

Carbohydrate Make-up of Minor Millets

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Starchy and non-starchy carbohydrates of samai, sanwa and panivaragu have been isolated and characterized. Starch isolated exhibited single stage swelling, moderate solubility in water, but a very high solubility in DMSO, and non-ionic character similar to several starches from *Leguminosae*. Treatment with mild alkali resulted in the separation of big-hexagonal, and small-spherical granules. Considerable retrogradation of the linear amylose fraction was observed. Hemicellulose A was shown to be a non-cellulosic glucan, whereas hemicellulose B was composed of hexoses, pentoses and uronic acids in varying proportions. The alkali-insoluble residues were exclusively composed of glucose and thus constituted the fibre fraction.

Kohlenhydratzusammensetzung von Kleinhirsearten. Stärke und Nichtstärke-Kohlenhydrate von Samai, Sanwa und Panivaragu wurden isoliert und charakterisiert. Die isolierte Stärke zeigte einstufige Quellung, mäßige Löslichkeit in Wasser, jedoch eine sehr hohe Löslichkeit in DMSO sowie nichtionischen Charakter, ähnlich verschiedenen Stärken aus Leguminosen. Milde Alkalibehandlung resultierte in der Trennung in groß-hexagonale und klein-sphärische Körner. Beträchtliche Retrogradation der linearen Amylosefraktion wurde beobachtet. Es wurde gezeigt, daß Hemicellulose A ein nicht-celluloseartiges Glucan ist, wogegen sich Hemicellulose B aus Hexosen, Pentosen und Uronsäuren in verschiedenen Mengenverhältnissen zusammensetzt. Die alkaliunlöslichen Reste waren ausschließlich aus Glucose zusammengesetzt und stellten somit die Faserfraktion dar.

1 Introduction

Scientific information on the carbohydrate make-up, particularly the starch component of minor millets is limited to only a few [1–7]. Some studies on the proximate composition of ragi (finger millet, *Eleusine coracana*) [1], navane (foxtail millet, *Setaria italica*) [1], and varagu (*Paspalum scrobiculatum*) [2] conducted in our laboratory have shown that starch is the principal carbohydrate of these millets. Apart from the possible subtle variations in molecular weight and chain length of starch components, *i. e.* amylose and amylopectin, no significant differences are discernible in several other physico-chemical properties such as peak-viscosity (P.V.) in Brabender amylogram, gelatinization temperature range, amylose/amylopectin ratios and solubility/swelling power behaviour in water and DMSO. The presence in these millets of various non-starchy carbohydrates ranged from 2 to 6% and all were shown to be mostly polysaccharide-complexes composed of hexoses, pentoses and uronic acids [1,2].

In view of finding useful technological applications for some of the minor millets it was felt that a systematic investigation on their carbohydrate make-up, particularly the digestible and non-digestible carbohydrates is highly desirable. The present communication describes the results of such a study on three minor millets, namely samai (*Panicum miliare*), sanwa (*Echinochloa frumentaceae*) and panivaragu (proso millet, *Panicum miliceum*). The *in vitro* digestibility of these starches in raw and uncooked form will be published elsewhere.

2 Materials

The millet grains were obtained from the University of Agricultural Sciences, Coimbatore, Tamil Nadu. They were dehulled and milled in a centrifugal sheller followed by aspiration in a Setake aspirator and used wherever necessary as 60 mesh powder.

3 Methods

3.1 General

Starch was estimated as glucose independently both by chemical [8] (phenol- H_2SO_4) and by enzymatic [9] (prior

amyolysis followed by glucose oxidase assay of the liberated glucose) methods. Moisture and ash were determined by AOAC methods [10] and protein by micro Kjeldahl ($N \times 6.25$) method.

3.2 Isolation of Carbohydrate Fractions

Preliminary extraction of millet flour with 70% ethyl alcohol yielded the soluble mono- and oligo-saccharides. The alcohol-insoluble residues obtained, thus were repeatedly extracted with water (containing 0.01-M $HgCl_2$ solution) to recover starch granules. The starch-free residues were then extracted with 10% NaOH according to the method of Whistler and Feather [11] and processed to recover hemicellulosic (A and B) and cellulosic fractions.

Sometimes starches were also isolated by steeping the grains in water (with 0.01-M $HgCl_2$) overnight, homogenizing in a waring blender and repeated sieving through different meshes. Crude starch was purified by stirring with dilute NaOH (pH 9 for 5 min) followed by repeated 0.2-M NaCl-toluene treatments.

3.3 Separation of Different Starch Granule Populations

Mild NaOH (to pH 9) treatments of aqueous starch suspensions (at room temperature) followed by a brief centrifugation (2000 rpm for 10–15 min) furnished an opalescent supernatant and purified starch sediment in each case. Light microscopy of the former revealed predominantly small-spherical granules; whereas the purified starch sediment contained exclusively big hexa- and polygonal granules.

3.4 Sugar Analyses

This was done by chromatographic methods as described earlier [2]. Alcohol-soluble sugars, after purification by successive passage through Dowex-1 \times 8 (OH^-) and Dowex-50 W (H^+) resins were analysed as such; whereas bound sugars were analysed after liberation by prior solubilization and preliminary hydrolysis with 72% H_2SO_4 (at 4°C for 1 h) followed by complete hydrolysis with 1-N acid (at about 95°C for 10 h).

3.5 Microscopy of Starch Granules

Zeiss photomicroscope was used to determine the shape, size and birefringence characteristics of different starch granule populations.

3.6 Viscosity Determination

The relative viscosity (η_r) of starch solutions prepared in 1-N KOH at 0.5% concentration was determined in an Ostwald U-shaped viscometer using the following equation [12] $\eta_r = t_s/t_0$ where t_s and t_0 are the flow time for starch solution and solvent respectively. The inherent viscosity η_i is the natural logarithm of η_r divided by the sample concentration in grams per 100 ml and was calculated by (1):

$$\eta_i (\text{dl/g}) = \frac{2.303 \times \log}{0.5 \text{ g} \times 100 \text{ ml}} \quad (1)$$

3.7 Miscellaneous Methods

Swelling and solubility behaviour of starches in water and DMSO, pasting characteristics in the Brabender amylograph, gelatinization temperature range, ionic character, and starch-I₂ blue colour determination were all done by the methods reported earlier [1, 3].

4 Results and Discussion

4.1 Digestible Carbohydrate (Starch)

Starch, the major carbohydrate of the millets, was isolated in an yield of 56–65%. From the results presented in Table 1 it is clear that both the chemical and enzymatic methods give comparable values for the glucose content in starch. The protein content of all the three starches was quite high and ranged from 3.4 to 5.5%. In spite of repeated purifications the protein content could not be reduced below 3%. However, millet starches are known for high protein contaminations for reasons not clearly known yet. The protein content of ragi and navane starches was ~1.4% whereas varagu starch contained unusually a low protein content (0.2%) [2].

Table 1.
Chemical Characteristics of Millet Starches.

	Sanwa	Samai	Panivaragu
Yield (%)	62.0	56.8	60.2
Moisture (%)	10.7	10.7	10.9
Ash (%)	1.1	1.4	1.1
Protein (% N × 6.25)	4.1	3.3	5.5
Total sugar (as glucose)			
a) phenol-H ₂ SO ₄ method	90.2	85.6	87.0
b) Enzymatic method	85.5	85.5	81.0
Granule size (μ)			
a) small-spherical	2.5–7.5	1.25–5	1.25–12.5
b) big-hexagonal	5–20	7.5–17.5	5–17.5
c) total	2.5–20	1.25–17.5	1.25–17.5
Hilum	Centric faint	faint	Centric
Gelatinization temperature ¹⁾ range (°C)	50–64–70	55–60–72	68–72–76
Amylose (%)	19.7	18.4	24.5
Amylopectin (%)	80.3	81.6	75.5

¹⁾ The values represent the initial, middle and final gelatinization temperatures.

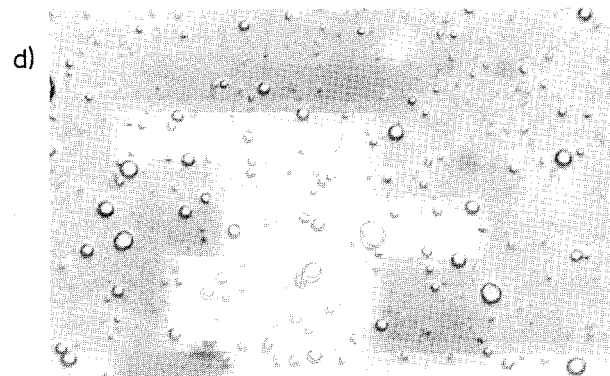
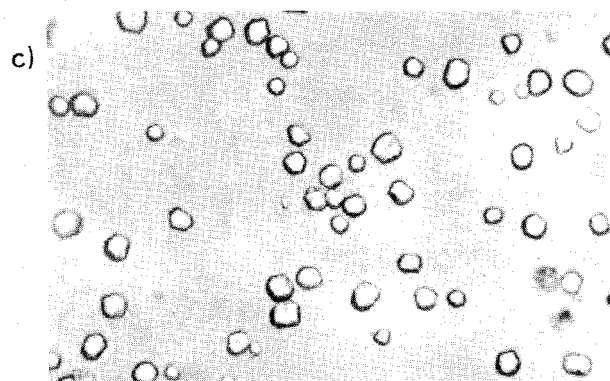
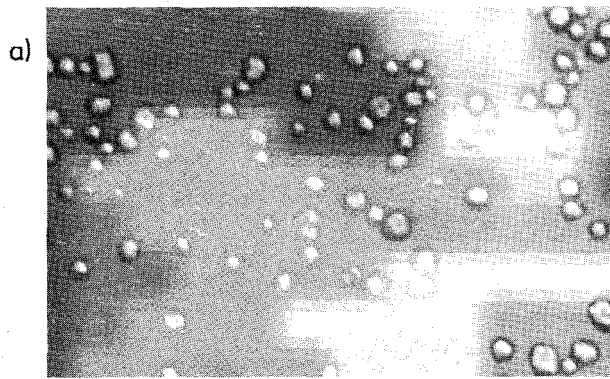
From the photomicrographs (Figs. 1, 2 and 3) it is evident that these starch granules possess mixed granule populations of different shapes and sizes, ranging from small-spherical granules (Figs. 1d, 2d and 3d; Table 1), to big hexa-polygonal granules (Figs. 1b/c, 2b/c and 3b/c; Table 1). The small-spherical granules possessed very poor birefringent characteristics for reasons not known yet. Attempts are under way to investigate the variations in physicochemical characteristics of these various granule populations. The separation of the former was possible during the starch purification by mild alkali treatment. However, repeated centrifugations even at 20,000 g for as long as 2 h did not completely sediment the small granules and hence the properties of these granule populations could not be studied separately. As shown in wheat starch [13] the pasting characteristics, differences in the digestibility and also the content of amylose all depend on the interactions between different sized granules. In the case of amylo-maize and dent-corn starches [14] the bigger filamentous granule (with a length to width ratio greater than 3.0) are shown to have a higher proportion of amylose than the other starch granule, *viz.*, spherical (L/W < 1.3) and ellipsoidal (L/W, 1.3 to 3.0). The size differences in the starch granules are more pronounced in legume starches than in cereal starches [15].

Considerable variations do exist in the temperature range of gelatinization (G.T.). Samai starch, though having a wider G.T. range has a very low initial value, *i.e.* 55°C in comparison to panivaragu starch having a higher initial value (68°C). Such gross variations in G.T., in turn the rate of swelling might possibly be due to (1) granule size distribution and (2) heterogeneous nature of bonding forces within the starch granules. Small sized granules are known to have higher G.T. than large sized granules [16]. It is therefore likely that in panivaragu starch the granules are tightly associated resulting in restricted but slow rate of swelling. A restricted swelling of legume starches at higher temperatures has previously been reported [17, 18].

The amylose content of starches as determined by the "blue value" method [19] ranged between 17–26%, slightly less than the value reported for several legume starches [20]. Brabender amylographic data (Fig. 4) indicated considerable viscosity characteristics. The peak viscosity (P.V.) of the three starches was around 700 B.U., while the viscosity at the set back (S.B.V.) was more for sanwa starch (1230 B.U.) followed by samai starch (1070 B.U.) and panivaragu starch (950 B.U.). On the other hand the 60 mesh flour of the millets exhibited different pasting characteristics, in that sanwa flour had higher P.V. of 380 B.U. and S.B.V. of 840 B.U., whereas samai and panivaragu flours had comparatively lower peak viscosity values. Samai flour had the highest S.B.V. of 1040 B.U. In comparison varagu [3] starch granules showed poor pasting characteristics, exhibiting a P.V. of 700 B.U. and a very low S.B.V. of about 400 B.U., as observed in several other starches [21, 22].

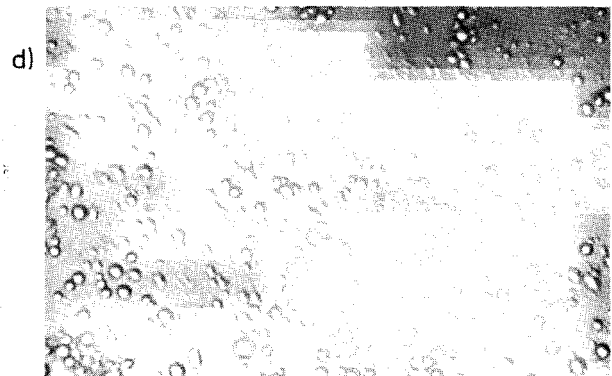
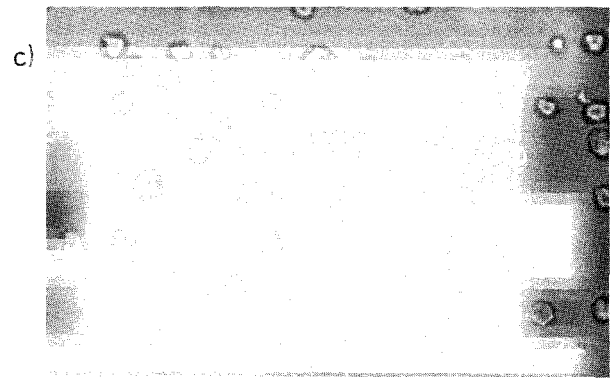
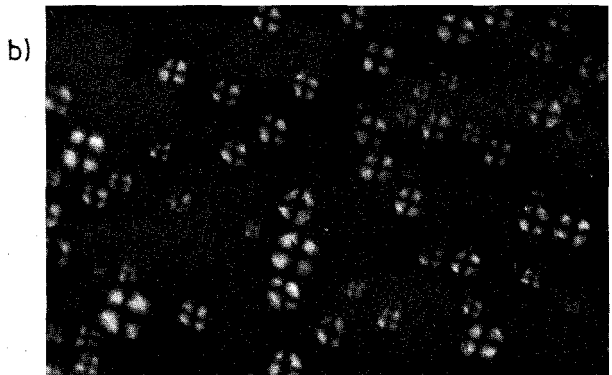
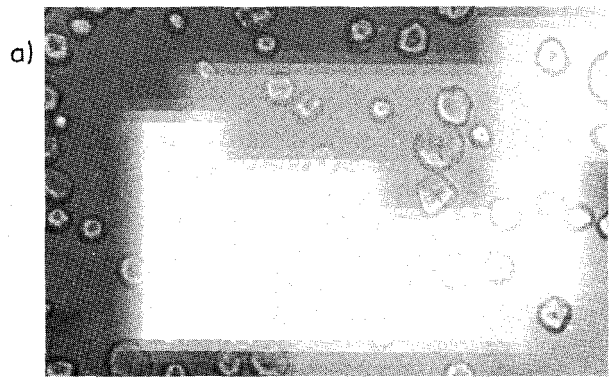
The swelling and solubility behaviour of the starches in water is shown in Figure 5. All the starches exhibited single stage swelling, panivaragu showing the lowest swelling power (~17.5 at 90°C). Their solubility at 90°C ranged from 46–74%, slightly higher than the values reported for other millet starches [1, 3]. Interestingly all the starches had very high solubilities in DMSO, almost 100% solubles even after 10 min. This is indicative of an easy penetration of solvent into the strongly bonded micellar granule structure. Varagu starch took ~30 h to reach this solubility [3] whereas starches from ragi and navane were comparatively less soluble [1].

Viscosity determinations revealed that (Table 2) the millet



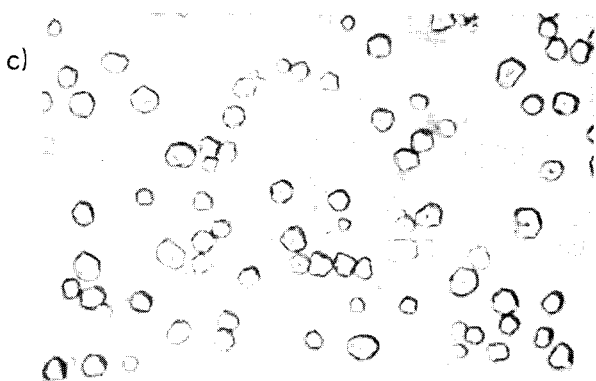
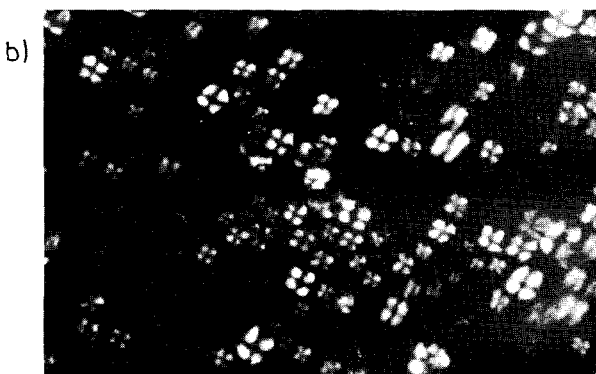
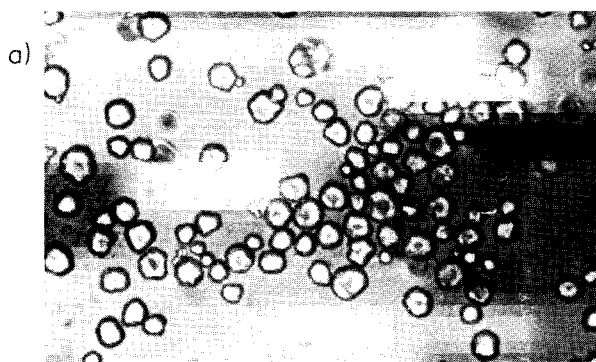
S 33.1

Figure 1. Photomicrographs of samai starch granules ($\times 400$). a) total granules, under ordinary light; b) total granules, under polarized light; c) big-hexagonal granules; d) small-spherical granules.



S 33.2

Figure 2. Photomicrographs of sanwa starch granules ($\times 400$). a) total granules, under ordinary light; b) total granules, under polarised light; c) big-hexagonal granules; d) small-spherical granules.



S 33.3

Figure 3. Photomicrographs of panivaragu starch granules ($\times 400$). a) total granules, under ordinary light; b) total granules, under polarised light; c) big-hexagonal granules; d) small-spherical granules.

starches in comparison to corn starch have a higher intrinsic and inherent viscosity in alkaline solutions. This in part is attributed to differences in molecular size and shape of the constituent fractions in starch granules.

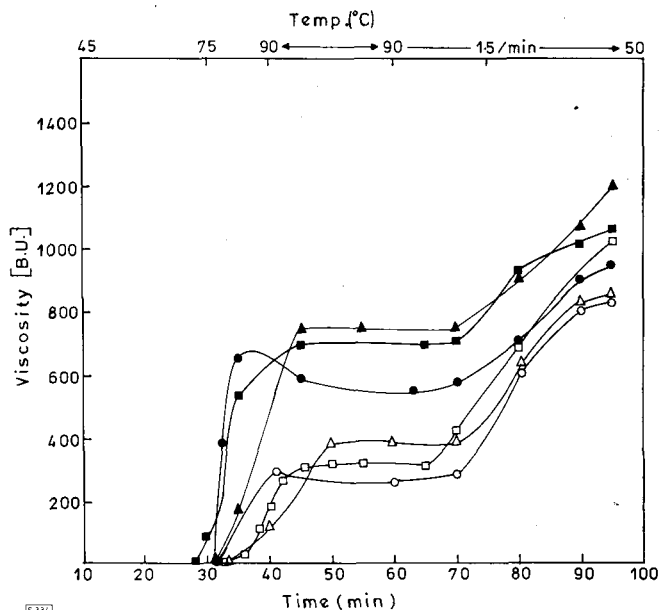


Figure 4. Brabender amylograms of millet starch and flour. \square - \square = Samai flour and starch, respectively; \triangle - \triangle = sanwa flour and starch, respectively; \circ - \bullet = panivaragu flour and starch, respectively.

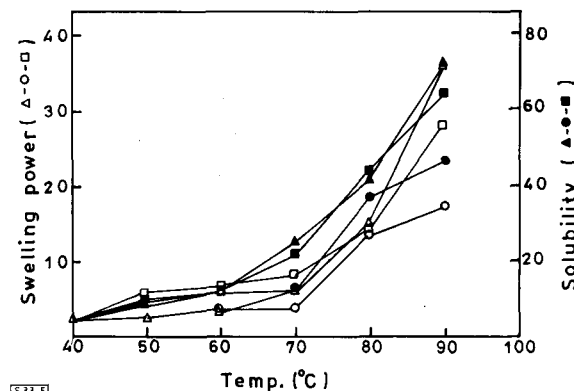


Figure 5. Swelling/solubility behaviour of millet starches. \triangle - \triangle = Samai starch, swelling/solubility; \square - \square = sanwa starch, swelling/solubility; \circ - \bullet = panivaragu starch, swelling/solubility.

Table 2. Intrinsic and Inherent Viscosity of Millet Starches.

	η_r	η_i (dl/g)
Panivaragu	2.06	0.144
Sanwa	2.07	0.145
Samai	1.84	0.123
Corn	2.14	0.152

4.2 Non-starchy Carbohydrates

The alcohol-soluble sugars of the millets obtained in 6–7% yield, were shown to be fructose and sucrose (1:1) with small amounts of raffinose. The quantitative sugar pattern was more or less similar in all the three millet extracts. *Becker and Lorenz* in their studies on proso and foxtail millet saccharides [4] have reported major amounts of sucrose followed by raffinose and myo-inositol together with trace amounts of glucose, fructose and galactose. The presence of myo-inositol might be attributed to phytic acid, a common constituent of stored cereal grains [23].

Table 3.
Composition of Non-digestible Carbohydrates of Samai, Sanwa and Panivaragu.

	Yield ¹⁾	Samai Sugars ²⁾ detected	Hexose: Pentose	Yield ¹⁾	Sanwa Sugars ²⁾ detected	Hexose: Pentose	Yield ¹⁾	Panivaragu Sugars ²⁾ detected	Hexose: Pentose
Hemicellulose A	0.65	Glc, Ara, Xyl (trace)	—	0.28	Glc, Ara, Xyl (trace)	—	0.21	Glc	—
Hemicellulose B	0.42	Glc, Ara, Xyl, Uronic acid (1:0.1:0.1:0.05)	1:0.2	0.16	Glc, Ara, Xyl, Uronic acid (2:1:1:0.2)	1:1	0.17	Glc, Gal, Ara, Xyl, Uronic acid (0.2:0.2:2:1:0.2)	0.4:3
Cellulose	0.88	Glc, small amount of Xylose	—	2.69	Glc, small amounts of Xylose	—	3.79	Glc	—

¹⁾ Percent yield based on the dryweight of the millet grain.

²⁾ The values in parenthesis represent relative proportion of sugars Glc (Glucose); Ara (Arabinose); Xyl (Xylose) etc.

Subsequent alkali extractions of the alcohol-insoluble residues furnished, in each case, hemicellulose A and B, and cellulose (alkali-insoluble residue, Table 3). Hemicellulose A was exclusively composed of glucose indicating it to be a non-cellulosic glucan-type polysaccharide. On the other hand, hemicellulose B was composed of residues of hexoses, pentoses and uronic acids. Sanwa hemicellulose B had glucose and arabinose/xylose in equal concentrations; whereas samai hemicellulose B had more of glucose (hexose/pentose ratio of 1:0.2). Contrary to these, the hemicellulose B from panivaragu contained more of pentoses than hexoses. Interestingly it also contained galactose as an additional hexose constituent. In the pentosans of finger millet (hamsa and purna varieties) galactose has been shown to be absent [6]; whereas in varagu hemicellulose B galactose and glucose were shown to be present in almost equal amounts.

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