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Production and characterisation of two wheat-bran fractions: an aleurone-rich and a pericarp-rich fraction

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Wheat bran is a good source of dietary fibre in the form of cell walls, but contains a number of different cell types. We describe a large-scale procedure for the production of an aleurone-rich and a pericarp-rich fraction from hard, Australian wheat. The fractions were characterised by field-emission scanning electron microscopy, by using a range of bright-field stains, colour reagents, and fluorochromes, and by chemical analysis of the walls. The aleurone fraction included the seed coat with its cuticle. Only the pericarp walls showed a histochemical reaction for lignin. The concentrations of ester-linked ferulic acid and $(1\rightarrow3)$,$(1\rightarrow4)$-β-glucans were greater in the aleurone-rich fraction than in the pericarp-rich fraction. The results are consistent with the arabinoxylans in the walls of the pericarp-rich fraction being more highly substituted with arabinose than those in the walls of the aleurone-rich fraction. When the fractions were fed as a dietary supplement to rats and walls were isolated from the faeces, it was found that the pericarp walls were not degraded, but the aleurone walls were partially degraded.

Keywords: Aleurone-rich fraction / Cell wall / Dietary fibre / Pericarp-rich fraction / Wheat bran

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1 Introduction

Dietary fibre in the form of plant cell walls is usually considered beneficial to health and is thought to protect against the development of a number of western diseases, especially cancer [1, 2]. A very important source of dietary fibre in industrialised countries is wheat bran, which contains about 70% of the dietary fibre of the whole grain [3, 4]. There is good evidence from animal and human intervention studies that supplementing the diet with wheat bran can protect against the development of colorectal cancer [1, 5] and wheat bran has been described as the 'gold standard' for treating constipation [6, 7].

Wheat bran is composed of the outer layers of the wheat grain and is a by-product of the roller milling of wheat grain to produce white flour [3, 8]. In roller milling, the bran is separated, as completely as possible, from the starchy endosperm, which is subsequently reduced to flour fineness. In practice, the wheat bran generated by this process contains some of the underlying starchy endosperm, as well as the bran layers. These bran layers consist of different cell types, which have walls with different chemical compositions [9–15]. The outer part of the bran comprises the pericarp or fruit wall, under this is a thin seed coat, which overlies the aleurone layer (Fig. 1). Which of these different cell types is the most important in terms of their walls conferring health benefits is unknown. Of course, the walls of one cell type may be beneficial in protecting against particular diseases, whereas the walls of another cell type may be more protective against another disease.

Although wheat bran is often referred to as "wheat fibre" (*e.g.*, [16]), it is important to recognize that wheat bran is not a dietary fibre as such; in addition to the walls, the cells have contents. Much of the rest of wheat bran can be accounted for as protein, starch (from contaminating starchy endosperm), moisture and ash, but a range of other components has also been described [1]. Several of these other components, including phenolic acids, flavonoids, lignans, and phytic acid, have been independently impli-

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Abbreviations: ARF, aleurone-rich fraction; **FESEM**, field-emission scanning electron microscopy; **PRF**, pericarp-rich fraction; **WB**, whole bran

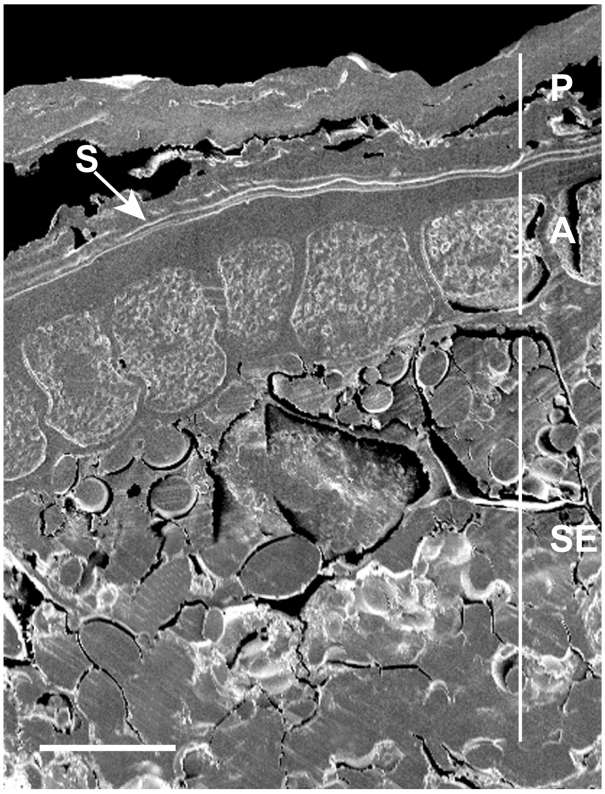

Figure 1. FESEM of a cross section of the outer (bran) layers of wheat (cv. Kotuku) showing the pericarp (P), seed-coat (S), and aleurone (A). The section also shows the underlying starchy endosperm cells (SE) which contain starch grains. Horizontal bar = 25 μm.

cated in protection against cancer. Indeed, these phytochemicals may account for many of the cancer-protective properties of wheat bran, although which are the most important is unknown. Furthermore, their concentrations in the different cell types of wheat bran probably vary widely, for example, it is known that phytic acid is concentrated in the aleurone cells [4, 17].

Because of the heterogeneity of wheat bran, it would be interesting to investigate the health benefits of bran fractions enriched in particular cell types. However, most of the studies on the chemical compositions of bran fractions have been done on small-scale fractionations. In the present study, we describe the production of two wheat-bran fractions, an aleurone-rich fraction (ARF) and a pericarp-rich fraction (PRF), in quantities sufficient for animal and human intervention studies. We characterised these fractions and whole bran (WB) obtained from the same wheat sample using field-emission scanning electron microscopy, and by histochemical and chemical techniques. We also fed rats a diet supplemented with these fractions and with the whole bran and examined microscopically the walls of different cell types in the faeces for evidence of microbial degradation.

2 Materials and methods

2.1 Experimental material

Hard, white, Australian wheat (*Triticum aestivum*), with a protein content of 12.5% (12% moisture basis), was processed by BRI Australia Ltd (North Ryde, NSW, Australia). The wheat was conditioned to 16% moisture for 18 h, and then an extra 1% moisture was added just before it went onto a PeriTec debranning machine VCW (Satake Corporation, Higashi-Hiroshima-shi, Hiroshima, Japan). The PeriTec debranning system has two stages: in the first, the grain is abraded; and in the second, friction polishing removes any bran fragments. Debranning is increasingly used before roller milling to remove 8 – 12% of the grain as it reduces the amount of milling subsequently required [18]. However, in the present study, the PeriTec was set so that only 2.5% of bran by weight was removed. Initial experiments had shown that this was the optimal setting for removing the pericarp without abrading the aleurone. This was the "pericarp-rich fraction" (PRF) and contained much of the germ as well as the pericarp. The debranned wheat was then put onto the first break of the pilot mill at BRI Australia [19]. This mill can replicate full-sized, commercial millings because the equipment is of commercial scale, except it has reduced roller width and sieve surface areas. The milling of both debranned and normal wheat used flow rates, and flows and sieving conditions designed to simulate Australian bakers' flour. The bran and pollard produced by this milling was mixed to form the "aleurone-rich fraction" (ARF). A further sample of the same wheat was milled on the pilot mill, without debranning, to produce entire bran, pollard and germ and is referred to as "wheat bran" (WB); the extraction rate of this sample was 77.3%, giving a bran yield of 22.7%. The outer (bran) layers of wheat grains cv. Kotuku were used for sectioning, followed by field-emission scanning electron microscopy (see Section 2.2).

2.2 Field-emission scanning electron microscopy (FESEM)

Material for FESEM was prepared as follows: WB, ARF, and PRF were frozen in liquid nitrogen and freeze-dried in an Edwards freeze dryer (Model EPD3, Crawley, UK); sections of WB, ARF, PRF, and of the outer (bran) layers of wheat grains were cut on a cryostat (see Section 2.3) and also freeze-dried. The freeze-dried material was placed on aluminium stubs using carbon tabs (ProSci Tech, Australia), sputter-coated with gold and palladium using a Polaron coater (Model E5000, Watford, UK), and examined using a

field-emission scanning electron microscope (Model XL30S FEG, Philips, Eindhoven, The Netherlands) at 5 kV.

2.3 Histochemistry

The WB, ARF, and PRF were examined by light microscopy after staining with a variety of histochemical reagents. The unfixed material was first embedded in O.C.T medium (Miles Inc., Elkart, IN, USA) (a neutral, water-soluble material) and cooled to –20°C. The blocks formed were mounted on stubs and transverse and longitudinal sections (14.5 μm thick) cut using a cryostat (Model Cryocut 1800, Leica, Nussloch, Germany). Sections were stained with the following bright-field stains: 1% alcian blue 8GX in 3% v/v aqueous acetic acid, pH 2.5 (stained for 2 min) [20]; freshly prepared 0.02% w/v ruthenium red in 1% w/v ammonium acetate (stained for 5 min) [21]; 0.05% w/v toluidine blue O in 0.02 M sodium benzoate buffer, pH 4.4 (stained for 1 min) [22]; and 0.1% w/v Sudan red 7B in a solution consisting of 50% v/v polyethylene glycol (PEG), 45% v/v glycerol, and 5% v/v water (stained for 1 h) [23]. Ruthenium red and alcian blue stain polyanions, such as pectic homogalacturonans, pink or purple [20, 21, 24, 25]. Toluidine blue O stains polychromatically: lignin stains green or blue-green; polyanions, such as pectic homogalacturonans, stain pink or purple [26]. Sudan Red 7B stains lipids, cuticles, and suberized walls [23]. Sections were stained with the following bright-field colour reagents: freshly prepared phloroglucinol-HCl, made by mixing 1 mL of 2% w/v phloroglucinol in 95% v/v aqueous ethanol with 2 mL 10 M HCl (sections were examined immediately) [27]; 0.5% w/v potassium permanganate for 10 min, then rinsed with water, 10% v/v HCl for 5 min, rinsed with water and mounted in 18 M ammonium hydroxide solution (the Mäule reagent) [28]; and iodine in potassium iodide (0.2 g iodine and 2 g potassium iodide in 100 mL of water) (sections were examined immediately). Phloroglucinol-HCl and the Mäule reagent give a red colour with lignin [29]; however, the Mäule reagent detects only syringyl lignin [28]. The solution of iodine detects starch [30]. Sections were also stained with the following fluorochromes: 0.01% w/v Calcofluor White M2R (Fluorescent Brightener 28) in water (stained for 1–2 min) [31]; 0.01% w/v ethidium bromide in water (stained for 10 min) [26]; and 0.01% w/v Nile Blue A in water (stained for 3 min) [32]. Autofluorescence of the cell walls in UV radiation was examined using sections mounted in water and in 0.1 M ammonium hydroxide [33, 34]. Calcofluor stains chitin and β-D-glucans [31]. Ethidium bromide stains lignin and DNA [26]. Nile Blue A has a similar specificity to Sudan Red 7B [32]. Control sections, mounted in the solvent for the stain, color reagent, or fluorochrome, were also examined. Bright-field microscopy was done using an Axioplan 2 Universal microscope (Carl Zeiss, Oberkochen, Germany) fitted with a halogen quartz lamp (20 W). Fluorescence microscopy was done using a D-7082 Universal microscope (Carl Zeiss), equipped for epi-illumination and fitted with a mercury vapour lamp (HBO 50). The following filter sets were used: for UV radiation (for autofluorescence, Calcofluor, and ethidium bromide), a G 365 excitation filter, a FT 395 chromatic beam splitter and a LP 420 barrier filter; and for blue light (for Nile Blue A), a BP 450–490 excitation filter, a FT 510 chromatic beam splitter and a LP 520 barrier filter. Photomicrographs were taken on Fujichrome Sensia II, 100 ASA, daylight colour film (Fuji Photo Film Co., Tokyo, Japan). The intensity of staining of the cell walls (Table 2) was based on human perception of intensity.

2.4 Chemical analyses

The WB, ARF, and PRF were dried over silica gel under vacuum and ground to pass a 0.4 mm screen using a Tecator Cyclone Sample Mill (Tecator AB, Högenäs, Sweden). They were then destarched to yield cell wall preparations using the method of Smith and Harris [35]. Briefly, this involved gelatinizing the starch by heating for 5 min at 80°C in Tris-maleate buffer (5 mM, pH 6.9), and then incubating with porcine pancreatic α-amylase (Type 1-A; Sigma, St. Louis, MO, USA). The suspensions were then washed by centrifugation ($100 \times g$, 5 min), filtered onto nylon mesh (pore size 11 μm), dried by washing successively with ethanol, methanol, and *n*-pentane, and stored under vacuum over silica gel. Starch could not be detected histochemically in the cell wall preparations. We did not attempt to remove protein. The neutral-monosaccharide compositions of the cell wall preparations were determined by acid hydrolysis using two methods: 2 M trifluoroacetic acid (TFA) (121°C, 1 h) [36] and a two-stage sulphuric acid procedure [37]. The resulting monosaccharides were converted to alditol acetates and analysed by capillary gas chromatography [34, 38]. With the TFA hydrolysis conditions the cellulose is only poorly hydrolysed and the monosaccharides released are derived from noncellulosic polysaccharides [39]. The (1→3),(1→4)-β-glucans in the cell wall preparations were quantified by a direct and specific enzymatic assay using the (1→3),(1→4)-β-glucan endo-hydrolase from *Bacillus subtilis* [35, 40]. The method was modified by first adding water (200 μL) to the dry cell walls (10 mg) and heating for 1 h at 121°C. Means of two determinations done on each of two samples were reported. The amounts of the hydroxycinnamic acids ferulic and *p*-coumaric acids released by saponifying the cell wall preparations with 1 M NaOH were determined by gas chromatography of their trimethylsilyl ethers as described by Carnachan and Harris [41]. Briefly, cellwall preparations (10 mg) were shaken with 1 M NaOH (1 mL) containing 3,4-dimethoxycinnamic acid (10 μg/mL) as the internal standard for 20 h at 20°C under Ar. The suspension was filtered, the filtrate

adjusted to pH 1.5 and the hydroxycinnamic acids extracted by shaking with diethyl ether (4 × 6 mL). The combined extracts were evaporated to dryness and the residues trimethylsilylated. The trimethylsilyl derivatives of the hydroxycinnamic acids were separated and quantified by capillary gas chromatography on a DB-1 column (30 m × 0.32 mm ID) (J&W Scientific, Folsom, CA, USA) [41]. The *Z*-isomers of ferulic and *p*-coumaric acids were quantified using the response factor of their *E*-isomer.

2.5 Rat feeding studies

Six-weeks-old female Wistar rats were provided with experimental diets. All experimental protocols were approved by the University of Auckland Animal Ethics Committee, Permit No. N500. Throughout the experiments, the rats were maintained at a constant environmental temperature of 22°C, with a 12 h light-dark cycle. The rats were kept (3 per cage) in cages (58 × 24.5 × 17 cm) with dropped-bottom wires and without sawdust, and were randomly allocated into groups. Four diets were used: a basal high-fat modification of the AIN-76™ diet [42] or this diet in which 100 g of the maize starch was replaced with WB, ARF, or PRF. Table 1 shows the compositions of these diets, which were prepared dry. Water (200 mL/kg of diet) was added to each diet, which was then molded into a ball. Diet (60 g/day) was added to each cage, and rats were provided with the diet, fed *ad libitum*, for four weeks. The amounts of uneaten food were recorded. Each rat was weighed three times per week, and carefully monitored for signs of ill health.

Table 1. Compositions of the rat diets[a)]

Component (g/kg)	Control diet	Diet supplemented with WB, ARF, or PRF
Casein	200	200
WB, ARF or PRF	0	100
Maize starch	350	250
Sucrose	200	200
Sunflower seed oil	100	100
Lard	100	100
AIN-76™ vitamin mix	10	10
AIN-76™ mineral mix	35	35
DL- Methionine	3	3
Choline bitartrate	2	2

a) The basic diet was a high-fat modification of the AIN-76™ diet [42].

2.6 FESEM examination of cell walls in the rat faeces

Rats were fed diets supplemented with the WB, ARF, and PRF and the faeces collected. These were treated to remove the bulk of the non-cell wall material in the faeces, enabling

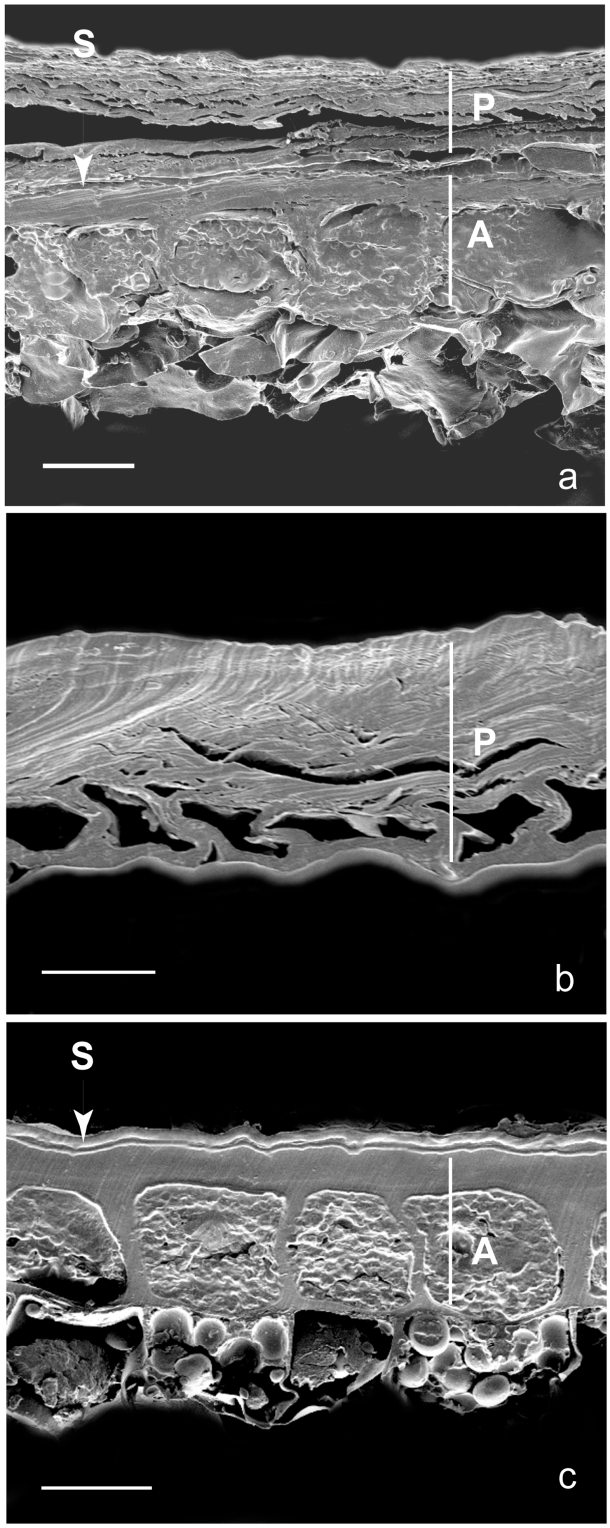

Figure 2. FESEM of cross sections of (a) WB, (b) PRF, and (c) ARF. The PRF has only pericarp cells; the ARF has only the aleurone and seedcoat, with some remnants of the starchy endosperm; the WB also has some remnants of the starchy endosperm. Abbreviations as in Fig. 1. Horizontal bars: a and c = 25 μm; b = 10 μm.

the cell walls to be examined by FESEM. Faeces (1 g fresh weight) were stirred in an aqueous solution of sodium dodecyl sulphate (SDS) (1.5% w/v; 20 mL) for 5 h at 4°C. The solid material was allowed to settle and the clear supernatant removed and discarded. The residue was resuspended in the SDS solution (20 mL, 4°C) and centrifuged ($100 \times g$, 5 min). The supernatant was further centrifuged ($400 \times g$, 5 min) and the supernatant discarded. The two pellets were resuspended in water, combined, and washed onto nylon mesh (pore size 11 μm). The residue on the mesh was washed with water (200 mL) and a small sample spread on a carbon tab. This was then frozen using liquid nitrogen, freeze-dried, coated, and examined using FESEM as described above.

3 Results

3.1 FESEM

Micrographs of the WB, PRF, and ARF are shown in Figs. 2a, b, and c, respectively. The PRF comprised only the pericarp and most of the ARF comprised only the seed coat (testa) and aleurone layer. About 5% of the ARF had some residual pericarp material (see Section 3.2). Remnants of starchy endosperm were present in the ARF and WB.

3.2 Histochemical staining properties of the walls

The results of the histochemical studies are shown in Table 2. Only the cell walls of the pericarp, including the epidermis, outer and inner pericarp, gave a positive (pink) colour reaction with phloroglucinol-HCl and stained with ethidium bromide indicating the presence of lignin (Figs. 3a–d). The staining with the ethidium bromide was particularly useful in characterising the fractions and showed that the PRF contained only pericarp walls, but about 5% of the ARF contained some residual pericarp material (Fig. 3d). The pericarp walls also gave a positive (pink) colour reaction with the Mäule reagent indicating the presence of syringyl lignin. The walls also autofluoresced blue in UV-radiation in both water and 0.1 M ammonium hydroxide (Fig. 3e) and stained blue or deep blue with toluidine blue O which is also consistent with the walls being lignified [33, 34]. Interestingly, these lignified walls stained either weakly or not at all with histochemical reagents known to bind to cell wall polysaccharides (Calcofluor white, alcian blue, and ruthenium red). In contrast, the walls of the aleurone cells showed no histochemical staining for lignin, but stained with those histochemical reagents known to bind to cell wall polysaccharides. Although no lignin was present in these walls, in UV-radiation, these walls autofluoresced blue in water and bright green in 0.1 M ammonium hydroxide (Fig. 3e), indicating the presence of ferulic acid ester-linked to the cell wall polysaccharides [33, 34]. The cuticle of the seed coat stained intensely with Sudan Red 7 B and Nile Blue A (Fig. 3f); the cuticle over the outer wall of the pericarp epidermis also stained, but only weakly.

3.3 Animal health and weight

All animals showed good health on all diets over the course of the study. There were no significant differences in the amounts of food eaten (data not shown).

3.4 FESEMs of walls in the rat faeces

Micrographs of walls from PRF and ARF that had passed through rats and had been recovered from the faeces (Figs. 4b, c; Figs. 5b–d) were compared with micrographs of equivalent, control fractions that had not been fed to rats (Fig. 4a; Fig. 5a). After passage through a rat, large numbers of bacteria, presumably originating from the large intestine, were seen adhering to the walls of the aleurone

Table 2. Histochemical staining properties of the walls of the WB[a)]

Cell type or structure	Bright-field stains			Bright-field colour reagents			Fluorochromes			
	Alcian Blue	Ruthenium Red	Toluidine Blue	Sudan Red 7B	Phloroglucinol-HCl	Mäule reagent	Auto-Fluorescence[b)]	Calcofluor	Nile Blue A	Ethidium bromide
Cuticle (on pericarp epidermis)	–	–	–	+p	–	–	+b	–	+y	–
Pericarp epidermis	–	–	+++db	–	+++p	+++p	+++b	–	–	+++r
Outer pericarp	–	–	+++db	–	+++p	+++p	+++b	+b	–	+++r
Inner pericarp	–	–	+++b	–	++p	+++b	++b	++b	–	+++r
Seed coat (with cuticle)	–	–	+++p	+++p	–	+p	++ly	+++b	+++y	–
Aleurone	++b	++p	++lb	–	–	–	+++g	+++b	–	–
Starchy endosperm (remnants)	+++b	+++p	+p	–	–	–	+g	+++b	–	–

a) The PRF was composed only of pericarp; the ARF was composed only of the seed coat and aleurone.
b) Fluorescence in 0.1 M ammonium hydroxide
Intensity of staining: +++ = intense; ++ = moderately intense; + = weak; – = no staining
Colour: b = blue; db = deep blue; lb = light blue; ly = light yellow; p = pink; r = red

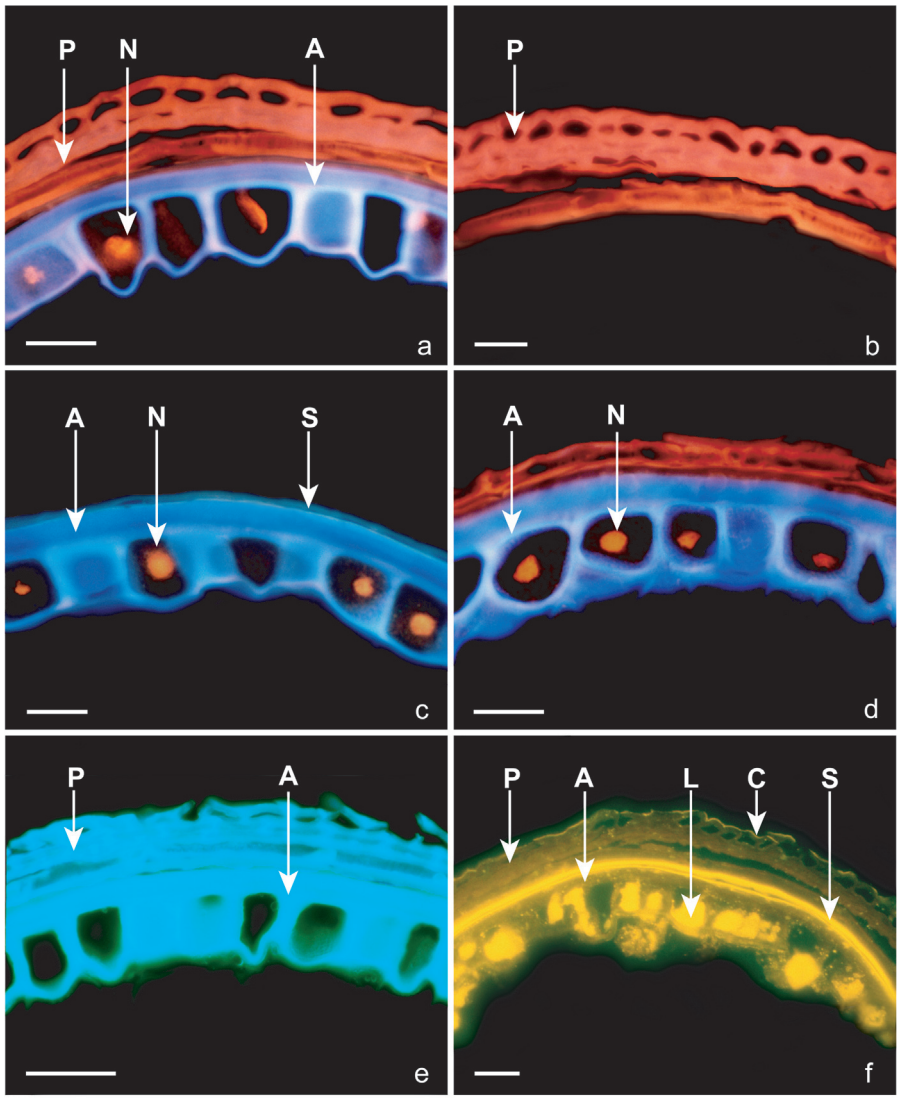

Figure 3. Fluorescence micrographs of sections of whole WB (a, e, f), PRF (b), and ARF (c, d). Sections (a – d) are stained with ethidium bromide. Most of the ARF has no adhering pericarp (c); however, about 5% of the ARF has some adhering pericarp, which fluoresces red (d) indicating the presence of lignin. When mounted in 0.1 M ammonium hydroxide (e): the aleurone walls fluoresce green in UV radiation, whereas the pericarp walls fluoresce blue. When stained with Nile Blue A (f), the cuticle on the seedcoat and lipid bodies in the aleurone cells fluoresce bright yellow; the cuticle on the epidermis of the pericarp fluoresces weakly. Abbreviations as in Fig. 1; C = cuticle over the pericarp epidermis, L = lipid bodies in the aleurone cells, N = nucleus. Bars = 10 μm.

cells in the ARF (Figs. 5b – d). The walls of these cells had also been partially degraded by bacterial enzymes, leading to the formation of holes (Fig. 5b, d), which would allow other bacteria to gain access and degrade the aleurone contents. In contrast, few bacteria were seen adhering to the walls of the PRF and these walls showed no evidence of being degraded (Figs. 4b, c). When the WB was examined after passage through a rat, similar results were found: the aleurone walls had large numbers of bacteria adhering to them and were partially degraded; the pericarp cell walls had few bacteria adhering to them and showed no signs of degradation (Fig. 4d).

3.5 Chemistry

The neutral-monosaccharide compositions of the cell wall preparations from the WB, ARF, and PRF are shown in Table 3. Xylose was the most abundant monosaccharide in the hydrolysates, and arabinose or glucose the second most abundant monosaccharide. Galactose and mannose were

Table 3. Neutral monosaccharide compositions of the bran walls[a)]

Walls	Acid hydrolysis	Monosaccharide (mol %)				
		Ara	Xyl	Man	Gal	Glc
WB	TFA	33.7	49.9	1.0	2.7	12.6
	H_2SO_4	26.8	43.9	1.1	2.1	26.1
ARF	TFA	30.4	51.6	1.3	2.4	14.3
	H_2SO_4	25.0	48.9	1.5	1.8	22.8
PRF	TFA	43.4	46.7	0.5	2.6	6.7
	H_2SO_4	28.7	38.0	0.7	1.9	30.7

a) Means of two determinations done on each of two samples. Replicates varied by >1%

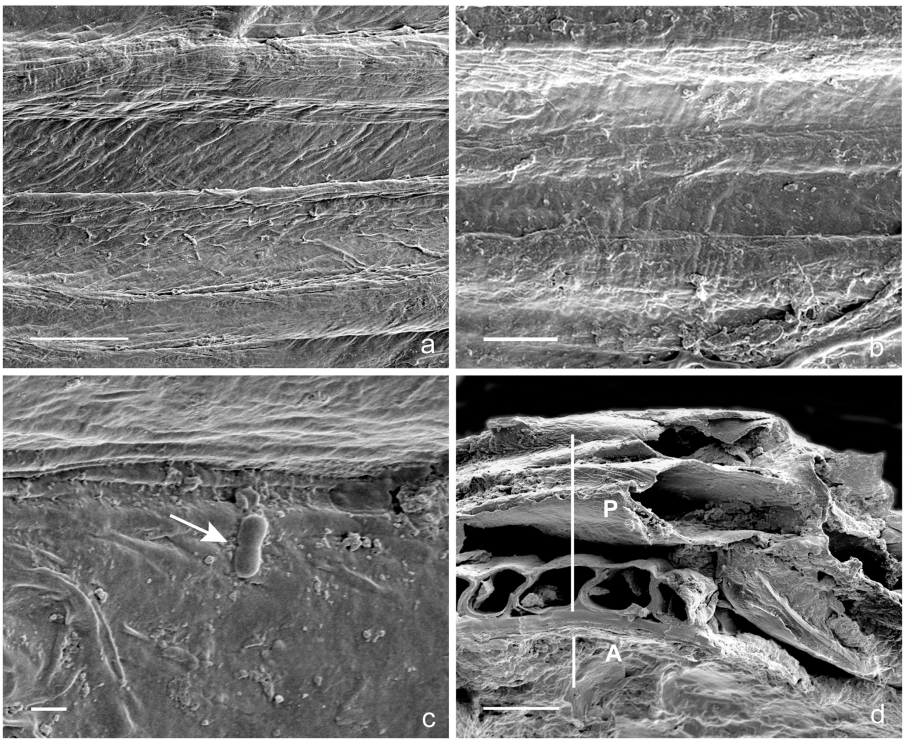

Figure 4. FESEM of the inner surfaces of the walls of (a–c) the PRF and (d) whole WB. The control, which had not passed through a rat is shown in (a); (b) shows identical material that had passed through a rat and had been recovered from the faeces. A higher magnification image of part of (b) is shown in (c); only a few bacteria are adhering to the walls and there is little evidence of wall degradation. Panel (d) shows WB that had passed through a rat; although there is little evidence of degradation of the pericarp walls, the aleurone walls are partially degraded. Bars: a and d = 20 μm; b = 10 μm; c = 2 μm.

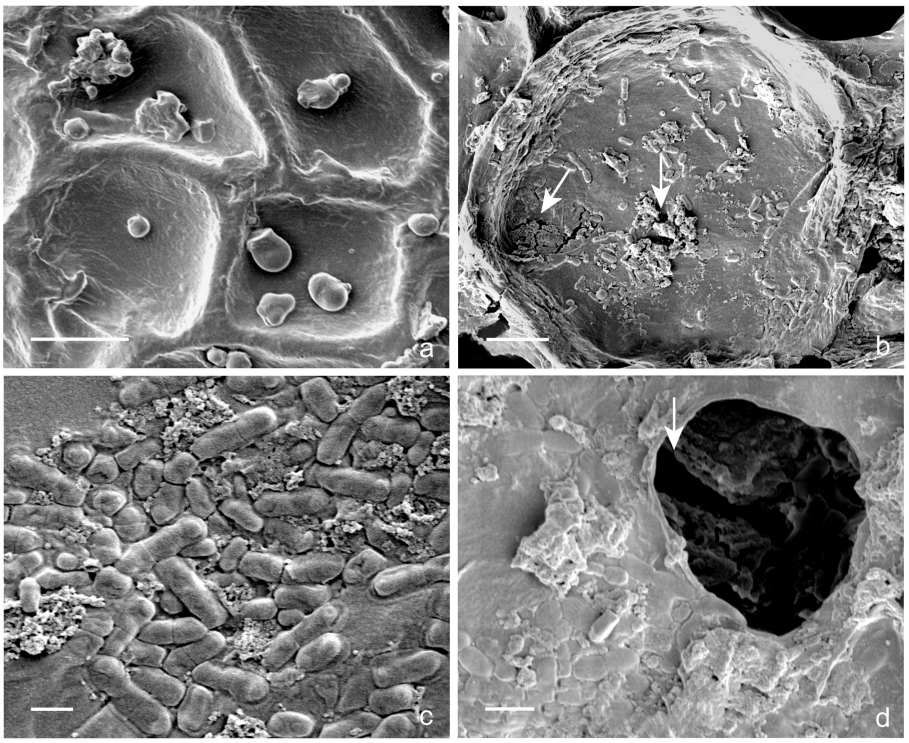

Figure 5. FESEM of the inner surfaces of the walls of the ARF. The control, which had not passed through a rat is shown in (a); (b–d) shows identical material that had passed through a rat and had been recovered from the faeces. Panels (b–d) show large numbers of bacteria adhering to the aleurone walls; they also show partial degradation of these walls, with holes present (arrows) (d). Bars: a = 20 μm; b = 10 μm; c and d = 2 μm.

present in small proportions. The ratio of arabinose to xylose was lowest in hydrolysates of the ARF (0.59 TFA; 0.51 sulphuric acid) and highest in hydrolysates of the PRF (0.93 TFA; 0.76 sulphuric acid). The sulphuric acid hydrolysates contained higher proportions of glucose than did the TFA hydrolysates. The highest proportion of glucose in the sulphuric acid hydrolysates was in the PRF and the lowest proportion was in the ARF. In contrast, the highest proportion of glucose in the TFA hydrolysates was in the ARF and the lowest proportion in the PRF.

Table 4. Concentrations[a] (mg/g) of ferulic and *p*-coumaric acids released from the walls by treatment with sodium hydroxide

Walls	Ferulic acid			*p*-Coumaric acid		
	E-	*Z*-	Total	*E*-	*Z*-	Total
WB	6.0	1.1	7.1	0.1	Tr[b]	0.1
ARF	6.6	1.6	8.2	0.1	–	0.1
PRF	4.0	0.9	4.9	0.1	–	0.1

a) Means of two determinations done on each of two samples. Replicates varied by >1%
b) Tr < 0.01 mg/g

The concentrations of (1→3),(1→4)-β-glucans in the cell wall preparations were as follows: WB 4.4%, ARF 5.1%, and PRF 1.3%. The concentrations of the hydroxycinnamic acids ferulic and *p*-coumaric acids in the cell wall preparations are shown in Table 4. Both acids occurred mainly as their *E*-isomer. Ferulic acid was the most abundant acid, with the highest concentration in the ARF walls and the lowest in the PRF walls. The concentrations of *p*-coumaric acid in the walls of the ARF, PRF, and WB were similar.

4 Discussion

The present study describes the large-scale production of two contrasting wheat bran fractions, an aleurone-rich fraction (ARF) and a pericarp-rich fraction (PRF). There have previously been a number of other studies on wheat bran fractions. Cheng *et al.* [43] used wheat bran from commercially milled soft wheat to obtain pericarp-seed coat and wheat aleurone fractions in sufficient quantities to carry out rat-feeding studies. They prepared these fractions by a sequence of impact milling, air elutriation, and electrostatic separation. Other researchers have obtained cell wall preparations from wheat bran on much smaller scales for chemical studies. For example, Ring and Selvendran [9] and DuPont and Selvendran [12] described the preparation of the walls of the outer-pericarp cells (beeswing bran) and Bacic and Stone [10] and Rhodes *et al.* [13] described the preparation of aleurone walls. Antoine *et al.* [14, 15] used manual dissection to obtain three fractions from wheat grains: an outer pericarp fraction, an aleurone fraction, and a fraction, referred to as "intermediate", containing the inner pericarp, testa and nucellar tissue.

The results of our studies can be compared with those obtained by others on the walls of wheat bran fractions. Bacic and Stone [11] analysed a purified preparation of wheat aleurone walls and reported that the polysaccharides comprised arabinoxylans (65%), (1→3),(1→4)-β-glucans (29%) and small proportions of cellulose (~2%), glucomannans (~2%), and callose (~1%). As found in the present study, Antoine *et al.* [14] reported that their aleurone cell wall fraction contained the highest proportion of (1→3),(1→4)-β-glucans. These glucans probably accounted for most of the staining of aleurone walls with Calcofluor, as has been reported previously [31]. It is not known which components of the aleurone walls were stained by the alcian blue or ruthenium. These dyes have been reported to bind to polyanions, such as pectic homogalacturonans [20, 24, 25], but such polysaccharides are either absent or present in only small proportions in these walls.

In contrast to the aleurone walls, the walls of an outer PRF (beeswing bran) comprised mostly or acidic arabinoxylans and cellulose, making up about 60% and 30% of the walls, respectively [9, 12]; no (1→3),(1→4)-β-glucans were reported in these walls. In the present study, the walls of the PRF contained only 1.2% (1→3),(1→4)-β-glucans. Our monosaccharide analyses of the walls of the fractions are also consistent with the walls of the PRF containing only small amounts of (1→3),(1→4)-β-glucans and much larger proportions of cellulose. The conditions of the TFA hydrolysis were such that only small proportions of cellulose were hydrolysed, but the sulphuric acid hydrolysis hydrolysed all the polysaccharides. Ring and Selvendran [9] found that the acidic arabinoxylans in the walls of their outer PRF were more highly substituted with arabinose (Ara : Xyl ratio of 1.29) than the arabinoxylans in the aleurone walls (Ara : Xyl ratio of 0.35) analysed by Bacic and Stone [11]. Antoine *et al.* [14] reported Ara : Xyl ratios of 0.38, 0.37, and 1.13 for the arabinoxylans in the walls of the aleurone, intermediate, and pericarp fractions, respectively. We also found a lower Ara : Xyl ratio for the arabinoxylans in the walls of our ARF (0.59) than our PRF (0.93), indicating a similar trend.

In contrast to aleurone walls, pericarp walls gave positive histochemical tests for lignin with phloroglucinol-HCl and the Mäule reagent. A positive reaction for lignin with phloroglucinol-HCl has previously been reported for wheat pericarp walls [44, 45]. However, definitive chemical proof has only recently been obtained for the presence of lignin in walls from wheat grains and from the pericarp. This proof was based on the detection of β-*O*-4-linkages connecting hydroxycinnamyl alcohol-derived units [14, 46].

Although the wheat aleurone walls gave negative histochemical tests for lignin, we found that they contained ester-linked ferulic acid as demonstrated by their autofluorescence and by chemical analysis. This confirmed the findings of Rhodes *et al.* [13] who also demonstrated the ferulic acid in these walls was ester-linked to arabinose residues of the arabinoxylans. As in our study, Antoine *et al.* [14] found the concentration of ester-linked ferulic acid in their aleurone-wall fraction (0.71%) was much higher than in their

pericarp-wall fraction (0.31%); the concentration in the walls of their "intermediate fraction" was intermediate (0.5%).

In addition to ferulic acid, the walls of wheat grains contain a range of ester-linked dehydrodimers of ferulic acid [14, 47, 48]. In the walls of wheat bran, Antoine *et al.* [14] found much higher concentrations of these dimers in the walls of their wheat pericarp fraction than in the walls of the other two fractions. A dehydrotrimer of ferulic acid, 4-*O*-8′, 5′-5″-dehydrotriferulic acid, has also recently been identified concentrated in the walls of wheat pericarp [15].

Our studies on the cell wall residues in the faeces of rats fed the wheat bran and wheat bran fractions indicated that the aleurone walls were partially degraded in the rat intestines, whereas the lignified walls of the pericarp showed little evidence of degradation. Similar results have been reported for experiments in which wheat bran was fed to rats [49] and to humans [44, 50] and in which wheat bran and isolated aleurone were incubated with human faecal bacteria [51, 52]. It is well known that lignification of the walls of forage grasses protects the wall polysaccharides from degradation in ruminant animals [53]. The large-scale production of wheat aleurone-rich and pericarp-rich fractions, which have walls with contrasting properties, will enable animal and human intervention studies to be carried out to determine the relative health benefits of the walls and phytochemicals of these two cell types in wheat bran.

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5 References

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