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EXTRACTION, QUANTIFICATION AND BIOCHEMICAL CHARACTERIZATION OF CEREAL INDUSTRY BY-PRODUCT CELL WALL

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Abstract

The current study was aimed to extract and purify the cell walls of cereal Industrial by-product's i.e. maize, wheat and barley bran, followed by their nutritional and biochemical properties. For the purpose, three varieties of wheat and barley were procured from Ayub Agriculture Research Institute, Faisalabad, Pakistan. Cell walls from industrial by-products were isolated and purified followed by the removal of starch and proteins from contaminating endosperm tissue. Moreover, their sugar and polysaccharide contents wall were determined through gas chromatography and alkaline treatment respectively. Results revealed that cell wall content is more in barley (43.18 ± 0.58 , 42.43 ± 1.07 and 42.08 ± 0.13 g/50g) than in wheat (39.30 ± 1.67 , 38.72 ± 0.45 and 37.45 ± 0.23 g/50g) and maize (34.00 ± 0.08 , 34.56 ± 0.39 and 33.76 ± 1.21 g/50 g). Moreover, barley industry by-product cell wall has higher total sugars and total phenolic acids contents whereas, wheat industry by-product cell wall showed lower followed by maize cell wall. Conclusively, cell wall of barley industry by-product is more nutritious than that of wheat and maize industry by-product cell walls.

Keywords: Cereal, Cell walls, Bran, Extraction, Nutrition, Purification

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Practical Applications

Cereals are the most abundant commodities widely used in the world as staple foods. Cereal bran cell wall have important bioactive components which have the ability to combat different life threatening diseases. Utilization of cell wall in cereal-based products can fulfill many objectives including improving product quality, shelf life, value addition, and maintaining good health of the consumers. Novel products with improved rheology can be introduced in market as functional foods against various diseases such as diabetes mellitus, cancer, bacterial infections. Cereal bran cell wall can be probed in the baking industry to enhance the shelf life and stability of the baked goods.

Introduction

Cereal bran is a composite plant material containing thick walled cells with different layers including aleurone, testa, pericarp and residual endosperm tissue. Cell walls of various types have different structural components such as higher contents of polysaccharides (mainly cellulose and heteroxylans), considerable amount of phenolic acids, proteins and lower lignin level and all of these components have covalent bridges, ionic and hydrogen bonding (Chanliaud et al, 1994; Carpita and Gibedut, 1993). Polysaccharides and some other components of cell wall including lignin and proteins are connected by phenolic acids through ester and ether bonds (Jung and Deetz, 1993). Among cereal brans, wheat bran is an outer layer of most important and commonly consumed cereal grain i.e. wheat (*Triticum aestivum* L). This wheat bran is separated from other parts of wheat grain (endosperm and germ) through milling (Tufail et al, 2020).

Wheat bran is an important by-product of the cereal industry produced in vast quantities worldwide, and it is estimated that more than a 100 million tons of bran are available in the European Union (Martel et al, 2010). Wheat bran consists of various components including 38 % non-starch polysaccharides (70 % arabinoxylans, 19 % cellulose and 6 % β -glucan), 19 % starch, 18 % protein, 6 % lignin, significant amounts of phenolic acids esterified to arabinofuranosyl residues (ferulic and p-coumaric acids) (Vangsoe et al, 2019; Parker, et al, 2005). Maize is another most important cereal with bran cell wall that contains different types of sugars such as feruloylated oligosaccharides on side chains of heteroxylans and each heteroxylan macromolecule carried -60 ferulic acid esters and can be cross-linked through approximately five diferulic bridges (Lau et al., 2019; Tufail et al, 2018).

Another most important cereal is barley (*Hordeum vulgare* L.) which is at fourth position in cereals in worldwide production. Nowadays, there is an increasing trend of barley utilization as

a food source in research owing to the presence of higher content of soluble dietary fibers, especially β -glucans (Bilgi and Celik, 2004). A large amount of functional constituents such as arabinoxylan, beta-glucan and pectin are present in barley. Among cereals, barley grain cell wall is rich in beta-glucans (Ahmed, et al, 2011). Besides nutritional and functional properties of cereal bran, its utilization at industrial level is still very low (Bayer et al, 2014).

Therefore, there is dire need to isolated and purify cell wall from cereal bran and to incorporate this cell wall in products to make them functional against various diseases. In this study, cell wall was extracted from cereal bran and then purified. After purification, it was characterized for its nutritional components mainly sugars and phenolic acids and this characterization was followed by the extraction of components of cereal bran cell wall. By keeping in view all the aforementioned aspects, the current study has been designed to extract and purify the cell walls of maize, barley and wheat bran followed by their nutritional characterization with special reference to monosaccharides and phenolic acids.

Materials and methods

Procurement of raw material

Commercial micronized (particle size $< 80 \mu\text{m}$) cereal bran (Pearl-11, MMRI-11 & YH-98 of maize, Gold-16, AARI-11 & Punjab-11 in wheat and Sultan-17, Jau-17 & Jau-87 of barley) were procured from Ayub Agriculture Research Institute Faisalabad, Pakistan.

Isolation and purification of bran cell walls

Cell wall from different cereal bran was isolated and purified followed by the removal of starch and proteins from contaminating endosperm tissue according to the method of (Brillouet, et al, 1988). For the purpose Megazyme Total Dietary Fiber Assay Kit Containing (Protease, Amyloglucosidase, Alpha Amylase) was used as enzymatic treatment. In brief, firstly bran (50 g) was suspended in 0.1 M phosphate buffer (pH 7.5, 1 l) with sodium lauryl sulfate (I “A), 2-mercaptoethanol (1%) and sodium azide (0.02%). Suspension in buffer was followed by the addition of 25 ml solution of 5 mg/ml alkaline protease and then stirring of this mixture was done for 2 h at 40°C. After centrifugation, the residue was rinsed with distilled water and suspended again in 1 l of distilled water. The mixture was boiled for 15 min, then 10 ml of Termamyl 120 L was added and the mixture was stirred for 1 h at 95°C. The residue was collected on a sintered glass filter, rinsed with hot water (500 ml) and then dried by solvent exchange (ethanol, acetone)

and kept overnight in an oven at 40°C. Residues were observed through microscope and presence of starch was checked by Iodine test and at the end while performing iodine test it did not shown blue color that was an indicator for the absence of starch. Pure Cell wall then freeze dried in powder form.

Monosaccharide analysis

Monosaccharides from cell walls of cereal brans were determined by Gas Chromatography (GC). Gas chromatograph equipped with a flame ionization detector (Agilent 6890) was used to determine monosaccharide content which acquired through extraction. A 30m long DB-5 fused silica capillary column with a film thickness of 1.0µm and a 0.25mm inside diameter was used. A multi-ramp column oven temperature program was used to separate the monosaccharides (Li, 1996). An initial temperature of 180°C was held for 4 min, then increased to 200°C at a rate of 1.5°C/min, held for 1 min, increased to 260°C at a rate of 10°C/min, held for 2 min, then increased to 290°C at a rate of 6°C/min increments and held for 8 min. The detector and injection port temperatures were 300°C and 280°C, respectively. Nitrogen was used as the carrier gas with a flow rate of approximately 1.5 ml/min. A 2.0µl split less sample was injected to quantitate individual sugars according to (Pettolino et al, 2012).

Total Phenolics

Total phenolics from cell walls of cereal brans were extracted by applying alkali treatment to the cereal bran cell walls with some changes according to the method of Waldron et al (1996) and coumaric acid, ferulic acid & diferulic acid standards were used. Ferulic acids were evaluated by HPLC (Perkin Elmer series 200) equipped with UV visible detector. The analytical column used was C 18 with Reverse phase.

Statistical analysis

At the end, obtained data of each parameter was analyze by applying statistical analysis using statistical package i.e. Statistix 8.1. Firstly, the data was subjected to complete randomized design (CRD) to compare varieties for their composition. Later, to probe the significance level of treatments over varieties, least significance difference was applied according to the methods of Steel *et al.* (1997).

Result and Discussion

Cell wall content of cereals

Table 1 explicated mean values regarding cell wall contents of different varieties of cereal brans i.e. Pearl-11, MMRI-11 & YH-98 of maize, Gold-16, AARI-11 & Punjab-11 of wheat and Sultan-17, Jau-17 & Jau-87 of barley. The highest cell wall contents were revealed by barley varieties whereas, both varieties of maize showed the lowest cell wall content. In barley, maximum results were shown by Jau-17 (43.18 ± 0.58 g/50g) and minimum cell wall content was shown by Jau-87 (42.08 ± 0.13 g/50g) followed by Sultan-17 (42.43 ± 1.07 g/50g). Furthermore, in case of maize, Pearl-11 exhibited 34.00 ± 0.08 g/50g, MMRI-11 showed 34.56 ± 0.39 g/50 g and YH-98 depicted 33.76 ± 1.21 g/50g cell wall contents. Moreover, in wheat, Gold-16, AARI-11 and Punjab-11, 39.30 ± 1.67 g/50g, 37.45 ± 0.23 g/50g and 38.72 ± 0.45 g/50g cell wall contents were present respectively. The results of maize bran cell wall were in accordance with the results of (Saulnier, et al, 1995) who probed the cell wall residue in the bran of maize and found that maize bran contained approximately 74 % cell wall content in which maximum constituents are non-starch polysaccharides.

Table 1 Place here

Total sugar content of bran cell wall of cereals

Mean values for total sugars content of bran cell walls of three different varieties of barley, wheat and maize are depicted in Table 2. The highest results were shown by xylose (239.87 ± 2.06 µg/mg dry cell wall material) whilst the lowest were exhibited by galactose (4.18 ± 1.29 % of dry weight). In case of maize bran cell wall, Arabinose (23.37 ± 2.34 %) and Xylose (35.90 ± 0.10 %) contents were more of MMRI-11 than in Pearl-11 (22.83 ± 2.11 and 35.12 ± 1.45 %) and YH-98 (22.12 ± 2.05 and 33.67 ± 0.89 %) respectively. Moreover, Glucose content was 22.11 ± 1.06 , 20.33 ± 0.12 and 19.67 ± 2.54 % in Pearl-11, YH-98 and MMRI-11, respectively. Furthermore, the contents of glucouronic acid and galactose of MMRI-11, Pearl-11 & YH-98 were 5.11 ± 0.03 , 4.87 ± 2.03 & 4.87 ± 2.03 % and 5.62 ± 1.78 , 4.18 ± 1.29 & 4.34 ± 1.57 %, respectively.

In case of wheat bran cell wall, the contents of glucose, galactose and glucouronic acid of Gold-16, AARI-11 & Punjab-11 were 49.26 ± 0.09 , 52.88 ± 0.02 & 51.39 ± 2.54 µg/mg dry cell wall material, 7.91 ± 0.94 , 7.12 ± 0.78 & 7.66 ± 1.29 µg/mg dry cell wall material and 19.54 ± 0.83 , 17.74 ± 1.40 & 17.98 ± 0.37 µg/mg dry cell wall material, respectively. Arabinose (147.23 ± 1.54 µg/mg dry cell wall material) and Xylose (217.56 ± 1.12 µg/mg dry cell wall material) contents were more in bran cell wall of Gold-16 than of AARI (133.45 ± 0.07 and 215.12 ± 3.21 µg/mg dry cell wall material) and Punjab-11 (138.22 ± 2.30 and 215.89 ± 1.48 µg/mg dry cell wall material),

respectively. Moreover, as far as the sugar content of barley bran cell wall is concerned, arabinose, xylose, galactose, glucouronic acid and glucose in Sultan-17 were 198.22 ± 2.14 , 239.87 ± 2.06 , 26.34 ± 0.19 , 61.78 ± 0.13 & 69.56 ± 0.01 $\mu\text{g}/\text{mg}$ dry cell wall material followed by Jau-17 (203.19 ± 0.04 , 236.55 ± 2.10 , 28.10 ± 2.29 , 65.24 ± 0.53 & 70.15 ± 0.04 $\mu\text{g}/\text{mg}$ dry cell wall material) and Jau-87 (186.07 ± 0.34 , 231.49 ± 0.01 , 21.78 ± 1.20 , 60.17 ± 0.02 & 75.23 ± 0.54 $\mu\text{g}/\text{mg}$ dry cell wall material), respectively.

Table 2 Place here

Total phenolic content of bran cell wall of cereals

In Table 3, mean values of total phenolic content of bran cell walls depicted that barley bran cell wall contained maximum total phenolic content followed by wheat bran cell wall and maize bran cell wall. In case of barley, p-Coumaric acid, ferulic acid & diferulic acid in Sultan-17 bran cell wall were 8.12 ± 0.60 , 15.5 ± 1.56 & 9.12 ± 2.45 % dry weight than Jau-17 bran cell wall (8.78 ± 0.09 , 13.78 ± 0.49 & 8.65 ± 1.78 % dry weight) and Jau-87 bran cell wall (7.45 ± 0.17 , 14.06 ± 0.38 & 9.56 ± 0.27 % dry weight). In case of wheat bran cell wall, Gold-16, AARI-11 & Punjab-11 contained 4.97 ± 3.58 , 3.58 ± 1.22 & 3.83 ± 0.12 % dry weight, 12.45 ± 0.54 , 10.23 ± 0.35 & 11.76 ± 0.06 % dry weight and 5.12 ± 0.09 , 4.78 ± 2.10 & 4.99 ± 1.24 % p-Coumaric acid, ferulic acid and diferulic acid, respectively. Moreover, in case of maize bran cell wall, p-Coumaric acid content was 0.46 ± 2.23 % for Pearl-11, 0.51 ± 0.67 % for MMRI-11 and 0.42 ± 0.10 % for YH-98. Whilst, maximum ferulic acid content (3.75 ± 1.12 %) was shown by MMRI-11 followed by YH-98 (3.51 ± 0.34 %) and Pearl-11 (3.28 ± 1.65 %). Furthermore, diferulic acid was 0.73 ± 2.98 % for Pearl-11, 3.51 ± 0.34 % for YH-98 and 0.40 ± 2.20 % for MMRI-11 (Ahmad et al., 2019).

Table 3 place here

Conclusion

It is evident from the present study that cell wall content is more in barley bran than in wheat and maize bran. Moreover, barley bran cell wall has higher total sugars and total phenolic acids contents followed by total sugars and total phenolic acids contents in wheat and maize bran cell wall. This aspect acts as a pillar to make barley bran cell wall nutritionally more important than wheat and maize bran cell wall. This open the door for the efficient utilization of cereal bran cell wall content especially barley bran cell wall as an ingredient in the development of various

functional foods. By keeping in view all the literature and current research, there is still some more research required regarding cell wall of cereal bran and their characterization.

Conflict of Interests

Authors declare that they have no conflict of interest.

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Table 1: Cell wall content of wheat, barley and maize industry by-products

Cereals	Maize industry by-products (g/50g)			Wheat industry by-products (g/50g)			Barley industry by-products (g/50g)		
	Pearl-11	MMRI-11	YH-98	Gold-16	AARI-11	Punjab-11	Sultan-17	Jau-17	Jau-87
Cell walls	34.00±0.08	34.56±0.39	33.76±1.21	39.30±1.67	37.45±0.23	38.72±0.45	42.43±1.07	43.18±0.58	42.08±0.13

Table 2: Total sugar content of wheat, barley and maize industry by-product's cell wall

Sugars		Arabinose	Xylose	Galactose	Glucouronic acid	Glucose
Maize industry by-products (% dry weight)	Pearl-11	22.83±2.11	35.12±1.45	5.62±1.78	4.87±2.03	22.11±1.06
	MMRI-11	23.37±2.34	35.90±0.10	4.18±1.29	5.11±0.03	19.67±2.54
	YH-98	22.12±2.05	33.67±0.89	4.34±1.57	4.54±0.78	20.33±0.12
Wheat industry by-products (µg/mg dry CWM)	Gold-16	147.23±1.54	217.56±1.12	7.91±0.94	19.54±0.83	49.26±0.09
	AARI-11	133.45±0.07	215.12±3.21	7.12±0.78	17.74±1.40	52.88±0.02
	Punjab-11	138.22±2.30	215.89±1.48	7.66±1.29	17.98±0.37	51.39±2.54
Barley industry by-products (µg/mg dry CWM)	Sultan-17	198.22±2.14	239.87±2.06	26.34±0.19	61.78±0.13	69.56±0.01
	Jau-17	203.19±0.04	236.55±2.10	28.10±2.29	65.24±0.53	70.15±0.04
	Jau-87	186.07±0.34	231.49±0.01	21.78±1.20	60.17±0.02	75.23±0.54

Table 3: Phenolic acid content (% dry weight) of wheat, barley and maize industries by-product's cell wall

Phenolic acids		p-Coumaric acid	Ferulic acid	Diferulic acid
Maize industry	Pearl-11	0.46±2.23	3.28±1.65	0.73±2.98
by-products	MMRI-11	0.51±0.67	3.75±1.12	0.40±2.20
	YH-98	0.42±0.10	3.51±0.34	0.45±1.84
Wheat industry	Gold-16	4.97±2.04	12.45±0.54	5.12±0.09
by-products	AARI-11	3.58±1.22	10.23±0.35	4.78±2.10
	Punjab-11	3.83±0.12	11.76±0.06	4.99±1.24
Barley industry	Sultan-17	8.12±0.60	15.5±1.56	9.12±2.45
by-products	Jau-17	8.78±0.09	13.78±0.49	8.65±1.78

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