Biochemical characterization of wheat straw cell wall with special reference to bioactive profile

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**ABSTRACT**

The core objective of the current study was to extract and characterize the wheat straw cell wall for its nutritional and bioactive profile. For the purpose, four different wheat straw varieties namely Ujala-16, Johar-16, Gold-16, and Galaxy-13 were procured from Ayub Agriculture Research Institute, Faisalabad, Pakistan. The whole research was conducted in three different phases. In first phase, nutritional composition and mineral profile of straw of different wheat varieties were determined. In second phase, wheat straw cell wall of different varieties was isolated and characterized for its important bioactive constituents, such as lignin, cellulose, hemicelluloses, phytosterol, and policosanol (PC) content. Results showed that straw of different wheat varieties contained 7.75–9.24, 3.98–5.06, 3.43–3.98, and 1.60–2.24 g/100 g moisture, ash, protein, and fat contents, respectively, whereas potassium, calcium, phosphorus, and magnesium were 1.19–2.03, 0.10–0.79, 0.10–0.98, 0.03–0.98 ppm, respectively. Moreover, lignocellulosic mass: cellulose 37.75–38.18 g/100 g raw material, lignin 15.67–16.07 g/100 g raw material, hemicelluloses 28.25–28.98 g/100 g raw material, was present in wheat straw and varied significantly among different varieties. In addition, phytosterol ranged from 854 to 1176 mg/kg in straw of different wheat cultivars, whilst PC from 196.09 to 236.48 mg/kg. Conclusively, wheat straw was an excellent source of many important bioactive moieties especially lignocelluloses and could have functional use.

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**Introduction**

In millennia, agro-industrial waste captured interest owing to its abundant availability, pollution reduction ability, low price, and lignocellulosic nature.\textsuperscript{[1]} It is important for the renewable energy, biofuels, and biochemical generation and is obtained from various sources, agricultural and forestry waste stream.\textsuperscript{[2]} The main industrial wastes include wheat straw, rice straw, corn straw, sugarcane, and sugarcane bagasse.\textsuperscript{[3,4]} Among agricultural residues, straw is most abundant and the cheapest pollution mitigator. Globally, wheat straw is most important by-product of wheat processing produced in larger quantity.\textsuperscript{[5,6]} About 529 million tons wheat straw is generated every year in all over the world.\textsuperscript{[7]} Moreover, in all over the world and Europe, it is the second most abundant and largest biomass feed stock after rice straw.\textsuperscript{[8,9]} The main fractions of wheat straw include nodes, internodes, and leaves. Physical characteristics revealed that the
main parts of wheat straw are internodes (68.5%), leaf-sheath (20.3%), leaf-blade (5.5%), nodes and fines (4.2%), and grains and debris (1.5%). As far as the nutritional composition is considered, wheat straw consists of high level of carbohydrates, proteins, minerals, vitamins, silica, acid detergent fibers, ash and important bioactive compounds. The macro- and micro-nutrient concentration depends upon the variety and cultivar, stage of plant growth, the nature of soil, fertilizer, and climatic situations.

Moreover, wheat straw contains important cellulosic and noncellulosic components that are present in complex structured cell wall. These components have varying degree of structural and functional complexity. Cellulosic part contains three main structural components: lignin (8–15%), cellulose (35–45%), and hemicelluloses (20–30%). These components are bonded by non-covalent forces and covalent cross-linkages. Among these structural components, cellulose is one of the main components and is considered as the most imperative biomass in the world. These cellulose chains are bonded together by hydrogen bond to form microfibrils. These microfibrils vary in diameter (nanometers) and length (millimeters) and are structural unit of cell wall. These are bonded by a gel matrix composed of hemicelluloses, lignin, and other carbohydrate polymers to form a bio-composite. Lignin is the second most abundant, three dimensional, natural polymers and forms 10–25% biomass of lignocelluloses. Three different types of substituted phenols for instance sinapyl alcohols, p-coumaroyl, and coniferyl by the polymerization of enzymes are responsible for the formation of linkages and functional groups. Lignins that extracted from hydroxycinnamoyl alcohols are generally known as syringyl, hydroxyphenyl, guaiacyl, and lignin, whereas hemicellulose is a complex polysaccharide that occurs in combination with cellulose extracted from the cell walls of lignocellulosic biomass. In contrast, hemicellulose consists of branched configuration that represents different structure of biomass of lignocellulose. It consists of four typical structurally different polysaccharide types: mixed-linkage beta-glucans, xyloglucans, xylans, and mannoglycans. Xylans and mannans are important hemicellulose in plant kingdom.

Wheat straw wax contains phytosterols mainly including stigmasterol, campesterol, β-sitosterol, cholesterol, ergosterol, and stigmastanol. The structure of all these phytosterol resembles to β-sitosterol. They are natural constituents of cell membrane of plants. Along with phytosterols, another bioactive compound present in wheat straw is policosanol (PC) which is the common name for a mixture of high molecular weight (20–36 carbon) aliphatic primary alcohols, which are constituents of plant epicuticular. Owing to the presence of these phytosterols and PCs, wheat straw is used to lower the cholesterol level and chances of coronary heart disease. The consent of the present study was to characterize the wheat straw for its nutritional and bioactive profile. The functional and nutraceutical properties of wheat straw are owing to its bioactive profile and these properties are valuable for industrialists to improve the quality of their products.

**Materials and methods**

**Procurement of raw materials**

Four different varieties of wheat straw, i.e., Ujala-16, Johar-16, Gold-16, and Galaxy-13, were procured from Ayub Agriculture Research Institute (AARI), Faisalabad.

**Isolation of cell wall**

Air-dried wheat straw was grounded roughly with 5.5 mm stainless steel balls in a 2-ml Sarstedt screw cap tube using an iWall, a grinding and dispensing robot (30 s). An alternative non-robotic low-throughput procedure (ball-mill, retschmill) could be used. A volume of 1.5 ml of 70% aqueous ethanol was added to the dispensed ground material and vortex thoroughly. Then, it was centrifuged at 10,000 rpm for 10 min to pellet the alcohol insoluble residue. Then, the supernatant was aspirated.
or decanted. A volume of 1.5 ml of chloroform/methanol (1:1 v/v) solution was added to the residue and tube was shaken thoroughly to resuspend the pellet. Then, it was centrifuged at 10,000 rpm for 10 min and the supernatant was aspirated or decanted.

Pellet was re-suspended in 500 μl of acetone. The solvent was evaporated with a stream of air at 35°C until dry. The pellet was resuspended in 1.5 ml of a 0.1-M sodium acetate buffer pH 5.0 to initiate the removal of starch from the sample. Sarstedt tubes were capped and heated for 20 min. at 80°C in a heating block. The suspension was cooled on ice. Thirty-five microliter of 0.01% sodium azide (NaN₃), 35 μl amylase (50 μg/mL H₂O; from Bacillus species, Sigma), 17 μl pullulanase (17.8 units from Bacillus acidopullulyticus; Sigma) were added to the pellet. The tube was capped and vortex thoroughly. The suspension was incubated over night at 37°C in the shaker. Positioning the tubes horizontally enhanced mixing. The tube was heated at 100°C for 10 min in a heating block to terminate digestion. Supernatant containing solubilized starch was centrifuged (10,000 rpm, 10 min) and discarded. The remaining pellet was washed three times by adding 1.5 ml water, vortexed, centrifuged, and decanted of the washing water. Pellet was resuspended in 500 μl of acetone. Solvent was evaporated with a stream of air at 35°C until dry. It may be necessary also to break up the material in the tube with a spatula for better drying. The dried material was present in the isolated cell wall (lignocellulosics).

**Chemical analysis**

Different wheat straw varieties were analyzed for moisture, crude protein, crude fat, and ash content. The mineral contents like Na, K, Ca, Mg, Fe, Cu, Zn, and Mn in wheat straw cell wall were determined by the method described in AOAC. Lignin was determined as “Klason lignin” from different varieties of wheat straw cell wall using the method described by Wood et al. Cellulose was determined in cell wall of different wheat straw varieties using the method described by Wada et al. Hemicelluloses in cell wall of different wheat straw varieties were also determined using the method described by Lawther et al.

**Phytosterol**

Phytosterol of wheat straw cell wall was analyzed through the method as described in Torigoe.

**PC**

PC content of wheat straw cell wall was determined through method as described by Irmak et al.

**Monosaccharide analysis**

Monosaccharides were estimated by gas chromatography of their alditol acetates obtained after sulfuric acid hydrolysis of the samples.

**Statistical analysis**

The data obtained for each parameter were subjected for completely randomized design and Latin square design to determine the level of significance.

**Results and discussion**

**Chemical analysis of wheat straw**

The different wheat straw varieties (i.e., Galaxy-13, Johar-16, Gold-16, Ujala-16) were analyzed (dry basis) for moisture, ash, crude protein, and crude fat contents and mean values have been
presented in Table 1. Results indicated that these four varieties varied significantly from each other regarding their chemical composition. Moisture content ranged from 7.75% to 9.24% in different varieties of wheat straw. Analysis revealed that Ujala-16 showed higher moisture content (9.24 ± 0.04%) while the minimum (7.75 ± 0.04%) was observed in Johar-16 followed by Gold-16 and Galaxy-13. Ujala-16 with 3.98 ± 0.04% again showed highest results for protein while Gold-16 had lowest (3.43 ± 0.05%). In case of ash, highest content (5.06 ± 0.04%) was shown by Johar-16 and lowest content was shown by Gold-16 (3.98 ± 0.03%) followed by Ujala-16 (4.78 ± 0.04%) and Galaxy-13 (4.03 ± 0.03%). Furthermore, the fat content analysis in wheat straw revealed that the highest fat content was present in Gold-16 (2.24 ± 0.04%) followed by Johar-16 (2.05 ± 0.05%) and Ujala-16 (1.92 ± 0.03%) whilst the lowest was found in Galaxy-13 (1.60 ± 0.02%). By keeping in mind the results of moisture, ash, crude protein, and crude fat content present in wheat straw of different varieties, Johar-16 and Ujala-16 are known as the best varieties owing to their lowest moisture and highest protein content, respectively, whereas for minimum ash and maximum crude fat, Gold-16 is acknowledged as best variety.

Results for moisture content in present study are in accordance to Yasin et al.\textsuperscript{[12]} who reported that wheat straw contained 6–11% moisture content. Shrivastava et al.\textsuperscript{[30]} studies showed that ash content for wheat straw was 3.71%. Results for crude protein are likewise to Yasin et al.\textsuperscript{[12]} who reported that wheat straw contained 3.6% of crude protein content. Results of present study are in accordance to Anderson and Hoffman\textsuperscript{[31]} who reported that wheat straw contained 1.6% fat content.

### Minerals analysis of wheat straw

The mineral content was observed to be significantly different among different wheat straw varieties (Table 2). The highest phosphorus content was observed in Gold-16 (0.98 ± 0.06%) followed by Ujala-16 (0.58 ± 0.03%) and Johar-16 (0.27 ± 0.02%) whilst the lowest was found in Galaxy-13 (0.10 ± 0.04%). As far as the calcium was concerned, Johar-16 showed highest content (0.79 ± 0.04%), while Gold-16 contained lowest content (0.58 ± 0.03%), whereas the potassium content in Gold-16, Ujala-16, Johar-16, and Galaxy-13 was 2.03 ± 0.03%, 1.43 ± 0.05%, 1.24 ± 0.06%, and 1.19 ± 0.02%. Moreover, maximum magnesium content was observed in Gold-16 (0.98 ± 0.05%) followed by Galaxy-13 (0.54 ± 0.02%) and Ujala-16 (0.13 ± 0.03%) whilst Johar-16 showed the minimum content (0.03 ± 0.02%). All the results related to mineral content revealed that Gold-16 is known as the best variety owing to its highest magnesium, phosphorus, and potassium content while Johar-16 is considered as best for maximum calcium content.

### Table 1. chemical analysis of wheat straw cell wall.

| Varieties  | Moisture (%) | Ash (%) | Crude protein (%) | Fat (%) |
|------------|--------------|---------|-------------------|---------|
| Galaxy-13  | 8.04 ± 0.03\textsuperscript{a} | 4.03 ± 0.03\textsuperscript{bc} | 3.78 ± 0.03\textsuperscript{c} | 1.60 ± 0.02\textsuperscript{b} |
| Ujala-16   | 9.24 ± 0.04\textsuperscript{a} | 4.78 ± 0.04\textsuperscript{ab} | 3.98 ± 0.04\textsuperscript{c} | 1.92 ± 0.03\textsuperscript{b} |
| Gold-16    | 8.58 ± 0.07\textsuperscript{b} | 3.98 ± 0.03\textsuperscript{c} | 3.43 ± 0.05\textsuperscript{b} | 2.24 ± 0.04\textsuperscript{b} |
| JOHAR-16   | 7.75 ± 0.04\textsuperscript{d} | 5.06 ± 0.04\textsuperscript{a} | 3.69 ± 0.03\textsuperscript{a} | 2.05 ± 0.05\textsuperscript{a} |

Mean ± SD for chemical analysis of wheat straw cell wall. Means carrying same letters are significantly identical.

### Table 2. minerals analysis of cell wall from different wheat straw varieties.

| Varieties  | P (ppm) | Ca (ppm) | K (ppm) | Mg (ppm) |
|------------|---------|----------|---------|----------|
| Galaxy-13  | 0.10 ± 0.04\textsuperscript{bc} | 0.21 ± 0.02\textsuperscript{b} | 1.19 ± 0.02\textsuperscript{a} | 0.54 ± 0.02\textsuperscript{b} |
| Ujala-16   | 0.58 ± 0.03\textsuperscript{b} | 0.58 ± 0.03\textsuperscript{b} | 1.43 ± 0.05\textsuperscript{a} | 0.13 ± 0.03\textsuperscript{b} |
| Gold-16    | 0.98 ± 0.06\textsuperscript{a} | 0.10 ± 0.02\textsuperscript{c} | 2.03 ± 0.03\textsuperscript{a} | 0.98 ± 0.05\textsuperscript{b} |
| JOHAR-16   | 0.27 ± 0.02\textsuperscript{a} | 0.79 ± 0.04\textsuperscript{a} | 1.24 ± 0.06\textsuperscript{a} | 0.03 ± 0.02\textsuperscript{a} |

Mean ± SD for mineral analysis of wheat straw cell wall. Means carrying same letters are significantly identical.
Results of present study are in accordance to Anderson and Hoffman[31] who reported that wheat straw contained 0.14% phosphorus, 0.23% calcium, 1.24% potassium, and 0.11% magnesium content.

**Lignin content**

Mean values regarding lignin content in different wheat straw varieties have been depicted in Table 3. Ujala-16 contained maximum lignin content, i.e., 16.07 ± 0.03% while Galaxy-13 was at the bottom with 15.67 ± 0.03% followed by Gold-16 and Johar-16. The results of present study are similar to the findings of Refs. [6,32]. They reported that lignin content in wheat straw was 14–17% whereas Watkins et al.[33] explicated that lignin content in wheat straw was 20.40%.

**Cellulose content**

Results regarding cellulose content of different wheat straw varieties revealed that Galaxy-13 showed highest value at 38.18 ± 0.05% whilst Johar-16 was the lowest with 37.75 ± 0.05%. Results of present study are in line with Lawther et al.[34,35]; they reported that wheat straw contained 37.19–38.55% of cellulose content. Another study of Zhang et al.[36] also showed nearly similar results, i.e., 38.2%.

**Hemicelluloses content**

Mean values for hemicelluloses content have been shown in Table 3. The hemicelluloses content was observed to be significant among different spring wheats. The highest hemicelluloses content was observed in Galaxy-13 (28.98 ± 0.05%) followed by Ujala-16 (28.77 ± 0.02%) and Gold-16 (28.59 ± 0.04%) whilst the lowest was found in Johar-16 (28.25 ± 0.03%). As per the results of hemicellulose content present in wheat straw of different varieties, Galaxy-13 is known as the best variety owing to its highest hemicellulose content. Shrivastava et al.[30] studies showed that hemicellulose content for wheat straw was 29.36% whereas Thakur et al.[37] reported that wheat straw contained 28.05 ± 0.58 hemicelluloses content. Zhang et al.[36] reported that the hemicelluloses content in wheat straw was 36.4%.

**Phytosterol and PC content of wheat straw**

The phytosterol and PC contents were observed to be significantly affected among different wheat straw varieties as shown in Table 4. Johar-16 was at the top (76.55 ± 0.04%) for phytosterol content while Galaxy-13 was at the bottom (66.15 ± 0.91%). In case of PC, Gold-16 contained the highest value (236.48 ± 0.13%) followed by Ujala-16 (225.83 ± 0.49%) and Johar-16 (206.58 ± 0.56%) whilst the minimum value was found in Galaxy-13 (196.09 ± 0.33%). In case of present study, by keeping in mind the results of phytosterol content present in wheat straw of different varieties, Johar-16 and Gold-16 are known as the best varieties owing to their highest phytosterol and PC content, respectively. Dunford and Edwards[38] studies showed that phytosterol content for wheat straw was 60–76% whereas PC was 137–274 mg/kg.

| Varieties   | Lignin (%)        | Cellulose (%)    | Hemicellulose (%) |
|------------|-------------------|------------------|-------------------|
| Galaxy-13  | 15.67 ± 0.03d     | 38.18 ± 0.05a    | 28.98 ± 0.05a     |
| Ujala-16   | 16.07 ± 0.05a     | 37.90 ± 0.04c    | 28.77 ± 0.02a     |
| Gold-16    | 15.88 ± 0.04b     | 38.07 ± 0.03b    | 28.59 ± 0.04b     |
| JOHAR-16   | 15.75 ± 0.03c     | 37.75 ± 0.05b    | 28.25 ± 0.03d     |

Mean ± SD for lignocellulosic mass of wheat straw cell wall. Means carrying same letters are significantly identical.
Monosaccharide content of wheat straw

Mean values for monosaccharides content in wheat straw of different spring varieties have been depicted in Table 5. The monosaccharides content was observed to be significant among different spring varieties. Analysis of xylan content revealed that Johar-16 is at the top with 24.37 ± 0.13% whilst Ujala-16 is at the bottom with 20.47 ± 0.37%, whereas xylan content in other two varieties, i.e., Gold-16 and Galaxy-13 was 23.55 ± 0.32% and 22.14 ± 0.05%. Wheat straw varieties were also analyzed for arabinose content. The results of means for arabinose content in wheat straw showed that wheat straw contained about 2.83 ± 0.02, 2.82 ± 0.08, 2.85 ± 0.04, and 2.91 ± 0.05 in Galaxy-13, Ujala-16, Gold-16, and Johar-16, respectively. These varieties were also investigated for glucan content. The value of 38.29 ± 0.05% for glucan content was found to be highest in Johar-16 and lowest at 36.19 ± 0.06% for Galaxy-13. However, other varieties regarding their xylose content remained in the sequence: Ujala-16 (37.09 ± 0.05%) and Gold-16 (37.65 ± 0.05%). Wheat straw varieties were investigated for arabinan content. Arabinan content in Johar-16, Ujala-16, Gold-16, and Galaxy-13 was 4.12 ± 0.10%, 3.65 ± 0.08%, 2.72 ± 0.07%, and 2.12 ± 0.07%. Arabinan content present in wheat straw of different varieties; Johar-16 is known as the best variety owing to its highest arabinan content. Various studies supported the findings of present research regarding monosaccharide contents like Kristensen et al.\cite{39} reported that result for monosaccharide content including xylan was 24.5%, arabinose was 2.8%, glucan was 36.3%, arabinan was 2.1%, and galactan was <0.6% each. Saeed et al.\cite{40} explicated that xylan, glucan, arabinan, galactan, mannan contents in wheat straw were 22.2 ± 0.3, 38.8 ± 0.5, 4.7 ± 0.1, 2.7 ± 0.1, 1.7 ± 0.2, respectively.

Conclusion

Conclusively, agro-industrial waste is the cheapest and largely generated lignocellulosic mass containing high contents of lignocellulose and starch. Cell wall of wheat straw is an excellent source of lignocelluloses, i.e., lignin, cellulose, hemicelluloses. This lignocellulosic nature makes wheat straw and its cell wall more functional and more useful. Additionally, it makes the cell wall of wheat straw an important resource for the production of renewable energy, biofuel, bioethanol, and biochemicals. Moreover, some important bioactive moieties such as PC and phytoestrogen are present in cell wall of wheat straw. These functional ingredients make the cell wall of wheat straw much functional toward common diseases mainly cholesterol lowering and cardiovascular diseases.

| Varieties | Xylan (g/100 g) | Arabinose (g/100 g) | Glucan (g/100 g) | Arabinan (g/100 g) |
|-----------|----------------|-------------------|-----------------|------------------|
| Galaxy-13 | 22.14 ± 0.05\(^c\) | 2.83 ± 0.02\(^a\) | 36.19 ± 0.06\(^d\) | 2.12 ± 0.07\(^d\) |
| Ujala-16  | 20.47 ± 0.37\(^d\) | 2.82 ± 0.08\(^a\) | 37.09 ± 0.05\(^c\) | 3.65 ± 0.08\(^b\) |
| Gold-16   | 23.55 ± 0.32\(^b\) | 2.85 ± 0.04\(^a\) | 37.65 ± 0.05\(^b\) | 2.72 ± 0.07\(^c\) |
| JOHAR-16  | 24.37 ± 0.13\(^a\) | 2.91 ± 0.05\(^a\) | 38.29 ± 0.05\(^a\) | 4.12 ± 0.10\(^a\) |

Mean ± SD for monosaccharide content of wheat straw cell wall. Means carrying same letters are significantly identical.
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