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Characteristics of native and enzymatically hydrolyzed *ragi* (*Eleusine coracana*) and rice (*Oryza sativa*) starches

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Abstract

The compositional, structural and enzymatic digestibility profiles of starches isolated from *ragi* and rice were determined. Although, the gelatinization temperature (73 ± 2 °C) and GPC elution profiles of both starches on Sepharose CL-2B gel were comparable, the peak (PV: 273 RVU) and set back (SB: 196 RVU) viscosities and also the thermic energy (TE: 34.78 cal/g) values for *ragi* starch were considerably higher than for rice starch (PV: 200 RVU; SB: 150 RVU and TE: 26.54 cal/g). Upon digestion with α-amylase (AA) (*human salivary*), β-amylase (BA) (*barley malt*), pullulanase (PN) (*Klebsiella pneumoniae*) and amyloglucosidase (AG) (*Aspergillus niger*), the hydrolysates from AA contained glucose, maltose and oligosaccharides of 15–20 DP prominently, whereas, those from BA, PN and AG contained only maltose, maltotriose and glucose, respectively. The molecular weight (M_W) and degree of crystallinity of the starch residues from the enzymatic digests of *ragi* starch were significantly higher than of rice starch. The SEM examination of the residues indicated high degree of fragmentation in the case of rice but only a few bigger chunks in case of *ragi*. These parameters may help to explain subtle differences in the digestibility and physiological properties that exist between *ragi* and rice starches.

Keywords: Carbohydrate digestibility; Starch crystallinity; X-ray diffraction; Thermal energy

1. Introduction

Cereals constitute the major source of dietary carbohydrates, proteins, vitamins and minerals particularly for the vegetarians worldwide. *Ragi* (Finger millet, *Eleusine coracana*), a low cost minor cereal forms staple food for a large section of population in Indian sub-continent and also in many of the African countries (Guptha, Appa Rao, & House, 1986) whereas, rice (*Oryza sativa*) forms the dietary component for nearly 50% of the world population, that too mainly in the Asian region. Nearly 80% of cereals comprise of carbohydrates, and the starch, a versatile natural polymer happens to be main component of cereal carbohydrates, and occurs as discrete granules of different sizes and shapes. It contains linear (amylose) and branched (amylopectin) chains with α 1-4 and, α 1-4 and α 1-6 glucose units, respectively. The starch granules undergo physical and chemical changes during processing, and influences the textural and many of the physico-chemical characteristics including digestibility of the food (Tharanathan, Muralikrishna, Salimath, & Raghavendra Rao, 1987; Tharanathan, 1995). Even though many aspects of the cereal starches have been studied, very little information is available with respect to the comparative digestibility of different cereal carbohydrates. *Ragi* is known for high sustaining power with excellent hypoglycemic and hypocholesterolemic characteristics whereas, rice is known for easy digestion (Gopalan, 1981; Madhusudan & Tharanathan, 1995). Dietary carbohydrates play a major role in regulating obesity and blood glucose attenuation. In recent years *diabetes mellitus* and obesity are turning out to be endemic at global level and to control their rapidity, search for food sources with hypoglycemic characteristics and satiety inducing qualities is gaining prominence. In this direction investigations on the physicochemical characteristics and enzymatic digestion

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profiles of *ragi* starches may be very remunerative for the management of obesity and *diabetes mellitus*, *as ragi* is known for slow digestion and hypoglycemic characteristics. Accordingly, the present communication is an attempt to understand the intrinsic physicochemical characteristics and enzymatic digestion of *ragi* starch in comparison with rice starch.

2. Materials and methods

2.1. Materials

Ragi variety GE 5153 and rice variety IR 20 were procured from the University of Agricultural Sciences, Bangalore, India. *Ragi* was deglumed, whereas rice was debranned in a pilot scale rice mill (McGill Miller, Century Elect. Co, St Louis, USA). α-Amylase (AA) (A0521, EC 3.2.1.1, 1 unit to liberate 1.0 mg maltose from starch in 3 min at pH 6.9 at 20 °C in phosphate buffer), β-amylase (BA) (A7130, EC 3.2.1.2, 1 unit to liberate 1.0 mg maltose from starch in 3 min at pH 4.8 at 20 °C in acetate buffer), amyloglucosidase (AG) (A7420, EC 3.2.1.3, 1 unit to liberate 1.0 mg glucose from starch in 3 min at pH 4.5 at 37 °C in acetate buffer) and pullulanase (PN) (P1067, EC 3.2.1.41, 1 unit to liberate 1.0 μmol of maltotriose from pullulan/min at pH 5.0 at 25 °C in acetate buffer) were obtained from Sigma Chemical Co., St Louis, MO, USA. Other reagents used were of highest purity available.

2.2. Isolation of starch

The deglumed *ragi* and milled rice were soaked in excess water for 16 h at ambient conditions and to the steep water $HgCl_2$ (100 ppm) was added to prevent incipient sprouting of grains during steeping. The steeped material was washed to free from leachates and mashed with 5 volumes of water in a waring blender and the slurry was screened through a sieve of 85-micron openings. The residue was again dispersed in water, wet ground and sieved, and the process was repeated till the residue was free from starch (negative to iodine test). The slurry containing starch was pooled and centrifuged, and the residue was washed with mild alkali followed by salt solution and toluene to free the starches from protein and pigment contamination. After ensuring the purity by microscopic examination, the starches were dried over methanol (Adkins & Greenwood, 1966).

2.3. Chemical composition

Moisture, protein and ash contents of the isolated starches were estimated following standard methods (AACC, 1995). Total and soluble amylose contents were determined by iodine blue complexing (0.2% iodine solution in 2% potassium iodide) as per Sowbhagya & Bhattacharya (1979) and the λ_{max} of the starch–iodine complex was recorded in a Shimadzu UV–Visible spectrophotometer.

2.4. Solubility and swelling characteristics

Starch samples (1 g) dispersed by vertexing in 10 ml water in a graduated test tube were equilibrated at 30–90 °C with 10 °C increments, for 30 min with occasional shaking, centrifuged at 3000 *g* and the supernatants were transferred to weighed Petri plates and the volume as well as the weight of the residue were recorded. The supernatants and residues were dried at 90 °C to constant weight and the solubility index and the swelling power were determined (Leach, McCowenan & Schoch, 1959).

2.5. Pasting profile

Starch samples (3.5 g, on 14% moisture basis) were mixed with water (25 ml) in a canister, heated in a rapid visco-analyser (RVA-model Newport Scientific Pvt. Ltd., NSW, Australia) at the rate of 5 °C per minute to 95 °C maintained at 95 °C for 7 min and cooled at the rate of 6 °C per minute to 50 °C. The changes in viscosity during heating, cooking and cooling were recorded and the gelatinization temperature, peak, breakdown and set back viscosity values were noted (Batey & Curtin, 2000).

2.6. Differential scanning calorimetry

To 2 mg starch taken in aluminum pans, 20 μl water was added; the pans were sealed, equilibrated for 1 h and scanned in a differential scanning calorimeter (Perkin–Elmer DSC 7 Robotic, USA) to record the calorigrams. The instrument was calibrated using indium and dodecane, and was programmed to rise 10 °C/min with a sensitivity of 0.005 cal/s. The thermal transition of starches in terms of temperature of onset (T_0), peak temperature (T_P) and end point of gelatinization (T_E) were recorded from the calorigrams. Based on the area of the triangle of the calorigrams, the ΔH (enthalpy) associated with gelatinization of starch was calculated (Rosa, Lopez, Trejo, & Falomir, 1989).

2.7. Digestibility

About 2 g starch samples dispersed in cold water [10% slurry concentration (w/v)] were heated to boiling in a water bath and cooled to room temperature. Soon after that, aliquots of the starch slurries (0.5 g each) were homogenized with suitable buffers namely 0.02 M phosphate buffer for AA, pH 6.9, 0.01 M acetate buffer of pH 4.8, 4.6 and 5.5 for BA, AG and PN, respectively, and subjected to enzymatic digestion (5 U AA, 25 U BA, 10 U AG and 0.2 U PN per mg starch) for 180 min at 37 °C. The enzymatic hydrolysis was arrested by adding ethanol (3 vol.) and the residue from the hydrolysate were separated

by centrifugation (5000*g*). The reducing sugar contents of the supernatants were estimated (Miller, 1959) and characterized by HPLC (CR4 A, Shimadzu) fitted with μ-bondapack amino column (4.1 mm × 30 cm) and eluted with acetonitrile and water (75:25) solvent system at a flow rate of 1 ml/min and the area under each peak was measured to quantify the individual sugars. Glucose, maltose and maltotriose were the reference sugars used. The residues were freeze-dried and used for molecular mass determination by gel permeation chromatography, SE-HPLC, morphological features by microscopic examination and also for the degree of crystallinity by X-ray diffraction.

2.8. Gel permeation chromatography

The isolated starches and also the residues prepared from the enzymatic hydrolysis were dispersed in aqueous dimethylsulphoxide (85%) and an aliquot containing 10 mg carbohydrate (dry weight) was fractionated by ascending chromatography on a Sepharose CL-2B gel (Pharmacia, Sweden) column (1.7 × 90 cm) at a flow rate of 12 ml/h, using water containing 0.02% sodium azide as an eluent. Total carbohydrate (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and the absorption maxima (λ_{max}) (iodine–polysaccharide complex) of the eluents were determined. The M_W of the fractionated carbohydrates was calculated by running dextran standards (Pharmacia, Sweden) on the same column (Chinnaswamy & Bhattacharya, 1986).

2.9. Size exclusion-high-performance liquid chromatography (SE-HPLC)

The residues obtained from enzyme digestion were solubilized in aqueous DMSO (85%), centrifuged and 10 μl of the supernatant was used for fractionation and M_W determination (Madhusudhan & Tharanathan, 1996) using size exclusion HPLC [Shimadzu HIC-6A ion chromatograph equipped with a Shimadzu RID-6A refractive index (RI) detector, SCL-6A system controller, CR 2A chromatopac integrator, and fitted with E-linear and E-1000 μ-Bondagel (SS, 30 cm × 3.9 mm) columns, (Waters Associates, Milford, USA)] connected in series with the guard column. Ultra pure water was the eluent (0.8 ml/min) used at 40 °C.

2.10. X-ray diffraction

Isolated starches and also the residues from the enzymatic digests were examined for their crystallinity using EG-7G solid state germanium liquid nitrogen cooled detector, Scintag XDS-2000 instrument equipped with a θ–θ goniometer at 25 mA and 30 kV. The samples were exposed for 5 h to Cokα filtered radiation (λ 1.54184 nm) (Cheetham & Tao, 1998). Diffractograms were scanned from 2 to 40°, which covers all the significant diffraction peaks of starch crystallites at a diffraction angle of 2*θ*. The degree of crystallinity was quantitatively estimated by following the method of Nara & Komiya (1983).

2.11. Scanning electron microscopy (SEM)

Native starches and also the residues from the enzymatic digests were mounted on metal stubs with the aid of double sided Scotch adhesive tape. The samples were gold coated (about 100 A°) in a KSE 24M high vacuum evaporator and scanned in a scanning electron microscope (LEO 435VP LEO Electron Microscopy Ltd., Cambridge, England) and the selected regions depicting distinct morphological features were photographed (Tharanathan & Ramadas Bhat, 1988).

3. Results and discussion

The yield of starches from *ragi* and rice were 42 and 59%, respectively on the grain mass basis. The low yield could be due to losses occurring during repeated washings to free the starches from pigments, seed coat and the cell wall materials during isolation. The protein and the ash content of *ragi* and rice starches were 0.14 and 0.11%, and 0.24 and 0.05%, respectively, which indicates their purity (Table 1). Although, the starchy material was repeatedly treated with saline and toluene, and also with methanol, the starches still contained a little protein. However, the treatment did not affect the integrity of the granules, but influence the protein and lipid contents of the starch on the enzymatic digestion was not ruled out (Sineviratne & Biliaderis, 1991). The amylose content of *ragi* and rice starch was 22 and 25%, and the λ_{max} of the iodine blue colored complex was 611 and 603 nm, respectively. This revealed the presence of considerable proportion of straight chain fractions, mainly the linear (amylose) component in the starches.

Table 1
Proximate composition (%) of *ragi* and rice starches[a]

	Ragi starch	Rice starch
Yield	42.0	59.0
Composition of isolated starch		
Moisture	9.10	8.90
Protein	0.14	0.24
Fat	0.20	0.30
Ash	0.11	0.05
Calcium (mg%)	12	10
Phosphorous (mg%)	50	40
Total carbohydrates	90.45	90.51
Starch components		
Amylose	22	25
Amylopectin	78	75
λ_{max} (nm)	611	603

[a] Average of two determinations, on dry weight basis

Table 2
Swelling power and solubility (%) of *Ragi* and rice starches

	Swelling power					Solubles				
Temperature (°C)	30	50	70	80	90	30	50	70	80	90
Ragi	1.4	1.9	6.4	10.2	13.6	0.75	0.82	0.91	1.24	1.48
Rice	1.8	2.5	9.0	10.7	11.4	0.58	0.69	0.76	0.95	1.23

3.1. Swelling and solubility characteristics

The changes that occur in starch granules during heating an aqueous slurry are dependent on the temperature and water availability in the system. Both *ragi* and rice starches exhibited single stage swelling, the swelling power at 30 and 80 °C being 1.4 and 10.2% for *ragi* and 1.8 and 10.7% for rice starches, respectively (Table 2). The low solubility pattern for *ragi* and rice starches (0.75 and 1.24, 0.58, and 0.95%, respectively) at pre- and post-gelatinization stages exhibits the presence of relatively strong bonding forces within the granules. It may be noted that the power of swelling was lower for *ragi* starch than for rice starch at pre-gelatinization stage but the trend reversed at post-gelatinization temperature. Swelling and solubility properties of the starches are greatly influenced by the species and facilitate understanding the nature of the associative bonding forces within the granules. Besides, the small variations in amylose and amylopectin content, between *ragi* and rice starches, the subtle architectural variations possibly present in the amylose of *ragi* starches could be the reason for its slow digestibility.

3.2. Pasting properties

The patterns of changes in viscosity of aqueous slurries of the starches recorded in RVA presented in Fig. 1 bring out the differences in pasting behavior between *ragi* and rice starches. There was a sharp increase in viscosity of *ragi* starch at about 70 °C and that of rice starch about 74 °C, which is considered as temperature of gelatinization. Gelatinization occurs when the internal crystalline structure of the granules collapse due to excessive swelling and the consequent loss of granular structure. The peak viscosity (PV) and the breakdown viscosity (BD) of *ragi* and rice starches were 273 and 196, and 200 and 150 RV units, respectively, indicating significantly higher PV for *ragi* starch as compared to rice starch, and this could be due to its greater water binding capacity and granular rigidity. On the other hand, the very limited fall in viscosity of *ragi* starch during cooking reveals lower levels of exudates from its granules even on prolonged heating after gelatinization. Pasting generally becomes prominent after the granules absorb sufficient water, swell and this happens after gelatinization in most of the native cereal starches. The swollen granules brush each other and in the process, the soluble components exude from the granules and cause rise in viscosity, and at the same time the fragile starch granules loose their identity and form homogenous mass (Atwell, Hood, Lineback, Marston, & Zobel, 1988). The relatively lower PV for ragi starch could be due to low levels of leaching especially that of amylose during cooking or in other words, the fragility of the swollen granules of *ragi* starch may be lower than rice starch granules. Besides, the soluble amylose re-associates during cooking resulting in increased viscosity of the slurry. These observations indicate that *ragi* and rice starches differ considerably not only in swelling but also in re-association pattern.

3.3. Differential scanning calorimetry (DSC)

The difference in the heat flow pattern between the *ragi* and rice starches during heating is vividly brought out by the DSC thermograms (Table 3). The quantum of heat flow recorded in terms of energy to gelatinize *ragi* starch was 34.8 cal/g which nearly 30% higher compared to rice starch (26.5 cal/g). In other words, the endothermic transition or the glass transition temperature for *ragi* starch is slightly higher than that of rice starch. DSC is a valuable tool in the study of starch characteristics (Stevens & Elton, 1971) as it provides a quantitative measurement of the enthalpy (ΔH), namely, the energy transformation that occurs during melting of crystallites in the starch granule and it provides

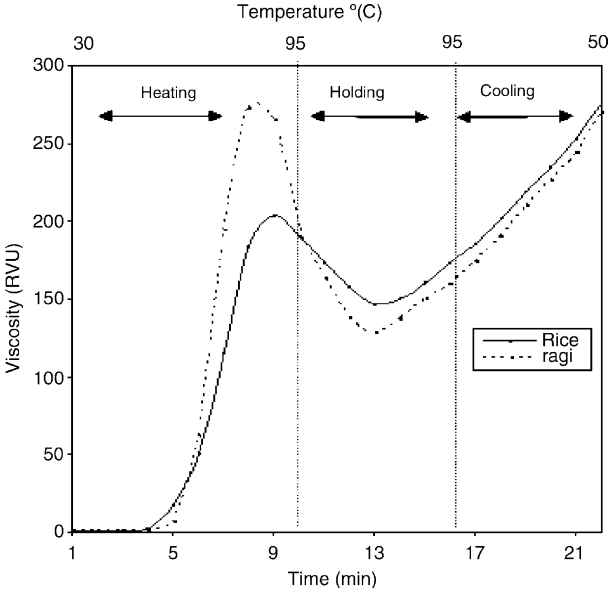


Fig. 1. Pasting characteristics of *ragi* and rice starches.

Table 3
DSC thermogram data of *ragi* and rice starches

Sample	Endothermic transition (°C)			Enthalpy of gelatinization [ΔH (cal/g)]
	T_0	T_p	T_e	
Ragi	64.5	69.0	75.0	34.78
Rice	60.0	63.0	67.5	26.54

a precise measurement of the temperature range over which these transformations occur (Longston & LeGrys, 1981). DSC facilitates measuring the energy required for gelatinization of starch or the energy required to melt the crystallinity or the loss of birefringence of the starch granules (Krueger, Knutson, Inglett & Walker, 1987). The higher energy needed to gelatinize *ragi* starch than rice starch denotes a stronger crystalline nature of the former than the latter.

3.4. Fractionation of starches

The starches solubilized in DMSO were fractionated into two main components by Sepharose CL-2B gel (Fig. 2), as the high molecular component of the starch elutes first as a void volume fraction (Fraction 1) and that of smaller M_W (Fraction 2) elutes subsequently. In the present investigation, the total carbohydrate content of fraction 1 and fraction 2 accounted for nearly 75 and 25% of the eluted starch, respectively. Generally, fraction 1 is considered as amylopectin and fraction 2 as amylose or the branched chain and straight chain polymers of maltose, respectively. Even though, this classification is accepted internationally (Chinnaswamy & Bhattacharya, 1986), there exists controversy over the behavior of fraction I, partially as amylose,

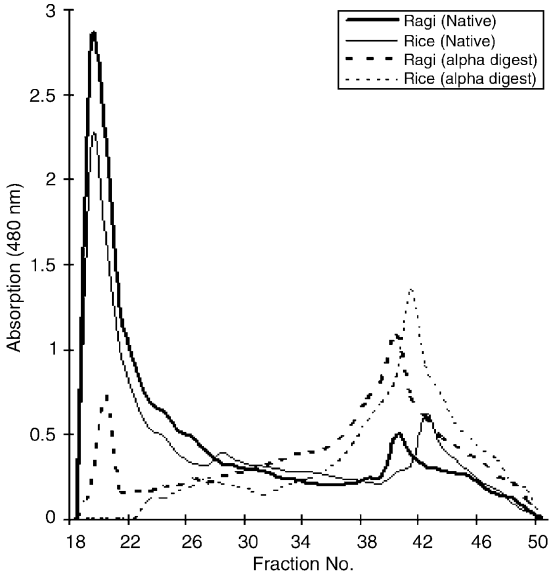
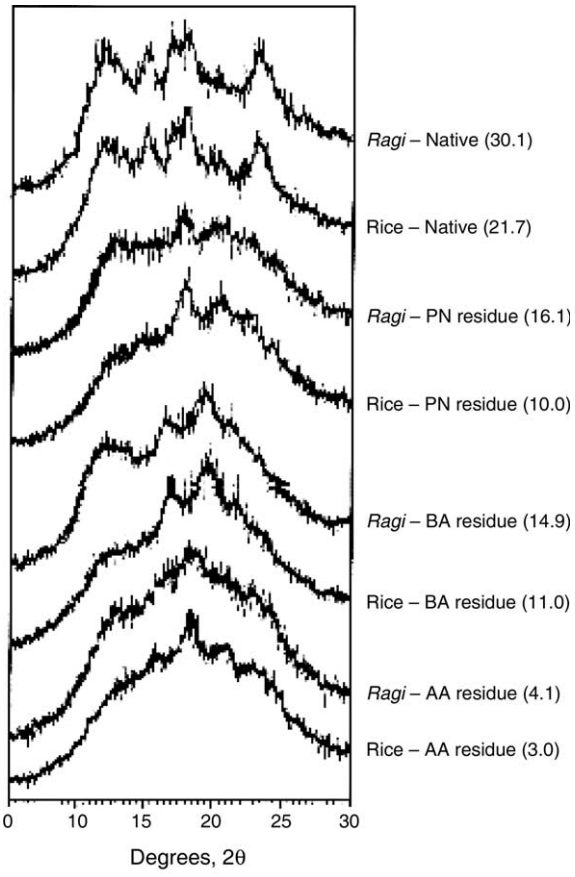


Fig. 2. Carbohydrate elution profiles of native as well as enzyme digests of *ragi* and rice starches on Sepharose CL-2B.

Table 4
The M_W of enzymatically hydrolyzed ragi and rice starch residues

	Ragi		Rice	
	M_W (kDa)	Yield (%)	M_W (kDa)	Yield (%)
α-Amylase	>500	1.80	415	0.22
digest	3.3	16.2	3.4	10.8
β-Amylase	>500	9.90	>500	4.70
digest	3.5	23.1	3.3	21.3
Pullalanase	>500	88	>500	54.4
digest			300.4	25.6
Amyloglucosi-	3.4	5.30	3.2	3.64
dase digest	2.8	0.72	2.7	0.40

mainly due to the presence of longer chain branches, which bind with iodine giving blue colour similar to amylose. The intermediate fraction, generally noticed in the processed samples was not prominent in these starches. The GPC profiles of *ragi* and rice starches were comparable as far as fraction 1 was concerned but fraction 2 of rice starch eluted slightly later than of *ragi* starch, which could be due to sparsely branched amylose molecules. This shows that the degree of branching as well as chain length of amylose of *ragi* and rice starches differ to a considerable extent. The AA digested residue of *ragi* starch still showed the presence of a small amount of high M_W component which



PN – Pullulanase; BA - β–Amylase; AA - α-Amylase. The values in parenthesis indicate degree of crystallinity

Fig. 3. X-ray diffractograms of native and enzymatically digested starches.

was eluted in the void volume (λ_{max} 560 nm) but this was not the case with the residues from AA digested rice starch. This shows that, rice starch is fully hydrolyzed by amylase into sugar or low M_W carbohydrates, dextrins, whereas *ragi* starch contained amylase resistant high M_W carbohydrates.

The major product obtained after enzymatic digestion of starches was reducing equivalents in the supernatant, whereas, Table 4 represents yield and M_W of the various residues obtained. Considerable variations in the M_W of the carbohydrate residues from α- and BAs, and also of PN digests were observed. The M_W of *ragi* starch digests was significantly higher than those of rice starches. While this was the case with fairly pure starch isolated from *ragi* and rice, the difference could be more pronounced when whole meals from *ragi* and rice were digested. This could be attributed to the association of non-starch molecules such as non-starch-polysaccharides, starch–lipid complexes, protein encapsulated granule matrix and also the phytochemicals namely phytate and polyphenols, all of them hindering amylolytic digestibility to various extents.

3.5. Degree of crystallinity

The X-ray diffractograms of native and the residues of enzymatically hydrolyzed digests of *ragi* and rice starches and their crystallinity values are presented in Fig. 3. While, the scattering angle, at which the diffraction intensities observed was 2θ, the 'd' spacing was used to discriminate the planes of different sites. The X-ray diffraction patterns were comparable to those reported earlier for cereal starches (Grenat, Rodosta, Anger, & Damaschum, 1993; Zobel, 1988). Both *ragi* and rice starches showed typical A- type

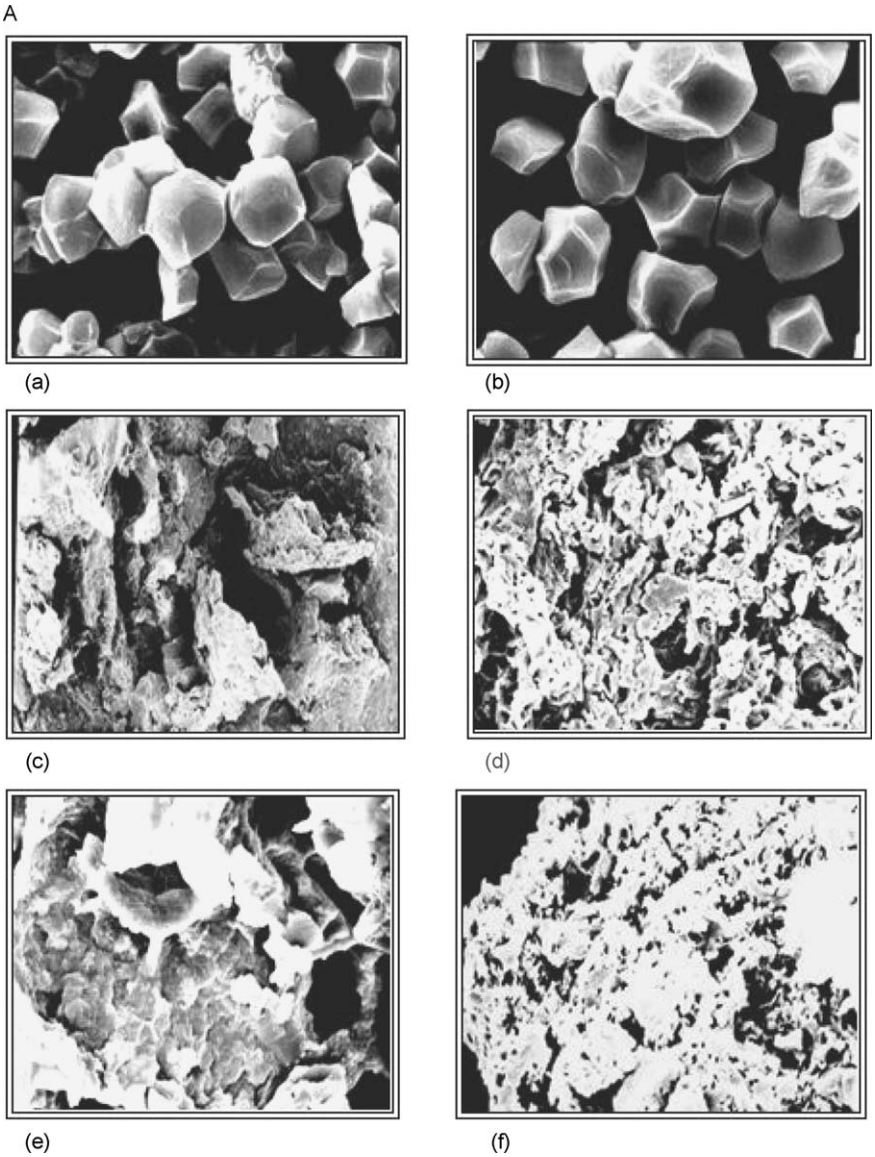


Fig. 4. (A) Scanning electron photomicrographs of native rice (a) and *ragi* (b); cooked rice (c) and *ragi* (d); residues of α-amylase digest rice (e) and *ragi* (f) starches. (B) Scanning electron photomicrographs of β-amylase digests, rice (a) and *ragi* (b); pullulanase digests rice (c) and *ragi* (d); amyloglucosidase digests rice (e) and *ragi* (f) starches.

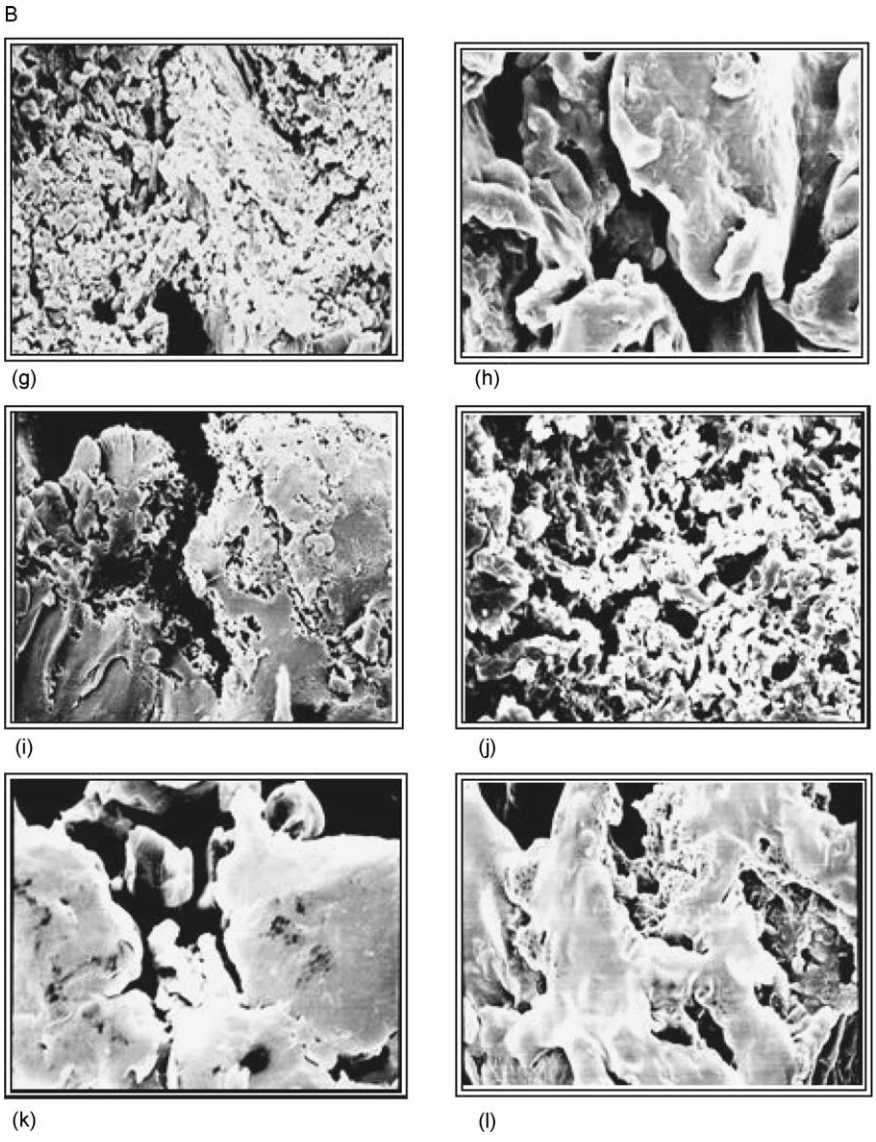


Fig. 4 (*continued*)

diffraction pattern with strong reflection at 15 and 23° (Wu & Sarko, 1978; Imberty & Perez, 1988; Imberty, Chanzy, Perez, Buleon, & Tran, 1988), but the degree of crystallinity of *ragi* starch was significantly higher (30.09%) than of rice starch (21.69%). It was also noted that the crystallinity of the residues from enzymatically hydrolyzed *ragi* starch also was significantly higher than rice starch residues.

3.6. Scanning electron microscopy

Scanning electron photomicrographs of the isolated, cooked and enzyme digested starches from *ragi* and rice are presented in Fig. 4A and B. Majority of native ragi starch granule appeared hexagonal but some of the granules were of irregular shape. Nearly 50% of *ragi* starch granules were of less than 5 μm size, 30% lying in between 5 and 10 μm and 20% larger than 10 μm. In the case of just gelatinized starch, one could see swollen granules, but the fully gelatinized as well as the enzyme digested starches had lost their granular structure completely and looked like 'cotton' or 'a cloudy mass'. Due to this, the distinct mode of enzymatic action on the native granules reported earlier (Tharanathan & Ramadas Bhat, 1988) was not visible in the residues. Nevertheless, some subtle differences in the morphological features of the residues were observed. The residues from the AA and AG digests exhibited highly fragmented topography, whereas, those of BA and PN digests were lumpy with a few of the lumps having surface erosions, visible pitting and indentations. The morphological features of the native rice starch differed considerably with respect to granule size and shape as it contained granules of irregular, pentagonal, hexagonal and spherical shapes. Among the enzyme digested rice starches, the residues from the AA and AG gave a highly fragmented

matter, whereas, the residues from ragi starch were largely compact with intermediate indentations. BA digestion did not produce any visible morphological changes but the PN-digested samples exhibited several smaller fragments. Thus it could be inferred that the degree of starch digestion is slightly lower in the case of *ragi* than rice, under identical conditions of enzymatic treatment.

4. Conclusion

The degree of crystallinity of *ragi* starch granules seems to be significantly higher than rice starch granules and this was also reflected by high energy required to gelatinize *ragi* starch as compared to rice starch. The differences in the amylolytic digestion pattern of these starches could be due to the rigid architecture with variable intermolecular forces in *ragi* than rice starch granules. These observations may possibly explain the slow digesting nature of *ragi* diets, which is of therapeutic advantage.

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