 1979; Paramahans *et al.*, 1980). According to Lund (1984), the gelatinization of starches may be influenced by time, temperature, moisture, ingredients and processing conditions. Freeman *et al.* (1968) have claimed that environmental conditions during grain maturation can cause differences in starch gelatinization temperatures even among cultivars.

Swelling and solubility characteristics

To study the nature of associative bonding forces within the granules, the swelling and solubility behaviour in an aqueous system has been investigated. The results are presented in Table 2. The data indicated that the starch showed two-stage swelling and solubility patterns similar to the results reported by Beleia *et al.* (1980).

TABLE 2
Swelling Power and Solubility (%) of Pearl Millet Starch[a]

Properties	*Temperature (°C)*						
	30	*40*	*50*	*60*	*70*	*80*	*90*
Swelling power	0·78	0·92	1·50	3·50	6·85	11·85	14·80
Solubility	0·38	0·85	1·25	4·75	9·82	14·45	20·50

[a]Each value represents the average of three determinations.

Pasting characteristics of the starch

Pasting characteristics of the starch were investigated with the Brabender viscoamylograph and the results are presented in Table 3. The pasting temperature of the starch was 70°C. The peak viscosity of the pearl millet starch (8%, w/v) was 640 BU, but it decreased considerably during the holding period of 30 min at 93°C. This may be due to the swollen

TABLE 3
Amylograph Pasting Characteristics of Starches

Starch	*Pasting temperature (°C)*	*Pasting peak viscosity (Brabender units, (BU))*	*Viscosity (BU) at 93°C for 30 min*	*Viscosity (BU) on cooling at 50°C for 30 min*
Pearl millet (BJ-104)[a]	70·0	640·0	528·0	885·0
Pearl millet[b]	76·5	400–500	340–400	460–620
Pearl millet[c]	72·0	560·0	640	780·0
Sorghum[c]	78·0	570·0	515·0	685·0
Sorghum (CSH-1)[d]	75·5	800·0	420·0	1228·0
Sorghum (IFQT)[e]				
non-waxy	68·5–77·5	750–1170	390–675	610–1030
Finger millet[f]	67·5–69·0	1580–1720	1200–1300	1730–1920
Foxtail millet[f]	67·5	1780·0	1540·0	2000·0
Corn starch[g]	77·0	370·0	450·0	700·0

[a]Each value represents the mean of three determinations (8%, w/v concentration of starch).
[b]Beleia *et al.* (1980) (using 9%, w/v concentration of starch) moisture basis).
[c]Badi *et al.* (1976) (using 11·25% concentration of starch).
[d]Wankhede *et al.* (1989) (8% w/v concentration of starch).
[e]Akingbala *et al.* (1981) (using 10% concentration of starch basis).
[f]Wankhede *et al.* (1979) (using 9·5% concentration of starch).
[g]Takeda *et al.* (1988) (using 9·5% concentration of starch).

starch granules subsequently fragmented and solubilized at higher temperatures. However, the viscosity of the starch paste increased abruptly to 885 BU while cooling from 93 to 50°C, presumably due to retrogradation. These results are in good agreement with the data reported by Badi *et al.* (1976) and Beleia *et al.* (1980) for pearl millet starches. Similar results on pasting behaviour of starches of foxtail and finger millets have been reported by Wankhede *et al.* (1979).

In-vitro digestibility of native and gelatinized starches of pearl millet by human salivary α-amylase

Percentage hydrolysis of both native and gelatinized starches of pearl millet by α-amylase are presented in Table 4. The data indicated that the gelatinized starch was hydrolysed comparatively more (75·5%) than the native pearl millet starch (52·5%) under similar experimental conditions. This may be attributed to the change in weakening of homogeneous bonding forces within the granules and certain alterations in the crystallinity of the starch during cooking of the starch paste. Similar results were reported by Wankhede *et al.* (1979) on the amylolysis of native and gelatinized starches of finger and foxtail millets. According to Modi and Kulkarni (1976), the gelatinized ragi starch was hydrolysed to the extent of 75·2% in 3 min. The data on the extent of hydrolysis of native and gelatinized pearl millet starches by purified glucoamylase are presented in Table 5. The results indicated that the native pearl millet starch was hydrolysed to a limited extent (60·5%) in 100 h of incubation. According to Tharanathan *et al.* (1980), the finger millet starch was hydrolysed to a very limited extent (~11%) by glucomylase-II. On the contrary, Wankhede *et al.* (1979) reported 60% hydrolysis of finger millet starch by partially purified glucoamylase. This may be attributed to the source of starches, enzyme origin, debranching ability of the particular enzyme

TABLE 4
In-vitro Amylolysis (%) of Pearl Millet Starch with Human Salivary α-amylase[a]

Starch	*Incubation time intervals (min) at 37°C*							
	0·5	*1·0*	*2·0*	*5·0*	*10·0*	*20·0*	*40·0*	*60·0*	*100·0*
Native starch	2·80	3·00	3·50	4·00	8·50	14·25	22·40	42·50	52·50
Gelatinized starch	4·82	7·50	9·55	20·00	25·28	35·00	50·20	65·50	75·48

[a]The values represent per cent maltose released during α-amylolysis; controls containing no enzymes indicated 0·01 and 0·04% hydrolysis of native and gelatinized pearl millet starches, under the experimental conditions employed.

TABLE 5
In-vitro Hydrolysis (%) of Pearl Millet Starch with Glucoamylase[a]

Starch	*Incubation time intervals (h) at 37°C*							
	0·5	*1·0*	*5·0*	*10·0*	*16·0*	*32·0*	*64·0*	*100·0*
Native pearl millet starch	0·08	3·25	3·95	4·20	6·50	8·00	8·50	10·80
Gelatinized pearl millet starch	8·75	10·50	15·40	30·75	50·55	53·50	60·20	60·50

[a]The values represent per cent glucose liberated during amylolysis; controls containing no enzymes indicated 0·02 and 0·05% hydrolysis of native and gelatinized pearl millet starches, under the experimental conditions employed.

and the contamination of glucoamylase with other amylases used by different investigators.

Amylolytic susceptibility of native starch granules as viewed by SEM

The mode of attack by human salivary α-amylase on the native pearl millet starch granules was investigated using scanning electron microscopy. The results are presented in Fig. 2. From the results (Fig. 2(a)–(d), it is clear that the enzyme has selectively attacked the granule probably starting from a single pin-hole and tunnelling into the interior portion of the granule leaving only an outer shell (Fig. 2(c)). However, it is not clear whether two halves of the granule visible in Fig. 2(b) are due to amylolysis or due to the mechanical damage caused during isolation and purification of the starch granules. These results are in good accordance with the mode of attack by α-amylase on ragi starch granules (Tharanathan *et*

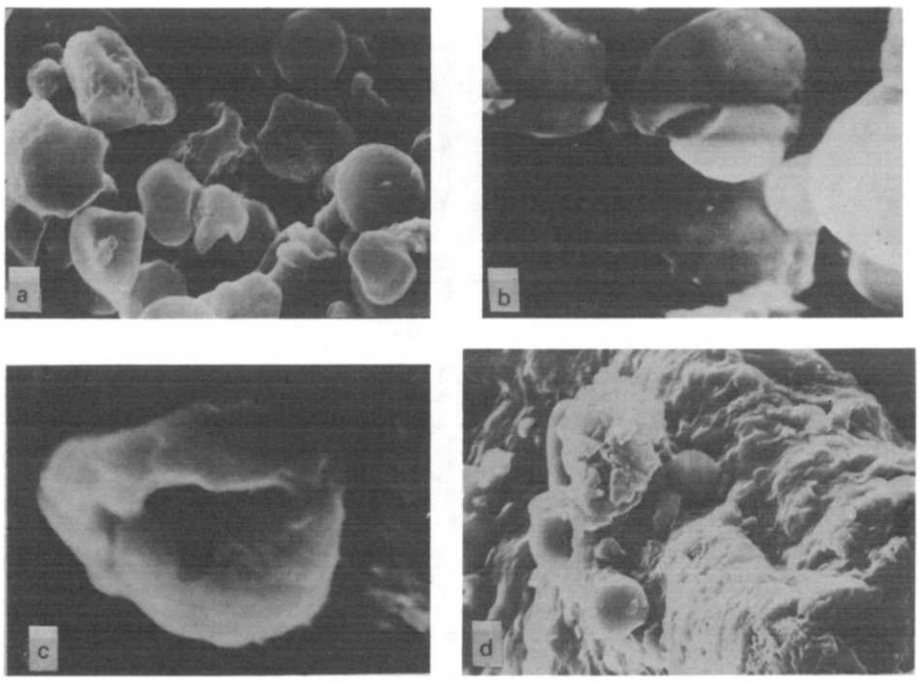

Fig. 2. Scanning electron photomicrographs (SEM) of pearl millet starch granules attacked by human salivary α-amylase; incubation periods and magnifications, (a) 6 min, 1600×; (b) 20 min, 3000×; (c) 40 min, 3200×; (d) 100 min, 1900×.

al., 1980). According to Beleia and Varriano-Marston (1981), α-amylase of pearl millet grains preferentially attacked large granules of wheat starch. The attack was mostly confined to the interior portion of the granules leaving only an outer shell similar in structure to a mushroom cap. According to Hoseney and Varriano-Marston (1980) the enzyme preferentially attacks the spherical granules of pearl millet. They have also reported that the starch hydrolysis was more vigorous at the centre of the granule than at the periphery.

The nature and pattern of attack by purified glucoamylase on native pearl millet starch granules as viewed by SEM are depicted in Fig. 3. The micrographs initially show a large number of pin-holes over the entire granule surface, eventually resulting in complete dissolution of the granules (Fig. 3(d)). This behaviour is in good agreement with the results reported by Tharanathan *et al.* (1980). In addition, it was also observed that some of the starch granules showed absolutely no attack.

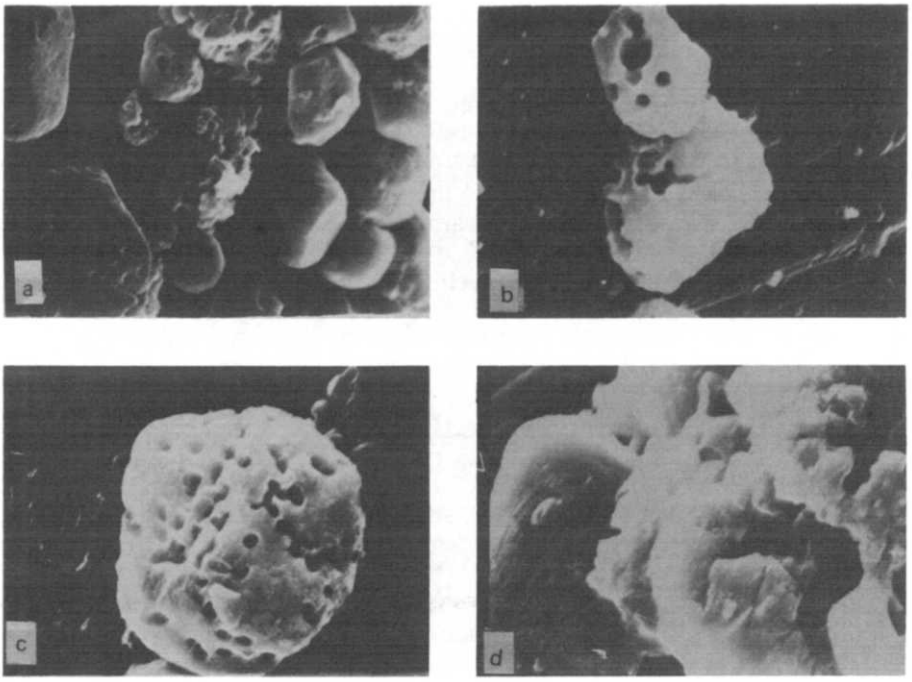

Fig. 3. Scanning electron photomicrograph (SEM) of pearl millet starch granules attacked by purified glucoamylase. Incubation periods and magnifications, (a) 1 h, 3000×; (b) 16 h, 1900×; (c) 64 h, 3200×; (d) 100 h, 3200×.

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