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0308-8146(95)00099-2

Food Chemistry, Vol. 56, No. 4, pp. 355–367, 1996

0308-8146/96 $15.00 + .00

Composition and physicochemical properties of starch from pearl millet grains

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(Received 6 February 1995; accepted 23 July 1995)

The physicochemical properties of starches isolated from three cultivars (ICTP 8203, ICMS 7703 and ICMH 356) of pearl millet grains were studied. The yield of the starch was in the range 53–56% on a whole grain basis. The starch granules were round or polygonal with numerous indentations and pores on their surface. The free, bound and total starch lipids ranged from 0.04 to 0.08%, from 0.40 and 0.47% and from 0.44 and 0.55%, respectively. In all starches, neutral lipids (NL) and phospholipids (PL), respectively, formed the major lipid class in the free and bound lipid extracts. The major fatty acid in the NL fraction being linolenic acid, whereas palmitic acid was the major fatty acid in PL and glycolipid fractions. The total amylose contents ranged from 28.8 to 31.9%, of which 14.6–17.2% were complexed by native lipid. The X-ray pattern was of the A-type. The intensities of the major diffraction peaks followed the order: ICMS > ICTP > ICMH. The swelling factor followed the order: ICTP > ICMH > ICMS. All three starches exhibited identical pasting temperatures, but they differed with respect to the Brabender viscosity at 95°C (ICMH > ICTP > ICMS), and the extent of increase in viscosity during the holding cycle at 95°C (ICMS > ICMH > ICTP). The gelatinization temperature ranges were: 61.2–70.5°C (ICTP), 60.9–67.5°C (ICMH) and 64.5–78.0°C (ICMS). The enthalpy of gelatinization (cal g^{-1}) were: 2.7 (ICTP), 2.5 (ICMH), 3.5 (ICMS). The extent of hydrolysis by porcine pancreatic α-amylase and 2.2 N HCl followed the order: ICTP > ICMH > ICTP. The percentage light transmission followed the order: ICTP > ICMH > ICMS. The stability towards freeze–thaw cycling followed the order: ICMS > ICMH > ICTP. The retrogradation endotherm was broader than the gelatinization endotherm (ICTP > ICMS > ICMH). In all starches, the melting temperature (T_p) of the retrogradation endotherm increased only marginally during storage. The increase in the enthalpy of retrogradation (ΔH_R), however, was much higher in ICTP than in ICMS and ICMH. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Pearl millet [*Pennisetum americanum* (L) Leeke] is a drought tolerant cereal crop grown primarily as a food grain in India and Africa. It is known as dark millet in Europe, Bajza in India, bubrush in Africa and Mands forage in the United States (Freeman & Bocan, 1973). Starch is the major component of the pearl millet grain (55–60%). There have been several reports on the properties of pearl millet starch (Badi *et al.*, 1976; Beleia *et al.*, 1980; Freeman & Bocan, 1973; Wankhede *et al.*, 1990; Abd Allah *et al.*, 1987). However, information is lacking on surface morphology, particle size analysis, lipid composition, lipid complexed amylose, retrogradation characteristics, paste clarity, granular susceptibility towards hydrolysis with acid (2.2 N HCl) and porcine pancreatic α-amylase, and on the properties of the enzyme digested granules.

The objectives of the present study were to isolate the starch fraction from three cultivars of pearl millet grains and to determine their physicochemical properties.

MATERIALS AND METHODS

Materials

Pearl millet grains (cultivars; ICTP 8203, ICMS 7703, ICMH 356) were grown at the International Crop Research Institute for the Semi Arid Tropics Center

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(Patancheru, India) in the post-rainy season of 1993. Wheat and corn starches were obtained from Sigma Chemical Co., St. Louis, MO, USA.

Crystalline porcine pancreatic alpha-amylase (EC 3.2.1.1), type 1-A, was obtained from Sigma Chemical Co. (St. Louis, MO). Chemicals and solvents were analytical grade. Solvents were distilled from glass before use.

Methods

Starch isolation

Isolation of starches from pearl millet seeds were carried out by the procedure of Beleia *et al.* (1980) and purification was carried out by procedures outlined in an earlier publication (Hoover *et al.*, 1991).

Chemical composition of starch

Quantitative estimations of moisture, ash, nitrogen and starch damage were performed by the standard AACC (1983) procedures. Starch lipids were analysed as follows: at ambient temperature (25–27°C) lipids (mainly unbound) were extracted from pearl millet 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 (mainly bound) were obtained by soxhlet extraction (7 h) with 100 ml of 3:1 *n*-propanol–water. Lipids were also extracted, after acid hydrolysis of pearl millet starch with 24% HCl at 70–80°C for 30 min and the hydrolysate then extracted three times with 1-hexane (Goshima *et al.*, 1985). The purification and fractionation of extracted lipids and quantification of lipid classes were carried out by procedures that have been described elsewhere (Vasanthan & Hoover, 1992a). Apparent and total amylose contents were determined by the method of Chrastil (1987).

Starch granule size analysis

Mounts for light microscopy of the starch samples were prepared from a dilute slurry of starch–ethanol (95% w/v) mixture. Images of the starch granules from a Leitz Laborlux K and D light microscope (Leitz Laborlux Wetzlar Gmloh, Wetzlar, Germany (×63 magnification) were analysed for particle size distribution on BioQuant System IV image analyser (Image Analyser Image Technology Corp., NY, USA) equipped with an image acquisition and processing station. In each measurement, an image of an optical micrometer was introduced for size calibration. Two thousand starch granules from each starch sample were measured for their diameter (the diameter of a circle of equal area to the particle) and the percentage of particles that occurred within the predetermined diameter ranges were obtained.

Granule morphology

Granule morphology of native starches was studied by scanning electron microscopy (SEM). Starch samples were mounted on circular aluminum stubs with double sticky tape and then coated with 20 nm of gold and examined and photographed in a Hitachi (S570) scanning electron microscope at an accelerated potential of 20 kV.

X-ray diffraction

The X-ray diffractograms were obtained with a Rigaku RU 200R X-ray diffractometer at a chart speed of 20 mm min^{-1}. The starch powder was scanned through the 2Θ range of 3–35°. Traces were obtained using a Cu-Kα 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^{-1}.

Swelling factor

The swelling factor of the starches when heated to 50–95°C in excess water was measured according to the method of Tester & Morrison (1990). This method measures only intragranular water and hence the true swelling factor at a given temperature. The swelling factor is reported as a ratio of the volume of swollen starch granules to the volume of the dry starch. Results used for calculations were means of triplicate measurements.

Extent of amylose leaching

Various concentrations of starches (15–20 mg) in water were heated (50–95°C) in volume-calibrated sealed tubes for 30 min. The tubes were then cooled at ambient temperature and centrifuged at 2000 ***g*** for 10 min. The supernatant liquid (1 ml) was withdrawn and its amylose content was determined by the method of Chrastil (1987). Results used for calculations were means of triplicate measurements.

Pasting behaviour

A Brabender viscoamylograph, Model VA-V equipped with a 700 cm cartridge was used to study pasting properties at a concentration of 6% (w/v). Two replicates were used for this determination.

Enzymatic digestibility

Enzymatic digestibility studies on native starches were done using a crystalline suspension of porcine pancreatic α-amylase in 2.9 M saturated sodium chloride containing 3 mM calcium chloride (Sigma Chemical Co., St. Louis, MO), in which the concentration of α-amylase was 30.0 mg ml^{-1} and the specific activity was 790 U mg^{-1} of protein. One unit was defined as the α-amylase activity which liberated 1 mg maltose in 3 min at 20°C at pH 6.9. The procedure used was essentially that of Knutson *et al.* (1982). Percentage hydrolysis was calculated as the amount (mg) of maltose released per 100 mg of dry starch. Controls without enzymes but subjected to the above experimental conditions were run concurrently. The above experiment was replicated twice.

For DSC analysis the granular residues were repeatedly washed with ethanol and once with chloroform before air drying. The above experiment was replicated twice.

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 described earlier.

Acid hydrolysis

The starches were hydrolysed with 2.2 HCl at 25°C (1.0 g starch/40 ml acid) for 20 days. The starch slurries were shaken by hand daily to resuspend the deposited granules. At various time intervals, aliquots of the reaction mixture were neutralized and centrifuged (2000*g*). The extent of hydrolysis was determined by expressing the solubilized carbohydrates as a percentage of the initial starch. Results used for calculations were means of triplicate measurements.

Light transmittance of starch pastes

The following procedure adapted from Craig *et al.* (1989) was used to prepare 1% starch pastes. Starch (50 mg db) was suspended in water (5 ml) in screw cap tubes and the pH adjusted by addition of 0.1 N HCl or NaOH as required. The tubes were then heated in a boiling water bath (with occasional shaking) for 30 min. After cooling to ambient temperature, the percentage transmittance (%*T*) at 650 nm was determined against a water blank in a Hewlett Packard spectrophotometer.

Gel preparation

Gels (40% w/v) were prepared as described by Krüsi & Neukom (1984). Pearl millet starches (4 g dry basis) were carefully weighed into circular aluminum moulds (diameter 3.0 cm, height 3.0 cm) with removable tops and bases and then mixed with 10 ml distilled water containing 0.02% $Na_2S_2O_3$ as a preservative. The moulds were then heated in a water bath for 35 min at 95°C. The resulting gels were allowed to cool within the moulds for 30 min at 4°C prior to storage at 25°C for 1 and 7 days.

Gel powder preparation

The procedure (with minor modifications) of Roulet *et al.* (1988) was used to convert the stored gels (at 25°C) to a powder prior to examination by DSC and X-ray diffraction. The gels were rinsed with water, cut into small pieces and mixed with 100 ml acetone. After homogenization using a polytron, the mixture was left to decant for 5 min. The liquid was discarded and the rest was transferred to screw cap tubes. Acetone was again added, the mixture centrifuged (3000*g*) and the supernatant discarded. This procedure was repeated three times and the remaining mass was dried in an air oven for 6 h at 30°C.

Differential scanning calorimetry (DSC)

DSC measurements on native and enzyme-treated starches were carried out using a Perkin-Elmer DSC-2 (Norwalk, CT) differential scanning calorimeter, with a 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 allowed to stand overnight at room temperature. The scanning temperature range and the heating rate were, respectively, 20–120°C and 10°C min^{-1}. The thermogram was recorded with water as reference. The transition temperatures reported are the onset (T_o), peak (T_p) and conclusion (T_c). 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}). Fusion of retrograded amylopectin (Hoover *et al.*, 1994*b*) after 4 and 7 days of storage was determined by weighing (3–4 mg dry basis) of the stored (at 25°C) gels (40% w/v) into DSC pans which were then sealed and scanned from 20 to 100°C at 5°C min^{-1}. All DSC experiments were replicated at least three times.

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°. Five cycles of freeze–thaw were performed. The excluded water was determined by centrifuging the tubes (30 mm diameter × 100 mm) at 1000*g* for 20 min after thawing. Values are the means of three replicates.

RESULTS AND DISCUSSION

Morphological granular characteristics of the starches

The starch granules appeared to be round or polygonal [Fig. 1(A), (C) and (E)]. The surface of many granules of the three starches had deep indentations and numerous pores [Fig. 1(B), (D) and (F)]. Pores were more evident in ICMH [Fig. 1(F)] than in the other two starches [Fig. 1(B) and (D)]. In all starches, the pores were present in both oval and polygonal granules and were scattered throughout the granule surface. However, in wheat [Fig. 2(A) and (B)] and corn [Fig. 2(C) and (D)] starches, the number of pores were much less (corn < wheat). Fannon *et al.* (1990) have also shown the presence of pores on the entire granule surfaces in corn (all cultivars) millet and sorghum. Whereas in wheat, rye and barley starches, they were present on the large granules, but only along the hemispherical groove. The above authors have shown that pores are real features of granular morphology related to the genetic make-up of the source plant and are not artifacts of the isolation, preparation or observation techniques.

Figure 3(A) and (C) shows the particle size distribution of the pearl millet starches. All three starches exhibited a normal distribution of sizes centred at about 10.5 μm.

Chemical composition of the starch

The yield of pearl millet starches was in the range 53.1–56.5% on a total seed basis (Table 1). The chemical composition showed that the starches contained 0.12–

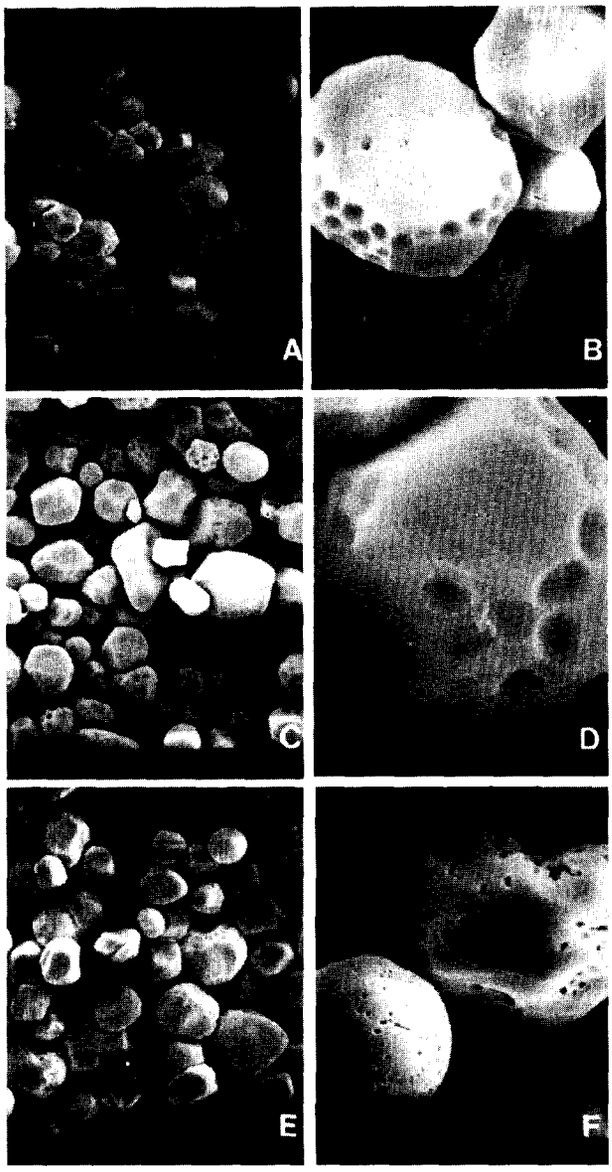

Fig. 1. Scanning electron micrographs of native pearl millet starches: (A) & (B) ICTP 9203; (C) & (D) ICMS 7703; (E) & (F) ICMH 356.

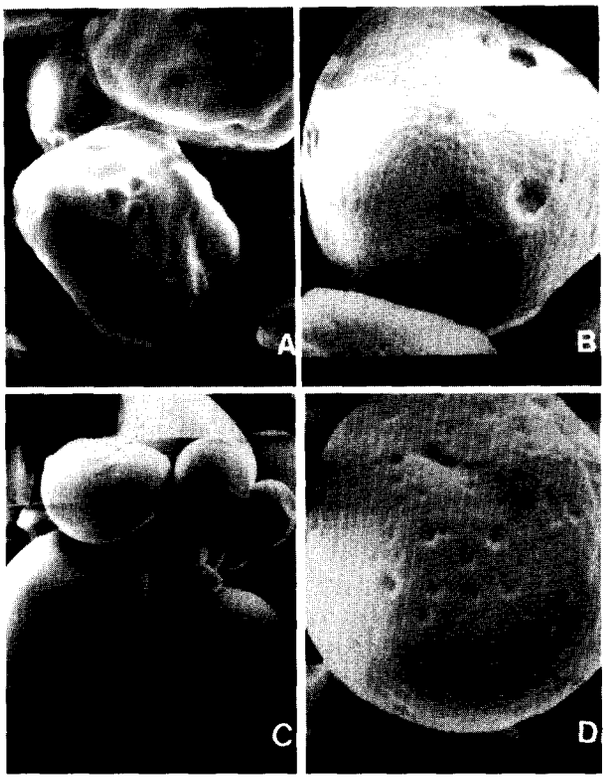

Fig. 2. Scanning electron micrographs of (A) & (B) wheat starch; (C) & (D) corn starch.

0.15% nitrogen and 0.02–0.03% ash. These low values indicated high purity and the absence of non-starch lipids. Therefore, the total lipids (0.45–0.51%) obtained by acid hydrolysis of the pearl millet starches mainly represent free and bound lipids. The above value for total lipids was less than that reported (0.90%) by Wankhede *et al.* (1990), but higher than that reported (0.09–0.19%) by Beleia *et al.* (1980) for six cultivars of pearl millet starches. The total lipid content of the pearl millet starches (Table 1) was less than that of corn, 0.80% (Hoover *et al.*, 1991), and wheat, 0.72% (Vasanthan & Hoover, 1992*a*) analysed by the same procedure. The free lipids (obtained by extraction with $CHCl_3$–CH_3OH (CM)) amounted to 0.04, 0.08 and 0.07% in ICTP, ICMS and ICMH, respectively, while the corresponding values for bound lipids [obtained by extraction of CM residues with hot *n*-propanol–water (PW)] were, respectively, 0.40, 0.47 and 0.43%. These values were much less than that reported for wheat [0.08 (free), 0.64 (bound)] and corn [0.04 (free), 0.76 (bound)] starches (Vasanthan & Hoover, 1992*a*,*b*).

It is plausible that both free and bound lipids may be present on the granule surface as well as within the granule interior. The bound lipids probably represent: (1) lipids which are present in the form of amylose inclusion complexes (Van Lonkhuysen & Blankestijn, 1974; Acker, 1977); (2) lipids which are linked via ionic or hydrogen bonding to hydroxyl groups of starch components; and (3) lipids which are trapped between starch chains (Morrison, 1981).

Chloroform–methanol (CM) extracted lipids

The proportion of lipid classes in the CM residues of the three pearl millet starches followed the order: neutral lipid (NL) > phospholipid (PL) > glycolipid (GL) (Table 2). The concentrations of solvent extracted lipids (SEXL) of NL were 5.7, 4.6 and 5.6%, respectively, in ICTP, ICMS and ICMH. The corresponding values for PL were 1.38, 1.04 and 1.40%, respectively. GL occurred only in trace quantities. The major NL (% total NL) in the CM extracts was free fatty acids (FFA) which amounted to 49.3, 49.0 and 50.7%, respectively, in ICTP, ICMS and ICMH (Table 3). The major PL (% total PL) in the CM extracts was lysophosphatidylcholine (LPC) which amounted to 57.1, 80.0 and 83.0%, respectively, in ICTP, ICMS and ICMH (Table 3). Linoleic (C18:2) and palmitic (C16:0) acids were the predominant fatty acids in the NL fractions of the CM extracts of the three starches.

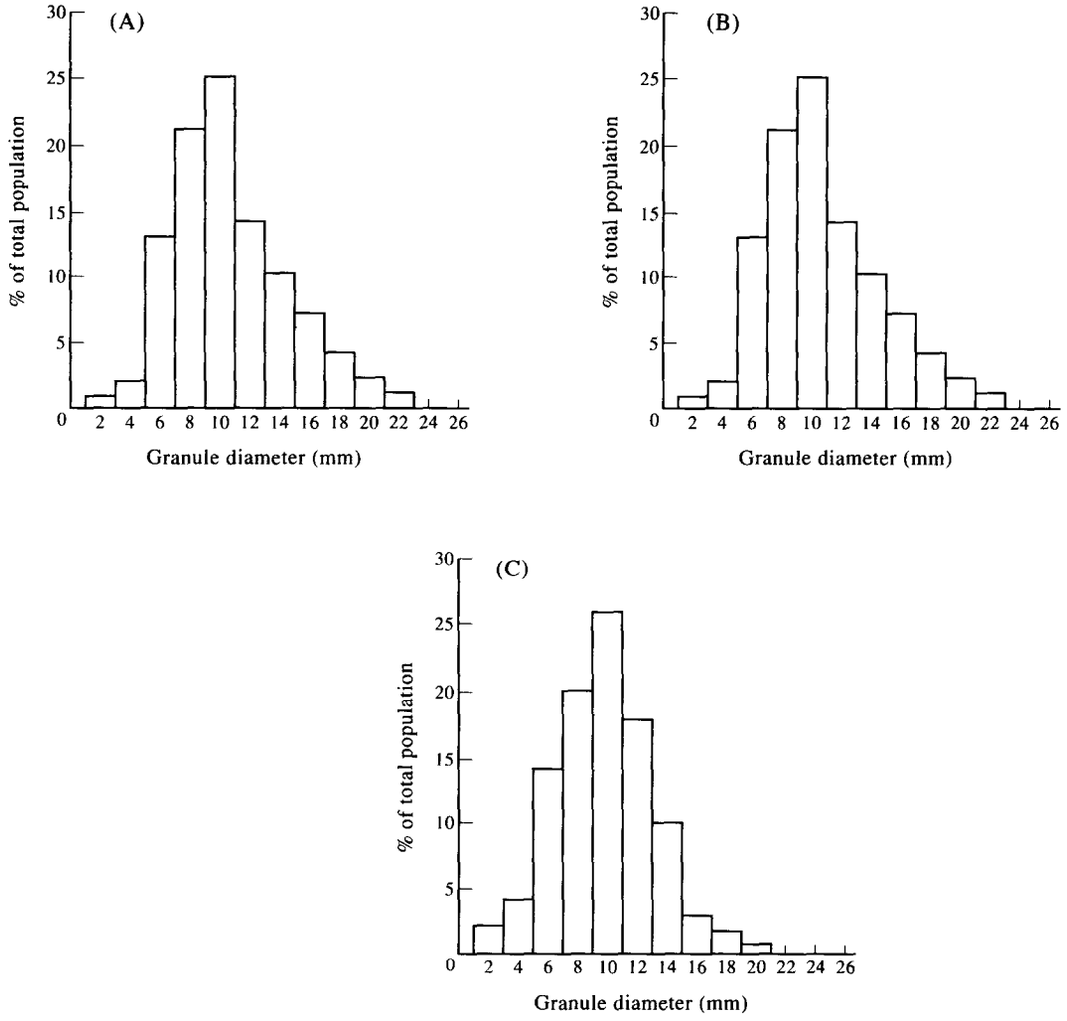

Fig. 3. Particle size distribution: (A) ICTP 8203; (B) ICMS 7703; and (C) ICMH 356.

Table 1. Proximate composition of pearl millet starches[a]

	ICTP 8203	ICMS 7703	ICMH 356
Starch yield (% initial material)	53.1	54.9	56.5
Moisture	8.3 ± 0.1	10.4 ± 0.1	9.7 ± 0.1
Ash	0.02 ± 0.01	0.03 ± 0.02	0.03 ± 0.01
Nitrogen	0.15 ± 0.01	0.17 ± 0.02	0.12 ± 0.01
Starch damage	1.5 ± 0.1	2.0 ± 0.1	2.6 ± 0.1
Lipid			
Acid hydrolyzed[b]	0.44 ± 0.06	0.55 ± 0.04	0.50 ± 0.05
Solvent extracted			
chloroform–methanol [CM][c]	0.04 ± 0.01	0.08 ± 0.01	0.07 ± 0.02
n-propanol–water [PW][d]	0.40 ± 0.03	0.47 ± 0.02	0.43 ± 0.03
Amylose content (% of total starch)			
Apparent[e]	24.6 ± 0.2	24.0 ± 0.3	27.2 ± 0.1
Total[f]	28.8 ± 0.4	29.0 ± 0.1	31.9 ± 0.1
Amylose complexed with native lipid (%)	14.6	17.2	14.8%

[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 from native starch by CM 2:1 (v/v) at 25°C (mainly unbound lipids).
[d]Lipids extracted by hot PW 3:1 (v/v) from the residue left after CM extraction (mainly bound lipids).
[e]Apparent and total amylose determined by I_2 binding before and after removal of bound lipids by hot PW extraction.
[f]((Total amylose–apparent amylose)/ total amylose) × 100.

Propanol–water (PW) extracted lipids

The lipid classes in the PW extracted lipids of all three starches followed the order: PL > NL > GL. The NL contents (% SEXL) were 34.9, 32.3 and 34.0%, respectively, in ICTP, ICMS and ICMH (Table 2). The major NL (% total NL) was FFA which was in the range 71–72% in the three starches (Tables 2 and 3). The PL levels (% SEXL) were 36.0, 39.8 and 35.0%, respectively, in ICTP, ICMS and ICMH (Tables 2 and 3). The major PL (Table 3) fraction (% total PL) in all three starches was LPC (~70%). The GL contents (% SEXL) were 6.3, 5.7 and 5.4%, respectively, in ICTP, ICMS and ICMH (Tables 1 and 2). The major GL (Table 3) fraction (% total GL) was digalactosyldiglyceride (DGDG) in ICTP and ICMS (~30%). Monogalactosyldiglyceride (MGDG), however, was the predominant GL (30%) in ICMH. In all three starches, the major fatty acid in the NL fraction was C18:2, whereas C16:0 was the major fatty acid in PL and GL fractions (Table 4). The monoacyl lipid content (% total starch lipids) in both CM and PW extracts was ~84.0% in ICTP and ICMH and 86.2% in ICMS (Table 3). These values were within the range reported for cereal starches (Vasanthan & Hoover, 1992*a*).

The apparent amylose contents (Table 1) were 24.6, 24.0 and 27.2%, respectively, in ICTP, ICMS and ICMH starches. The corresponding values for the total amylose content were 28.8, 29.0 and 31.9%, respectively, which exceeded those of wheat (27.3%) (Hoover

Table 2. Lipid classes in chloroform–methanol and *n*-propanol–water extracts (mg per 100 g dry starch)[a]

Starch source and extraction method	Neutral	Phospholipid	Glycolipid
ICTP 8205			
CM[b]	29	7	tr
PW[c]	178	263	32
ICMS 7703			
CM[b]	22	5	tr
PW[c]	165	280	28
ICMH 356			
CM[b]	26	7	tr
PW[c]	170	270	27

[a]Values are averages of three determinations.
[b]Lipids extracted from native starch by chloroform–methanol 2:1 (v/v) at 25°C.
[c]Lipids extracted by hot *n*-propanol–water 3:1 (v/v) from the residue left after CM extraction.

Table 3. Lipids class[a] (mg per 100 g dry starch) component composition in solvent extracts of pearl millet starches

	ICTP 8203		ICMS 7703		ICMH 356	
	CM[b]	PW[c]	CM[b]	PW[c]	CM[b]	PW[c]
Neutral lipids[d,e]						
FFA	14.3	127.8	10.8	117.5	13.2	120.5
MG	5.1	tr	3.1	3.2	3.8	2.5
DG	1.8	18.2	2.0	13.5	1.8	17.0
TG	3.5	8.9	3.1	6.7	3.2	8.4
FS	3.0	16.0	1.6	13.3	2.6	15.6
SE	1.0	6.2	1.4	5.8	1.4	6.0
Glycolipids[d,e]						
MGMG	tr[f]	7.5	tr	7.0	tr	6.5
MGDG	tr	9.0	tr	8.2	tr	8.0
DGDG	tr	9.5	tr	8.7	tr	7.0
DGMG	tr	3.0	tr	2.0	tr	3.0
Cer I	tr	3.0	tr	2.1	tr	2.5
Cer II	tr	tr	tr	tr	tr	tr
Phospholipids[d,e]						
LPC	4.0	67.4	4.0	195	5.8	175
LPE	1.9	8.0	1.0	72.5	tr	78
PC	1.2	4.5	tr	9.0	1.2	11.5
PE	tr	tr	tr	3.0	tr	4.8
PS	tr	tr	tr	1.4	tr	tr
PG	tr	tr	tr	tr	tr	tr
PA	tr	tr	tr	tr	tr	tr

[a]Based on densitometric absorbance.
[b]Lipids extracted from native starch by chloroform–methanol 2:1 (v/v) at 25°C.
[c]Lipids extracted by hot *n*-propanol–water 3:1 (v/v) from the residue left after CM extraction.
[d]FFA, free fatty acid; MG, monoacylglycerol; DG, diacylglycerol; TG, triacylglycerol; FS, free sterol; SE, sterol ester; MGMG, monogalactosylmonoglyceride; MGDG, monogalactosyldiglyceride; DGDG, digalactosyldiglyceride; Cer I, cerebroside I; Cer II, cerebroside II; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PG, phosphatidylglycerol; PA, phosphatidic acid.
[e]Values are average of three determinations.
[f]Trace = less than 0.5% of total lipid class.

& Vasanthan, 1994*a*) and corn (26.0%) (Hoover *et al.*, 1991) starches.

The amount of lipids complexed with amylose (Table 1) was higher in ICMS (17.2%) than in ICTP (14.6%) and ICMH (14.8) starches. The above values were lower than that reported for wheat (22.7%) (Hoover & Vasanthan, 1994*a*) and corn (21.3%) (unpublished results) starches.

X-ray diffraction

The X-ray spectra of the pearl millet starches were of the A type representative of cereal starches (Table 5). At approximately the same moisture content, the intensities of the major *d*-spacings was much higher in ICMS than in the other two starches (Table 5). This seems to suggest that starch crystallites in ICMS are better oriented than those in ICTP and ICMH to diffract X-rays, and/or the number of crystallites that diffract X-rays are of a higher order of magnitude in ICMS than in the other two starches. The X-ray intensities of the pearl millet starches were generally higher than those reported for wheat (Hoover & Vasanthan, 1994*a*) and corn (unpublished results) starches.

Swelling factor and amylose leaching

The swelling factor (SF) and amylose leaching (AML) of the pearl millet starches at temperatures in the range 50–95°C are presented in Table 6. The SF and AML

Table 4. Fatty and composition (area %) of the major lipid classes in CM and PW extracts of pearl millet starches

Starch source, lipid class and extraction medium	16:0	18:0	18:1	18:2	18:3	20:0	Other[a]
ICIP 8205							
Neutral lipids							
CM[c]	38.7	4.5	12.5	41.5	1.4	1.1	0.3
PW[d]	39.3	5.2	8.1	44.5	2.1	0.6	0.2
Glycolipids							
CM[c]	tr[b]	tr	tr	tr	tr	tr	tr
PW[d]	42.2	3.8	14.2	37.5	0.8	1.0	0.5
Phospholipids							
CM[c]	39.8	4.5	13.6	37.6	2.0	1.6	0.9
PW[d]	45.6	3.0	12.6	35.5	1.2	1.4	0.7
ICMS 7703							
Neutral lipids							
CM[c]	37.5	3.8	11.8	42.7	1.9	2.0	0.3
PW[d]	39.2	4.8	8.0	44.6	2.0	1.2	tr
Glycolipids							
CM[c]	tr	tr	tr	tr	tr	tr	tr
PW[d]	43.3	3.5	13.8	36.5	0.6	1.3	1.0
Phospholipids							
CM[c]	37.3	4.0	12.0	40.9	3.0	1.8	1.0
PW[d]	43.8	3.7	10.4	38.9	1.0	1.2	1.0
ICMH 356							
Neutral lipids							
CM[c]	36.2	5.0	11.2	43.2	1.2	1.6	1.3
pW[d]	38.0	6.1	7.0	45.8	1.5	0.8	0.8
Glycolipids							
CM[c]	tr	tr	tr	tr	tr	tr	tr
pW[d]	44.0	5.0	14.0	36.0	1.0	1.0	0.5
Phospholipids							
CM[c]	37.6	4.2	14.0	39.2	2.5	1.5	1.0
pW[d]	41.5	3.0	13.0	37.6	2.0	1.4	1.5

[a]Includes 14:0 and 22:0.
[b]trace = less than 0.1%.
[c]Lipids extracted from native starch by chloroform–methanol 2:1 (v/v) at 25°C.
[d]Lipids extracted by hot *n*-propanol–water 3:1 (v/v) from the residue left after CM extraction.

Table 5. X-ray diffraction spacings and intensities of the major peaks of pearl millet starches

Starch source	Moisture content (%)	Interplanar spacings (*d*) in Å with intensities (CPS)[a]			
ICTP 8203	9.5%	5.86 (1220)	5.18 (1574)	4.93 (1670)	3.85 (1349)
ICMS 7703	9.2%	5.86 (1677)	5.11 (1750)	4.88 (1850)	3.83 (1425)
ICMH 356	9.7%	5.88 (1104)	5.17 (1422)	4.95 (1616)	3.84 (1292)

[a]Counts per s.

increased with rise in temperature. The increase in SF and AML were most predominant between 60 and 70°C. At all temperatures, the SF followed the order: ICTP > ICMH > ICMS, while, the corresponding order for AML was: ICMH > ICTP > ICMS (Table 6). The SF of ICTP and ICMH were closer to that of wheat and corn starches than that of ICMS. For instance, at 95°C (Table 6), the SF was, respectively, 30.6, 28.2 and 23.1 for ICTP, ICMH and ICMS starches. While the corresponding values for wheat (Hoover & Vasanthan, 1994*a*) and corn (unpublished results) starches are, respectively, 28.0 and 30.1%. At 95°C, the extent of AML (Table 6) in all pearl millet starches [ICTP (17.1%), ICMS (15.8%), ICMH (17.6%)] were higher than in wheat (10%) (Hoover & Vasanthan, 1994*a*) and corn (27%) (unpublished results) starches.

The low SF of ICMS could be attributed to its higher content of lipid complexed amylose chains (Table 1) and/or to the presence of a larger number of crystallites within the granule interior (Table 5). The SF of ICTP and ICMH are fairly close, likely because these two starches did not differ significantly with respect to the amount of lipid-complexed amylose chains (Table 1), and to the amount of starch crystallites (Table 5). The low degree of AML in ICMS is a reflection of its higher content of lipid-complexed amylose chains. The extent of AML is slightly higher in ICMH (in spite of its higher content of amylose-complexed lipids) than in ICTP (Table 6), likely owing to the higher total amylose content of the former (Table 1).

Pasting properties

The pasting properties of pearl millet starches are presented in Table 7. The three starches showed identical pasting temperatures (90°C), which were higher than those of wheat (86°C) (Hoover & Vasanthan, 1994*a*) and corn (81°C) (Hoover *et al.*, 1991) starches. The pearl millet starches differed from each other with respect to the 95°C viscosity (ICMH > ICTP > ICMS). This suggests that the 95°C viscosity is probably influenced by the interplay of the following factors: (1) the degree of crystallinity [ICMS > ICTP > ICMH (Table 5)]; (2) the extent of amylose leaching [ICMH > ICTP > ICMS (Table 6)]; and (3) the amount of lipid amylose [ICMS > ICMH > ICTP (Table 1)].

In all three starches, the viscosity increased during the holding cycle (Table 1) at 95°C [ICMS (60 BU) > ICMH (30 BU) > ICTP (2 BU)]. The higher increase for ICMS probably reflects stronger bonding forces within the granule interior (Table 5). Increases during the holding cycle have also been reported by Badi *et al.* (1976) (80 BU) and Freeman & Bocan (1973) (20 BU) for pearl millet starches from other cultivars. In some cultivars however, a decrease in viscosity was found to occur during the holding cycle [Beleia *et al.*, 1980 (0–100 BU); Wankhede *et al.*, 1990 (72 BU); Freeman & Bocan, 1973 (260 BU)]. Wheat and corn starches were shown to exhibit a viscosity decrease of 5 BU (Hoover & Vasanthan, 1994*a*) and 10 BU (Hoover *et al.*, 1991), respectively, during the holding cycle. The pasting behaviour of pearl millet starches used in this study was

Table 6. Effect of temperature (°C) on the swelling factor (SF) and amylose leaching (AML) in pearl millet starches[a]

Starch source	50	60	70	80	90	95
ICTP 8203						
SF[b]	3.4 ± 0.2	4.4 ± 0.3	12.6 ± 0.2	15.5 ± 0.1	28.6 ± 0.1	30.6 ± 0.3
AML[c]	0.26 ± 0.03	0.50 ± 0.02	7.80 ± 0.02	7.80 ± 0.02	10.40 ± 0.04	17.10 ± 0.10
ICMS 7703						
SF[b]	—	—	8.0 ± 0.2	10.2 ± 0.2	18.0 ± 0.4	23.1 ± 0.4
AML[c]	0.20 ± 0.02	0.45 ± 0.03	4.50 ± 0.10	7.20 ± 0.05	9.45 ± 0.05	15.80 ± 0.20
ICMH 356						
SF[b]	2.9 ± 0.3	3.9 ± 0.4	11.9 ± 0.5	14.9 ± 0.3	25.6 ± 0.2	28.2 ± 0.1
AML[c]	0.30 ± 0.07	0.55 ± 0.07	5.40 ± 0.05	8.00 ± 0.02	9.60 ± 0.05	17.60 ± 0.05

[a]The data represents the means of three measurements.
[b]Swelling factor.
[c]Amylose leaching.

Table 7. Pasting characteristics of pearl millet starches[a,b]

Starch source	Pasting temperature (°C)	Highest viscosity reached during heating to 95°C (BU)[c]	Viscosity at 95°C (BU)	Viscosity after 30 min at 95°C (BU)	Viscosity at 50°C (BU)
ICTP 8203	90.0	167	167	169	273
ICMS 7703	90.0	100	100	160	345
ICMH 356	89.3	180	180	210	430

[a]Each value represents the mean of two determinations.
[b]Starch concentration (6% w/v) and pH 5.5.
[c]Brabender units.

rather surprising since a high pasting temperature and resistance to shear and temperature (during the holding cycle) are normally exhibited only by cross-linked starches and legume starches. This suggests that, in these starches, the bonding forces within the granule interior are of a higher order of magnitude than in corn and wheat starches.

Gelatinization temperatures

The gelatinization transition temperatures [T_o (onset); T_p (midpoint), T_c (conclusion)] and the enthalpy of gelatinization (ΔH) of the pearl millet starches are presented in Table 8. The gelatinization temperature range of ICTP (61.2–70.5°C) was close to that of ICMH (60.9–67.5°C). However, that of ICMS (64.5–78.0°C) was much higher than the other two starches. A similar trend was also observed with respect to ΔH, where the values were 2.7, 3.5 and 2.5 cal g^{-1}, respectively, in ICTP, ICMS and ICMH.

Wheat and corn starches have been reported to exhibit gelatinization temperatures in the range 56–66°C (Hoover & Vasanthan, 1994*a*) and 64–72°C (Hoover *et al.*, 1991), respectively. The values for ΔH were 2.1 cal g^{-1} (Gudmundsson & Eliasson, 1989) and 2.3 cal g^{-1} (Hoover *et al.*, 1991), respectively, for wheat and corn starches.

It is difficult to compare our results (Table 8) with reported values for pearl millet starches, since in the latter studies, T_o, T_p and T_c were determined using the Kofler hot stage microscope. However, even by this technique, differences in starch gelatinization temperatures were shown to exist among cultivars (Beleia *et al.*, 1980; Malleshi *et al.*, 1986; Wankhede *et al.*, 1990). The higher T_o, T_p and T_c of ICMS suggests that the crystallite size and/or crystallite association within its granules are of a higher order of magnitude than in ICTP, ICMH, wheat and corn starches.

Cooke & Gidley (1992) 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. Therefore, the differences in ΔH between pearl millet starches probably reflect differences in the number (ICMS > ICTP > ICMH) of double helices (within the amorphous and crystalline regions of the granule) that unravel and melt during gelatinization.

***In vitro* digestibility of native starches by porcine pancreatic α-amylase**

The extent of α-amylase hydrolysis of the pearl millet starches is presented in Fig. 4 and the mode of action of α-amylase on granules of ICTP at intervals of 2, 7 and 9 h are presented in Fig. 5(A)–(C). The extent of hydrolysis followed the order: ICTP > ICMH > ICMS. For instance, after 48 h of hydrolysis, ICTP, ICMH and ICMS were hydrolysed to the extent of 76.1, 74.4 and 70.0%, respectively. In comparison with pearl millet starches (Fig. 4), corn (23% in 9 h) (Hoover *et al.*, 1991) and wheat (63% in 72 h) (Hoover & Vasanthan, 1994*a*) starches are digested more slowly by porcine pancreatic α-amylase.

The above results cannot be compared with digestibility studies on starches from other pearl millet cultivars (Wankhede *et al.*, 1990) because of differences in enzyme source (human salivary α-amylase).

After enzyme hydrolysis (2–24 h), the granular residue was analysed by DSC (Table 9). There was a progressive small reduction in the melting enthalpies (ΔH) of pearl millet starch residues (ICTP > ICMH > ICMS) as hydrolysis with the α-amylase proceeded. It is likely that the smaller reduction in ΔH for ICMS reflected its greater resistance towards enzyme hydrolysis (Fig. 4). Marginal decreases were also observed for T_p (ICTP ~ ICMS ~ ICMH). This suggests that the perfecting

Table 8. Gelatinization[a] and retrogradation[b] characteristics of pearl millet starches

Starch source	Storage time (days) at 25°C	Transition temperatures[c] (°C)			T_c–T_o (°C)	ΔH^d (cal g^{-1})	ΔH_R^e (cal g^{-1})
		T_o	T_p	T_c			
ICTP 8203	0	61.2	68.0	75.0	13.8	2.7	—
	4	45.0	51.0	64.0	19.0	—	1.06
	7	46.0	53.0	66.0	20.0	—	1.56
ICMS 7703	0	64.5	70.0	78.0	13.5	3.5	—
	4	44.5	50.5	60.5	16.0	—	1.10
	7	45.0	54.0	61.5	16.5	—	1.45
ICMH 356	0	60.9	67.5	74.0	13.1	2.5	—
	4	46.0	52.5	63.0	17.0	—	1.20
	7	46.5	53.5	63.5	17.0	—	1.56

[a]Starch:water ratio, 1:3 (w/w dry basis).
[b]Starch:water ratio, 40:60 (w/w dry basis).
[c]For native starches, T_o, T_p and T_c indicate the temperatures of the onset, midpoint and end of gelatinization, respectively. Whereas for retrograded starches, T_o, T_p and T_c indicate the temperatures of the onset, midpoint and end of melting of crystallites formed during storage at (25°C). Average standard deviation = 0.1°C (n = 3).
[d]Enthalpy of gelatinization. Average standard deviation = 0.1 (n = 3).
[e]Enthalpy of retrogradation. Average standard deviation = 0.1 (n = 3).

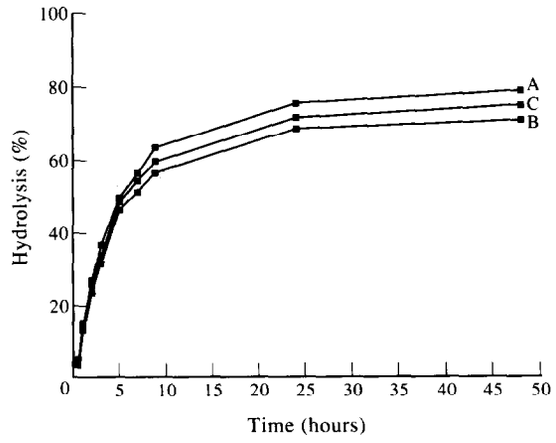

Fig. 4. The extent of hydrolysis of pearl millet starches by porcine pancreatic α-amylase. (A) ICTP; (B) ICMS; and (C) ICMH.

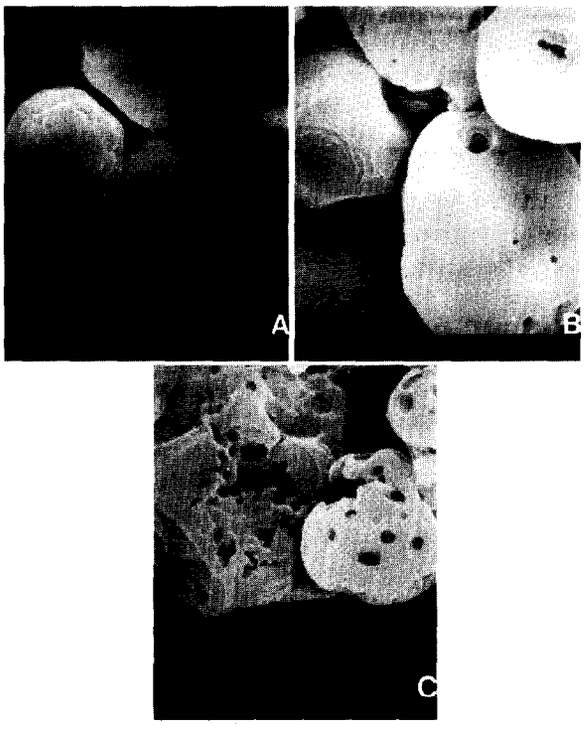

Fig. 5. Scanning electron micrographs of α-amylase-hydrolysed native starch granules of ICTP 8203. (A) ICTP after 2 h hydrolysis; (B) ICTP after 4 h hydrolysis; and (C) ICTP after 9 h hydrolysis.

and ordering of the amylopectin crystallites in the granular residues are very nearly the same as in the native granule.

The mode of attack by α-amylase on pearl millet starches was investigated using SEM. The mode of action was similar for all three starches. Therefore, only the results for ICTP are presented in Fig. 5. The surfaces of the granules were extensively eroded and were covered with numerous craters of varying size and depth [Fig. 5(C)] as if the α-amylase had entered the granule and preferentially hydrolysed the interior portion.

Acid hydrolysis

The solubilization patterns of pearl millet starches are presented in Fig. 6. The extent of hydrolysis followed the order: ICTP > ICMH > ICMS. At the end of 20 days, the degrees of hydrolysis were 88.2, 80.5 and 84.0%, respectively, for ICTP, ICMS and ICMH. During the same time, corn and wheat starches were hydrolysed to the extent of 76% (unpublished results) and 82% (Hoover & Vasanthan, 1994*a*), respectively.

The faster hydrolysis rate during the initial 5 days has been shown to correspond to the destruction of amorphous regions of the granule. During the second stage, the crystalline region is slowly degraded (French, 1984; Kainuma & French, 1971; Robin *et al.*, 1974). It is evident from the results presented in Fig. 6 that the amorphous and crystalline regions of the starch granule are more highly ordered in ICMS than in ICTP and ICMH.

Light transmittance

The results of transmittance (%T) measurements differed at all pH levels among the three starches (ICTP > ICMH > ICMS) (Fig. 7). At pH 12, the %T of the starches were 86, 74 and 81%, respectively, for ICTP, ICMS and ICMH. At this same pH, wheat starch exhibited a %T of 90 (Hoover & Vasanthan, 1992). Craig *et al.* (1989) postulated that when a beam of light passes through native starch granules, a large proportion of the light is reflected back and the starch appears white and opaque due to the surface of the granule being larger than the wavelength of light. They also

Table 9. DSC gelatinization parameters of enzyme treated granular residues following hydrolysis with porcine pancreatic α-amylase

Starch source	DSC parameter	Hydrolysis time (h)				
		2	5	7	9	24
ICTP 8203	T_p[a]	68.0 ± 0.2[c]	67.5 ± 0.2	67.5 ± 0.2	67.0 ± 0.1	67.0 ± 0.2	67.0 ± 0.2
	ΔH[b]	2.7 ± 0.1	2.5 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.0 ± 0.1	1.8 ± 0.2
ICMS 7703	T_p	70.0 ± 0.1	70.0 ± 0.1	70.0 ± 0.5	69.5 ± 0.1	69.5 ± 0.1	69.0 ± 0.2
	ΔH	3.5 ± 0.2	3.5 ± 0.2	3.3 ± 0.1	3.3 ± 1.0	3.1 ± 0.1	3.0 ± 0.1
ICMH356	T_p	67.5 ± 0.1	67.5 ± 0.1	66.8 ± 0.2	66.5 ± 0.2	66.0 ± 0.1	66.0 ± 0.2
	ΔH	2.5 ± 0.1	2.5 ± 0.2	2.4 ± 0.1	2.2 ± 0.2	2.0 ± 0.1	1.9 ± 0.1

[a]Peak temperature (°C).
[b]Enthalpy of gelatinization (cal g^{-1}).
[c]Mean ± SD ($n = 3$).

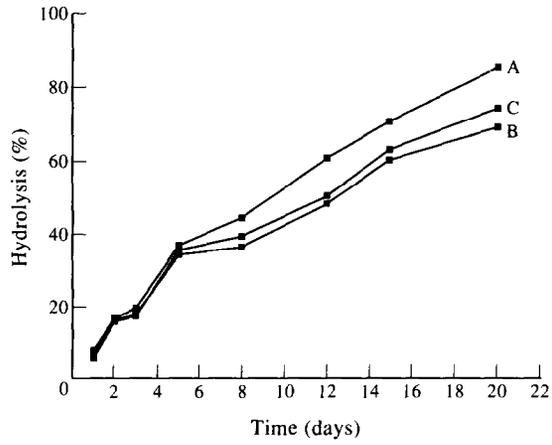

Fig. 6. Heterogenous acid hydrolysis of pearl millet starches. (A) ICTP; (B) ICMS; and (C) ICMH.

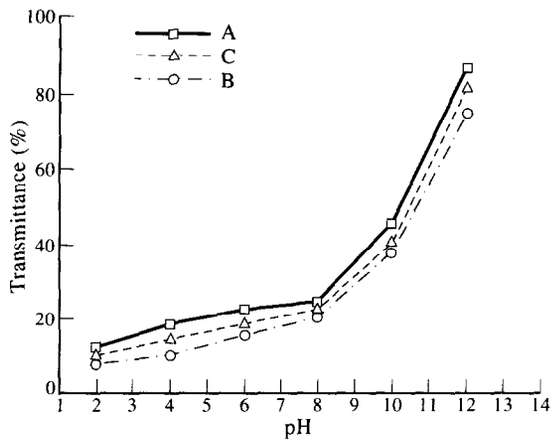

Fig. 7. Effect of pH on light transmittance of pearl millet starches. (A) ICTP; (B) ICMS; and (C) ICMH.

proposed that the separation of starch chains during gelatinization decreases the reflecting ability of starch granules and, thus, increases the $\%T$ of a starch paste. Swinkels (1985) and Craig *et al.* (1989) showed that amylose–lipid inclusion complexes decrease the $\%T$ of starch pastes. The latter authors also showed that $\%T$ increases with the degree of swelling. Our results are in agreement with the findings of Craig *et al.* (1989), since the lowest $\%T$ was shown by the starch paste (ICMS) which had the highest content of lipid-complexed amylose (Table 1) and the lowest degree of swelling (Table 5). The effect of pH on $\%T$ has been described elsewhere (Hoover & Vasanthan, 1992).

Retrogradation of starch gels

The extent of retrogradation during gel storage was monitored by determining changes in freeze–thaw stability and retrogradation enthalpy.

Freeze–thaw stability

The freeze–thaw stability of a starch gel is evaluated by the amount (%) of water released (syneresis) when starch chains retrograde (reassociate) during the freeze–thaw cycles. The degree of syneresis of the starch gels (ICTP > ICMH > ICMS) are presented in Table 10. The % syneresis was much lower in pearl millet starch gels than in previously reported data on corn and wheat starches. For instance, after 2 days storage at −16°C, the % syneresis in pearl millet starch gels ranged from 20.4 to 28.8% (Table 10). However, during this same period (at −16°C), the corresponding values for corn (Hoover *et al.*, 1991) and wheat (Hoover & Vasanthan, 1992) starches were 65 and 51%, respectively. Several researchers (Gidley & Bulpin, 1989; Ring *et al.*, 1987; Wu & Seib, 1990) have postulated that the outer A branches of amylopectin influence the extent of recrystallization. Wu & Seib (1990) attributed differences in freeze–thaw stability between waxy barley (WB) and waxy maize (WM) starches (WB > WM) to differences in the degree of polymerization (DP) of the A chains of amylopectin [WB (DP 12), WM (DP 15)]. They postulated that branch chains of DP 12 are less prone to recrystallization than chains with a DP of 15. In non-waxy starches, both amylose and amylopectin crystallization influence the degree of syneresis. Gidley & Bulpin (1989) showed that the kinetics of aggregation of amylose chains and the variation of gel strength with amylose concentration show a dependence on amylose chain length. These authors showed that precipitation and gelation occur for amylose chain lengths of 250–660 residues, whereas for longer chains (> 1100 residues), gelation predominates over precipitation. Thus, differences in freeze–thaw stability (Table 10) probably reflect differences in the amylopectin (A chains) and/or amylose chain lengths of the pearl millet starches.

Differential scanning calorimetry

The retrogradation endotherm of the pearl millet starches are presented in Table 8. In all three starches, T_o,

Table 10. Freeze–thaw stability of pearl millet starches

Starch source	Syneresis (%)[a]					
	Number of freeze–thaw cycles					
	1	2	3	4	5	6
ICTP 8203	21.0 ± 1.5	28.5 ± 1.0	33.6 ± 1.5	36.5 ± 1.5	44.0 ± 2.0	51.0 ± 1.5
ICMS 7703	15.0 ± 1.5	20.4 ± 0.8	26.4 ± 1.2	29.5 ± 1.2	33.2 ± 1.6	37.5 ± 1.7
ICMH 356	18.0 ± 1.3	25.0 ± 0.8	29.5 ± 1.5	32.0 ± 1.2	36.0 ± 1.4	41.5 ± 1.2

[a]The data represent the mean of three determinations.

T_p and T_c of the retrograded gels were lower than those for the gelatinization endotherm (Table 8), and T_c–T_o for retrogradation was broader (ICTP > ICMS ~ ICMH) than for gelatinization (Table 8). The enthalpy of retrogradation (ΔH_R) increased (Table 8) on storage (ICTP > ICMS ~ ICMH). The broadening of T_c–T_o on retrogradation implies that the retrogradation endotherm probably reflects melting of crystallites of different stability, size or perfection formed by different types of starch chain associations (amylose–amylopectin and/or amylopectin–amylopectin) during gel storage. The increase in ΔH_R (Table 8) on storage reflects the formation of double helices between A chains of amylopectin (Hoover *et al.*, 1994*b*). The results suggest that ICTP retrogrades faster than ICMS and ICMH. This seems plausible since the difference in T_c–T_o (between the retrogradation and gelatinization endotherm) and the increase in ΔH_R (on storage) is more pronounced in ICTP.

CONCLUSION

This study showed that the starches from the three pearl millet cultivars differed widely in swelling factor, amylose leaching, X-ray diffraction intensities, gelatinization transition temperatures, enthalpy of gelatinization, light transmittance, paste temperatures, susceptibility towards hydrolysis by α-amylase and acid, and in the extent of retrogradation. These differences are probably influenced by differences in the amount of lipid-complexed amylose molecules, the magnitude of interaction between and among starch chains within the native granule, and the chain lengths of amylose and amylopectin.

Further work is now in progress on the fine structure of the pearl millet starch components in order to obtain a more precise insight into the granular structure.

ACKNOWLEDGEMENTS

The senior author expresses his appreciation to the Natural Science and Engineering Research Council of Canada for supporting the work through an operating grant. Thanks are also due to the International Crop Research Institute for the Semi Arid Tropics Center (Patancheru, India) for providing pearl millet grains.

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