Isolation and characterization of cereal cell walls

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ABSTRACT
The present study was aimed to isolate and characterize cereal cell walls with special reference to monosaccharide and total phenolic content. For the purpose, two varieties of each cereal, i.e. wheat (Ujala-16 and FSD-08), barley (Jau-87 and Haider-93), and sorghum (Sorghum-11 and JS-02) were procured from Ayub Agriculture Research Institute, Faisalabad. In phase I, endospermic cell walls were isolated from cereals through popping method. In phase II, isolated cell walls were analyzed for monosaccharides, beta-glucan, and total phenolics according to their respective methods. Higher cell wall contents were shown by both varieties of barley (8.32–8.99 g/100 g) followed by wheat (5.21–5.68 g/100 g) and sorghum (3.88–4.02 g/100 g) varieties. Results regarding monosaccharides of endospermic cell walls revealed that these cell walls had arabinose, xylose, mannose, galactose, and glucose with glucose absence in wheat and mannose and galactose absence in sorghum. Furthermore, barley varieties were higher in beta-glucan and total phenolics content followed by wheat and sorghum. Conclusively, barley cell wall was considered more nutritious as compared to wheat and sorghum.

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Introduction
Cereals are the widespread crop throughout the temperate and tropical regions of the world belonging to the family Gramineae, where the species wheat, rice, and maize provide over 50% of the world’s plant-derived food energy.[1] Among the cereals cultivated in Europe, wheat is considered as the largest crop followed by barley and oat in the second and third places, respectively.[2] The cereal grain consists of three major portions, germ, endosperm, and bran, consisting of various layers to protect the grain.[3] The cereal grain is a complex structure possessing a cell wall with varying composition and properties.[4] It comprises mainly of starch along with proteins and non-starch polysaccharides, accounting for about 70–80%, 10–15%, and 3–8%, respectively.[5] The major non-cellulosic components are arabinoxylan and (1,3;1,4)-β-D-glucan, with lower levels of xyloglucans, glucomannans, and pectic polysaccharides. β-glucans and arabinoxylans are structural components of cell walls of many cereals such as wheat, oat, barley, and rye. Barley and oats are well known as the richest source of beta-glucan, whereas rye and wheat cell walls being the richest source of arabinoxylan.[6] These polysaccharides captured interest in both human and animal nutrition owing to their type and composition.[7,8] The physicochemical properties of arabinoxylan and β-glucan are influenced by the variation in their structural features which in turn influence many aspects of the end uses of cereals.[9] Cellulosic components of cereals cell walls are cellulose and lignins. Cellulose and lignin content in the cell wall of grains are higher than in the cell wall of endosperm. Owing to the less load-bearing role, there is a possibility of low cellulose and lignin contents in the endospermic wall.[10,11]
The composition of cereal endosperm flour was probed and found that it contained 70–80% starch, 5–15% protein, and 0.5–4% nonstarch polysaccharides mainly arabinoxylan and beta-glucan and to a lesser degree arabinogalactan. In a study, soluble and insoluble cell wall tiny proportions from rye, wheat, and hull-less barley endosperm flours were characterized and evaluated for the cross-linkage of cell wall and components. It was found that \( \beta \)-glucan has weak bonding with cell wall whereas arabinoxylan is strongly attached to the cell wall. Extraction of these foremost polymers from the cereal endosperm cell wall is not pretentious by inherent solution possessions of the constituent polysaccharides, but they are more probably affected by physical and chemical cross-linking contained by the cell wall. Maningat isolated and characterized the rice bran and germ cell walls with special reference to dietary fiber constituent and found that polysaccharides such as glucans, arabinoxylans, and pectins were 56–62% in cell wall followed by 8.49% arabinose, 3–35% xylose, 2–17% galactose, 1–8% fructose, 4–78% glucose, 1–17% mannose, and 0–1% rhamnose by using column chromatography.

Muller and Fulcher solubilized and digested the cell wall of oat endosperm through hot water and enzymatic treatment trailed by the analysis of monosaccharides through high pressure liquid chromatography (HPLC) and found an outer layer containing insoluble polysaccharides skeleton (cellulose and glucomannan) followed by an inner larger layer of soluble polysaccharides (arabinoxylan and beta-glucan). Parker et al. studied the effect of popping on the endosperm cell walls of sorghum and maize and found that popping increases the grain size along with its endosperm foam attached to the pericarp and embryo tissue. This popping method makes the protein and starch more susceptible to removal through digestive enzymes and improves their accessibility. By keeping in view all aforementioned perspectives, current investigation was designed to isolate and characterize wheat, barley, and sorghum endospermic cell walls for their sugar and total phenolic content.

**Material and methods**

**Procurement of raw material**

Two commercially available varieties of each cereals, i.e. wheat (Ujala-16 and FSD-08), barley (Jau-87 and Haider-93), and sorghum (Sorghum-11 and JS-02), were procured from Wheat Research Institute, Ayub Agriculture Research Institute, Faisalabad.

**Isolation of cell walls**

The cell wall was isolated from whole grains according to the method of Gartaula et al. In brief, firstly, all the endogenous enzymes were inactivated through heating of whole grains of wheat, barley, and sorghum at 130°C in a hot air oven for 90 min. After heating, these grains were cooled in a desiccator and transferred to a beaker for soaking in water (200 ml/100 g grains). After soaking, the beaker was placed in the refrigerator at 2°C for 5 days and the water was changed every day. After 5 days of soaking, grains were checked for softness and it was noticed that they were soft enough to be press by two fingers tips. After the softness test, the distal end of each soaked grain was cut with a pair of scissors and the proximal end pressed such that the white endosperm 'pops' out from the grain. This method is known as 'popping' method. Through popping, endosperm was achieved which was sieved through 250-micron sieve by using water. The purpose of sieving was to refine the popped endosperm and to remove impurities and any peripheral parts of grains that may have entered into the popped endosperm while cutting. This sieving was followed by addition of phosphate buffered saline (PBS) buffer at the rate of 40 ml per gram and thorough manual mixing of buffer in the residue to avoid clumps.

After the addition of buffer, popped endosperm was treated with enzymes at two different temperatures: one with the highest incubation temperature of 70°C and the next at 37°C to mimic the human physiological temperature. At first temperature, to gelatinize the starch and to denature proteins, the slurry of popped endosperm was heated at 70°C on a hot plate for 1 h. The enzyme
used at this step was heat stable alpha-amylase (50 µl per gram residue), and after enzyme addition, incubation was done for further 3 h. After heating at 70°C, the temperature was lowered to 60°C. Then to hydrolyze proteins and residual starch and oligosaccharides, addition of two enzymes, i.e. protease (60 µl per gram) and amyloglucosidase (100 µl per gram), and again incubation for 4 h were done.

In the second experiment, the popped endosperm slurry was incubated at 37°C for 48 h instead of 70°C and enzymes were added in the same concentration as in the first experiment. One main difference was the addition of sodium azide in buffer 0.02%. The level of digestion was tested by adding iodine in the slurry. After both experiments of digestion, the slurry was cooled and to remove smaller particulate matter, sieving with 20-micron sieve by using running water was done. After sieving, the non-digestible cell walls remain over the sieve and then centrifugation at 3200 g for 10 min was done. Washing of this residue was done twice with 70% ethanol and acetone and left to air-dry at room temperature.

Characterization of cereal cell walls

Monosaccharide analysis

Monosaccharides from cell walls of cereals were determined by gas chromatography. Firstly, sulfuric acid hydrolysis of cell walls of cereals was done and then alditol acetates were obtained. In detail, 250 µl of 72% sulfuric acid was used for the hydrolyzation of 5 mg of cereal cell walls at room temperature for 1 h. Then, this mixture was diluted with the addition of deionized water (2.75 ml). After dilution, incubation of mixture was done in an oven for 3 h at 100°C for further hydrolyzation. Then, cooling and neutralization of mixture were done with the addition of ammonia and 25 µl of internal standard (myo-inositol) and mixing was done. After neutralization, in another tube, 20 µl of this neutralized solution was added. Later, this solution was lowered with the addition of 200 µl of 20 mg/ml sodium borodeuteride at 40°C for 90 min in dimethyl sulphoxide. Then, 20 µl of acetic acid was used to destroy the reductant and mixed. Then, 25 µl 1-methylimidazole and 250 µl of acetic anhydride were added to acetylate it. Ten minutes of rest was given to the solution and then 2 ml of water was added in it followed by mixing. This step was followed by the addition of 1 ml dichloromethane to this solution and mixing was done. Extract alditol acetates were added in the solution and the mixture was allowed to stand for the purpose of separation. Washing of solution with water was done two times with 2 ml water, and then drying was done below a stream of nitrogen, re-formed in 500 µl of dichloromethane, and run on gas chromatography/mass spectrometry (column BPX40). Experiments were repeated for accuracy.

Total beta-glucan analysis

The cell walls of cereal grains were assessed for total beta-glucan contents by using a scaled-down mixed-linkage beta-glucan assay kit. According to the mixed-linkage beta-glucan assay kit, the reagents were made. Briefly, 10 mg of cell walls of cereal grains were weighed and placed in the 2-ml Eppendorf tubes. After this, 50 µl of 50% ethanol was used to wet samples for dispersion. Then, addition of 800 µl 20 mM sodium phosphate buffer (pH 6.5) was done in the samples and the mixture was vortexed. These Eppendorf tubes were placed in thermomixer for heating at 90°C/1000 rpm for 30 min. After heating, cooling of samples was done at 50°C and then this sample was placed in an incubator for 2 h at 50°C/1000 rpm with 100 µl of lichenase. Then, addition of 800 µl 200 mM sodium acetate buffer (pH 4.0) was made and vortexed. After that, mixture was centrifuged at 7000 g/10 min. Digestion of 100 µl of the supernatant taken with 100 µl beta-glucosidase was done at 50°C for 20 min. Then, 1.5 ml GOPOD reagent was placed in each reagent and placed in an incubator for 30 min at 50°C. Later, a spectrophotometer was used to measure glucose that was released and it was compared with the standard glucose at 510 nm. Analyses were done three times for confirmation.
Total phenolics

Total phenolics from cell walls of cereals were extracted by applying alkali treatment to the cereal cell walls with some changes according to the method of Waldron et al.\textsuperscript{[22]}. In detail, firstly, in a 2-ml Eppendorf tube, about 10 mg of the cell walls of cereals and 800 µl of 2M NaOH were added. For mixing of cell wall residue and NaOH, the tube was kept in a thermomixer and mixing was done for 24 h at 25°C. After mixing, pH of the content was reduced to below 2 with the addition of concentrated HCl to acidify the content. After acidification, content was centrifuged for 10 min for pellet separation. The supernatant obtained from centrifugation was added in another tube followed by three times extraction of phenolics by using 1 ml ethyl acetate for each time. After extraction of phenolics with ethyl acetate, this extract was put into 15-ml corning tubes and evaporated under vacuum. This residue with phenolic compounds was redissolved in 200 µl 50% MeOH, and this residue was used for the estimation of total phenolic compounds. Moreover, for the determination of total phenolic content, Folin–Ciocalteu assay was followed with some modifications.\textsuperscript{[23,24]} In detail, blank of 25 µl, standards of ferulic acid standards or the diluted (1:4) methanolic extracts and 200 µl of 10% (v/v) freshly prepared Folin–Ciocalteu reagent were placed in a 2-ml microtube and mixed. Fifteen minutes were given to this residue for reaction. Later, in each tube, 800 µl of 0.7 M sodium carbonate was added. UV/Vis spectrophotometer (UV-1700, Shimadzu Corporation, Japan) was used to check the absorbance of the mixture at 725 nm in contradiction of 50% MeOH as a blank. The investigations were done in triplicate.

Statistical analysis

At the end, the obtained data of each parameter was analyzed by applying statistical analysis using statistical package, i.e. Statistix 8.1. The data was subjected to complete randomized design to compare varieties for their composition followed by least significance difference according to the methods of Steel et al.\textsuperscript{[25]}.

Results and discussion

Cell wall content of cereals

Table\textsuperscript{1} explicates mean values regarding cell wall contents of different varieties of cereals, i.e. Ujala-16 and FSD-08, Jau-87 and Haider-93, and Sorghum-11 and JS-02. The highest cell wall contents were revealed by barley varieties whereas both varieties of sorghum showed the lowest cell wall content. In wheat, maximum results were shown by Ujala-16 and minimum cell wall content was shown by FSD-08. Furthermore, in the case of barley, Jau-87 exhibited 8.32 ± 0.21 g/100 g and Haider-93 showed 8.99 ± 0.23 g/100 g cell wall contents. Moreover, in Sorghum-11 and JS-02, 4.02 ± 0.12 g/100 g and 3.88 ± 0.07 g/100 g cell wall contents were present, respectively. The results of wheat endospermic cell wall were in accordance with the results of Dervilly et al.\textsuperscript{[26]} who probed the cell wall residue in the endosperm of wheat and found that wheat endosperm contained approximately 2–7% cell wall content in which maximum constituents are non-starch polysaccharides.

Monosaccharides content of endospermic cell wall of cereals

Mean values for the monosaccharide content of endospermic cell walls of two different varieties of wheat are depicted in Table\textsuperscript{2}. The highest results were shown by xylose (49.62 ± 0.12%) whilst the

\begin{table}[h]
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\begin{tabular}{lcccc}
\hline
Cereals & Wheat & Barley & Sorghum \\
\hline
 & Ujala-16 & FSD-08 & Jau-87 & Haider-93 & Sorghum-11 & JS-02 \\
Cell wall & 5.68 ± 0.15\textsuperscript{a} & 5.21 ± 0.19\textsuperscript{b} & 8.32 ± 0.21\textsuperscript{b} & 8.99 ± 0.23\textsuperscript{a} & 4.02 ± 0.12\textsuperscript{a} & 3.88 ± 0.07\textsuperscript{a} \\
\hline
\end{tabular}
\caption{Mean values for cell wall contents (g/100 g) of different varieties of cereals.}
\end{table}

Mean values carrying same letters are significantly identical.
lowest were exhibited by galactose (2.52 ± 0.03% of total neutral sugar). Arabinose (33.93 ± 0.06%) and xylose (49.62 ± 0.12%) contents were more in the endospermic cell wall of Ujala-16 than in FSD-08 that are 33.59 ± 0.11% and 45.54 ± 0.13%, respectively. Moreover, mannose content was 6.58 ± 0.01% and 6.85 ± 0.13% in endospermic cell walls of Ujala-16 and FSD-08, respectively. Furthermore, the contents of galactose in endospermic cell walls of Ujala-16 and FSD-08 were 2.52 ± 0.03% and 2.55 ± 0.04%, respectively, whereas glucose contents were 1.98 ± 0.34% and 1.64 ± 0.08% in endospermic cell walls of Ujala-16 and FSD-08, respectively. These investigations were similar to the results of various studies.\[27–30\] These studies showed that cell wall of wheat endosperm contained 34%, 53.5%, 7%, and 2.5% of arabinose, xylose, mannose, and galactose, respectively.

Table 2 depicts mean values for monosaccharide content of endospermic cell walls of Jau-87 and Haider-93. Among monosaccharides, glucose revealed maximum results (77.11 ± 0.13%) whilst mannose showed lower results (2.00 ± 0.04%). In Jau-87, arabinose, xylose, mannose, galactose, and glucose were 8.98 ± 0.01%, 9.94 ± 0.07%, 2.00 ± 0.04%, 2.72 ± 0.06%, and 77.11 ± 0.13%, and in Haider-93, these sugars were 8.75 ± 0.03%, 10.10 ± 0.06%, 1.88 ± 0.01%, 3.07 ± 0.05%, and 77.51 ± 0.04%, respectively. The results were in accordance with the studies of Nandini and Salimath\[31\] and Bacic and Stone\[29,30\] who probed the monosaccharides content in cell wall of barley endosperm and found that endospermic cell wall of barley contained 9%, 10%, 2%, 3%, and 79% of total neutral sugar for arabinose, xylose, mannose, galactose, and glucose, respectively.

Mean values for the monosaccharide content of endospermic cell walls of two different varieties of sorghum are depicted in Table 2. Among monosaccharides, highest results were shown by arabinose followed by xylose and glucose in both sorghum varieties. The contents of arabinose in sorghum-11 and JS-02 were 2.25 ± 0.01% and 2.10 ± 0.04%, respectively, whereas xylose contents were 2.47 ± 0.06% and 2.52 ± 0.03%, respectively. Moreover, glucose content was more in Sorghum-11 (0.90 ± 0.02%) than in JS-02 (0.77 ± 0.03%). The results revealed that mannose (0.19 ± 0.18%, 0.34 ± 0.02%) and galactose (0.21 ± 0.06%, 0.08 ± 0.06%) were present in Sorghum-11 and JS-02, respectively. The results of the present study were in line with the results of McCleary and Glemie-Holmes.\[32\] The results of this study revealed that main monosaccharides in the endospermic cell wall of sorghum were arabinose (1.3%), xylose (1.1%), and glucose (0.8%).

**Beta-glucan content of endospermic cell wall of cereals**

Table 3 explicates mean values regarding beta-glucan contents of endospermic cell wall contents of different varieties of cereals, i.e. Ujala-16 and FSD-08 of wheat, Jau-87 and Haider-93 of barley, and Sorghum-11 and JS-02 of sorghum. Highest beta-glucan contents were revealed by both varieties of barley whereas, both varieties of sorghum had lowest beta-glucan content. In wheat, maximum results were shown by Ujala-16 (4.77 ± 0.04 g/100 g) and minimum beta-glucan in endospermic cell wall content was shown by FSD-08 (4.59 ± 0.08). Furthermore, in the case of barley, Jau-87 had 38.41 ± 0.08 g/100 g and Haider-93 had 39.83 ± 0.06 g/100 g beta-glucan in the endospermic cell wall. Moreover, 0.17 ± 0.03 g/100 g and 0.19 ± 0.08 g/100 g beta-glucan contents were present in Sorghum-11 and JS-02, respectively. Many studies showed similar results to the results of the present study. Havrlentova and Krai\[33\] evaluated beta-glucan content in white flour of barley and wheat

| Cereals | Varieties | Arabinose | Xylose | Mannose | Galactose | Glucose |
|---------|-----------|-----------|--------|---------|-----------|---------|
| Wheat   | Ujala-16  | 33.93 ± 0.06a | 49.62 ± 0.12a | 6.58 ± 0.01b | 2.52 ± 0.03b | 1.98 ± 0.34a |
|         | FSD-08    | 33.59 ± 0.11b | 45.54 ± 0.13b | 6.85 ± 0.13b | 2.55 ± 0.04b | 1.64 ± 0.08b |
| Barley  | Jau-87    | 8.86 ± 0.01b | 9.94 ± 0.07a | 2.00 ± 0.04a | 2.72 ± 0.06b | 77.11 ± 0.13b |
|         | Haider-93 | 8.75 ± 0.03b | 10.10 ± 0.06b | 1.88 ± 0.01a | 3.07 ± 0.05a | 77.51 ± 0.04b |
| Sorghum | Sorghum-11| 2.25 ± 0.01a | 2.47 ± 0.06b | 0.19 ± 0.18b | 0.21 ± 0.06a | 0.90 ± 0.02a |
|         | JS-02     | 2.10 ± 0.04b | 2.52 ± 0.03a | 0.34 ± 0.02b | 0.08 ± 0.05b | 0.77 ± 0.03b |

Mean values carrying same letters are significantly identical.
and it was found that beta-glucan content was 41.6 and 4.8 g/kg in barley and wheat, respectively. Moreover, in a study, Moraes et al.\textsuperscript{[34]} exhibited beta-glucan contents of whole sorghum flour and sorghum bran which were 0.12 ± 0.01 and 0.21 ± 0.01 g/100 g, respectively.

**Total phenolic content of endospermic cell wall of cereals**

In Table 3, mean values of total phenolic content of endospermic cell walls depicted that barley endospermic cell wall contained maximum total phenolic content, whereas minimum total phenolic content was showed by sorghum varieties. In the case of wheat, Ujala-16 and FSD-08 contained 0.14 ± 0.01 and 0.19 ± 0.02 mg gallic acid/g endospermic cell wall of wheat of total phenolic content, respectively. Moreover, in case of barley, total phenolic content was 0.53 ± 0.08 mg gallic acid/g endospermic cell wall of barley for Jau-87 and 0.61 ± 0.05 mg gallic acid/g endospermic cell wall of barley for Haider-93. However, maximum total phenolic content (0.10 ± 0.03 mg gallic acid/g endospermic cell wall of sorghum) was shown by Sorghum-11 and minimum total phenolic content mg gallic acid/g endospermic cell wall of sorghum was exhibited by JS-02 (0.05 ± 0.04 mg gallic acid/g endospermic cell wall of sorghum) in sorghum. The results of the current study were in accordance with the results of Bonoli et al.\textsuperscript{[35]} and Cardoso et al.\textsuperscript{[36]} who probed the total phenolic content in white flour of barley and sorghum and found 0.13–0.68 mg gallic acids/g flour of barley and 6.0 ± 0.1 μg gallic acid equivalents/g in dry basis in sorghum, respectively.

**Conclusion**

Isolation of endospermic cell walls from different cereals revealed that barley contained higher cell wall content than wheat and sorghum. Moreover, the cell wall of barley was rich in beta-glucan, whereas the cell wall of wheat had higher arabinose and xylose content. Furthermore, with reference to total phenolics, barley endospermic cell wall was more important as compared to wheat and sorghum endospermic cell walls. Keeping in view the results of current investigation, extraction followed by incorporation of components of cereal (especially barley) endosperm cell wall in cereal-based baked products should be done and the resultant novel products should be introduced in market for functional and nutritional attributes because consumers are more conscious toward their diet and wish for natural remedies.

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