Structural and nutritional properties of psyllium husk arabinoxylans with special reference to their antioxidant potential

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ABSTRACT
The current research aimed to extract and characterize arabinoxylans (AX) from psyllium seeds husk for their nutritional and structural properties. For this purpose, psyllium husk (PH) was procured from the local market and subjected for the nutritional profile, dietary fiber (DF) composition, and AXs extraction. The extraction of AXs from psyllium seed husk was done through enzymatic method and Fourier transform infrared (FTIR) spectroscopy was used for its structural characterization. Further, the antioxidant activity of AXs was also elucidated using ferric reducing antioxidant power (FRAP) and diphenyl picrylhydrayl (DPPH) assays. Results showed that psyllium seed husk is a good source of DFs (61.5 ± 2.6%) and the proximate composition including moisture, ash, crude fat, crude protein, crude fibers, and Nitrogen free extract (NFE) was 4.9 ± 0.01, 4.1 ± 0.01, 1.2 ± 0.01, 3.9 ± 0.05, 20.23 ± 0.4 and 78.2 ± 4.01 g/100 g, respectively. Furthermore, AXs content was 68.29 ± 3.4 g/100 g of PH. Different monosaccharides including xylose, arabinose, rhamnose, glucose, and galactose contents in PHAXs were 64.23 ± 1.04%, 13.38 ± 0.16%, 1.43 ± 0.04%, 1.89 ± 0.05%, and 3.64 ± 0.07%, respectively. FTIR absorption peaks from 900 to 1100 cm⁻¹ showed spectra of AXs with the highest peak from 980 to 1015 cm⁻¹ reflecting arabinose and xylose ratio. Additionally, the substitution positions on the xylan backbone were 2800–3400 cm⁻¹ of O-H stretching vibrations and peaks of C-H stretching at 2853 and 2923 cm⁻¹. The antioxidant activity of PHAXs exhibited that the FRAP value of extracted AXs was 1.39 ± 0.06 μmol Fe²+/g and the DPPH value was 3.01 ± 0.15 Trolox mg/g. It is depicted that PH is a good source of AXs (non-starch polysaccharide) with better antioxidant potential.

INTRODUCTION
Dietary fibers (DFs) are a complex combination of polysaccharides, i.e. cellulose, hemicellulose, and lignin, which are non-digestible carbohydrates and unable to assimilate.¹,² Plants are good source of DFs which are made up of a complex polymer of phenylpropanoid subunits. Further, soluble fibers are an edible fraction of plant fibers that are resistant to digestion but fermented by colonic bacteria and produce short-chain fatty acids (SCFAs) in the large intestine, while the insoluble fraction of DFs are unable to digest and passed from the gastrointestinal (GI) tract. Moreover, these DFs are non-starch
polysaccharides having functional and nutraceutical properties and chiefly composed of hemicellulose, pectin, gums, mucilage, and lignin.\[3\]

Furthermore, DFs have a reliable effect on the maintenance of body weight by giving bulk to the food in the GI tract,\[4\] as well as increasing satiety.\[5\] Non-starch polysaccharides are also beneficial to the body in the acute to chronic gut malfunctioning including specially constipation by inducing GI peristalsis, which absorbs toxins from the stomach and facilitates their excretion.\[6\] Additionally, these non-starch polysaccharides help enhance gut microflora by serving as a prebiotic in the human GI tract. In many clinical trials and efficacies, DFs are claimed as supernatural food which plays a significant role to combat various metabolic syndromes.\[7\]

Psyllium plant (\textit{Plantago ovata}) is one of 200 \textit{Plantago} species and is grown in most parts of the world, especially in South Asian countries.\[8\] Psyllium husk (PH) has been used as a water-soluble, gel-reducing material and is commonly used in Asian countries as herbal medicine to treat metabolic disorders, high blood pressure, and hypercholesterolemia. The highly branched heteroxylans, with the backbone of 1–4 D-xylopyranose, are the most common polysaccharide in PH, comprising 46.8% arabinose and 24.1% xylose on a dry basis.\[9\] The bioactive components of PH are polysaccharides, especially arabinoxylans (AXs), which comprise 55–60% of the total weight. Previous studies on AXs reported about its antioxidant, antidiabetic, antilipidemic, and cardioprotective properties.\[10\] These moieties also have many functional and technological aspects; due to these properties, AXs are now also be used as functional components as well as to produce different value-added products.\[9,11,12\]

PH extract (non-starch polysaccharides) improves the functionality of gut microflora due to its health-promoting properties.\[13\] Further, these fibers, as prebiotics, promote the functionality of the gut by increasing the formation of SCFAs like butyrate and propionate.\[14\] Non-starch polysaccharides retain water in the small intestine, increasing water flow into the ascending colon which softens and increases the stool frequency as well.\[15\] The effects of PH DFs on host physiology have been widely documented by Lin et al.,\[16\] who reported that these fibers have a greater physical barrier coming from the gel-forming characteristics. The current research aimed to extract and explore the AXs from PH and characterize their structural (Fourier transform infrared [FTIR]) and antioxidant properties by using their respective methods.

\textbf{Materials and methods}

\textit{Procurement of raw material}

PH was purchased from SB Departmental Store Faisalabad, Pakistan. Enzymes including Glucosidase from \textit{Aspergillus Niger}, a-amyrase, protease, lipase (Megazyme enzyme assay kit K-TDFR-100A, Bray Business Park Bray, Co. Wicklow A98 YV29, Ireland) and all analytical grade chemicals and buffers were procured from Sigma-Aldrich (Schnelldorf, Germany). Analysis was performed in the Advance Food Analysis Lab, Department of Food Science, Central High Tech Lab, Government College University, Faisalabad, and Cereal Technology Lab, Ayub Agriculture Research Institute, Faisalabad.

\textbf{Nutritional composition}

The nutritional composition including moisture, ash, crude fat, crude protein, crude fiber, and NFE of the PH was performed with their respective methods, with the methods prescribed by AACC.\[17\] All the samples were analyzed in triplicates, and values have been written as mean along with their standard deviation.

\textbf{Total dietary fibers (soluble and insoluble dietary fibers)}

Total dietary (soluble and insoluble fractions) were assessed by using the Megazyme total DFs assay kit and followed the AACC standard method no. 32–05.01. 1 g dried sample was subjected to sequential
enzymatic digestion using heat stable α-amylase, protease, and amyloglucosidase. Each enzyme was exposed to a specific temperature and pH to maximize the enzymatic activity. Insoluble DF (IDF) was then filtered and washed with hot distilled water. A combined filtrate and water solution was precipitated with 95% ethanol to determine the soluble DF (SDF), and then the precipitate is filtered and dried. Both SDF and IDF residues were corrected for protein, ash, and blank, for the final calculation of SDF and IDF values.

**Extraction of psyllium husk arabinoxylans**

**Extraction of arabinoxylans**

AXs were extracted from grounded PH sample through the method used by Saeed et al.\(^\text{[18]}\) and Lynch et al.\(^\text{[19]}\) with major modifications. Briefly, 100 g defatted sample was subjected for extraction. Sample was defatted with organic solvent (n-hexane) after which sample was placed in oven at 60°C for 60 min to remove the moisture and n-hexane residues from the sample. Dried sample was then added in 1000 ml deionized water and boiled for 60 min on a hot plate using a magnetic stirrer at 600 rpm for starch gelatinization and protein denaturation. Then, phosphate-buffered saline (40 ml/g) was added in the solution to achieve the pH of 5.5. The mixture was mixed thoroughly in the buffer manually to prevent the formation of clumps. 50 µl/g Thermostable α-amylase (40°C for 90 min), 60 µl/g protease (4 h at 60°C) and 100 µl/g glucoamylase (60°C for 90 min). Degree of starch degradation was assessed through iodine solution as an indicator in 1 ml slurry sample, and 0.02% sodium azide was added to prevent microbial spoilage. After the completion of enzymatic degradation for 7 h, the mixture was allowed to cool and centrifuged (15 min at 12000 rpm) for 20 min to obtain the supernatant. The total AXs were finally recovered in the supernatant and recuperated with absolute ethanol and acetone. The solution was then placed at 4°C for 4 h and the residues were then freeze-dried and converted into powdered form to obtain purified AXs.

**Neutral sugar content of arabinoxylans**

Neutral sugars of the PHAXs were assessed by following the method used by Qaisrani et al.\(^\text{[20]}\) with slight modifications throughout the application of gas chromatography-mass spectrometry (GC-MS). Helium is used as a carrier gas at a constant flow rate of 1.3 ml/min. Monosaccharide standards including rhamnose, glucose, galactose, arabinose, and xylose and all analytical grade chemicals were purchased from Sigma Aldrich and Merck Corp. Yield of AXs was quantified by using the following equation:

\[
\text{Total AX} = (\text{Xylose} + \text{Arabinose}) \times 0.88
\]

**Fourier transform infrared spectroscopy**

Detection of functional infrared compounds based on their spectrum was identified by using the FTIR (Shimadzu Fourier transform infrared spectrophotometer-FTIR 8400 S) with an absorbance spectrum of 4000–400 cm\(^{-1}\). In FTIR, dried PHAX sample was placed and detected the functional groups through continuous infrared waves, which are connected to a computer-based system. After the detection of functional moieties, FTIR spectra of the sample are based on the molecular binding, chemical structures, and functional groups.

**Antioxidant activity of psyllium husk arabinoxylan**

**Diphenyl picrylhydrazyl assay**

The antioxidant activity of the PHAXs was evaluated by using the diphenyl picryl hydrazyl (DPPH) assay through the method illustrated by Yen and Chen,\(^\text{[21]}\) with little amendments in which 0.1 mL ethanol extracted sample was individually added in DPPH solution (0.12 mM) in a test tube with 1:4
and placed for 20 min in a dark. Furthermore, Trolox absorbance at 517 nm was recorded by using a ultraviolet/visible spectrophotometer alongside control and blank according to Jemaa et al.\textsuperscript{[21]}

**Ferric reducing antioxidant power assay**

Ferric reducing antioxidant power (FRAP) test was performed using spectrophotometer according to the method of Rao and Muralikrishna\textsuperscript{[22]} with some modifications. For this purpose, the prepared FRAP reagent was mixed in a 300 mmol L\(^{-1}\) acetate buffer (pH 3.6) with a 20 mmol L\(^{-1}\) FeCl\(_3\) \(\cdot\) 6H\(_2\) O and 10 mmol L\(^{-1}\) TPTZ (in 40 mmol L\(^{-1}\) HCl) at a ratio of 10:1:1 (v/v). The reagent was heated to 37°C, and then, the AXs extract (0.5 mL) was added to the FRAP reagent (4.5 mL) and placed for 20 min in dark. The absorbance at 575 nm was noted against distilled water after incubation for 5 min using a spectrophotometer. An aqueous solution of FeSO\(_4\) \(\cdot\) 7H\(_2\)O was used for the calibration. The results of FRAP assay was expressed as \(\mu\)mol Fe2+/1 g sample.

**Statistical analysis**

The samples were analyzed in triplicates for statistical evaluation and Microsoft excel-2016 and the results were written in the form of means and their standard deviations.

**Results and discussions**

**Biochemical analysis**

Results regarding the biochemical composition of PH are presented in Table 1, which are based on its moisture, ash, crude fat, crude protein, crude fiber, and NFE content with the resulting values including 4.9 ± 0.01, 4.1 ± 0.01, 1.2 ± 0.01, 3.9 ± 0.05, 20.23 ± 0.40, and 78.2 ± 4.01 g/100 g of PH sample, respectively. Current results are closely corroborated with the findings of Qaisrani et al.,\textsuperscript{[20]} who reported that moisture, ash, crude protein, crude fat, crude fiber, and NFE is present in 6.43 ± 0.05%, 3.85 ± 0.04%, 2.08 ± 0.06%, 0.09 ± 0.01%, 3.83 ± 0.02%, and 83.72 ± 0.08%, respectively.

Moisture content has a prodigious impact on assessing the quality of food products as well as their physical and chemical properties regarding safety perspectives. In the current study, the moisture content was 4.9 g/100 g of PH sample, due to which PH is categorized into nonperishable food with better storage stability. Similarly, ash-inorganic residue, in food products, recovered after the thorough scorching of the organic compound,\textsuperscript{[23]} which in the current research, was 4.1 g/100 g of the sample. Further, the crude fiber content in PH provides bulk volume, which facilitates the consumer in satiety, demonstrates healing for the patients diagnosed with irritable bowel syndrome (IBS), and flourishes the gut microflora by serving as a healthy prebiotic source. Based on solubility, DF is generally categorized into two major fractions including SDF and ISDF. PH is classified as a superfood that contains both fractions and facilitates the body in both constipation as well as diarrhea. In many in vivo studies, many researchers reported that DFs have hepatoprotective as well as antioxidant potential. Food with higher fiber content usually presents an unequal size and irregular shape, which is due to the structural constituents of fibers. PH significantly affects physicochemical properties like

| Nutritional components | Composition (%) |
|------------------------|-----------------|
| Moisture               | 4.9 ± 0.01      |
| Ash                    | 4.1 ± 0.01      |
| Crude Fat              | 1.2 ± 0.01      |
| Crude Protein          | 3.9 ± 0.05      |
| Crude Fiber            | 20.23 ± 0.4     |
| NFE                    | 78.2 ± 4.01     |
| TDF                    | 61.5 ± 2.6      |
water holding capacity (WHC) and oil retention capacity (ORC) with the potential to improve intestinal health, fecal transition, softening of stool, and mainly colon cancer.\textsuperscript{[24,25]}

**Extraction of psyllium husk arabinoxylan**

The AXs content in PH was 49.81 ± 2.5 g/100 g. However, previous studies strengthen the current results that AXs in PH are present between the range of 45% and 60%. Furthermore, it was also indicated that the major fractions were of arabinose and xylose while minor fractions include some other sugars including rhamnose, galactose, and glucose. Different researchers extracted the AXs from the psyllium seed husk; however, variations in their results were observed. It was also concluded that, extraction technique is the major factor that can affect the yield of AXs. Fischer et al.\textsuperscript{[26]} reported that 62.5% of AXs yield with 0.41 arabinose to xylose ratio through alkali hydrolysis. Further, temperature, pH, and suspension concentration also reported to have a significant effect on the yield of AXs as alkali digestion yield up to 77% of the AXs whereas acid hydrolysis shows the yield of 97%. Further, cereals are also a good source of AXs and various researchers have extracted AXs from different cereals and their byproducts.\textsuperscript{[27]}

**Neutral sugar contents**

Table 2 demonstrates the neutral sugar content present in PHAXs. In the current study, AXs were subjected for their neutral sugar content including arabinose, xylose, galactose, rhamnose, and glucose were characterized with the application of GC-MS. In the results, arabinose (13.38%) and xylose (64.23%) comprised the major proportion with a mutual ratio of 4.80 and other monosaccharide sugars including glucose (1.89%), galactose (3.64%), and rhamnose (1.43%) are present in trace amounts. Moreover, a similar study\textsuperscript{[28]} reported the same results with the major fractions of arabinose and xylose. Extracted AXs content (49.81 ± 2.5 g/100 g) in the current research strengthens the proclamation that arabinose and xylose comprise the major concentration as compared to other sugars. Moreover, Fischer et al.\textsuperscript{[26]} reported similar results with arabinose and xylose contents were 22.6% and 74.6% respectively. Higher arabinose to xylose ratio is a good interpreter of the gel-forming capacity of the non-starch polysaccharides due to the presence of small micropores present on its surface, which enhance its WHC as well as its ORC and trigger its gel formation. Furthermore, the gel-forming capacity of AXs has a potential application as matrices targeted nutrient delivery in food and non-food applications.\textsuperscript{[29]} The current research depicted that extracted fractions are good source of polysaccharides. Furthermore, non-starch polysaccharides are also present in AXs moieties that have various functional and nutraceutical and medicinal properties.

**Fourier transform infrared spectroscopy**

FTIR spectra of PHAX is shown in Figure 1. The spectra result were recorded from 600 to 4000 cm\textsuperscript{-1}. The absorption peaks from 900 to 1100 cm\textsuperscript{-1} showed spectra of AXs with the highest peak from 980 to 1015 cm\textsuperscript{-1} reflecting arabinose and xylose ratio. The substitution positions on the xylan backbone were 2800 to 3400 cm\textsuperscript{-1} of O-H stretching vibrations and peaks of C-H stretching at 2853 and 2923 cm\textsuperscript{-1}. The peak at 1744 cm\textsuperscript{-1} was assigned to C = O stretching in the carboxyl group of uronic acid. The peak at 1140–1150 cm\textsuperscript{-1} was consigned to the C-O-C vibration of glycosidic bonds.

**Table 2. Monosaccharide composition of psyllium husk arabinoxylans.**

| Parameters | Yield/Purity of arabinoxylans | Rha | Glu | Gal | Ara | Xyl |
|------------|-------------------------------|-----|-----|-----|-----|-----|
| Composition (%) | 68.29 ± 3.4% | 1.43 ± 0.04 | 1.89 ± 0.05 | 3.64 ± 0.07 | 13.38 ± 0.16 | 64.23 ± 1.04 |

Rha: Rhamnose, Glu: Glucose, Gal: Galactose, Ara: Arabinose, Xyl: Xylose
According to previous research of Ren et al.,\cite{30} the spectra peak of arabino glucuronoxylan due to ring vibrations, C-OH stretching vibrations of side groups, and C-O-C glycosidic bond vibration was observed showing peaks at 1162/1152 and 1037/1027 cm\(^{-1}\). It has been proved that the psyllium polysaccharide is constituted of \(\beta\)-xylose in pyranose form in the backbone and both \(\alpha\)-arabinose in furanose form and \(\beta\)-xylose in sidechains substituting on C-3 or/and C-2. The current results explored that xylose is substituted by arabinose ratio and forms a branched arabinose structure. The spectra of AXs from the 1020–920 cm\(^{-1}\) range reflect the A/X ratio and substitution positions on xylan backbones. These band shifts may be rationalized with the strong 1015 cm\(^{-1}\) interactions probably a higher degree of cross-linking.

**Antioxidant potential of psyllium husk arabinoxylan**

Antioxidant activity of AXs from PH were assessed through DPPH and FRAP assay. The mean values of both antioxidant assays are shown in Figure 2. The mean values regarding the DPPH and FRAP assays were 3.01 \(\pm\) 0.15 Trolox mg/g and 1.39 \(\pm\) 0.06 \(\mu\)mol Fe\(^{2+}\)/g, respectively. Further, Tili et al.\cite{31,32} reported that folk medicinal plant (P. ovata) extract showed FRAP was 0.17 \(\pm\) 0.012 and 0.20 \(\pm\) 0.006 EC 50 mg/ml of 70% methanol and 70% acetone extracts, respectively, and DPPH assay showed the value 0.56 \(\pm\) 0.01 and 0.48 \(\pm\) 0.004 EC 50 mg/ml of 70% methanol and 70% acetone extracts, respectively. Another study of Sagar et al.\cite{33} who assessed the antioxidant activity through FRAP assay and reported the results as 1.68 \(\mu\)mol Fe (II) eq/g, which are closely corroborated the findings of current research.

Polyphenols are the mainly associated with the scavenging potential; however, in the current study, in vitro assessment through two different antioxidant assays showed lower antioxidant activity as compared to cereal-based AXs, which according to different previous studies showed that the presence of hydroxycinnamic class of phenolic acids in cereals, which are the major contributor in better in vitro antioxidant oxidant activity due to their electron donating ability. However, in different in vivo and in vitro studies, it has been claimed that AXs have a soothing effect on the GI tract\cite{34–36} by developing a symbiotic relation with gut microbiota which upon fermentation of PH DFs produce a compounds which regulate the body mechanisms and prevent the abnormalities in metabolic cycles specifically cellular oxidative stress. P. ovata has been claimed as the food with better nutritional and therapeutic potential due to its better impact on gut health.\cite{8} However, now its derivatives (AXs and their gels) are also being characterized with better characteristics which showed the smoothening effect on gastric health regulation.
Conclusion

In the current study, AXs were extracted from PH, and for the purpose, it was concluded that PH contains 68.29 ± 3.4% AXs, which categorize PH as a better source for AXs. The structure of AXs was validated through FTIR spectroscopy and the spectra at different wavelength was recorded. The monosaccharides composition of extracted AXs showed the major fractions of arabinose and xylose from which AXs are composed. Antioxidant activity of PHAXs was assessed through two commonly used assays including DPPH and FRAP which showed the satisfactory results; however, their activity was found lower as compared to cereal-based AXs, which can also be associated with the presence of polyphenols. Conclusively, it was perceived in the current study that PH is a suitable source of DFs and beneficial to use in various health and food properties due to their nutritional and structural properties. Further, their gut-friendly nature also convenient in the production of SCFAs by gut-microbiota.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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