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Studies on the physicochemical properties of native, defatted, and heat–moisture treated pigeon pea (*Cajanus cajan L*) starch

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ABSTRACT

Starch from pigeon pea (*Cajanus cajan L*) was isolated and some of the important characteristics determined. The yield of starch was 29.7% on a whole seed basis. The shape of the starch granule was oval to elliptical to irregular, with granules 8–32 μm in diameter. Scanning electron micrographs revealed the presence of smooth surfaces with a large number of grooves. The gelatinization temperature-range was 347–354–360 K and the total amylose content was 29.3%, of which 2.7% was complexed by native lipids. The starch exhibited a restricted two-stage swelling pattern and a moderate solubility in water. The viscoamylographic examination of the starch paste (6% w/v) showed the absence of a peak viscosity, a low 95°C viscosity (80 Brabender units, BU) and an increase in consistency (140 BU) during the holding cycle at 95°. Native granules were very resistance to hydrolysis by porcine pancreatic alpha amylase and 2.2 M HCl. The X-ray diffraction pattern was of the C-type. The results showed that the starch chains in the amorphous regions of pigeon pea were more highly associated than those of other legume starches. Defatting (hot 3:1 1-propanol–water) and heat–moisture treatment (100°C, 16 h, 30% moisture) increased the gelatinization temperature and broadened the gelatinization temperature range, with relatively little change in gelatinization enthalpy. Defatting and heat–moisture treatment also increased the pasting temperature and susceptibility towards alpha-amylase, but decreased the swelling factor, amylose leaching, and paste consistencies at 95 and 50°C. However, the relative crystallinity remained unchanged. Acid hydrolysis increased on defatting but decreased on heat–moisture treatment. Defatting caused greater changes to the granular surface than heat–moisture treatment.

INTRODUCTION

Grain legumes constitute an important part of the human diet in many parts of the world. They supply significant amounts of energy through carbohydrates, proteins, fiber, lipid (leguminous oil seeds), minerals, and vitamins[1]. Although the major component of legume seeds in general is starch, greater attention has been given to their proteins. However, utilization of the starch will be economically

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important if legume protein concentrates are used in foods. Legume starches have been of great interest to nutritionists, since they have been found to exhibit a lower glycemic index than the cereals[2], thereby helping in the dietary control of diabetes as well as arterial disease. Pigeon pea (*Cajanus cajan L*) is one of the most commonly consumed food legumes in India, East Africa, and Latin America. India alone contributes over 90% of the present world pigeon pea production. In many of these countries, pigeon pea is consumed after boiling in the form of dhal (decorticated split cotyledons)[3]. Singh et al.[3] have suggested that pigeon pea starch was as good for noodle preparation as mung bean starch.

Although there have been some reports[3] on the pasting, gelatinization, swelling power, and gel strengths of pigeon pea starch, detailed information on proximate composition, crystallinity, freeze–thaw stability, thermal properties, and granular susceptibility to acidic and enzymic hydrolysis is lacking.

The objective of this study was to isolate the starch fraction from pigeon pea seeds and to determine its physicochemical properties in the native state and after physical modification (lipid removal and heat–moisture treatment). It is hoped that this investigation may lead to greater utilization of pigeon pea starch in the food industry.

EXPERIMENTAL

Materials.—Pigeon pea seeds (variety ICPL 87) were obtained from the International Crop Research Institute for the Semi Arid Tropics Center (Patancheru, India). These seeds were sown on 11th June 1990 and harvested on 10th October 1990. Starch was isolated from pigeon pea seeds by the procedure of Schoch and Maywald[4] with minor modifications. About 300 g of beans were steeped in 200 mL of water containing 0.01% sodium metabisulfite for 20 h at 40°C. The swollen seeds were rinsed with water and homogenized in a Waring blender for 5 min. The homogenate was passed four times through a 20-μm polypropylene screen. The residue was homogenized four more times and the combined extracts passed four times through a 70-μm polypropylene screen. The filtrate was allowed to sediment at room temperature for 10 h and the supernatant discarded.

The sediment was suspended in an excess of 0.02% NaOH and, after being kept for 1 h, the supernatant was removed. This procedure was repeated six times. The final sediment was suspended in distilled water, passed through a 70-μm polypropylene screen, neutralized to pH 7.0 with HCl, filtered on a Buchner funnel, and thoroughly washed on the filter with distilled water. The filter cake was dried overnight at 30°C.

Crystalline porcine pancreatic alpha amylase (EC 3.2.1.1.), type 1-A was obtained from Sigma Chemical Co (St. Louis, MO).

Chemical composition of starch.—Quantitative estimations of moisture, ash, nitrogen, and starch damage were performed by the standard AACC[5] procedures. Starch lipids were analyzed as follows: at ambient temperature (25–27°C), lipids

were extracted from pigeon pea starch (5 g dry basis) with 100 mL of 2:1 $CHCl_3$–CH_3OH under vigorous agitation in a wrist-action shaker for 1 h. At elevated temperatures (90–100°C) lipids were obtained by Soxhlet extraction (7 h) with 100 mL of 3:1 1-propanol–water. Lipids were also extracted, after acid hydrolysis of pigeon pea starch with 24% HCl at 70–80°C for 30 min, and the hydrolyzate then extracted three times with 1-hexane[6]. The purification and quantification of extracted lipids were carried out by procedures described elsewhere[7].

Apparent and total amylose content was determined by the blue value method of Gilbert and Spragg[8]. Calculation for amylose content was by the method of Williams et al.[9].

Swelling factor.—The swelling factor of the starches when heated to 50–95°C in an excess of water was measured according to the method of Tester and Morrison[10]. This method measures only intragranular water and hence the true swelling factor at a given temperature. The swelling factor is reported as the ratio of the volume of swollen starch granules to the volume of the dry starch.

Extent of amylose leaching.—Various concentrations of the starches (15–20 mg) in water were heated in volume calibrated sealed tubes (50–95°C) for 30 min. The tubes were then cooled to ambient temperature and centrifuged at 3600 rpm for 10 min. The supernatant liquid (1 mL) was withdrawn and its amylose content was determined by the method of Chrastil[11].

X-Ray diffraction.—X-Ray diffractograms were obtained with a Rigaku RU 200R X-ray diffractometer with a chart speed of 20 mm/min. The starch powder was scanned through the 2θ range of 3–35°. Traces were obtained by using a Cu $K\alpha$ radiation detector with a nickel filter and a scintillation counter operating under the following conditions: 40 KV, 50 mA, 1°/1° divergence slit/scattering slit, 0.30 mm receiving slit, 1 s time constant, and scanning rate of 3°/min. Relative crystallinity was measured by the method of Nara et al.[12]. Quartz was used as the 100% reference crystal.

Differential scanning calorimetry (DSC).—Gelatinization temperatures were measured and recorded on a Perkin—Elmer DSC-2 (Norwalk, CT) differential scanning calorimeter, with thermal analysis data station. Water (8.0 μL) was added with a microsyringe to starch (2.5 mg) in the DSC pans, which were then sealed, reweighed, and kept overnight at room temperature. The scanning temperature range and the heating rate were respectively 20–120°C and 10°C/min. The thermogram was recorded with water as reference. The transition temperatures reported are the onset (T_o), peak (T_p), and conclusion (T_c) of the gelatinization endotherm. Indium was used for calibration. The enthalpy (ΔH) was estimated by integrating the area between the thermogram and a base line under the peak and was expressed in terms of calories per unit weight of dry starch (cal/g^1). All DSC experiments were replicated at least twice.

Pasting properties.—A Brabender viscoamylograph (model VA-V) equipped with a 700-cm cartridge was used to determine the pasting properties at a

concentration of 6% w/v db and pH 5.5. The starch dispersions were stirred at 75 rpm and heated at 1.5°C/min to 95°C, held at this temperature for 30 min, and cooled to 50°C.

Freeze–thaw stability.—The gels (6% db) were subjected to cold storage at 4°C for 16 h (to increase nucleation) and then frozen at −16°C. To measure freeze–thaw stability, the gels frozen at −16°C for 24 h, were thawed at 25°C for 6 h and then refrozen at −16°C. Five cycles of freeze–thaw were performed. The excluded water was determined by centrifuging the tubes (30 diam. × 100 mm) at 1000 g for 20 min after thawing.

Acid hydrolysis.—The starches were hydrolyzed with 2.2 M HCl at 35°C (1.0 g starch/40 mL acid) for 25 days. The starch slurries were shaken by hand daily to resuspend the deposited granules. At 24-h intervals, aliquots of the mixtures were neutralized and centrifuged (3500 rpm) and the supernatant liquid was assayed for total carbohydrate[13]. The extent of hydrolysis was determined by expressing the solubilized carbohydrates as a percentage of the initial starch.

The kinetics of acid hydrolysis are presented according to Robin et al.[14] by plotting log $(100/100 - x)$ vs. time (t), where x is the percent of hydrolyzed starch. Linear regression analysis showed a good fit of the experimental data for the first stage of hydrolysis with a first-order type of process.

Consequently, the apparent first-order rate constants were calculated using the equation:

$$k = \frac{2.303}{t} \log \frac{100}{100 - x}$$

where k = initial hydrolysis rate constant.

Enzymic hydrolysis.—The extent of hydrolysis by porcine pancreatic alpha amylase was determined following previously described methods[15].

Scanning electron microscopy (SEM).—Granule morphology and the mode of action of alpha amylase were studied by SEM. Starch samples were mounted on circular aluminium stubs with double-sided sticky tape and then coated with 20-nm layer gold an examined and photographed in a Hitachi (S 570) SEM at an accelerating potential of 20 KV. Enzyme-digested granules were prepared for SEM by rapidly freezing in liquid nitrogen and freeze drying at 80°C. The dried samples were prepared for viewing as just described.

Defatting.—Lipids were extracted at ambient temperatures (25–27°C) from pigeon peak starch (5 g db) with 100 mL of 2:1 $CHCl_3$–CH_3OH under vigorous agitation in a wrist-action shaker for 1 h. At elevated temperatures (90–100°C) lipids were extracted by refluxing (7 h) with 100 mL of 3:1 1-propanol–water. Lipids were also extracted after hydrolysis of pigeon pea starch with 24% HCl for 30 min at 70–80°C, and the hydrolyzate then extracted three times with 1-hexane[6]. The purification of extracted lipids was carried out by procedures that have been described elsewhere[7].

Heat–moisture treatment.—The method of heat–moisture treatment was essentially that of Sair[16]. The moisture content was brought to 30%. The sealed samples

(in glass jars) were heated in an air oven at 100°C for periods up to 16 h. After they were cooled, the jars were opened, and the starch samples air dried to a uniform moisture content (~ 10%).

RESULTS

Morphological granular characteristics of the starch.—The starch granules ranged from oval to elliptical to irregular in shape with characteristic dimensions in the range 6–34 μm (Fig. 1A). The surface appeared to be smooth and showed the presence of grooves when viewed under the scanning electron microscope (Fig. 1B). Grooves have not been reported on granules of other legume starches.

Chemical composition of the starch.—The data on composition and yield are presented in Table I. The purity of the starch was judged on the basis of composition and microscopic observations. The yield of pigeon pea starch was 29.7% on total seed basis. This was lower than the value (49.3%) reported by Singh et al.[3]. The chemical composition showed that the starch contained 0.02% nitrogen and 0.03% ash. These low values indicated high purity and the absence of nonstarch lipids (lipid associated with endosperm proteins). Therefore, total lipids (obtained by acid hydrolysis) in pigeon pea (0.13%) mainly represent free and bound starch lipids. The free lipids (obtained by extraction with $CHCl_3$–CH_3OH) amounted to 0.03%, while the corresponding value for bound lipids (obtained by extraction of $CHCl_3$–CH_3OH residues with 1-propanol–water was 0.1%. The total lipid content was similar to those reported for other legume starches[17].

Fig. 1. Scanning electron micrographs of native pigeon pea starch (A and B).

TABLE I

Chemical composition (%) and some of the properties of pigeon pea starch [a]

Characteristics	Composition (%)
Yield (% initial material)	29.70
Moisture	10.90
Ash	0.03
Nitrogen	0.02
Starch damage	2.00
Lipid	
Acid hydrolyzed [b]	0.13
Solvent extracted	
Chloroform–Methanol [c]	0.03
1-propanol–water [d]	0.10
Amylose content [e] (% of total starch)	
Apparent	28.50
Total	29.30
Amylose complexed by native lipid	2.70
Starch granule characteristics	
Granule shape	oval to elliptical to irregular
Granule size (μm)	8–32

[a] All data reported on dry basis and represent the mean of three determinations. [b] Lipids obtained by acid hydrolysis (24% HCl) of the native starch (total lipids). [c] Lipids extracted by 2:1 chloroform–methanol at 25°C (mainly unbound lipids). [d] Lipids extracted by 1:1 1-propanol–water from the residue left after chloroform–methanol extraction (mainly bound lipids). [e] Apparent and total amylose determined by I_2 binding before and after removal of bound lipids by hot 1-propanol–water extraction. [f] $\frac{\text{Total amylose} - \text{apparent amylose} \times 100}{\text{Total amylose}}$.

The total amylose content of legume starches have been reported to be in the range 24–65% (ref. 17). The corresponding value for pigeon pea starch was 29.3% of which 2.7% was complexed by native lipids (Table I). However, Singh et al.[3] reported a value of 46.4%, as the total amylose content of pigeon pea starch (variety C-11). This major discrepancy in data may have been due mainly to varietal differences[18], rather than to differences in the methods used for amylose determination (using the method followed by Singh et al.[3], we obtained a value of 28.9% as the total amylose content for native pigeon pea starch) or to the physiological state of the seeds.

X-Ray diffraction.—Native, defatted, and heat–moisture treated pigeon pea starches showed the characteristic "C" pattern of legume starches. The pattern in native (Fig. 2A) and defatted starch (Fig. 2B) was characterized by two strong intensity lines at 5.21 and 5.14 Å and by three medium lines at 5.96, 590, 3.88 Å (Figs. 2A and B). The corresponding values for heat–moisture treated starch (Fig. 2C) was 5.28, 5.22, 5.98, 5.91, and 3.81 Å, respectively. The relative crystallinity of native pigeon pea starch (0.68) was higher than the value reported for potato starch (0.52)[19] but was comparable to that of wheat (0.67), corn (0.63), and lentil (0.64) starches[19]. The relative crystallinity of pigeon pea starch remained unchanged on defatting and heat–moisture treatment. However, that of wheat, corn,

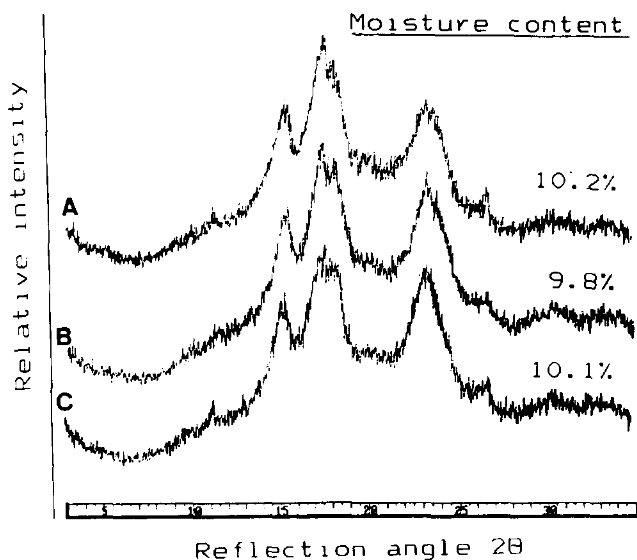

Fig. 2. X-Ray diffraction patterns of native (A), defatted (B), and heat–moisture treated (C) pigeon pea starch.

lentil, and potato starches have been found to increase on defatting[19], whereas a decrease in relative crystallinity has been observed on heat–moisture treatment of potato and wheat starches[20].

Swelling factor and amylose leaching.—The swelling factor (SF) and amylose leacing (AML) of native, defatted, and heat–moisture treated pigeon pea starches in the temperature range 50–95°C are presented in Figs. 3 and 4, respectively. The SF and AML of native pigeon peak starch was within the range reported for other legume starches[17,19,21]. The SF and AML of native, defatted, and heat–moisture treated starches exhibited a restricted two-stage swelling pattern which is indicative of strong bonding (between or among starch components) by two sets of

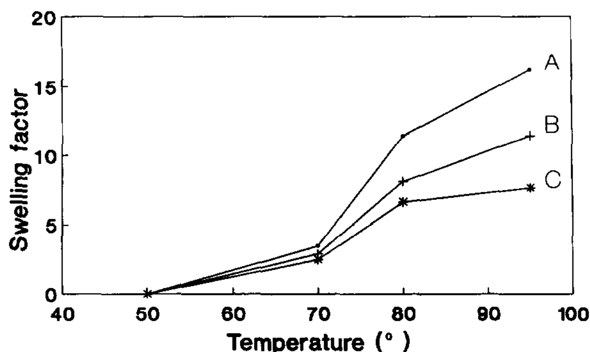

Fig. 3. Swelling factor of native (A), defatted (B), and heat–moisture treated (C) pigeon pea starch.

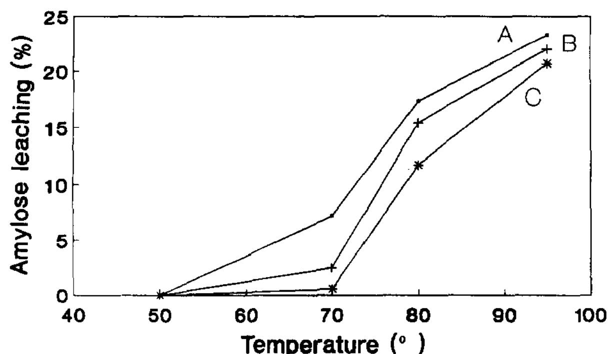

Fig. 4. Amylose leaching in native (A), defatted (B), and heat–moisture treated (C) pigeon peak starch.

forces, which relax at different temperatures. Irrespective of the prior treatments, all starches showed a rapid increase in swelling at ~ 72°C. However, the rate and extent of swelling decreased in the order: native > defatted > heat–moisture treated. Defatting and heat–moisture treatment decreased the SF of native starch (at 95°C) by 29.3 and 53%, respectively. The decrease in SF on defatting was much higher than those observed for cereal starches[19,21], but lower than those for potato and lentil starches[19]. The effect of heat–moisture treatment on the SF of legume starches has not been reported previously, and therefore no comparisons are possible. However, the SF decrease on heat–moisture treatment of pigeon pea starch was higher than that observed for wheat starch[21], but was comparable to that of potato starch[21]. At 70°C, the extent of amylose leaching was 7.0, 2.5, and 0.6%, respectively (Fig. 4), in native defatted and heat-moisture treated starches. The corresponding values at 95°C were 24, 22, and 20.8%.

Gelatinization temperatures.—The gelatinization parameters of native, defatted, and heat–moisture treated starches are presented in Table II. The gelatinization temperature of native pigeon pea starch (347–354–360 K) was higher than that reported by Singh et al.[3] (338–344–349 K) and was also higher than that of other legume starches[17]. The discrepancy between our results and those of Singh et al.[3]

TABLE II

Gelatinization temperatures of native, defatted, and heat–moisture treated pigeon pea starches [a]

Treatment	Transition temperatures (K)				Enthalpy ΔH [e] (cal/g)
	T_0	T_p	T_c	T [d]	
Native [b]	347	354	360	13	2.6
Defatted [b]	351	356	365	14	2.6
Heat–moisture treated [b]	352	358	368	16	2.6

[a] Average standard deviation, 0.1 ($n = 3$). [b] Water:starch ratio, 3:1. [c] T_o, T_p, and T_c indicate the temperatures of the onset, mid point, and end of gelatinization respectively. [d] $T = T_c - T_o$. [e] ΔH = Enthalpy of gelatinization.

TABLE III

Pasting properties of native, defatted, and heat–moisture treated pigeon pea starch

Sample	Pasting temperature (°C)	Viscosity at 95°C (BU [a])	Viscosity after 30 min at 95°C (BU)	Viscosity at 50°C (BU)
Native	89	80	220	360
Defatted	91	20	120	220
Heat–moisture treated	> 95	[b]	10	25

[a] Brabender units. [b] Too low to be recorded.

may have been due to varietal differences or to different measurement techniques (DSC vs. microscopy). The gelatinization temperature range (13 K) and the enthalpy (ΔH) of gelatinization (2.6 cal/g) was comparable to that of other legume starches[17]. Defatting and heat–moisture treatment increased transition temperatures (the effect on T_c being greater than on T_o or T_p) and caused a broadening of the endothermic peak (Table II). These changes were greater on heat–moisture treatment. However, ΔH remained unchanged.

Defatting has also been reported[19] to increase the transition temperatures of potato and lentil starches, but not those of wheat, corn, and cassava[19]. The increase in transition temperatures on heat–moisture treatment was similar to that observed for potato[20,21] and wheat starches[21]. However, unlike pigeon pea starch (Table II), the ΔH of potato starch has been reported to decrease on heat–moisture treatment[22].

Pasting characteristics.—The pasting characteristics of the starches at a concentration of 6% (w/v) and pH 5.5 were investigated with the Brabender viscoamylograph and the results are presented in Table III. At this pH and concentration most legume starches[4] exhibit pasting temperatures in the region 65–75°C, 95°C viscosities greater than 100 BU and a gradual increase in consistency (40–69 BU) during the holding period at (95°C). However, the pasting curve of native pigeon pea starch was not typical of legume starches. The high pasting temperature (87°C), low 95°C paste viscosity (80 BU) and the high increase in viscosity (140 BU) during the holding period (at 95°C) is indicative of strong associative bonding forces within the granule. Defatting and heat–moisture treatment increased the pasting temperature and decreased the viscosities at 95 and 50°C (Table III). These changes were more marked on heat–moisture treatment. Similar changes on defatting have also been reported for lentil[19], lima bean[15], wheat[19], and corn[19] starches. The changes in pasting temperature and viscosities on heat–moisture treatment of pigeon pea starch was much higher than that reported for cereal[20] and root[20] starches.

In vitro digestibility of native, defatted, and heat–moisture treated pigeon pea starches by porcine pancreatic alpha amylase.—The extent of alpha amylase hydrolysis of native, defatted, and heat–moisture treated starches is presented in Fig. 5.

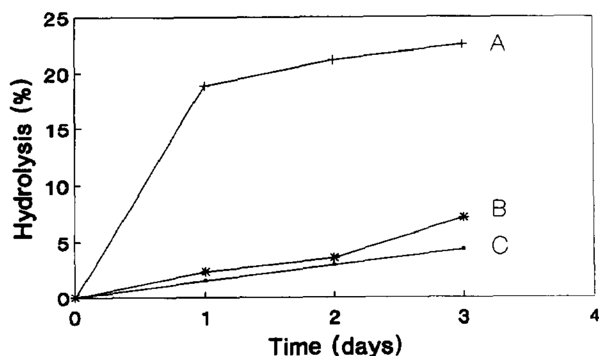

Fig. 5. Time course of hydrolysis of defatted (A), heat-moisture treated (B), and native (C) pigeon pea starch by porcine pancreatic α amylase.

The extent of hydrolysis of native pigeon pea starch (1.9% in 24 h) was comparable to that of lima bean starch[15] (1.8% in 24 h), but was much lower than that of other legume starches (24–28% in 7 h)[23]. After 72 h, the extent of hydrolysis of native pigeon pea starch was only 4.3% (Fig. 5). Defatting increased the extent of hydrolysis of pigeon pea starch (Fig. 5) from 4.3 to 22.5% (72 h). During this same time interval, heat-moisture treated granules (Fig. 5) was hydrolysed only to the extent of 7.1%. Increased susceptibility towards alpha amylase hydrolysis has also been observed on defatted wheat, corn, lentil, and potato starches[19], and on heat-moisture treated wheat starch[21,24].

The surfaces of native, defatted, and heat-moisture treated starches (Fig. 6), and the mode of attack by alpha amylase on the above granules (Figs. 7 and 8) was investigated by SEM. The surface of native granules appeared quite smooth (Figs. 6A and B). However, defatted granules (Figs. 6C and D) exhibited highly roughened surfaces, which were covered with numerous fissures of varying size. In contrast the slightly roughened surfaces of heat-moisture treated granules (Figs. 6E and F) were devoid of any fissures. The attack of alpha amylase (72 h) on native pigeon pea starch manifested itself in only superficial surface erosion of the granules (Figs. 7A and B). Morphological changes were not discernible, and the result is in agreement with the low amylolysis rates (Fig. 5). During the same time period, granules of defatted starch were more extensively attacked by alpha amylase (Fig. 7C) than those of native starch (Fig. 7A). Many of these granules were split along the region of the grooves (Figs. 7C and 8A). The surfaces of defatted granules were extensively eroded and were covered with numerous craters of varying size and depth (Fig. 7D) as if the alpha amylase had entered the granule and preferentially hydrolysed the interior portion. In Fig. 8B, the interior portion can be seen wherein the layered internal structures are apparent. It is likely, that the extensive attack by alpha amylase in the form termed "tunnelling" on defatted granules (Fig. 7D) may have begun in the region of the fissures (Fig. 6D) on the granule surface and widened as it penetrated inwards (Fig. 7D) resulting eventually in granule splitting.

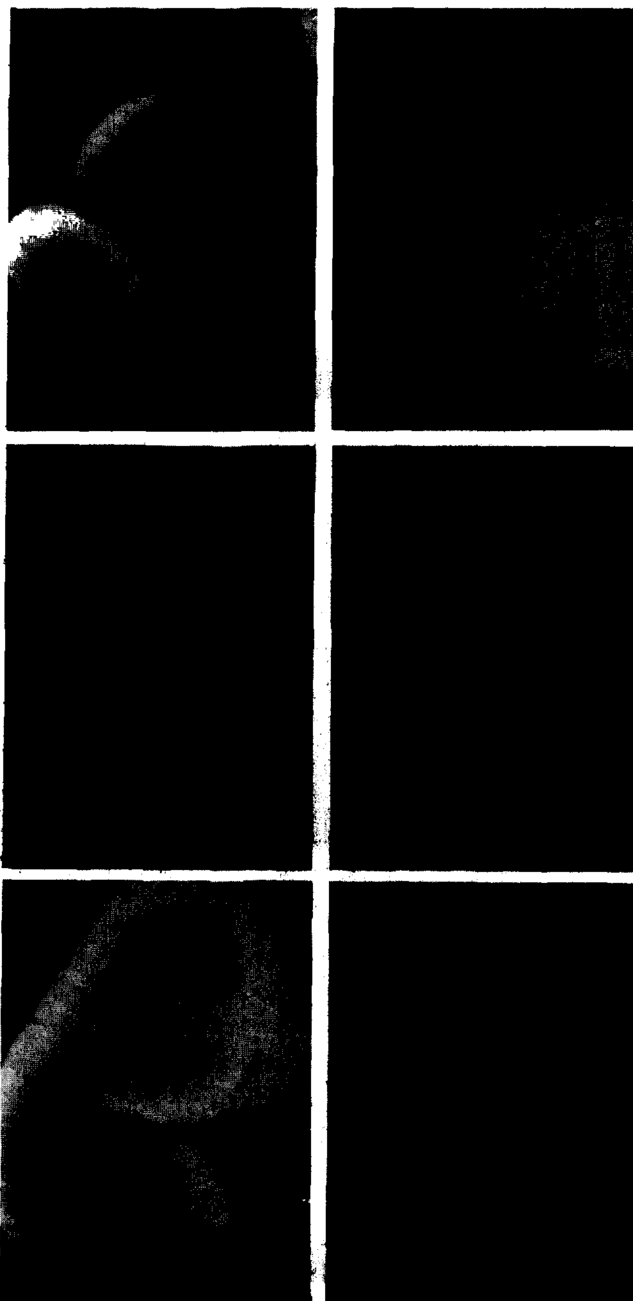

Fig. 6. Scanning electron micrographs of the surfaces of native (A and B), defatted (C and D), and heat–moisture treated (E and F) pigeon pea starch.

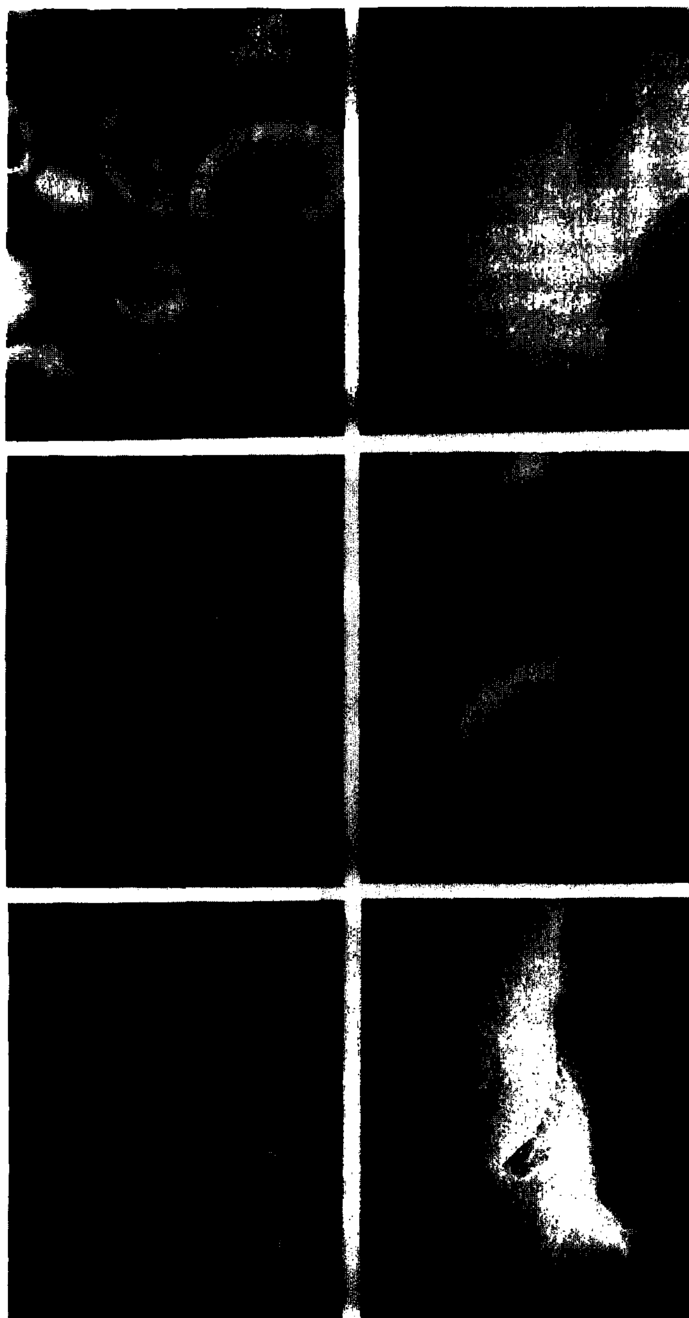

Fig. 7. Scanning electron micrographs of alpha-amylase-hydrolyzed (72 h) pigeon pea starch granules: A and B, native starch (4.3% solubilized); C and D, defatted starch (22.5% solubilized); E and F heat-moisture treated starch (7.1% solubilized).

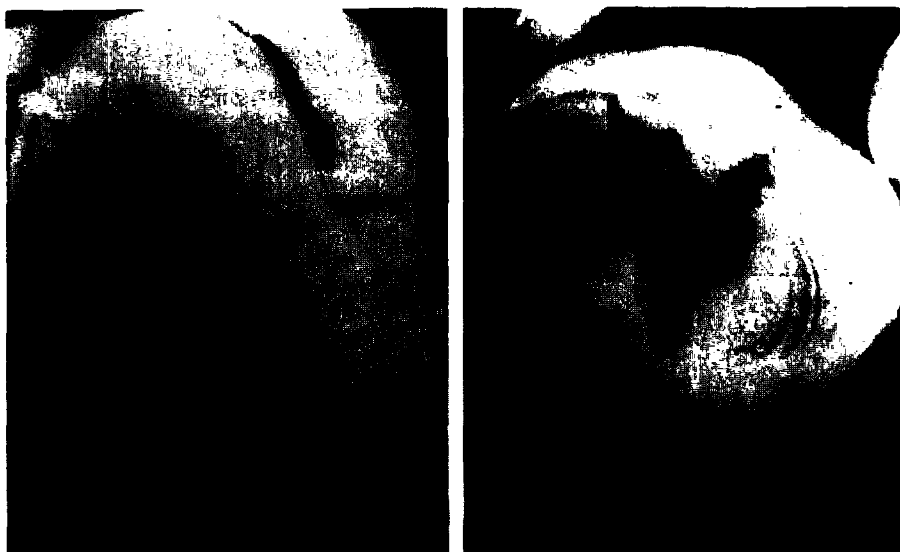

Fig. 8. Scanning electron micrographs of alpha amylase-hydrolyzed defatted pigeon pea starch. (A) Granule splitting along the region of the groove. (B) The layered internal surface of the split granule.

The attack of alpha amylase on heat–moisture treated granules (Fig. 7E), although less extensive than on defatted starch, also produced craters of varying size (Fig. 7F) which however, were smaller than those seen on defatted starch.

Acid hydrolysis.—The solubilization pattern of native, defatted, and heat-moisture treated pigeon starch granules are presented in Fig. 9. All starches exhibited a two-stage solubilization pattern. A relatively higher rate was observed during the first 6 days, followed by a lower rate between 6 and 14 days (Figs. 9A and B).

At the end of the 6th day of hydrolysis (corresponding to the degradation of the amorphous regions of the granule) native pigeon pea starch was hydrolyzed to the extent of 22.6% (Fig. 9A). However, during this same time period, native granules of lentil[25], mung bean[25], wrinkled pea[25], and lima bean[15] have been shown to be hydrolyzed to the extent of 65, 45, 27, and 22.4%. A comparison of these values indicated that the extent of acid hydrolysis is not dependent on amylose content, but is more likely influenced by the degree of association of the starch chains within the amorphous regions of the granule.

The percentage solubilization increased on defatting (Fig. 9A). The average increase was 15.4% during the first 8 days and 3.3% thereafter. Similar increases in acid hydrolysis on defatting has also been observed for potato[19] and lentil[19] starches, but not for cereal starches[19].

Heat–moisture treatment decreased the extent of solubilization (Fig. 9A). The average decrease was 29% during the first 4 days, and 10% thereafter.

The hydrolysis rate constants of the starches, calculated from plots log $(100/100 - x)$ vs. time (Fig. 9B) are presented in Table IV. The k (apparent rate constant

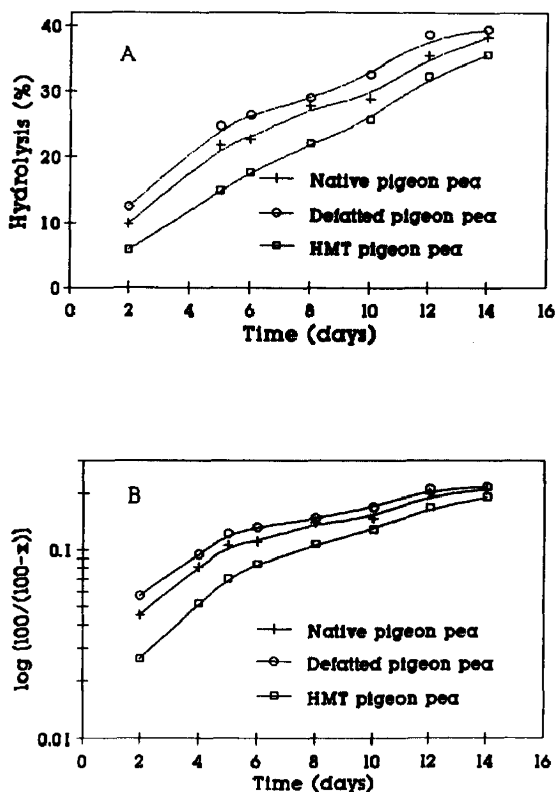

Fig. 9. (A) Heterogeneous acid hydrolysis of native (+), defatted (−), and heat–moisture treated pigeon pea starch in 2.2 M HCl at 35°C. (B) Starch hydrolysis data plotted as $\log_{10}$ (100/100 − X) vs. time.

for the first stage of the hydrolysis curve), corresponding to the amorphous parts of the granule showed considerable differences among the various treatments. Heat–moisture treated pigeon pea starch proved to be the most resistance ($k = 7.1 \times 10^{-2}$ in days^{-1}), followed in decreasing order by native ($k = 9.9 \times 10^{-2}$ in days^{-1}) and defatted ($k = 10.6 \times 10^{-2}$ in days^{-1}) starches. The apparent rate constant for native pigeon pea starch was much lower than that reported for other legume starches [$k = (7.1\text{–}14.6) \times 10^{-2}$ days^{-1}][25].

TABLE IV

Acid hydrolysis rate constants of native, defatted, and heat–moisture treated pigeon pea starch

Sample	$k \times 10^{-2}$ (days^{-1}) [a]
Native	9.9
Defatted	10.6
Heat–moisture treated	9.1

[a] k is the apparent rate constant for the first stage of hydrolysis curve, (100/100 − x) vs. time, calculated from the equation $k = (2.303/t) \log (100/100 - x)$.

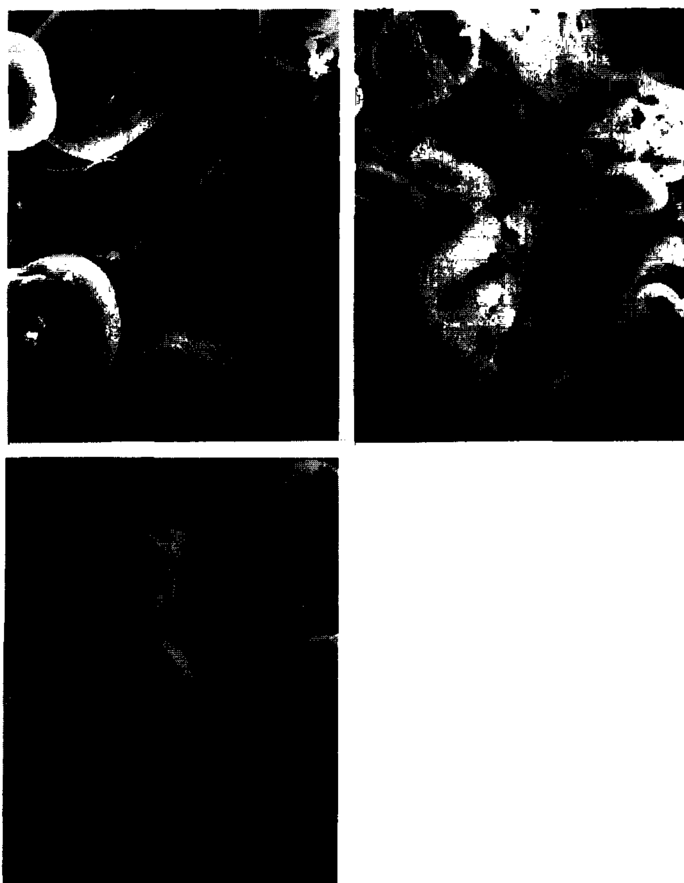

Fig. 10. Scanning electron micrographs of native (A), defatted (B), and heat–moisture treated (C) pigeon pea starch hydrolyzed by 2.2 M HCl at 35°C.

The mode of attack by H_3O^+ on native, defatted, and heat-moisture treated pigeon pea starches was investigated using SEM. The results are presented in Figs. 10A–C. After 5 h of hydrolysis, a large number of defatted granules were deformed and eroded over the entire surface (Fig. 10B). Some showed surface terraces which are exposed edges of a layered internal structure (Fig. 10C). However, during this period of time, native (Fig. 10A) and heat-moisture treated (Fig. 10C) starches were less heavily eroded, with the extent of erosion being higher in the former.

Freeze–thaw stability.—The freeze–thaw stability of starches are generally determined by estimation of the amount of water exuded from the starch gel system stored at low temperatures. The amount of water exuded would be the result of increased inter- and intra-molecular hydrogen bonding due to aggregation of starch chains (retrogradation) during frozen storage.

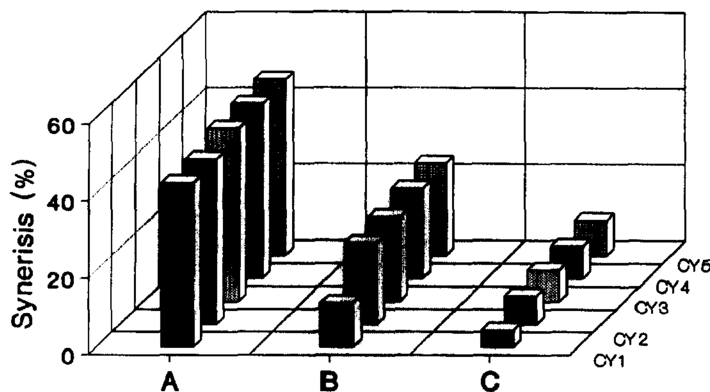

Fig. 11. Freeze–thaw stability of native (A), defatted (B), and heat–moisture treated (C) pigeon pea starches. CY1–CY5 represent the number of freeze–thaw cycles.

The percentage exudate (syneresis) from native, defatted, and heat–moisture treated pigeon pea starch gels stored at −16°C (after five freeze–thaw cyles) was respectively, 46.1, 24.1, and 9.2% (Fig. 11).

DISCUSSION

Cooke and Gidley[26] showed by X-ray spectroscopy and ^{13}C solid-state NMR, that ΔH values reflect mainly the loss of double-helical order rather than crystalline register. They postulated that the forces holding the starch granule are largely at the double-helical level and that the observed crystallinity may function as a means of achieving closer packing rather than as a means of providing structural stability. Therefore, the unchanged values for ΔH (Table II) and crystallinity (Fig. 2) suggests that in pigeon pea starch, the degree of association between double helices (within the crystalline architecture of the granule) does not decrease or increase on defatting or heat–moisture treatment. The increased gelatinization transition temperature (Table II), the decrease in swelling factor (Fig. 3) and amylose leaching (Fig. 4), and the high pasting temperatures and decreased viscosities (Table III), suggest that the moisture and thermal energy during defatting and heat–moisture treatment increases starch chain mobility in the amorphous regions of the granule, thereby, enabling associations to occur between amylose chains and/or between amylose and the outer chain branches of amylopectin [the X-ray diffraction data (Fig. 2) indicates that these newly formed crystallites are probably not large enough or perfect enough to diffract X-rays significantly]. Therefore, at the same volume fraction of water ($\nu = 0.86$), the starch chains in the amorphous regions of defatted and heat–moisture treated granules, would require a higher input of thermal energy (due to greater rigidity of the starch chains in the amorphous regions) than those of native starch (Table II) to exceed the glass transition temperature (T_g) of the amorphous regions (the

water added outside the granule acts to depress the Tg of continous amorphous regions permitting sufficient mobility for the melting of the metastable crystallites[27]). The increase in T_o, T_p, and T_c (Table II), on defatting and heat–moisture treatment is thus explained.

It has been reported that hydrolysis by alpha amylase occurs predominantly in the amorphous regions of the granule[28,29]. Several investigators[23,30,31] have postulated that one of the many factors that determine the differences in the in vitro digestibility of native starches is the extent of molecular association between starch components. Since the granule size, surface area, crystal type, (C), relative crystallinity, starch damage, amylose/amylopectin ratio, lipid content, and the percentage of amylose complexed lipids of native pigeon pea starch was comparable to that of other legume starches, the observed differences in hydrolysis probably reflect differences in the degree of accessibility of alpha amylase into the amorphous regions (this being more difficult in the case of native pigeon pea starch, due to greater interaction between starch chains within the amorphous regions).

Vasanthan and Hoover[19] have suggested that the increase in alpha amylase hydrolysis on defatting is probably due to a change in amylose conformation (V helix to random coil), with the result that a large surface area becomes available for enzyme action. Previous studies[31,32] have shown that amylose–lipid complexes show decreased susceptibility to alpha amylase digestion. However, in pigeon pea starch, a change in amylose conformation on defatting is less likely, since it contains only trace quantities of complexed lipids (Table I). The increase in hydrolysis on defatting has also been attributed to the action of alpha amylase on those amylose chains (which in the native granule were a part of the crystalline structure), which were released into the amorphous regions during defatting[19]. Such a mechanism was shown to be accompanied by a change in relative crystallinity[19]. However, since the relative crystallinity of pigeon pea starch remained unchanged on defatting (Fig. 2), the increase in hydrolysis cannot be explained on this basis.

Lorenz and Kulp[20] have postulated that heat–moisture treated cereal and tuber starches are more susceptible to alpha-amylase action than their native counterparts (due to a change in the orientation of the starch crystallites and/or to a certain degree of granular degradation). This postulate may have been based on the reduction in relative crystallinity which has been reported to occur during heat–moisture treatment of the above starches[20]. However, in our study, changes in relative crystallinity were not observed on heat–moisture treatment (Fig. 2). Therefore, the observed increases in digestibility cannot be ascribed to changes in size, number, or reorientation of starch crystallites. The results from swelling factor (Fig. 3) amylose leaching (Fig. 4) and DSC (Table II) measurements proved conclusively that starch chains within the amorphous regions of the granule interact with each other during defatting and heat–moisture treatment. Therefore, since alpha amylase preferentially hydrolyzes the amorphous regions of the granule, the extent of hydrolysis of defatted and heat–moisture treated starches should

have been theoretically less than that of native starch. We therefore, postulate that the greater susceptibility of pigeon pea starch to alpha amylase attack on defatting and heat–moisture treatment, probably reflects to a large extent the increased accessibility of alpha-amylase to the granule interior (as a result of modifications to the granule surface, Figs. 6D and F). It is likely, that the concentration of alpha amylase within the granule interior of defatted and heat–moisture treated starches may have been sufficiently high to hydrolyze even the aggregated starch chains within the amorphous regions.

During treatment of starch granules with mineral acids at temperatures below the gelatinization temperature, the amorphous regions are more rapidly attacked than are the crystalline regions[33]. As shown earlier the extent of association of starch chains within the amorphous regions of the granules followed the decreasing order: heat–moisture treated > defatted > native starch. On this basis, the extent and rate of acid hydrolysis of native starch should have theoretically been greater than that of defatted starch. Therefore, the observed rates of acid hydrolysis (defatted > native) seems to suggest that the concentration of H_3O^+ within the granule interior may have been higher in defatted (due to rapid diffusion of H_3O^+ via the fissures on the granule surface) than in native starch. The results suggest that at the concentration of H_3O^+ prevailing within the granule interior of defatted starch, even the aggregated starch chains in the amorphous regions are readily hydrolyzed. Enzymic studies showed that both defatted (Fig. 7C) and heat–moisture treated (Fig. 7E) pigeon pea starches were more susceptible than native starch (Fig. 7A) to hydrolysis by alpha amylase. This indicated that the surface of native starch was less permeable than those of defatted and heat-moisture treated starches to penetration by alpha amylase. On this basis, the granules of heat–moisture treated starch should have been hydrolyzed by acid to a greater extent than those of native starch. However, the observed rates of acid hydrolysis (native > heat–moisture treated) seems to suggest, that even though more acid may have penetrated into the granule interior of heat–moisture treated as compared to native starch, the concentration of H_3O^+ may not have been sufficiently high enough to effectively hydrolyze the aggregated starch chains in the amorphous regions of heat–moisture treated granules.

The comparison of the effect of acid and enzyme hydrolysis on native and heat–moisture treated granules seems to indicate that aggregated starch chains are more susceptible to enzyme than to acid hydrolysis.

Due to bonding forces in native pigeon pea starches being weaker in magnitude than those in defatted and heat–moisture treated starches, additional interactions between or among starch components during gel storage would be more extensive in native starch. This would then explain the higher degree of synerisis (Fig. 11) shown by native starch.

It is evident from these results that the gel from native pigeon pea starch offers very little resistance to freeze–thaw damage and would be unsuitable as a thickening agent in precooked frozen foods. However, the substitution of heat–moisture

treated pigeon peak starch for native pigeon starch in baked foods may permit texture variations and improvememt in their low-temperature stability.

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