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Legume and cereal starches—why differences in digestibility?— Part II. Isolation and characterization of starches from rice (*O. sativa*) and ragi (finger millet, *E. coracana*)

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Starches were isolated from rice and ragi (finger millet) flours and studied for their physicochemical and digestibility properties. Both the starches were poorly birefringent and non-ionic in nature. They exhibited single stage swelling and low solubility in water and showed ~100% (for rice) and ~65% (for ragi) solubility in DMSO after 60 h. Gas liquid chromatography (GLC) analysis of fatty acid methyl ester (FAME) derivatives of the isolated starch lipid fractions revealed the predominance of C16:0 in rice starch and both C16:0 and C18:2 in ragi starch. The amylose content of starch isolates ranged between 22 and 30%. The ragi starch isolates (I/II) exhibited a slightly higher hot paste viscosity (300 BU) than those of rice starch isolates (~200 BU). Their setback viscosity increase was minimal. *In vitro* digestibility studies showed rice starch to be more digestible; in the native state, pancreatic α-amylase digested rice starch I to ~60% and ragi starch I to ~56%; whereas in the gelatinized state, glucoamylase digested the former to ~88% and the latter to ~70%.

INTRODUCTION

Cereals are an important component of the human diet throughout the world, particularly in tropical and subtropical regions. Cereals constitute the major source of carbohydrates (both available and unavailable), proteins, vitamins and minerals. Rice (*Oryza sativa*) is the most widely consumed cereal grain in South Asia in general, and ragi (finger millet, *Eleusine coracana*) is one of the staple foods in South India, particularly. In India, they are consumed as much by rural as by urban communities and by rich (especially rice) and poor (especially ragi) alike. Compared to legumes, cereals take less time to cook, are easily digested and cause no flatulence, due to the lack of raffinose series oligosaccharides (Tharanathan *et al.*, 1987).

The purpose of this study was to determine the relationship between the compositional/structural variations of cereal and legume starches with their wide *in vitro* digestibility differences. As a prelude to this, the isolation and physicochemical characterization of rice and ragi starches are described in this communication.

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MATERIALS AND METHODS

Materials

Locally available varieties of rice (Rc) and ragi (Rg) were purchased in the market. After cleaning, they were sun dried and finely ground in a plate mill to pass through 60 and 170 mesh sieves. Starch isolates I–IV were obtained by the differential sedimentation and centrifugation steps as reported earlier (Madhusudhan *et al.*, 1993).

Methods

Chemical composition

Starch content (glucose (Glc) × 0.9) was determined by digestion with glucoamylase followed by glucose oxidase assay of the released glucose (Dahlqvist, 1964), total sugar by modified phenol–H_2SO_4 (Rao & Pattabiraman, 1989), reducing sugar as described by Nelson (1944) and nitrogen/protein (N × 6.25) by the micro-Kjeldahl methods. Amylose content was determined by the Chrastil (1987) method. Free lipids (FL) were extracted with 1-propanol and water

saturated 1-butanol and the internally bound lipids (BL) from the defatted granules by 6 N HCl hydrolysis (100°C/2 h) followed by repeated petroleum ether (40–60°C) extractions. Fatty acid methyl esters (FAME) were prepared by reaction with dry methanolic-hydrogen chloride (4 N, 120°C/4 h, Metcalfe *et al.*, 1966) and analyzed by gas liquid chromatography (GLC) on 10% DEGS on Chromosorb W (100–120 mesh) column (SS, 6 ft × 1/8 inch i.d.) operating at 180°C isothermal.

Starch isolates (10 mg) were acid hydrolyzed (2 N H_2SO_4, 100°C for 6 h), neutralized (solid $BaCO_3$) and derivatized into alditol acetates (Paramahans & Tharanathan, 1980). Subsequent GLC was done in a Packard model 437 GC fitted with a flame ionization detector and a 5 ft × 1/8 inch SS column containing 3% OV-225 on Chromosorb W (HP, 80–100 mesh). The column was operated by the isothermal mode at 190°C and nitrogen was the carrier gas used (15 ml min^{-1}). The quantitation of the resolved components was done by the attached Packard model 604 recording data processor. Myoinositol was the internal standard used.

Physicochemical characteristics

The size, shape and birefringent characteristics of starch isolates were determined using a Carl–Zeiss photo-microscope, under ordinary and polarized light. The λ_{max} of the blue coloured complex with I_2–KI solution was read in a Shimadzu UV-Vis 160 A spectrophotometer. Solubility and swelling power in water, solubility in DMSO, ionic nature and hot paste viscosity in a Brabender viscograph model E and the amylolytic susceptibility with pancreatic α-amylase and glucoamylase of native granules and gelatinized starch suspension were studied as before (Madhusudhan *et al.*, 1993).

SDS–PAGE pattern of starch granule proteins

The granule-bound proteins were extracted from starch solution in 0.1 M NaOH (500 mg ml^{-1}) with SDS (1%, 3 ml) by heating at 95°C for ~30 min. SDS–PAGE was done according to the procedure of Laemmlis (1970) at a constant voltage (100 V) for 3 h and the separated protein bands were stained with Coomassie blue R-250. Molecular weights of the individual protein bands were computed by comparison with marker proteins of known molecular weight values.

Amino acids, liberated into vapour phase by hydrolysis with 6 N HCl (100°C for 24 h) were derivatized with phenylisothiocyanate and then separated and quantified by reverse phase HPLC using PICO-TAG columns (Waters Associates, Milford, USA).

RESULTS AND DISCUSSION

The microscopic examination indicated that Rc starches were of relatively smaller size than Rg starch isolates and both were poorly birefringent. From the relative granule size distribution shown in Fig. 1, it may be seen that the population density of larger granules (4–8 μm) was more in Rg than Rc starch. The granule size varied from 1.0 to 4.5 μm for Rc and 1 to 9 μm for Rg starch, and their shapes included small spherical to hexa-/polygonal granules. Hilum was seen only on a few big granules.

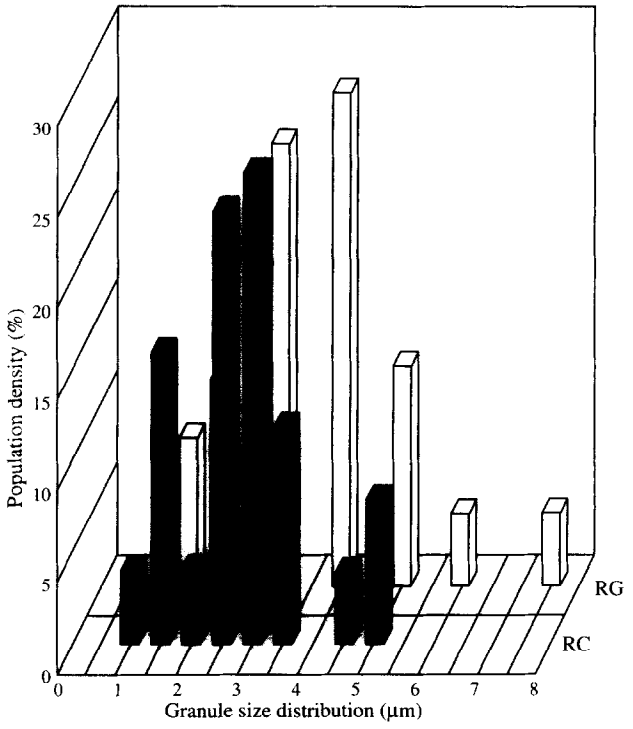

Fig. 1. Granule size distribution of Rc and Rg starches.

From Table 1, it may be inferred that the starch isolate I, in both the cereals are of relatively much higher purity. From isolate I to IV, the contents of nitrogen and protein increased. However, the later isolates contained considerable amounts of non-starch polysaccharides (~10–20%) as judged by the differences in the total carbohydrate content (as glucose) between the chemical and enzymatic methods. Isolate IV of rice starch was very low in starch content (only 10%) compared to Rg starch, and its purification was found to be extremely difficult. GLC analysis as alditol acetates of Rc starch isolate IV revealed the presence of small amounts (~10%) of rhamnose, arabinose and xylose, together with a huge glucose peak, derived from starch as well as may be the cellulosic material in it. No such sugar peaks except glucose, could be detected in isolates I/II.

The total lipid content of Rc and Rg starch isolate I was ~0.8%. They were richer in free lipids (~0.6%) than internal bound lipids (~0.2%). Their GLC analysis revealed the predominance of C16:0 in Rc and both C16:0 and C18:2 in Rg (Table 2). The ratio of saturated to unsaturated fatty acids was 1:0.77 and 1:0.81, respectively in Rc and Rg starch. The profile of minor (< 5%) fatty acids of these lipid fractions is shown in

Table 1. Proximate composition (%) of the starch isolates of rice and ragi

Source Isolates	Rice starch				Ragi starch			
	I	II	III	IV	I	II	III	IV
Yield	45.4	9.6	7.3	15.2	41.1	8.2	18.3	7.3
Moisture	8.9	8.9	9.7	9.9	8.9	8.9	9.1	9.4
Lipid								
Free	0.6	0.6	ND	ND	0.7	0.5	ND	ND
Bound	0.2	0.2	ND	ND	0.2	0.2	ND	ND
Protein								
Nitrogen (N)	0.05	0.05	0.60	0.70	0.14	0.17	0.40	0.60
N × 6.25	0.32	0.33	3.75	4.40	0.87	1.06	2.50	3.75
Total carbohydrates	91.0	85.0	90.0	30.0	90.0	89.5	83.0	45.0
Starch by TGO[a]	89.2	82.9	70.8	10.5	87.4	85.5	72.1	35.0
NSP[b]	1.8	2.1	19.2	19.5	2.6	4.0	10.9	10.0
Starch components								
Amylose	30.0	28.0	25.0	8.0	28.0	26.0	22.0	9.0
Amylopectin[c]	70.0	72.0	75.0	92.0	72.0	74.0	78.0	91.0
λ_{max}(nm)	619	ND	ND	ND	616	ND	ND	ND

[a] (Glc × 0.9)
[b] NSP = Total carbohydrate—starch
[c] 100—amylose; ND, not determined.

Table 2. Fatty acids (%) in free and bound lipid fractions of starches

Source	Rice starch								Ragi starch							
Fraction	Free lipids				Bound lipids				Free lipids				Bound lipids			
Isolates	I	II	III	IV	I	II	III	IV	I	II	III	IV	I	II	III	IV
C14:0	5.3	*	*	*	*	*	5.5	5.2	—	—	—	—	*	*	*	—
C16:0	44.1	40.0	46.1	38.0	26.9	50.8	51.8	42.8	35.8	29.8	23.4	35.0	27.7	39.0	21.0	39.2
C18:0	—	—	—	—	—	—	—	12.3	—	—	—	—	—	—	—	—
C18:1	—	—	—	—	—	—	7.6	13.7	—	—	*	—	—	—	—	—
C18:2	20.4	23.8	35.4	24.0	35.5	29.4	10.8	17.3	52.2	50.5	49.0	54.0	25.5	39.2	38.2	49.2
C18:3	27.0	23.1	22.1	27.4	22.5	16.3	*	—	7.5	7.1	12.2	7.8	27.0	*	20.1	5.7
C20:0	—	—	—	—	—	—	5.8	—	—	10.2	7.0	—	8.6	—	—	—
Unidentified fatty acids	—	—	—	—	8.8	—	8.7	*	—	—	*	—	—	*	9.5	16.4

* < 5.0%; —, not present.

Fig. 2. From the retention time characteristics, the unidentified peaks could probably represent the long chain fatty acids. It has been reported that high amylose starches contain more lipids than normal starches, and thus a positive correlation between the amylose and lipid contents has been proposed (Morrison, 1988). High amounts of lipids confer resistance to mechanical damage and α-amylolysis of starch. The lower glucose and insulin responses in man after ingestion of high amylose rice compared to rice with no amylose have been attributed to the presence of amylose–lipid complexes in the former (Goddard *et al.*, 1984). Nutritionally, the starch lipids are beneficial as they provide a considerable proportion of essential fatty acids, especially for vegetarians whose staple food constitutes cereals and legumes.

The total protein content of isolates I was low (<0.5%) compared to isolates IV (~5%). It is suggested that the starch granule surface may act as ion-exchanger to which the protein by virtue of its basic character may adhere (Lowy *et al.*, 1981). Rigorous conditions are indeed required to extract the firmly bound protein(s) in the granule. Nevertheless, the glycoprotein nature of starch and glycogen proposed by Whelan (1992) lends support to the findings of small amounts of protein even after repeated purification steps. A covalently bound protein–maltosaccharide molecule, named glycogenin, has been identified in rabbit muscle glycogen preparation. Glycogenin acts as an endogenous primer for the synthesis of glycogen and starch molecules. Starch–protein interactions, especially in the form of a matrix encapsulating (some) starch granules have been shown to reduce the overall digestibility *in vitro* (Slack *et al.*, 1979) and also in the

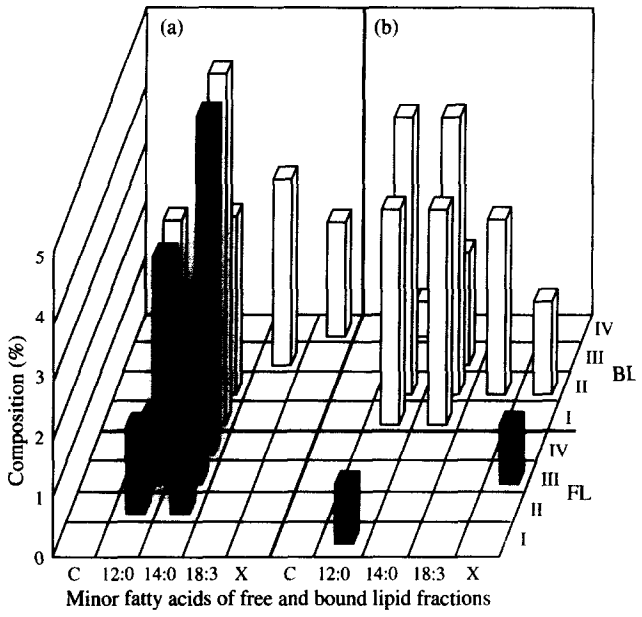

Fig. 2. Minor fatty acids of FL and BL fractions of Rc(a) and Rg(b) starches; X, unidentified fatty acids.

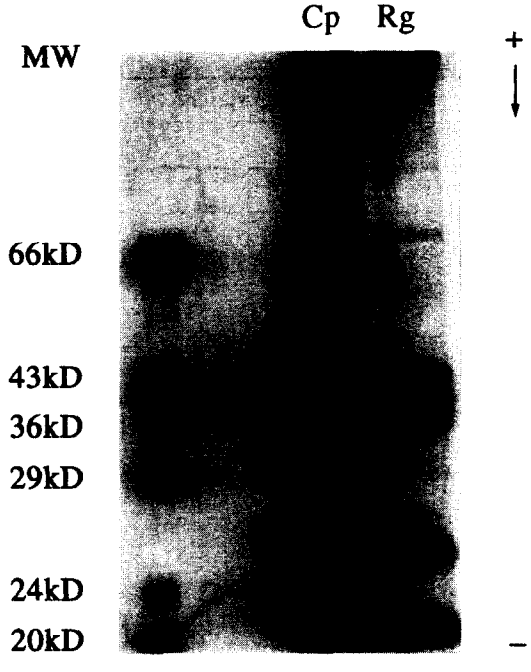

Fig. 3. SDS–PAGE patterns of starch granule proteins extracted from Rg and chickpea starches.

human gastrointestinal tract, as reported for barley (Twish, 1970), maize (McNeill *et al.*, 1975) and sorghum (Würsch *et al.*, 1986). *In vitro* prior denaturation or predigestion of the protein with pepsin, however, has resulted in an increased accessibility of α-amylase to the starchy substrate (Malouf *et al.*, 1992).

The SDS–PAGE pattern of Rg starch protein together with that of a legume starch (chickpea, *Cicer arietinum*) protein for comparison, is shown in Fig. 3. This contrasts with what was observed in wheat starch

Table 3. Amino acid composition (pmole %) of starch-bound proteins

Amino acid	Source	
	Chickpea starch	Ragi starch
Asp	20.5	10.5
Glu	21.7	12.8
Ser	6.7	7.7
Gly	7.3	13.5
Arg	5.7	3.7
Thr	3.3	4.0
Ala	5.2	9.1
Pro	4.9	7.2
Tyr	1.0	2.8
Val	4.4	6.0
Met	—	3.2
Cys	—	3.7
His	1.5	—
Ileu	3.5	2.3
Leu	6.3	5.8
Lys	3.9	4.7
Phe	3.9	2.6

where SDS treatment left proteins of ~59 kDa and larger unextracted, which later could be removed only after pronase treatment (Malouf *et al.*, 1992). SDS at 95°C removed low molecular weight proteins (~20–43 kDa) from Rg starch. Considerable differences were seen in the amino acid composition of these starch-bound proteins (Table 3). Rg starch protein contained fewer aspartic and glutamic acids, but more glycine. Histidine was absent in Rg starch protein unlike both methionine and cysteine in chickpea starch protein. The content of hydrophobic amino acids was high in Rg starch protein.

The amylose content of isolates I–III ranged between 22 and 30% in both starches. The λ_{max} of the resulting blue-coloured complex was ~620 nm, slightly less than that of pure amylose fraction (~630–650 nm).

Similar to other cereal and millet starches (Muralikrishna *et al.*, 1982; McNeill *et al.*, 1975), both rice and ragi starches were non-ionic and exhibited a single stage swelling and solubility pattern in water (Fig. 4). In DMSO, the rice starch attained almost a total solubility in just ~60 h compared to only ~61% solubility for Rg starch isolate I (Fig. 5). The higher DMSO solubility of Rc starch shows an easy penetration of solvent molecules into the granular matrix, due to heterogeneous bonding forces. A very high solubility of >90% in just 20 h is reported for pigweed starch (Goering, 1978). DMSO solubility is also a measure of amylolytic susceptibility of starches, the higher the solubility the better is the enzymic digestibility (Leach & Schoch, 1962).

The pasting characteristics of starches at different reference points are given in Table 4. The Rg starch isolates I/II exhibited a slightly higher hot paste viscosity (~300 BU) than those of Rc starch isolates

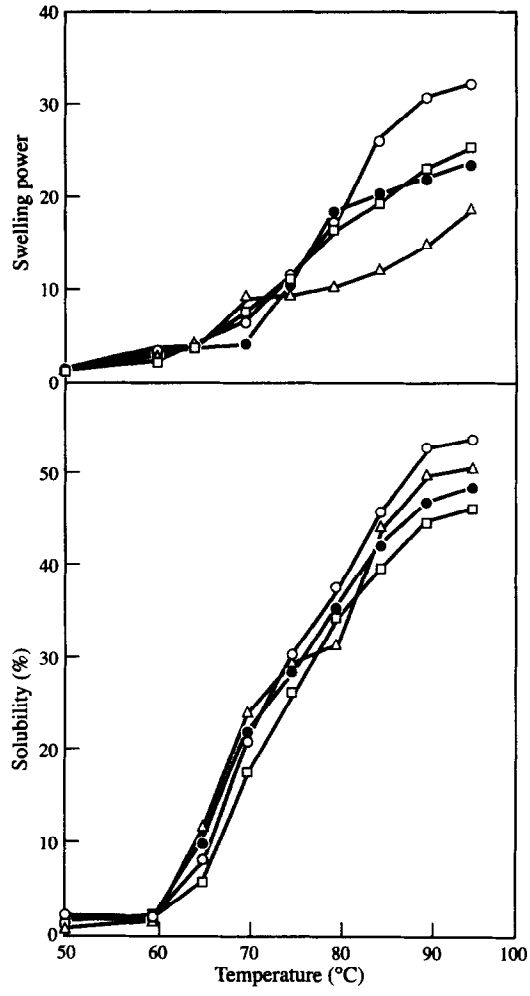

Fig. 4. Swelling power and % solubility in water of Rc and Rg starch isolates; RcS-I, -○-; RcS-II, -△-; RgS-I, -●- and RgS-II, -□-.

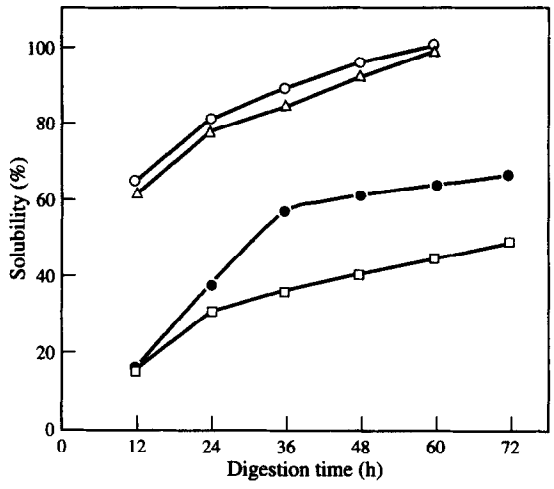

Fig. 5. Solubility in DMSO of Rc and Rg starch isolates, RcS-I, -○-; RcS-II, -△-; RgS-I, -●- and RgS-II, -□-.

Table 4. Pasting characteristics (BU) of starch isolates

Isolate	PT (°C)	P	H	C	C–H	H/P	C/P	C/H
Rc I	76.0	250	220	360	140	0.88	1.44	1.64
Rc II	83.5	200	200	320	120	1.00	1.60	1.60
Rg I	86.5	330	310	420	110	0.94	1.27	1.35
Rg II	80.5	280	250	380	130	0.89	1.36	1.52

PT = Pasting temperature
P = Peak viscosity
H = Hot paste viscosity
C = Cold paste fiscosity
C–H = Set back
C/P = Set back ratio
C/H = Total set back ratio
H/P = Break down ratio

Table 5. *In vitro* digestibility of Rc and Rg starch isolates

Starch		% hydrolysis		
		Native		Gelatinized glucoamylase
		Glucoamylase	Pancreatic α-amylase	
Corn starch		5.7	57.1	72.9
Rice starch	I	6.6	59.2	78.3
	II	ND	ND	71.5
	III	ND	ND	63.1
	IV	ND	ND	44.3
Ragi starch	I	5.8	55.8	70.2
	II	ND	ND	66.1
	III	ND	ND	51.0
	IV	ND	ND	30.8

(~200 BU). Their setback viscosity (C–H) increase was also minimal indicating a rather low retrogradation tendency due to restricted swelling by the strong granular matrix. Both starches showed negligible breakdown ratio (H/P) and, in particular, Rg starch isolates showed a lower total setback ratio (C/H) in comparison to that of Rc starches, which fits in well with its (Rg) low swelling power in water and DMSO, and also its low digestibility values. Differences in the pasting temperature and viscosity of starches, in general, are attributable to the adherance of hydrophobic proteins and surface lipids on the granules, which would affect the ability of granules to swell. In fact, removal of surface lipids by solvent extraction permits substantial granule swelling even at low pasting temperature (Sosulski *et al.*, 1989).

The *in vitro* digestibility of starches, in their native and cooked forms is shown in Table 5. Rg starch granules were slightly less digestible with pancreatic α-amylase than the Rc starches. In the native state, amylolysis with pancreatic α-amylase was better than with glucoamylase. In gelatinized form the Rc starch isolate I was hydrolysed by glucoamylase to ~78%, a value slightly higher than that for corn starch (~73%) used as control. The greater digestibility of Rc starch can also be correlated with its high solubility in water

and DMSO, and slightly lower protein (and total lipid) contents. Owing to a higher surface area and, therefore, better adsorptive capabilities the small sized Rc starch has been reported to be more digestible *in vitro* (Meredith, 1980). The slight differences in the digestibility of these starches could be accounted for, in part by the damaged starch formed during plate milling and also by the resistant starch formed during sample preparation steps (unpublished data). Resistant starch is nothing but retrograded amylose (Gruchala & Pomeranz, 1993). High amylose varieties of maize are poorly digested in both humans and mice, and it has been shown that amylomaize starch is far more resistant to swelling and gelatinization than normal starches (Wolf *et al.*, 1977). Enhancement of digestibility of gelatinized starches has been attributed to the swelling and rupturing of starch granules, which facilitate an easy access for the enzyme to effect hydrolysis (Lee *et al.*, 1985). In general, starch digestibility is essentially dependent on the source as well as on the nature of starch *per se*.

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