*Phytochemistry*, Vol 22, No 1, pp 151–159, 1983
Printed in Great Britain

0031-9422/83/010151-09$03 00/0


# PROPERTIES OF CELL WALL PREPARATIONS OF MILLED RICE

CYNTHIA G PASCUAL and BIENVENIDO O JULIANO

Chemistry Department, The International Rice Research Institute, Los Baños, Laguna, Philippines

(*Revised received* 22 *June* 1982)

**Key Word Index**—*Oryza sativa*, Gramineae, milled rice cell walls, non-starch polysaccharides

**Abstract**—Cell walls were prepared from milled rices of eight varieties, differing in direction of grain expansion during cooking (length-wise or girth-wise), by SDS-β-mercaptoethanol extraction, starch gelatinization at 80° and *Bacillus licheniformis* α-amylase treatment at 70° The cell wall preparations were recovered at 0 3–0 7% of milled rice and had similar neutral sugar composition and solubility fractions in water and alkali Polysaccharides containing uronic acid, arabinose, xylose and glucose, and phenols were preferentially extracted with $H_2O$ at 100° and dilute alkali in detailed studies of IR32 cell walls Iodine–potassium iodide staining polysaccharide was eluted from DEAE-cellulose CC of aqueous, oxalate and 4 M potassium hydroxide fractions of IR32 walls with 0 25 M sodium hydroxide Proteins in cell wall preparations were mainly protein bodies and were not bound to polysaccharides based on gel electrophoresis and TCA precipitation The cell wall preparations had a hardening effect on waxy-rice starch gel

## INTRODUCTION

Although a minor fraction of milled rice [1], the cell wall is important in maintaining grain integrity during milling and cooking The water-insoluble wall maintains grain integrity and probably influences the relative direction of grain expansion during cooking (lengthwise vs girthwise) as a result of starch granule swelling [2] The water-soluble fraction of cell wall has been shown to affect the pasting viscosity of rice flours [3] Cellulase treatment of milled rice results in a lower pasting viscosity of the flour suggesting changes in cell wall properties during aging of freshly harvested rice may be involved in the increase in flour viscosity during aging [4]

Recent characterization studies of milled-rice cell walls [5–8] reflect the renewed interest on this minor fraction in view of its complexing ability with starch and as a dietary fibre source [9, 10] Commercial amylase [11] and protease [12] preparations used for preparing cell walls may contain contaminant glycohydrolases which can degrade the cell walls during preparation The cell walls of milled rice are relatively fragile and not amenable to the non-aqueous method of isolation of whole wall developed for barley and wheat [13] Because rice is boiled before consumption, fractionation into water-soluble and -insoluble substances has practical value This paper presents our study of the properties of IR32 milled rice cell wall preparations and those of other varieties differing in elongation characteristics during cooking in view of the above advances in methodology since our earlier study [14] A companion paper [15] deals with the cell walls of bran and germ Studies on the water-soluble fraction are in progress [16]

## RESULTS

### *Preparation of milled-rice cell walls*

Protein extraction preceded starch gelatinization since heating reduces protein extractability [17] Four extractions with 0 5% SDS–0 6% β-mercaptoethanol (ME) at a rice–solvent ratio of 1 20 (w/v) gave better extraction of protein from IR32 and IR36 than seven extractions with a 1 2 (w/v) rice–solvent ratio (> 99% vs 91–98%) Residual protein in the cell wall preparations represented 0 2–2 3% of milled-rice protein

Heating to 80–85° effectively gelatinized the starch granules even of samples with intermediate final gelatinization temperature (70–74°) (D25-4, Palman 246, IR36, and IR32) making them susceptible to bacterial α-amylase action The 0 05 M sodium acetate buffer was preferred to calcium phosphate buffer for α-amylolysis as additional $Ca^{2+}$ was not required for the stability of the *Bacillus licheniformis* α-amylase Residual $Ca^{2+}$ content of cell wall preparations ranged from 0 7 to 1 9% in the latter buffer. Preheating the α-amylase at 80° for 15 min effectively inactivated the glucanase activity [18] The resulting destarched cell walls were negative for blue staining with iodine–potassium iodide reagent and for raw starch granules with birefringence (Maltese cross) under polarized light

### *Properties of the cell wall preparations*

Four varieties each of rice expanding during cooking mainly lengthwise (elongation ratio > 1 65) and mainly girthwise (elongation ratio < 1 6) gave overlapping values for gel consistency (a measure of gel viscosity in 0 2 N potassium hydroxide) [19], crude fibre, and neutral detergent fibre (Table 1) Recovery of cell wall preparations from these eight varieties were lower than the neutral detergent fibre content but higher than the crude fibre content of the milled rice, except Calrose which had 0 54% neutral detergent fibre, but yielded 0 57% cell wall preparation Basmati 370 gave the lowest recovery of 0 26% cell wall with the lowest protein content of 6%

Polysaccharides were the major component of the cell wall preparations of which polyuronic acid was a fraction (Table 1) The polysaccharide figure was underestimated

Table 1 Summary of properties of eight varieties of milled rice differing in grain elongation during cooking and in their cell wall preparations

| Property | Elongating rice* Range | Elongating rice* Mean | Non-elongating rice† Range | Non-elongating rice† Mean |
|---|---|---|---|---|
| Milled rice | | | | |
| Elongation ratio | 1 68–2 10 | 1 82 | 1 42–1 53 | 1 46 |
| Gel consistency (mm) | 43–80 | 60 | 34–100 | 69 |
| Crude fibre (%) | 0 12–0 17 | 0 15 | 0 10–0 22 | 0 17 |
| Neutral detergent fibre (%) | 0 54–0 84 | 0 72 | 0 62–0 75 | 0 66 |
| Cell wall preparations | | | | |
| Recovery from milled rice (%) | 0 26–0 70 | 0 52 | 0 40–0 58 | 0 48 |
| Polyuronic acid content (%) | 3–5 | 4 | 2–5 | 4 |
| Polysaccharide content (%) | 46–72 | 60 | 43–68 | 59 |
| Protein content (%) | 6–36 | 21 | 9–42 | 22 |
| Neutral sugar ratio | | | | |
| Rha | 2–4 | 2 | 2–3 | 2 |
| Fuc | trace–1 | <1 | trace | tr |
| Ara | 26–32 | 30 | 21–31 | 26 |
| Xyl | 29–36 | 32 | 27–40 | 33 |
| Man | 19–22 | 20 | 1–29 | 14 |
| Glc | 9–17 | 12 | 14–30 | 20 |
| Gal | 3–5 | 4 | 3–4 | 3 |
| Solubility fractions | | | | |
| Hot-$H_2O$ | 6–9 | 8 | 6–14 | 10 |
| 4 M KOH, TCA-soluble | 40–54 | 48 | 37–43 | 40 |
| 4 M KOH, TCA-insoluble | 2–6 | 3 | 3–17 | 10 |
| 6 M NaOH–0 81 M $H_3BO_3$ | 13–15 | 14 | 5–14 | 9 |
| Residue | 22–30 | 27 | 21–41 | 34 |
| IR29 starch gel consistency (mm)‡ | | | | |
| Whole preparation | 49–52 | 50 | 41–55 | 48 |
| Hot-$H_2O$ fraction | 46–55 | 52 | 34–60 | 46 |
| 4 M KOH, TCA-soluble fraction | 40–58 | 49 | 49–60 | 56 |

*Basmati 370, Dumzard, D25-4 and Calrose

†Palman 246, IR36, IR32 and UPL-Ri-1

‡Control gel consistency for IR29 starch (100 mg/1 6 ml $H_2O$) (mean of 1 and 2 mg adjuncts) 58 mm for whole, 62 mm for $H_2O$ fraction and 65 mm for 4 M KOH fraction runs

because of the poor colour development of uronic acid with phenol–sulphuric acid [20] The principal neutral sugars were arabinose, xylose, glucose and mannose together with galactose, rhamnose and fucose An exception was the waxy sample (1 5% amylose) UPL-Ri-1 which had only 1% mannose All these sugars were reported by Shibuya and Iwasaki [6] for their milled rice cell walls but fucose was not detected by Mod *et al* [5], and Cartaño and Juliano [14], using PC, did not detect rhamnose, fucose and mannose The elongating and non-elongating rices gave similar carbohydrate composition of their cell wall preparations

Staining with Congo red showed the walls to be in the form of glassy, pleated sheets with some striations Cell walls of D25-4 and Basmati 370, elongating rices, had smoother surfaces on SEM than those of IR36, a non-elongating rice Spherical protein bodies in aggregates were noted on the wall surface, the relative abundance closely following the protein content of the preparation, as confirmed by SEM Shibuya and Iwasaki [6] also reported on protein body contamination of their milled-rice cell wall preparation

Fluorescence microscopy under UV excitation revealed intense green or blue autofluorescence of the walls suggestive of phenolics [21] The phenols were identified by TLC to be mainly ferulic acid (blue fluorescence) plus a major unknown spot with $R_{fa}$ of 0 61 (yellow-green fluorescence) in benzene–methanol–acetic acid (45 8 4) and a minor spot with $R_{fa}$ of 1 07 Hydrolysis with dilute hydrochloric acid at 80° increased the $R_{fa}$ value of the major unknown phenol to 0 88 but yielded, in addition, two minor spots with $R_{fa}$ 0 94 and 1 09 Addition of alkali to saponify phenols caused immediate bright yellow colouration of the samples

The cell wall preparations showed very variable protein contents (Table 1) They also differed in amino acid composition, particularly lysine The three preparations with low lysine contents (1 0–2 2 g/16 8 g N) also had lower aspartic acid (7 2–9 2% vs 11 0–14 0%), glycine (3 6–4 4% vs 5 0–6 8%) and arginine (5 1–6 6% vs 7 1–8 7%) but higher glutamic acid (24 3–28 0% vs 12 7–20 7%) than the five with high lysine content

(4 9–8 5 g/16 8 g N) [18] Only Basmati 370 had a high lysine content (5 5%) among the four elongating rices Since rice endosperm protein bodies have 3 5–4 0 g lysine/16 g N [17], the wide variation in lysine content was unexpected The *B lichenformis* α-amylase preparation purified three times by ammonium sulphate precipitation had 7% lysine, 6% arginine, 14% aspartic acid, 11% glutamic acid and 6% glycine [18] Its adsorption on the cell wall may explain in part this variability in protein composition among samples Undigested core protein of milled rice protein bodies are also poor (1–2%) in lysine [17]

In contrast to the findings of Shibuya and Iwasaki [6], the proteins in the cell wall preparations had only a trace of hydroxyproline [18] Higher values were obtained with preparations using relatively impure α-amylase by Mod *et al* [5] of 5 9% in the water-soluble fraction and 0 3–1 1% in the 0 5 M sodium hydroxide fraction The cell wall preparation of Shibuya and Iwasaki [6] was treated with *R niveus* glucoamylase and *A melleus* protease and had 19 mol % hydroxyproline

Analytical and SDS disc gel electrophoresis of whole cell wall preparation and its fractions showed protein bands which did not stain for carbohydrates suggesting the absence of linkage between protein and polysaccharides [18] Most of the protein did not enter the electrophoretic gel in the 4 M potassium hydroxide, TCA-soluble and 6 M sodium hydroxide extracts suggestive of rice glutelin Some protein staining was noted at the position of the tracking dye for the 4 M potassium hydroxide, TCA-soluble fraction, which did not stain for carbohydrates Similar results were obtained with SDS-PAGE wherein the protein bands did not stain for carbohydrates suggesting the absence of glycoproteins, and confirming the SEM observation of the presence of contaminant protein bodies (mainly glutelin) [17] Cartaño and Juliano [14] also reported the protein in milled rice hemicellulose to be contaminant protein Shibuya and Iwasaki [6] suggested the presence of glycoproteins in milled-rice cell wall based on the high hydroxyproline content of their preparation

The elongating and non-elongating rices overlapped in the ratio of their cell wall preparations which were soluble in hot water, 4 M potassium hydroxide, 6 M sodium hydroxide–0 81 M boric acid and insoluble residue (Table 1) The major fraction was the 4 M potassium hydroxide-soluble polysaccharides (37–54%) which included proteins (2–17%) precipitated by TCA These polysaccharides were initially insoluble in neutral solvents but became water-soluble on extraction as observed also by Cartaño and Juliano [14] However, the elongating rices tended to have more 4 M potassium hydroxide, TCA-soluble and 6 M sodium hydroxide–0 81 M boric acid-soluble fractions but less of the residue or insoluble fraction than non-elongating rices, consistent with the slightly lower mean content of crude fibre of the elongating rices The mean level of insoluble fraction amounted to 0 14% of milled rice for elongating rices and 0 16% of non-elongating milled rice which were very close to their mean crude fibre contents of 0 15 and 0 17%, respectively (Table 1)

*Complexing with amylopectin*

The whole cell wall preparations at 1–2% of waxy rice starch (1–2 mg added to 100 mg starch/1 6 ml water) had a hardening effect on the gel of IR29 waxy rice starch as reflected in a lower gel consistency value (Table 1) Elongating and non-elongating rices overlapped in the gel consistency values of IR29 starch with added cell wall Cell walls of IR36 and waxy UPL-Ri-1, both non-elongating rices, had the greatest effect, with a gel consistency of 41 mm as compared to 58 mm for the control IR36 milled rice cell wall also had the hardest gel consistency value in 0 2 M potassium hydroxide of 34 mm The hot water-soluble fractions also had a hardening effect on the IR29 starch gel, with the IR36 sample being the most effective (gel consistency 34 mm) The 4 M potassium hydroxide fractions also had a slight hardening effect on IR29 starch gel with Calrose having the hardest gel (40 mm)

*Solubility fractionation of IR32 cell walls*

Preliminary studies on IR36 milled rice cell walls showed that although the 0 5 M sodium hydroxide solvent of Cartaño and Juliano [14] preferentially extracted polysaccharides rich in arabinose, xylose and uronic acid, and proteins (mainly glutelin) [18], the residue or insoluble fraction still had 8% arabinose, 12% xylose and 22% mannose in addition to 57% glucose [18] Better fractionation was achieved by the method of Anderson and Stone [22] and a modified extraction procedure was employed in subsequent study on IR32 cell wall preparations [18] Hot-water extraction was added before hot-ammonium oxalate extraction since this fraction probably represents residual water-soluble polysaccharides which did not dissolve during incubation in 70–80° water during cell wall preparation Subsequent 8 M urea extraction was deleted since it was a minor fraction but rich in glucose The 0 5 M potassium hydroxide solvent was deleted and 4 M rather than 4 27 M potassium hydroxide was used to extract most of the pentosans with minimum mannose contamination The 6 M sodium hydroxide–0 81 M boric acid was retained to extract mannose-containing polysaccharides Because of the similarity in properties of cell wall preparations of the eight varieties (Table 1), only IR32 cell walls were studied in detail

Solubility fractionation of IR32 milled rice cell wall preparation resulted in fractions differing in polyuronic acid content and neutral sugar composition (Table 2) Hot-water extracted polysaccharides were rich in uronic acids, glucose, arabinose and xylose The hot oxalate fraction was rich in uronic acids and arabinoxylans The 4 M potassium hydroxide fraction consisted mainly of arabinoxylans and glucans but was already poor in polyuronic acid In contrast to the 0 5 M potassium hydroxide extract which had only 1% mannose [18], the 4 M potassium hydroxide and TCA-soluble fraction already had 11% mannose in neutral sugars The protein-rich 4 M potassium hydroxide-soluble, TCA-insoluble fraction was rich in glucose TCA precipitation of protein from the 4 M potassium hydroxide extract with very small amount of carbohydrates again suggested that the protein was mainly protein-body protein or glutelin soluble in alkali [17, 23] The 6 M sodium hydroxide–0 81 M boric acid fraction was rich in mannose Galactose polysaccharides were more soluble in neutral solvents than the mannose polysaccharides The residue was essentially all glucose, principally cellulose The arabinoxylans in the water and oxalate fractions of IR32 and IR36 cell wall had higher Ara–Xyl ratios (1 3–1 6) than in the alkali-soluble fractions (0 5–1 2)

Table 2 Recovery and composition of IR32 milled rice cell wall preparation and its solubility fractions

| Fraction* | Wt (%) | Total polysaccharides (%) | Polyuronic acids (%) | Protein (%) | Neutral sugar composition (wt %) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Rha | Fuc | Ara | Xyl | Man | Glc | Gal |
| Whole | 100 | 62 | 3 | 18 | <1 | <1 | 13 | 14 | 15 | 49 | 8 |
| Hot $H_2O$-soluble | 7 | 60 (8)† | 18 | 7 | <1 | 1 | 29 | 19 | 1 | 34 | 12 |
| Hot 0 25% $(NH_4)_2C_2O_4$ soluble | 8 | 34 (5) | 12 | 38 | 6 | 1 | 37 | 28 | 2 | 9 | 16 |
| 4 M KOH, TCA-soluble | 18 | 63 (20) | 5 | 16 | trace | <1 | 28 | 30 | 11 | 37 | 4 |
| 4 M KOH-soluble, TCA-insoluble | 28 | 12 (6) | trace | 54 | trace | trace | 11 | 7 | 8 | 69 | 5 |
| 6 M NaOH–0 81 M $H_3BO_3$-soluble | 10 | 90 (16) | trace | 1 | trace | trace | 3 | 4 | 72 | 19 | 2 |
| Residue | 28 | 90 (45) | trace | <1 | trace | trace | trace | trace | 4 | 96 | trace |

*With 0 013 M $NaBH_4$ and under $N_2$ atmosphere, except for $H_2O$ and $(NH_4)_2C_2O_4$ extraction
†Percentage of recovered total carbohydrates

β-Glucan assay of the hot-water and 4 M potassium hydroxide fractions using β-glucanase [24] verified that a major portion of the glucose fraction was mixed 1,3 1,4-β-glucan Immunodiffusion assay using Yariv antigen [25] specific for β-glucan showed a faint precipitation reaction for the water-soluble fraction and no reaction for the oxalate, 4 M potassium hydroxide and 6 M sodium hydroxide–0 81 M boric acid fractions Anderson *et al* [24] reported the presence of mixed β-glucans in brown and milled rice, a major part of which is hot-water-soluble

The uronic acid was identified by TLC to be a mixture of glucuronic and galacturonic acids, which was also the case for rice bran and germ polyuronic acids [15] The quantity of glucuronic acid tended to be greater than that of galacturonic acid in the hydrolysates By contrast, Shibuya and Iwasaki [6] reported only galacturonic acid in milled rice pectin

*DEAE-cellulose and gel-filtration chromatography*

DEAE-cellulose-phosphate fractionation of the hot-water fraction of IR32 milled rice cell wall preparation resulted in eight polysaccharide peaks (Fig 1) The fractions eluted with 0 10, 0 25 and 0 50 M phosphate were particularly rich in uronic acid (31, 45 and 48 %, respectively, of the total carbohydrates) WS1 was rich in glucose, WS3 in arabinoxylans, WS5 in arabinoxylans and galactose, and WS8 in arabinoxylans, glucose and mannose (Table 3) The WS6 or 0 50 M phosphate fraction was also rich (23 %) in galactose Ara–Xyl ratio ranged from 0 5 to 3 2 and was highest in the WS5 and lowest in the WS8 subfraction Rhamnose content was highest in the neutral sugars of the 0 25 and 0 50 M phosphate eluates as compared to the others (trace–4 %) The WS8 subfraction stained deep blue with iodine–potassium iodide reagent and probably contained the iodine-staining xyloglucan complex previously reported in the 4 N potassium hydroxide fraction of Japanese milled-rice cell walls [6, 7]

Subfractions of DEAE-cellulose-phosphate chromatography of the hot oxalate fraction of IR32 milled rice cell walls gave seven subfractions [18] Again the fractions rich in polyuronic acids were those eluted with 0 25 and 0 50 M phosphate (Fig 1) with a uronic acid content in total polysaccharides of 39 and 52 %, respectively Both 0 25 and 0 50 M phosphate eluates were rich in galactose Arabinoxylans were the major polysaccharide in all but the A7 subfraction The Ara–Xyl ratio was >1 for all subfractions except the 0 25 M sodium hydroxide eluates Glucose content was highest in the A7 subfraction and mannose was highest in both 0 25 M sodium hydroxide eluates Iodine-staining of the 0 25 M sodium hydroxide fractions was less than those of the WS8 fraction of water-soluble polysaccharides The 0 50 M phosphate subfraction had 14 % rhamnose Thus, DEAE-cellulose chromatographic separation was similar for both the hot-water and hot-ammonium oxalate fractions of IR32 cell walls Shibuya and Iwasaki [6] reported a higher uronic acid content for their hot-oxalate fractions of Japanese rice cell walls

DEAE-cellulose ($BO_3^{3-}$ form) chromatography [26] of the 4 M potassium hydroxide, TCA-soluble fraction of IR32 milled-rice cell walls gave seven fractions, the major portion of which was only eluted with 0 25 M sodium hydroxide (Table 3) The $BO_3^{3-}$ eluates were rich in arabinoxylans with an Ara–Xyl ratio of 0 8–1 9 The second 0 30 M $BO_3^{3-}$ eluate, H3, and H4 were richer in mannose and glucose than the earlier $BO_3^{3-}$ eluates The 0 25 M sodium hydroxide eluates were rich in glucose together with xylose and mannose This fraction was reported by Shibuya and Iwasaki [6] to contain the iodine-staining xyloglucan of milled rice cell walls The iodine-staining of this fraction was less than that of the WS8 fraction of water-soluble IR32 cell wall polysaccharides

Gel filtration on Sepharose 4B-CL of these water, oxalate and 4 M potassium hydroxide, TCA-soluble fractions of IR32 milled-rice cell walls showed polydispersity of the polysaccharide extracts The MW range of the

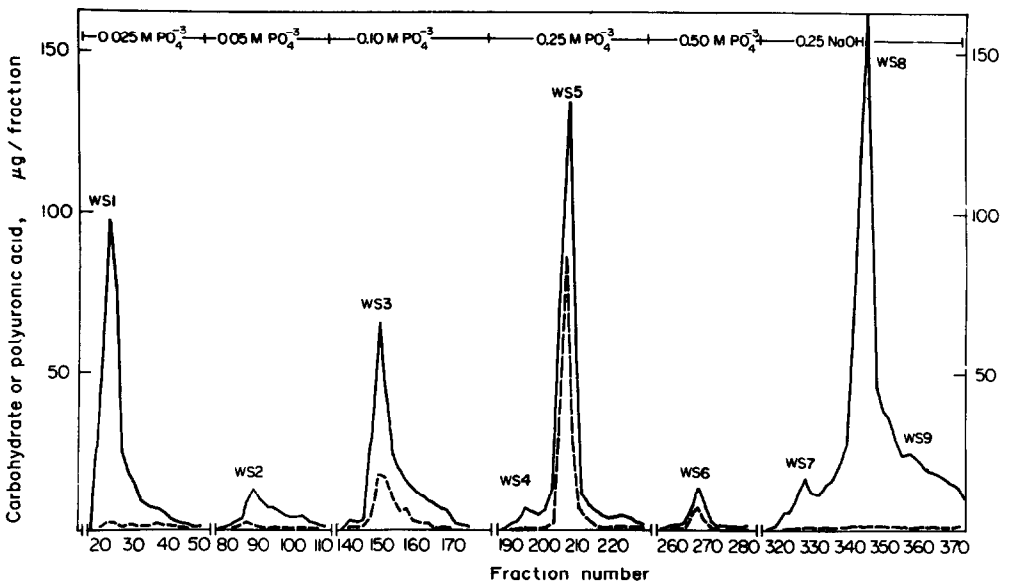


Fig 1 DEAE cellulose (2 6 × 38 cm, Pi form) chromatography of the hot $H_2O$-soluble fraction of IR32 milled rice cell wall preparation (7 ml/fraction) ——, Total carbohydrate, – – –, total polyuronic acid

Table 3 Recovery and composition of the major DEAE-cellulose (P1 form) subfractions of the hot-$H_2O$ and hot-$(NH_4)_2C_2O_4$ extracts and of the major DEAE-cellulose ($BO_3^{3-}$ form) subfractions of 4 M KOH, TCA-soluble extract of IR32 milled rice cell wall preparations

| Cell wall solubility subfraction | % recovered polysaccharides | Total polysaccharides (%) | Polyuronic acid (%) | Neutral sugar composition (wt %) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Rha | Fuc | Ara | Xyl | Man | Glc | Gal |
| Hot $H_2O$-soluble fraction | | | | | | | | | | |
| WS1 0 025 M P1 | 16 | 39 | 3 | <1 | 1 | 19 | 8 | 1 | 63 | 4 |
| WS3 0 10 M P1 | 14 | 23 | 7 | 2 | 1 | 50 | 31 | <1 | 6 | 9 |
| WS5 0 25 M P1 | 21 | 39 | 18 | 8 | 2 | 47 | 15 | <1 | 4 | 24 |
| WS8 0 25 M NaOH | 42 | 49 | 2 | trace | trace | 15 | 28 | 10 | 47 | trace |
| Hot $(NH_4)_2C_2O_4$-soluble fraction | | | | | | | | | | |
| A1 0 025 M P1 | 6 | 23 | 4 | 5 | 7 | 35 | 21 | 1 | 26 | 5 |
| A3 0 10 M P1 | 8 | 38 | 7 | 2 | 2 | 43 | 34 | 4 | 8 | 7 |
| A4 0 25 M P1 | 14 | 33 | 13 | 5 | 2 | 42 | 20 | 1 | 10 | 19 |
| A5 0 50 M P1 | 13 | 28 | 14 | 14 | 3 | 30 | 13 | 2 | 16 | 22 |
| A6 0 25 M NaOH | 28 | 34 | 2 | 1 | 1 | 18 | 34 | 17 | 28 | 1 |
| A7 0 25 M NaOH | 27 | 19 | 2 | trace | trace | 7 | 24 | 21 | 48 | trace |
| 4 M KOH, TCA-soluble fraction | | | | | | | | | | |
| H1 0 10 M $BO_3^{3-}$ | 8 | 10 | n d | trace | 2 | 45 | 41 | 2 | 9 | 1 |
| H2 0 30 M $BO_3^{3-}$ | 17 | 20 | n d | trace | 1 | 39 | 50 | 4 | 5 | 1 |
| H4 0 50 M $BO_3^{3-}$ | 10 | 9 | n d | trace | 1 | 32 | 26 | 20 | 17 | 4 |
| H5 0 25 M NaOH | 28 | 42 | n d | trace | trace | 8 | 22 | 9 | 61 | trace |
| H6 0 25 M NaOH | 25 | 60 | n d | trace | trace | 4 | 14 | 15 | 67 | trace |
| H7 0 25 M NaOH | 11 | 66 | n d | trace | trace | 5 | 15 | 17 | 63 | trace |

n d , Not determined

unresolved peaks was 1 3–64 × $10^4$ for the hot-water fraction (five peaks), up to 1 04 × $10^6$ for the hot 0 25% ammonium oxalate fraction (eight peaks) and up to 1 9 × $10^6$ (void volume) for the 4 M potassium hydroxide-soluble fraction (eight peaks) UV absorption at 280 nm was highest in the MW fractions with peaks at MW 13 000 for the water fraction, MWs 4100 and 53 000 for the oxalate fraction, and MW 10 200 for the 4 M potassium hydroxide fraction

## DISCUSSION

Varietal differences in the recovery and composition of cell wall preparations were not simply related to differences in grain elongation during cooking of milled rice Differences in direction of grain swelling during cooking possibly relate to relative thickness of cell walls in the radial and longitudinal axes of the varieties rather than compositional factors Preliminary histological study of cooked grains showed large internal fissures through the walls perpendicular to the longitudinal axis at regular intervals in Basmati 370 rice (elongating), which were absent in Palman (non-elongating) [27] In cross section, cooked Palman grains showed, along the lateral axis, an elongated core of small isodiametric cells surrounded by indistinct cells without enclosing cell walls Cooked Basmati had intact cells of small size throughout the cross section Further morphological studies on the volume expansion phenomenon of these varieties are needed

Even with the possible contamination of milled rice cell wall preparation with residual scutellum cell walls [1], milled rice cell walls differ from bran and germ cell walls in the nature of their protein fraction The proteins of milled-rice wall preparations are not glycoproteins but probably protein-body proteins together with denatured *B licheniformis* α-amylase adsorbed on protein Yoshizawa *et al* [8] observed that adsorption of α-amylase to milled-rice cell walls involved the protein fraction of the wall, mainly glutelin The high lysine content of some of the preparations is probably due to denatured α-amylase, with 7% lysine Thus, protease treatment may be utilized in the preparation of milled-rice cell walls, provided the enzyme is free of carbohydrases [12] Shibuya and Iwasaki [6] and Yoshizawa *et al* [8] obtained low-protein cell wall preparations by protease treatment By contrast, the proteins of bran and germ cell wall preparations are glycoprotein based on gel electrophoresis [5, 15] with a detectable hydroxyproline content of 0 4–1 6% [15] and 0 5–0 7% [5]

The hardening effect of the cell wall preparations and its solubility fractions on IR29 waxy rice starch gel (Table 1) indicate complexing ability Cold-water soluble nonstarch polysaccharides of milled rice had either a hardening effect or no effect [16] IR32 bran cold- and hot-water extracts hardened the IR29 starch gel but the cell wall preparation had a softening effect [15] Complexing reactions of cell wall with starch and cations [28] such as $Ca^{2+}$ in the α-amylolysis buffer and bile acids [29, 30], particularly of bran, have been attributed either to polyuronic acid, lignin or the neutral polysaccharide fraction [9, 10]. The water-soluble fractions are rich in polyuronic acid, phenolics, arabinoxylans and β-glucans and are dissolved during rice boiling (Tables 2 and 3) Ferulate has been reported to be bound to a water-soluble polysaccharide of rice bran with a MW 5–10 × $10^4$ [3] Normand *et al* [30] reported the cold-water-soluble hemicelluloses of brown rice as containing bran-bound bile acids whereas milled-rice hemicelluloses did not Among the neutral polysaccharides, β-glucans of barley are known to complex with fluorescing agents [31] and rice xyloglucan complexes with iodine–potassium iodide [6, 7] Verification of the relative importance of these various cell wall polysaccharides in altering the texture, and possibly nutritional value, of cooked rice awaits further study

Solubility fractionation and DEAE-cellulose chromatography of the more soluble fractions were partially successful in separating the component polysaccharides of milled-rice cell wall (Tables 2 and 3) Neutral solvents preferentially extracted β-glucans, polyuronic acids and galactose-rich polysaccharides and phenols Dilute (0 5–4 M) potassium hydroxide further extracted the arabinoxylans Mannose polysaccharides were extracted with 6 M sodium hydroxide–0 81 M boric acid and the residue was essentially cellulose Amyloid-type xyloglucan was extracted with hot-water, oxalate and 4 M potassium hydroxide DEAE-cellulose-phosphate chromatography showed elution of amyloid- and mannose-rich polysaccharides with 0 25 M sodium hydroxide, polyuronic acid, and rhamnose and galactose polysaccharides with the 0 25–0 50 M phosphate eluate and glucans with 0 025 M phosphate (Table 3) Arabinoxylans with high a Ara–Xyl ratio are eluted with phosphate and those with low a Ara–Xyl ratio are eluted by 0 25 M sodium hydroxide Different results were obtained with the 4 M potassium hydroxide extract fractionated on DEAE-cellulose $BO_3^{3-}$ Only the 0 25 M sodium hydroxide eluates were high in glucose, the 0 5 M $BO_3^{3-}$ fraction was richest in galactose, and the 0 10 and 0 30 M $BO_3^{3-}$ eluates were richest in arabinoxylans

## EXPERIMENTAL

Samples of IR32, IR36 and UPL-Ri-1 rough rices were obtained from the 1976, 1978 and 1979 crops of the International Rice Research Institute experimental farm Basmati 370 and Palman rough rice were obtained from the 1977 crop of the Rice Research Institute, Kala Shah Kaku, Lahore, Pakistan, Dumzard rought rice from the 1978 crop of the Rice Research Station, Rasht, Iran, and D25-4 (Nga Kywe) rough rice from the 1979 crop of the Hmarobi Central Agricultural Research Farm, Burma The rough rice was dehulled with a Satake testing husker THU-35 and the brown rice milled to 13% bran removal (overmilled) with a Satake TM-05 Grain Testing Mill The resulting milled rices were aspirated in a South Dakota seed blower and wiped with cheese cloth to ensure removal of contaminating bran Calrose milled rice was from the 1978 crop of the New South Wales Department of Agriculture, Yanco, N S W, Australia

*Isolation of rice endosperm cell walls* Milled-rice samples (200 g) were soaked in 2 vols. $H_2O$ for 4 hr at 0° to soften the grain and were then homogenized with a Silverson homogenizer Model L2R for 20 min at 25° Homogenization was repeated until all particles passed through an 80-mesh nylon sieve The slurry was centrifuged for 20 min at 1000 *g*, the supernatant discarded, and the residual flour extracted with 0 5% SDS (BDH Chemicals, U K) and 0 6% β-mercaptoethanol in a w/v ratio of 1 20 Extraction was for 3-hr periods, × 4 at 25° with constant stirring Dispersion of the slurry was effected with a Lightnin Model L mixer After the last extraction the residue was washed with $H_2O$ by constant shaking in a reciprocal Eberbach shaker for 20 min

and then centrifuged at 1000 *g* The whole residue was used for subsequent destarching procedure

The washed flour sample was then resuspended in 10 vols 0 5 M Pi buffer, pH 5 2, containing 170 ppm $Ca^{2+}$ as $CaCl_2$ $2H_2O$ or in 0 05 M NaOAc buffer, pH 5 2, without added $Ca^{2+}$ ions The suspension was stirred and heated at 80–85° in a water bath for 1 hr to gelatinize the starch, and then cooled to 70°, after which 30 ml of 10 × diluted α-amylase from *Bacillus licheniformis* (Termamyl 60L, Novo Industries) was added The diluted enzyme had been previously heated at 80° for 15 min to inactivate any β-glucanase present [18] and then filtered through Whatman No 1 filter paper Incubation was continued until the suspension was negative for starch by the $I_2$–KI blue reaction The digests were cooled to 25° and allowed to stand until all the insoluble residue had settled after which the supernatant liquid was carefully siphoned out The residue was resuspended in 2 l $H_2O$ and then carefully filtered through a 31-μm nylon sieve The procedure was repeated until the filtrate was clear The resulting cell wall preparation was then lyophilized

*Light and fluorescence microscopy* Separate samples of the cell wall preparations were stained with 1% aq Congo red, 0 2% $I_2$ in 2% KI, and Coomassie Brilliant Blue (CBB) G-250 (150 mg in 150 ml 55% $H_3PO_4$–75 ml 95% EtOH) [32] and examined under a Leitz light microscope at 1250 × magnification Samples for fluorescence examination were dry-mounted on glass slides and examined with a Leitz Laborlux 11 fluorescent microscope equipped with exciter filters in the UV (340–380 nm) and blue (450–490 nm) regions

*Fractionation procedures* The IR32 cell wall (10 g) was fractionated by a modified procedure of that in ref [22], consisting of the following sequential extraction twice with 200 ml $H_2O$ at 100°, under reflux for 5 hr each, once with 400 ml 0 25% $(NH_4)_2C_2O_4$ at 90° under reflux for 24 hr, once with 400 ml 4 M KOH–0 013 M $NaBH_4$ at 25° for 24 hr under a continuous $N_2$ stream and once with 200 ml 6 M NaOH–0 81 M $H_3BO_3$–0 013 M $NaBH_4$ at 25° for 24 hr under $N_2$ The 4 M KOH extract was further treated with TCA to pH 3 7 to ppt the extracted proteins which were then removed by centrifugation at 10 000 *g* All extracts and the residue were dialysed against $H_2O$ for 3–4 days at 4° using a cellulose membrane (cut off MW 2000–3000)

*Ion-exchange chromatography* Samples (100 mg in 6 ml 0 025 M Pi buffer, pH 6 0) of the $H_2O$ and oxalate fractions of IR32 cell walls were chromatographed on DEAE-cellulose (Cellex D, BioRad Industries) Pi form column (2 6 × 98 cm) employing sequential elution with increasing concns of Pi buffer followed with 0 25 M NaOH [6] The 4 M KOH, TCA-soluble fraction of IR32 cell walls (100 mg in 6 ml 0 01 M $BO_3^{3-}$ buffer, pH 9 2) was chromatographed on a DEAE-cellulose $BO_3^{3-}$ column (2 6 × 38 cm) according to the procedure of ref [26] In all cases 7-ml fractions were collected at a downward flow rate of 1 drop/3 sec Fractions were assayed for total carbohydrates and uronic acids according to the procedures of ref [15] Fractions corresponding to peaks were pooled, dialysed against $H_2O$ for 2–3 days at 4° using a cellulose membrane and then lyophilized

*Gel filtration chromatography* MW range approximation of selected fractions was determined by gel filtration on a Sepharose 4B-CL column (2 6 × 90 cm) using 0 3% NaCl containing 0 05% $NaN_3$ as eluant [15]

*TLC and GC* Samples for TLC and GC analyses were first hydrolysed by the Saeman method as described in ref [33] The uronic acid residues were identified by TLC on Si gel 60 precoated plates (Merck) using *iso*-PrOH–$Me_2CO$–1 M lactic acid (2 2 1) [34], and on Si gel plates impregnated with 0 3 M $Na_2HPO_4$ using *n*-BuOH–EtOH–0 1 N HCl (1 10 5) [35], as developing solvents The spots were visualized with an aniline–diphenylamine reagent in $Me_2CO$–$H_3PO_4$ [34] The neutral sugars were identified and quantitated by GC of their respective aldononitrile acetate derivatives according to ref [15]

Cell wall samples for the phenolic study were defatted with $CHCl_3$–MeOH (2 1) and $H_2O$-satd *n*-BuOH according to ref [36] The defatted samples were extracted for phenolic compounds by saponification with aq 0 5 M KOH according to ref [37] The phenolic compounds were investigated by TLC on Si gel $F_{254}$ plates using two solvent systems, hexane–amyl alcohol–HOAc (10 16 0 25) [38] and $C_6H_6$–MeOH–HOAc (90 25 8) [37] The spots were observed under UV before and after exposure to $NH_3$ vapor

*Immunodiffusion* Gel immunodiffusion examinations were carried out in Petri dishes using 3-mm thick 1 5% special Noble agar containing 0 85% NaCl, 0 1% $NaN_3$ and 0 001 M $Ca^{2+}$ [25] The centre and sample wells were 1 cm apart and had 0 9 and 0 6 cm diameters, respectively Yariv antigen specific for β-D-glucan (*ca* 1 mg/ml in 1% NaCl) was placed in the centre well Test samples (*ca* 2 mg/ml) were suspended in 1% NaCl, warmed at 50° to facilitate soln, and centrifuged to remove insoluble materials before placing in the sample wells Plates were kept at 0–4° until precipitin lines were observed (3–7 days)

*Determination of β-glucan content* Cell wall preparations were assayed for β-glucans using a specific bacterial 1,3 1,4-β-D-glucan hydrolase according to ref [24] The procedure was slightly modified—the total carbohydrate content [20] of the hydrolysate (corrected for by a control) was determined instead of the liberated glucose

Analytical methods were essentially as described in ref [15] Milled-rice was analysed for gelatinization temp type by the alkali test [39], amylose content by $I_2$ colorimetry [40], gel consistency [19] and elongation ratio (length of 10 cooked–10 raw grains) on boiling for 15 min grain which had been presoaked in cold $H_2O$ for 30 min [41]

*Acknowledgements*—V G Novenario supervised the gel consistency tests C C Manimgat and E P Alabata assisted in the preparation of crude cell wall preparations of the other varieties We thank Professor B A Stone for his interest and stimulation of this work and for the barley β-glucan, Yariv antigen and the β-glucan hydrolase Rice samples were obtained from A B Blakeney, New South Wales Department of Agriculture, Yanco, N S W, Australia, Myint Thein, Agricultural Research Division, Agriculture Corporation, Rangoon, Burma, A M Chawdhry, Rice Research Institute, Kala Shah Kaku, Lahore, Pakistan, and M Shafi Chaudry, Rice Research Station, Rasht, Iran

## REFERENCES

1 Juliano, B O (1980) in *Rice Production and Utilization* (Luh, B S, ed) p 403 Avi, Westport
2 Juliano, B O (1979) in *Chemical Aspects of Rice Grain Quality* p 69 International Rice Research Institute, Los Baños, Laguna, Philippines
3 Mod, R R, Normand, F L, Ory, R L and Conkerton, E J (1981) *J Food Sci* **46**, 571
4 Shibuya, N (1980) *Grains J* (Philippines) **5**, 21
5 Mod, R R, Conkerton, E J, Ory, R L and Normand, F L (1978) *J Agric Food Chem* **26**, 1031
6 Shibuya, N and Iwasaki, T (1978) *Agric Biol Chem* **42**, 2259
7 Shibuya, N and Misaki, A (1978) *Agric Biol Chem* **42**, 2267
8 Yoshizawa, K, Momose, H and Hasuo, T (1980) *Nippon Jozo Kyokai Zasshi* **75**, 428
9 National Institutes of Health (1978) *Am J Clin Nutr* **31** (10, supplement), S1

10 Inglett, G E and Falkehag, S I (eds) (1979) *Dietary Fibers Chemistry and Nutrition* Academic Press, New York
11 Huber, D J and Nevins, D J (1977) *Plant Physiol* **60**, 300
12 Davies, T E, Jr and Domer, J E (1977) *Analyt Biochem* **80**, 593
13 International Rice Research Institute (1980) *Annual Report for* 1979 p 25 Los Baños, Laguna, Philippines
14 Cartaño, A V and Juliano, B O (1970) *J Agric Food Chem* **18**, 40
15 Maningat, C C and Juliano, B O (1982) *Phytochemistry* **21**, 2509
16 International Rice Research Institute (1981) *Annual Report for* 1980 p 21 Los Baños, Laguna, Philippines
17 Tanaka, Y, Resurreccion, A P, Juliano, B O and Bechtel, D B (1978) *Agric Biol Chem* **42**, 2015
18 Pascual, C G (1981) M Sc Thesis, University of the Philippines, Los Baños, Philippines
19 Cagampang, G B, Perez, C M and Juliano, B O (1973) *J Sci Food Agric* **24**, 1589
20 Dubois, M, Gilles, K A, Hamilton, J K, Rebers, P A and Smith, F (1956) *Analyt Chem* **28**, 350
21 Fincher, G B (1976) *J Inst Brew (London)* **82**, 347
22 Anderson, R L and Stone, B A (1978) *Aust J Biol Sci* **31**, 573
23 Juliano, B O and Boulter, D (1976) *Phytochemistry* **15**, 1601
24 Anderson, R L, Cook, J A and Stone, B A (1978) *J Inst Brew (London)* **84**, 233
25 Baldo, B A, Neukom, H, Stone, B A and Uhlenbruck, G (1978) *Aust J Biol Sci* **31**, 149
26 Gremli, H and Juliano, B O (1970) *Carbohydr Res* **12**, 273
27 International Rice Research Institute (1979) *Annual Report for* 1978 p 24 Los Baños, Laguna, Philippines
28 Rasper, V F (1979) in *Dietary Fibers Chemistry and Nutrition* (Inglett, G E and Falkehag, S I, eds) p 93 Academic Press, New York
29 Vijayagopalan, P and Kurup, P A (1973) *Atherosclerosis* **18**, 379
30 Normand, F L, Ory, R L and Mod, R R (1979) in *Dietary Fibers Chemistry and Nutrition* (Inglett, G E and Falkehag, S I, eds) p 203 Academic Press, New York
31 Wood, P J and Fulcher, R G (1978) *Cereal Chem* **55**, 952
32 Bradford, M M (1976) *Analyt Biochem* **72**, 248
33 Selvendran, R R, March, J F and Ring, S G (1979) *Analyt Biochem* **96**, 282
34 Hansen, S A (1975) *J Chromatogr* **105**, 388
35 Ovodov, Y S, Evtushenko, E V, Vaskovsky, V E, Ovodova, R G and Solo'eva, T F (1967) *J Chromatogr* **26**, 111
36 Choudhury, N H and Juliano, B O (1980) *Phytochemistry* **19**, 1063
37 Fausch, H, Kundig, W and Neukom, H (1963) *Nature (London)* **199**, 287
38 Hartley, R D (1971) *J Chromatogr* **54**, 335
39 Little, R R, Hilder, G B and Dawson, E H (1958) *Cereal Chem* **35**, 111
40 Perez, C M and Juliano, B O (1978) *Starke* **30**, 424
41 Kongseree, N and Juliano, B O (1972) *J Agric Food Chem* **20**, 714