Profiling and characterization of oat cultivars (*Avena sativa L.*) with respect to bioactive compounds, pesticide residues and mycotoxin

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ABSTRACT

Oat predominately cultivated as forage crop and is not explored for its value-added bioactive moieties. This study was aimed to characterize five selected oat (*Avena sativa L.*) cultivars with respect to organic sugars, antioxidant activities, pesticide residues, mycotoxin and phenolic acids as measured by HPLC. Five indigenous oat cultivars (Avon, PD2LV65, S2000, SGD2011, SGD81) were profiled for seven phenolic acids (gallic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, chlorogenic acid and ferulic acid). Highest concentrations of vanillic acid, gallic acid, caffeic acid, syringic acid, ferulic acid, p-coumaric acid and chlorogenic acid were detected in the SGD2011, SGD81, S2000, SGD2011, S2000, SGD2011 and PD2LV65 respectively. TPC in case of all oat cultivars ranged 50.88–101.56 mg/100 g. Among all oat cultivars, SGD-81 exhibited the highest DPPH activity, TPC, TFC and TFOC contents whereas, SGD-2011 showed the highest total anthocyanin content followed by AVON. S2000 showed the highest concentration of mycotoxin M1 (18.51 ppm), while lowest M1 content (10.51 ppm) were exhibited by SGD-81. Abamectin and permethrin pesticide residues were undetected in oat cultivars whereas, Dieldrin was only detected in SGD-81 and chloropyrifos was not detected in S2000 and SGD-81. Mandipropanamid, parathion methyl, chlorthalonil, indoxacarb and lufernon were detected in the highest concentrations in SGD2011 and S2000 oat cultivars. Moreover, fructose, glucose and sucrose were found in highest amounts in S2000, PD2LV65 and SGD2011, respectively. Conclusively, it was evident from the results that SGD-81, SGD2011 and S2000 could be utilized as source of important and promising phytochemicals of nutraceutical significance. Moreover, significant potential of these oat cultivars can be harnessed owing to value addition and exploitation in formulations of functional food ingredients and foodstuffs.

ARTICLE HISTORY

Received 8 March 2021
Revised 6 July 2021
Accepted 8 July 2021

KEYWORDS

Phenolic acid; pesticide residue; Phytochemical; Organic sugars; HPLC; Mycotoxin

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Introduction

Cereals and their products are widely used as food ingredients in raw or processed form. In 2016, its market worth was 37 USD billion and it is supposed to reach 51 USD billion USD in 2023.[1] In Pakistan, oat is cultivated as one of the Rabbi fodder crop of cereals origin grown in winter season in all areas of rain-fed and irrigated regions. In terms of compositional profile, oat comprises of high protein and highly saturated fatty acid contents. On an average, oat compositional profile largely contains starch (63.2%), protein (15.9%) and fat (7.1%). Oat particularly exhibits significance for its use as a fodder crop in livestock feed. [2] Throughout the world, oat has been cultivated on wide range of soil types as versatile cereal crop. Moreover, it has also been demonstrated by the previous researches that oat exhibits high tolerance to acidic soil up to pH of 4.5 whereas, oat yield significantly improved by cultivation in soil having pH in range of 5.3–5.7.[3] According to the Ayub Agricultural Research Institute, Faisalabad and Fodder Research Institute, Sargodha, the potential yields of all oat varieties including Avon, PD2LV65, SGD81, SGD2011 and S2000 have been reported as 657.89, 759, 708.51, 880.56 and 809.71 maund/acre, respectively.[4,5] SGD-2011 has been recognized for its salient features of high green fodder yield with more tillers number and may yield fodder yield up to 87 tons/hectare. Significant features of S2000 include its high yield property as well as lodging-tolerance, resistance to insect pest, high adaptability as well as early maturity, and may produce green fodder yield up to 80 tons/hectare. PD2LV65 was first released in 1983 and has been characterized with respect to broad leaves, medium height with green fodder yield up to 75 tons/hectare. Green fodder yield for SGD-81 and Avon has been reported as 70 tons/hectare and 65 tons/hectare, respectively. Both SGD-81 and Avon were released firstly in 1983. SGD-81 is late maturing variety, whereas Avon is early maturing and both varieties exhibit resistance to insect pest and diseases.[5]

Cereals are loaded with potential important macro and micro nutrients including vitamin, dietary fiber, minerals, phytoestrogens and antioxidants. [6] Agrochemicals for better and enhanced productivity are widely used to ensure effective plant protection; however excessive chemicals and pesticides usage impart deleterious effect on foodstuffs and crop plants.[7] Different agrochemicals are used in agriculture; these chemical substances are potentially injurious to health and environment owing to imparting of adverse effects. Assessment of pesticide residues in agricultural produce made it possible to asses this hazardous effect. HPLC is widely used for quantification of pesticide residues in cereals and cereal-based processed products.[8] Mycotoxins are the natural toxins which are biosynthesized in food and feedstuffs because of toxigenic fungi during storage.[9] Phenolic acids constitute natural defense system of plants by safeguarding from UV radiation and pathogenicity. These are more diverse in their functions, such as protein synthesis, nutrient uptake, photosynthesis, allelopathy and enzymatic activity. Phenolic acids also have multiple health benefits including anti-mutagenic, anti-carcinogenic, anti-inflammatory and antioxidant activities. It also regulates various enzymatic functions. Cinamic acids (caffeic, p-coumaric, ferulic and sinapic acids) and benzoic acid-based phenolic acids (protocatechuic, p-hydroxybenzoic and vanillic acids) are most commonly distributed in cereals and considered secondary metabolites derived from flavonoids. Their acidic nature differentiate them from other polyphenols and are esterified by acid hydrolysis. Phenolic acids bind with polysaccharides through chelation, covalent bond, crosslinking or bridges. Largest fraction of total phenolics is concentrated in outer layer of grain, while endospermic part contains its least fraction. Cereals are the richest source of phenolic acids.[10,11] It is evident from studies that whole grains have potential role in management and prevention of various metabolic disorders including diabetes and CVDs. Most predominately, phenolic acids are found in bound form in cell wall of outer layer of grain along with other flavonoids. Cereals are abundant in ferulic acid and usually found in husk (75%), endosperm (15%) and aleuronic layer (10%).[12] Wheat is one of the most common food crops being studied for potential phenolic acids. Oat predominately cultivated as a forage crop and is not explored for its value-added bioactive moieties. In recently published literature, profiling and characterization of avenanthramides in commercial oat products have been carried out originated from oat brans, flakes, rolled oats, oatcakes and concentrate.[10,13]
This study was aimed at profiling and characterization of five selected oat cultivars (Avon, PD2L65, S2000, SGD2011 and SGD81) with respect to organic sugars, antioxidant activities, pesticide residues, mycotoxin and phenolic acids by HPLC. To the best of our knowledge, no work has been carried out yet on exploration and profiling of bioactive and phenolic compounds of selected oat varieties and this study explored oat as a functional crop in terms of its applications in various feed, food and nutraceutical industries. We believe that characterization of selected oat cultivars for phenolic compounds will open new horizon for commercialization and breeding of value-added cultivars rich in beneficial bioactive compounds and their potential application in formulation of functional foods. Selected five oat cultivars were analyzed for mycotoxin (M1) quantification and their biochemical parameters, such as total flavonols contents (TFoC), total phenolic content (TPC), total flavonoid content (TFC), free radical scavenging activity through 1,1-diphenyl-2-picrylhydrazyl (DPPH), total anthocyanin content (TAC) to select the best oat varieties through HPLC analysis.

Materials and methods

Procurement and grinding of seeds

Seeds of the selected cultivars were procured from the Fodder Research Institute, Sargodha Pakistan. All the cultivars were grown at Sargodha, Pakistan having latitude 32°09′60.00″ N and longitude 72°29′59.99″ E. Seeds were cleaned from foreign material and ground using coffee grinder then sieved using 50-mesh sieve followed by storage in air-tight polyethylene bags. These were stored at −20°C storage temperature until further analysis.

Flour extract preparation

Fiber sample was extracted by methanol (95% v/v). Methanol and fiber was taken in 10:1 proportion as narrated by Guan et al.[14] In an orbital shaker, the mixture was placed for 24 h. After shaking, the samples were filtered by Whatman filter paper no. 1. And then in rotary evaporator, the filtrate was dried and further diluted with 95% methanol to make total volume up to 50 mL. Until further analysis, it was kept in a refrigerator.

Total phenolic contents (TPC)

By using Folin Ciocalteu (F-C) reagent as described by Chen et al.,[15] total phenolic contents (TPC) of the fiber samples were determined. 2.5 mL of 10% F-C reagent and 0.5 mL of extract were mixed together. After 5 min of interval, 2.5 mL of sodium carbonate (7.5%, w/v) was added to mixture and then it was incubated at room temperature and allowed to stand for time period of 2 h. The absorbance of the samples was recorded at 765 nm with spectrophotometer (Model: SP-3000 plus Optima, Japan). TPC was expressed as milligram gallic acid equivalents (mg GAE.100 g⁻¹ DW). Absorbance of blank (without extract) was also taken and subtracted from the sample’s absorbance. Gallic acid’s standard curve prepared by using different concentrations of GAE. The outcome was expressed as mg GAE.100 g⁻¹.

\[
\text{Total phenolic content (mgGAE.100g}^{-1}) = (\text{sample absorbance} + 0.058)/0.102
\]

Total flavonoids contents (TFC)

Total flavonoids content (TFC) were determined as per the reported method of Nongalleima et al.[16] Briefly, 1 mL of fiber extract, 1.2 mL of deionized water, 0.1 mL of CH₃COONa (10%) and 0.1 mL of AlCl₃ (10%) were mixed together. Then, the samples were kept under darkness for 2.5 h. After 2 h, absorbance was recorded using spectrophotometer (Model: SP-3000 plus Optima, Japan) at 415 nm.
Blank was also run for all ingredients except extract and subtracted from final value. By using different strengths of quercetin solution, standard curve was prepared and expressed as quercetin equivalent as showed in results of TFoC. Total flavonoids of the fiber samples were determined as per described method of Ibrahim et al.\textsuperscript{[2]} Standard curve was obtained using different concentrations of quercetin solution and expressed as mg quercetin equivalent.

\[
\text{TFC (mg of quercetin equivalent)} = \frac{(\text{sample absorbance} + 0.006)}{0.091}
\]

**Total flavonols contents (TFoC)**

By procedure described by Ibrahim et al.,\textsuperscript{[2]} total flavonol content (TFoC) of fiber samples were determined. Briefly, 0.5 mL of the fiber, 0.5 mL of AlCl\textsubscript{3} (20%) and 1.5 mL of CH\textsubscript{3}COONa (10%) were mixed together. For 2.5 h, the samples were kept in dark and then, the absorbance was recorded at 440 nm by spectrophotometer (Model: SP-3000 plus Optima, Japan). The following equation was used for result calculation and expressed as mg of quercetin equivalent.

\[
\text{TFoC (mg of quercetin equivalent)} = \frac{(\text{sample absorbance} + 0.006)}{0.091}
\]

**Total anthocyanin contents (TAC)**

According to method described by Yodpitak et al.,\textsuperscript{[17]} TAC of fiber samples were determined. Two solutions were prepared: first was 0.4 M solution of CH\textsubscript{3}COONa having pH 4 and the second was 0.025 M solution of KCl having pH up to 1. Both reagents in an amount of 2 mL were added in one glass cuvette and 200 \( \mu \)L of extract was taken in another glass cuvette followed by absorbance measurement spectrophotometrically at 510 and 700 nm respectively. Blank was also run in terms of taking 3 mL of the distilled water. The results were expressed as mg.C3G.kg\textsuperscript{-1}.

**DPPH radical scavenging activity**

DPPH-RSA of the fiber sample was determined according to method described by Bei et al.\textsuperscript{[18]} Briefly, 0.1 mL of extract, 2.9 mL of the freshly prepared DPPH solution were thoroughly mixed. Then, in luminar chamber, the sample was kept in light for 1 h. After that, absorbance of samples was taken at 515 nm using spectrophotometer (Optima, Japan). By dissolving 24 mg DPPH in 100 mL of pure methanol, the stock solution was prepared. Absorbance of stock solution was adjusted to 0.980 ± 0.2 at 517 nm. Blank was also run using 2.9 mL of freshly prepared DPPH working solution and 0.1 mL of methanol at 515 nm.

\[
\text{DPPHRadicalscaavengingactivity(\%)} = 100 \times \left(\frac{A_0 - A_t}{A_0}\right).
\]

where, \(A_t\) is the absorbance of sample while \(A_0\) is absorbance of blank.

**Phenolic acids analysis through high-performance liquid chromatography**

**Acid and \(\alpha\)-Amylase hydrolysis.** Sample of selected oat cultivars were prepared as per method described by Du et al.\textsuperscript{[19]} About 1 mL of 0.2 N H\textsubscript{2}SO\textsubscript{4} was taken in test tube then mixed with 0.1 g flour. The mixture was heated in boiling water bath for 1 h. Then, samples were cooled (pH 4.5) in an ice-water bath for 10 min for termination of hydrolysis. Then, 0.2 mL of 2.5 M aqueous sodium acetate solution containing 2% (w/v) \(\alpha\)-amylase was also added. Samples were then incubated at 30°C for 1 h and centrifuged at 10000 \( \times \) g for 10 min. HPLC analysis was carried subsequently to analyze the supernatants.

**Phenolic acid profiling.** Phenolic acids profiling of selected oat cultivars was carried out using 200 series- HPLC system (Perkin Elmer, USA) equipped with a Kinetex Core-Shell RP-C18 column
(Inertsil ODS-SP: 250 mm × 4.6 mm, 5 μm) and DAD detector. Acetonitrile and HPLC grade water with 0.1% formic acid (v/v) were employed as the mobile phase. Gradient elution from 5% to 40% acetonitrile solvent in 40 min was applied. The column was equilibrated between two runs for a period of 5 min with 90% isocratic acetonitrile. According to comparison to retention times of external standards at 275 nm, the following phenolic acids were identified; vanillic, caffeic, syringic, p-coumaric, ferulic, gallic and chlorehogenic acid. Quantification of individual phenolic acids was carried out using a 5-point calibration curve (R² ≥ 0.99) following retention times and DAD absorption spectra of external standards. The injection volume was 10 μL. Identification of phenolic acids was accomplished by comparing the retention times of peaks in the samples to those of the standards under the same HPLC conditions. Quantification analysis of phenolic acids was carried out in triplicate (n = 3).

**Quantification of pesticide residue**

For pesticide residue quantification, each sample (50 g) was taken from the well-homogenized sample in 500 mL Erlenmeyer flasks with glass stopper and 75 mL of analytical grade ethyl acetate. 2.5 g sodium chloride (Merck, Darmstadt, Germany) and 10 g Na₂SO₄ (anhydrous) were added in each flask and stoppered. Flask was put in an orbital shaker for 1 h. By using Whatman filter paper No. 1, the solution was then filtered for removal of organic layer of CH₃COOH. Contents were evaporated by using rotary evaporator ( Büchi R-3000, Büchi Labortechnic AG, Postfach, Switzerland), and then was diluted with 1 mL of analytical grade acetone. ¹⁹

**Organic sugars quantification**

Organic sugar quantification was carried out according to reported method of Allwood et al. ²⁰ HPLC-grade acetonitrile bought from Thermo Fisher Scientific (USA) and HPLC-grade methanol bought from Kermel Chemical Reagent Co. Ltd. (Tianjin, China) were utilized. In this study, deionized water was also used (Milli-Q, Millipore Corp., Milford, MA, USA) of 18.2 MΩ/cm resistivity. Standard stock solutions of fructose, sucrose, and glucose were prepared by dissolving 2.97 g of sucrose, 1.648 g of fructose, and 0.902 g of glucose in 100 mL of water. By pipetting 0.4, 0.8, 1.2, 1.6 and 2.0 mL of the above-mixed standard stock solutions, the working solutions of sugars were prepared into five 2-mL amber glass volumetric flasks and then making up to the mark with distilled water, respectively. Calibration standard solutions were prepared by pipetting 1.0 mL of the IS stock solutions into five 2.0-mL amber glass volumetric flasks and making up to the mark with 1.0 mL of the above organic sugars working solutions separately. Mobile phase (75% acetonitrile in water) was obtained by adding 250 mL deionized water to 750 mL acetonitrile and mixing well aided by sonication.

**Mycotoxin determination**

Flour (1 g) of each sample was mixed in 5 mL of methanol and then the matrix was sonicated at 20 KHz as described by Irakli et al. ²¹

**Statistical analysis**

All measurements were performed in triplicate (n = 3). The data were analyzed using a one-way analysis of variance (ANOVA) under completely randomized design using SPSS ver. 18.0 software (SPSS Inc., Chicago, IL, USA), while significant differences in means were determined using Duncan’s multiple-range test (p < .05).


**Results and discussion**

**Phenolic acid profiling**

Phenolic acid profiling of selected oat cultivars was carried out and the results of phenolic acids contents are tabulated in Table 5. All the selected cultivars varied significantly \((p ≤ 0.05)\) with respect to all profiled phenolic acids. Oat cultivar SGD2011 excelled in vanillic acid content \((5.19 ± 0.26 \text{ mg.100 g}^{-1} \text{ DW})\), while least fraction was observed in oat cultivars Avon \((2.99 ± 0.11 \text{ mg.100 g}^{-1} \text{ DW})\). Oat cultivar SGD 81 exhibited the highest gallic acid contents \((103 ± 0.9 \text{ mg.100 g}^{-1} \text{ DW})\) and least were observed in oat cultivar S2000 \((29.94 ± 0.12 \text{ mg.100 g}^{-1} \text{ DW})\). Oat cultivar S2000 exhibited the significantly high \((p < .05)\) caffeic acid contents \((16.68 ± 0.20 \text{ mg.100 g}^{-1} \text{ DW})\) and least fraction of caffeic acid was observed in Avon \((13.18 ± 0.16 \text{ mg.100 g}^{-1} \text{ DW})\). Oat cultivar S2000 showed maximum syringic acid contents \((44.84 ± 0.06 \text{ mg.100 g}^{-1} \text{ DW})\). The highest fraction of ferulic acid was observed in S2000 \((147.13 ± 0.15 \text{ mg.100 g}^{-1} \text{ DW})\) and least was observed in SGD81 \((143.95 ± 0.18 \text{ mg.100 g}^{-1} \text{ DW})\). The maximum fraction of \(p\)-coumaric acid was observed in SGD2011 \((4.56 ± 0.33 \text{ mg.100 g}^{-1} \text{ DW})\) and the least \(p\)-coumaric acid contents were observed in Avon \((2.24 ± 0.05 \text{ mg.100 g}^{-1} \text{ DW})\). Chloregenic acid was only observed in two oat cultivars PD2LV65 \((3.65 ± 0.16 \text{ mg.100 g}^{-1} \text{ DW})\) and the least fraction was observed in oat cultivar Avon \((0.43 ± 0.12 \text{ mg.100 g}^{-1} \text{ DW})\). Chloregenic acid was not detected in selected oat cultivars named SGD81, SGD2011 and S2000. Phenolic acid profiling of selected oat cultivars showed significant \((p < .05)\) differences which might be attributed to the genetic diversity among all the cultivars. As oat is not native to the Pakistan and was introduced from various localities across the globe, therefore heat and water stress are abiotic factors which might cause triggering of antioxidant compounds production. Low rain fall and hot weather during the ripening stage of crop tend to produce phenolic acids. Conclusively, the HPLC profiling showed the variations in phenolic compounds contents in different oat cultivars. This information might be helpful in selection of oat cultivars for extracting phenolic compounds from oat fiber, oat bran and oatmeal. Among all oat cultivars, SGD81 and SGD2011 exhibited the highest concentrations of phenolic acids. Standard curves for phenolic acids, pesticide residues, mycotoxin and organic sugars (fructose, glucose and sucrose) are shown in Figs. 1, 2, 3 & 4, respectively. Moreover, retention times and peak heights of standard phenolic acids, pesticide residues, mycotoxin and organic sugars standard solutions are provided in Tables 1, 2, 3 and 4 respectively.

*Figure 1. Phenolic acid standard curve.*
Phytochemical quantification

Oat ranks 4th among cereal crops which is predominately cultivated as a forage crop. These bioactive compounds exert significant impact on human health by prevention and management of multiple metabolic disorders. Outermost layer of the seed contains phenolic compounds in rich amounts. Cereals are richly loaded with the plant bioactives including phenolic compounds. They inhibit lipid peroxidation and also suppress the side chain reactions. Total phenolic contents, flavonoids,
Figure 4. Standard curve of organic sugars (fructose, glucose and sucrose).

Table 1. Retention time and peak height of standard phenolic acids.

| Phenolic acids   | Retention time | Peak height | Concentration (ppm) |
|------------------|----------------|-------------|---------------------|
| Gallic acid      | 3.51           | 75          | 1000                |
| Vanillic acid    | 9.02           | 336         | 1000                |
| Caffeic acid     | 9.57           | 75          | 1000                |
| Syringic acid    | 9.96           | 24          | 1000                |
| p-Coumaric acid  | 14.96          | 438         | 1000                |
| Chlorogenic acid | 15.4           | 44          | 1000                |
| Ferulic acid     | 16.37          | 70          | 1000                |

Table 2. Pesticide residues standard retention time and peak height.

| Pesticide         | Retention time | Peak height | Concentration (ppm) |
|-------------------|----------------|-------------|---------------------|
| Mandipropamid     | 2.56           | 145         | 500                 |
| Parathion methyl  | 2.80           | 70          | 500                 |
| Chlorthalonil     | 3.07           | 38          | 500                 |
| Indoxacarb        | 3.67           | 30          | 500                 |
| Lufernon          | 4.29           | 111         | 500                 |
| Chlorpyrifos      | 6.24           | 17          | 500                 |
| Dieldrin          | 6.85           | 18          | 500                 |
| Abamectin         | 13.97          | 10          | 500                 |
| Permethrin        | 16.03          | 43          | 500                 |
| Bifenthrin        | 24.88          | 105         | 500                 |

Table 3. Mycotoxin standard solution retention time and peak height.

| Mycotoxin | Retention time | Peak height | Concentration (ppm) |
|-----------|----------------|-------------|---------------------|
| M₁        | 2.35–2.45      | 8           | 1                   |

Table 4. Organic sugars standard solutions retention times and peak heights.

| Sugars   | Retention time | Peak height | Concentration (ppm) |
|----------|----------------|-------------|---------------------|
| Fructose | 2.25–2.45      | 25          | 300                 |
| Sucrose  | 2.7–2.85       | 11          | 300                 |
| Glucose  | 3.3–3.5        | 12          | 300                 |
Table 5. Phenolic acid profiling of selected oat cultivars. Unit: mg/100 g DW.

| Varieties | Vanillic acid | Gallic acid | Caffeic acid | Syringic acid | Ferulic acid | p-Coumaric acid | Chlorogenic acid |
|-----------|----------------|-------------|--------------|--------------|--------------|-----------------|-----------------|
| Avon      | 2.99 ± 0.11c   | 62.50 ± 0.44b | 13.18 ± 0.16d | 42.26 ± 0.16c | 144.77 ± 0.13d | 2.24 ± 0.15a   | 0.43 ± 0.12b    |
| PD2LV65   | 4.31 ± 0.25b   | 37.18 ± 0.23d | 14.33 ± 0.22d | 44.71 ± 0.07c | 145.31 ± 0.25c | 3.24 ± 0.12d   | 3.64 ± 0.16b    |
| SGD81     | 3.78 ± 1.14e   | 103 ± 0.9b   | 15.54 ± 0.35c | 43.56 ± 0.08d | 143.95 ± 0.18b | 4.26 ± 0.09b   | ND              |
| SGD2011   | 5.19 ± 0.26e   | 47.05 ± 0.05c | 16.13 ± 0.13b | 45.18 ± 0.07d | 146.75 ± 0.19b | 4.56 ± 0.33a   | ND              |
| S2000     | 3.24 ± 0.13d   | 29.94 ± 0.12e | 16.68 ± 0.20b | 44.84 ± 0.06d | 147.13 ± 0.15c | 3.40 ± 0.15c   | ND              |

Figure 5. Total phenolic content (TPC) (a): %DPPH-RSA (b): Total flavonoid contents (TFC) (c): Total flavonol contents (TFoC) (d): Total anthocyanin contents (TAC) of oat cultivars (e).

Antioxidant activity, total flavonol and anthocyanin contents of the selected oat cultivars are shown in Figure 5. Total phenolic contents (TPC) among selected oat cultivars vary significantly \((p < .05)\). TPC varied from 36.07 to 101.56 mg GAE/100 g DW. The highest TPC contents were shown by the SGD-81 while the least 36.07 mg GAE/100 g DW were found in S2000. Total flavonoid contents (TFC) among the selected oat cultivars also exhibited significant \((p < .05)\) variations ranging from 754.16 to 1147.08 mg GAE/100 g DW.

Each value represents the mean ± standard deviation \((n = 3)\). Means with different letters showed significant difference \((p < .05)\). Values bearing different alphabet differ significantly at significance level of \(p < .05\).

Furthermore, all oat cultivars showed significant \((p < .05)\) variations of radical scavenging activity (RSA) and showed variability in range of 24.33–55.88%. The highest % RSA was exhibited by the SGD-81 cultivar, while the lowest % RSA was observed in SGD-2011 cultivar. Smuda et al.\(^{[23]}\) studied the % RSA in range of 39.3 ± 0.1 to 70.6 ± 2.00% of different cereals milling products. Varietal differences might be the cause of slight variations in RSA of oat cultivars. Moreover, different analytical protocols might also be the possible cause of variations in RSA results. In addition, TFC were affected by color of the flour from different cultivars and warm weather. SGD-81 exhibited higher TFC as compared to TFC observed in all other cultivars and yellow flour. TPC in case of all oat cultivars ranged 50.88–101.56 mg/100 g. Among all oat cultivars, SGD-81 exhibited the highest DPPH activity, TPC, TFC, TFoC contents whereas, SGD-2011 showed the highest total anthocyanin content followed by Avon.
Mycotoxin quantification

Mycotoxin quantification of selected oat cultivars was carried out and the results are tabulated in Table 6. Selected cultivars varied significantly ($p \leq 0.05$) with respect to mycotoxin. The highest fraction of M1 was observed in oat cultivar S2000 (18.50 ± 0.3 ppm). SGD-2011 showed the lowest M1 fraction (12.21 ± 0.21 ppm). Interaction of different environmental factors (pH, temperature, water activity, poor hygiene and mechanical damage, etc.) might also exert their influences on mycotoxin formation and existence in cereals during storage.

Extreme variations in temperature and moisture contents of grain during harvesting, storage, processing may contribute to increased fungal contamination. Mycotoxin analysis might be helpful in risk-based monitoring of cereals by food processors, food safety authorities and food industries. It might help in fungicide selection and application and purchasing of cereal commodities. The results might also be helpful in further analytical modeling and assurance of quality control in cereals processing and manufacturing of cereal-based byproducts. Conclusively, S2000 variety showed the highest M1 mycotoxin content as compared to others. It can be implied that oats among all cereals exhibits high susceptibility to pathogenic fungal infestations. In this regard, several fungal species have been reported to cause severe attack on oat crop plants during storage, such as *Penicillium, Aspergillus, Alternaria* and *Fusarium spp.* If environmental conditions become highly favorable then fungal contamination may lead to generation of plethora of mycotoxins which may cause severe toxicological effects upon consumption of oats by humans or animals though ingestion of contaminated foodstuffs and feed respectively.

Pesticide residues

The result regarding pesticide residues are shown in Table 7. Significant ($p \leq 0.05$) differences were shown by oat cultivars with respect to mandipropamid, parathion methyl, chorthalonil, indoxacarb, lufenuron and chloropyrifos. While pesticide residues of dieldrin, abamectin, permethrin and bifenthrin were not detected in selected oat cultivars. The highest fraction of mandipropamid was shown by oat cultivar SGD2011 (21.85 ± 2.4 ppm) and oat cultivar Avon showed least fraction of manidipropamid (0.81 ± 0.11 ppm).

The highest fraction of parthiomethyl was contained by SGD2011 (6.17 ± 1.6 ppm), while the least fraction was observed in Avon (0.67 ± 0.08 ppm). The highest fraction of chorthalonil was observed in oat cultivar S2000 (2.57 ± 0.57 ppm) and least was observed in oat cultivar Avon (0.63 ± 0.06). Chorthalonil was not detected in oat cultivar PD2LV65, SGD2011 and SGD81. The highest fraction of indoxacarb was observed in SGD2011 (10.4 ± 1.2 ppm) and least was observed in oat cultivar Avon (1.58 ± 0.17 ppm). The highest fraction of lufenuron was observed in oat cultivar Avon (1.26 ± 0.14 ppm) and least was shown by SGD2011 (0.43 ± 0.02 ppm), while lufenuron was not detected in oat cultivar PD2LV65 and SGD81. The highest fraction of chloropyrifos was observed in oat cultivar Avon (7.58 ± 0.45 ppm) and least was observed in PD2LV65 (5.61 ± 0.10 ppm). Dieldrin was only detected in oat cultivar SGD2011 (46.67 ± 0.9 ppm). Quantification of the pesticide residues in selected oat cultivars defines their safe use in different food

| Oat cultivars | M1 (ppm) |
|---------------|----------|
| Avon          | 14.80 ± 0.20 $^c$ |
| PD2LV65       | 17.20 ± 0.26 $^b$ |
| S2000         | 18.50 ± 0.3 $^b$ |
| SGD2011       | 12.21 ± 0.20 $^a$ |
| SGD81         | 10.50 ± 0.20 $^a$ |

Each value represents the mean ± standard deviation ($n = 3$). Means with different letters showed significant difference ($p < 0.05$).
Table 7. Pesticide residues detected in selected oat cultivars. **Unit: ppm.**

| Cultivars | Mandipropamid | Parathion methyl | Chlorthalonil | Indoxacarb | Lufemon | Chlorpyrifos | Dieldrin | Abamectin | Permethrin | Bifenthrin |
|-----------|---------------|------------------|--------------|------------|---------|--------------|---------|-----------|------------|-----------|
| Avon      | 0.81 ± 0.10<sup>d</sup> | 0.67 ± 0.08<sup>d</sup> | 0.63 ± 0.06<sup>b</sup> | 1.58 ± 0.17<sup>c</sup> | 1.26 ± 0.14<sup>a</sup> | 7.58 ± 0.45<sup>a</sup> | ND      | ND        | ND         | ND        |
| PD2LV65   | ND            | ND               | ND           | ND         | ND      | 5.61 ± 0.10<sup>c</sup> | ND      | ND        | ND         | ND        |
| S2000     | 6.24 ± 0.33<sup>b</sup> | 1.91 ± 0.12<sup>c</sup> | 2.75 ± 0.57<sup>a</sup> | ND         | 0.84 ± 0.08<sup>b</sup> | ND      | ND        | ND         | ND        |
| SGD2011   | 21.850 ± 2.4<sup>a</sup> | 6.17 ± 1.6<sup>a</sup> | ND           | 10.4 ± 1.2<sup>a</sup> | 0.43 ± 0.02<sup>c</sup> | 7.06 ± 0.06<sup>a</sup> | 46.67 ± 0.9<sup>a</sup> | ND        | ND        | ND        |
| SGD81     | 2.06 ± 0.07<sup>c</sup> | 1.94 ± 0.10<sup>b</sup> | ND           | 3.76 ± 0.20<sup>b</sup> | ND      | ND           | ND      | ND        | ND         | ND        |

Each value represents the mean ± standard deviation (n = 3). Means with different letters showed significant differences (p < 0.05). Values bearing different alphabet differ significantly at p < 0.05.
Table 8. Organic sugars quantification of selected oat cultivars.

| Cultivars | Fructose (μg/g) | Glucose (μg/g) | Sucrose (μg/g) |
|-----------|----------------|----------------|---------------|
| Avon      | 33.45 ± 0.09a  | 6.34 ± 0.11b   | 3.84 ± 0.09d  |
| PD2LV65   | 33.16 ± 0.15a  | 30.03 ± 0.15a  | 11.69 ± 0.06b |
| S2000     | 33.60 ± 0.12b  | 13.64 ± 0.06c  | 2.83 ± 0.07e  |
| SGD2011   | 26.26 ± 0.25d  | 18.16 ± 0.15b  | 14.23 ± 0.06b |
| SGD81     | 12.15 ± 0.13a  | 4.36 ± 0.18f   | 3.16 ± 0.06a  |

Each value represents the mean ± standard deviation (n = 3). Means with different letters showed significant difference (p < 0.05). Values bearing different statistical lettering differ significantly at p < 0.05.

and feed industries. Dieldrin is more resistant pesticide and that is why its use is banned in agricultural farming of crop plants due to its residual and toxic effect on human health. The analyzed residues were in acceptable range, hence this was helpful to ensure food safety for intended consumers. This information might be of potential use for determining pesticide residues in oat varieties. Especially in case of cereals, it is always challenging for determining pesticide residues owing to presence of higher proportions of fatty acids and interferents which may hamper the analytical throughput in case of oat analysis.26

Organic sugars

Quantification of organic sugars of selected oat cultivars was carried out and the results are tabulated in Table 8. The results indicated that fructose contents in oat cultivar S2000, Avon, PD2LV65, SGD2011 and SGD81 were 33.60 ± 0.12 μg/g, 33.45 ± 0.09 μg/g, 33.16 ± 0.15 μg/g, 26.26 ± 0.25 μg/g and 12.15 ± 0.13 μg/g respectively. The highest fraction of glucose contents were observed in oat cultivar PD2LV65 (30.03 ± 0.15 μg/g) and the least was observed in oat cultivar Avon (6.34 ± 0.11 μg/g). The highest fraction of sucrose was observed in oat cultivar SGD2011 (14.23 ± 0.06 μg/g) and the least was observed in S2000 (2.83 ± 0.7 μg/g). Conclusively, cereals are employed in manufacturing of baked products and organic sugars have potential roles in elevating the gelatinization temperature of starches, help in entrapment of air bubbles and cause development of light texture in oatmeals and oatmeal-derived bakery products. Organic sugars bind water owing to humectant properties and may potentially affect preservation of oat products as well as textural attributes.27

Conclusion

Food processors and industrial stakeholders are always keenly interested in exploitation of cost-effective raw materials. Cereals and their byproducts are widely used as food in raw or processed forms. Largest fraction of total phenolics is concentrated in outer layer of grain, while endosperm part contains its least fraction. Cereals are the richest source of phenolic acids. Oat predominately cultivated as forage crop and is not explored for its value-added bioactive moieties. This study was aimed at profiling and characterization of five selected oat (Avena sativa L.) cultivars with respect to organic sugars, antioxidant activities, pesticide residues, mycotoxin and phenolic acids by HPLC. This study was carried out for exploration of oat as indigenous material with cost-effectiveness to be exploited in formulation of nutraceuticals, food and feed industry. Five indigenous oat cultivars (Avon, PD2LV65, S2000, SGD2011 and SGD81) were profiled for seven phenolic acids (gallic acid, vanillic acid, caffeic acid, syringic acid, p-coumaric acid, chlorogenic acid and ferulic acid) by HPLC. TPC in selected oat cultivars were reported in range from 50.88 to 101.56 mg/100 g. % DPPH radical-scavenging activity ranged 29.77–55.89%. TFC was found to be in range of 754.16–1147.08 mg/100 g. TFOC were observed from 548.33 to 697.5 mg/100 g. Anthocyanin contents were reported from 0.5 to 2.87 mg C3G/kg. Vanillic acid, gallic acid, caffeic acid, syringic acid were found in ranges of 2.99–5.19 mg/100 g, 29.94–103 mg/100 g, 13.16–16.68 mg/100 g and 42.26–44.84 mg/100 g respectively. Whereas, ferulic acid, p-coumaric acid and chlorogenic acid were observed in ranges of 143.95–147.13 mg/100 g, 2.24–4.56 mg/100 g and 0.43–3.64 mg/100 g respectively. Mycotoxin varied from 10.50 to 18.50 ppm in all oat cultivars. Residue of
pesticides including mandipropamid (0.81–21.85 ppm), parathion methyl (0.67–6.17 ppm), chlorthalonil (0.63–2.75 ppm), indoxacarb (1.58–10.4 ppm), lufernnon (0.43–1.26 ppm), chloropyrifos (5.61–7.58 ppm) were detected in selected oat cultivars. While pesticide residues of dieldrin, abamectin, permethrin and bifenthrin were not detected in selected oat cultivars. In case of organic sugars, fructose, glucose and sucrose contents were found in ranges of 12.15–33.60 μg/g, 4.36–30.03 μg/g and 3.16–14.23 μg/g respectively. To the best of our knowledge, no work has been carried out yet on exploration and profiling of bioactive and phenolic compounds of selected oat varieties and this study will help to explore oat as a functional crop to have its application in various feed, food and nutraceutical industries. We believe that characterization of selected oat cultivars for phenolic compounds will open new horizon for commercialization and breeding of value-added cultivars rich in beneficial bioactive compounds and their potential application in formulation of functional foods. Conclusively, it was evident from the results that SGD-81, SGD2011 and SG2000 could be utilized as source of important and promising phytochemicals of nutraceutical significance. Moreover, significant potential of these oat cultivars can be harnessed owing to value addition and exploitation in formulations of functional food ingredients and foodstuffs as functional cereal crop rather than only utilized as the forage crop. Keeping in view the Pakistani perspective, this is a novel study as systematic intervention as no such research work has been previously carried out. The implications of this study may open new horizons for food processing industry in terms of isolation and extraction of bioactive compounds of nutritional significance for intended use by the general public to mitigate increasing incidence of chronic maladies owing to nutritional deficiency and poor food security. The findings of this research may be of pertinent relevance to the food scientists, food processors keenly involved in research and development and exploration of nutraceutical potential of phytochemicals from oat or oat-based food ingredients.

Conflicts of interest/Competing interests

Authors have no conflict of interest to declare.

Acknowledgements

This work was supported by Foundation of Ph.D. Research Project, Jilin Medical University (No. JYBS2019010), Jilin Province, China; Foundation of Science and Technology Department of Jilin Province (No. 20200404175YY), Foundation of the Education Department of Jilin Province (No. JJKH20170416K), Jy2018zx04), Foundation of Health and Family Planning Commission of Jilin Province (No. 2019J066), Foundation of Suzhou Institute of medical engineering, Chinese Academy of Sciences-Jilin Science and technology cooperation project (No.E0550101), Jilin Collaborative Innovation Center for Antibody Engineering, Jilin Medical University (No.20180623045TC).

Funding

There was no funding received for execution of this study.

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References

[1] Perdon, A. A.; Holopainen-Mantila, U. Cereal Grains and Other Ingredients. In A. Perdon, S. Schonauer & K. Poutanen (Eds.), Breakfast Cereals and How They are Made; AACC International Press: Washington, United States. 2020; pp 73–96. doi:10.1016/b978-0-12-812043-9.00004-7
[2] Ibrahim, M. S.; Ahmad, A.; Sohail, A.; Asad, M. J. Nutritional and Functional Characterization of Different Oat (Avena Sativa L.) Cultivars. Int. J. Food Prop. 2020, 23(1), 1373–1385. DOI: 10.1080/10942912.2020.1806297.
[3] Shvachko, N. A.; Loskutov, I. G.; Semilet, T. V.; Popov, V. S.; Kovaleva, O. N.; Konarev, A. V. Bioactive Components in Oat and Barley Grain as a Promising Breeding Trend for Functional Food Production. *Molecules.* 2021, 26(8), 2260. DOI: 10.20944/preprints202103.0154.v1.

[4] Ayub Agriculture Research Institute (AARI). Government of Punjab, Pakistan Oat Fodders: 2021. https://aari.punjab.gov.pk/oats_fodder (accessed June 26, 2021).

[5] Fodder Research Institute (FRI), Sargodha. Research Divisions Fodder Research Program. NARC, Islamabad. 2021, http://www.parc.gov.pk/index.php/en/research-divisions/137-narc/crop-sciences-institute/714-fodder-program (accessed June 25, 2021).

[6] Mir, N. A.; Riar, C. S.; Singh, S. Nutritional Constituents of Pseudo Cereals and Their Potential Use in Food Systems: A Review. *Trends Food Sci. Technol.* 2018, 75, 170–180. DOI: 10.1016/j.tifs.2018.03.016.

[7] Guha, T.; Gopal, G.; Kundu, R.; Mukherjee, A. Nanocomposites for Delivering Agrochemicals: A Comprehensive Review. *J. Agric. Food Chem.* 2020, 68(12), 3691–3702. DOI: 10.1021/acs.jafc.9b06982.

[8] Nieto, C. H. D.; Granero, A. M.; Zon, M. A.; Fernández, H. Sterigmatocystin: A Mycotoxin To Be Seriously Considered. *Food Chem. Toxicol.* 2018, 118, 460–470. DOI: 10.1016/j.fct.2018.05.057.

[9] Farha, W.; Abd El-Aty, A. M.; Rahman, M. M.; Jeong, J. H.; Shin, H. C.; Wang, J.; Shim, J. H. Analytical Approach, Dissipation Pattern and Risk Assessment of Pesticide Residue in Green Leafy Vegetables: A Comprehensive Review. *Biomed. Chromatogr.* 2018, 32(1), e1434. DOI: 10.1002/bmc.4134.

[10] Rashmi, H. B.; Negi, P. S. Phenolic Acids from Vegetables: A Review on Processing Stability and Health Benefits. *Food Res. Int.* 2020, 136, 109298. DOI: 10.1016/j.foodres.2020.109298.

[11] Amer, K.; Shahbaz, H. M.; Kwon, J. H. Green Extraction Methods for Polyphenols from Plant Matrices and Their Byproducts: A Review. *Comp. Rev. Food Sci. Food S.* 2017, 16(2), 295–315. DOI:10.1111/1541-4337.12253.

[12] Stuper-Szablewska, K.; Kurasia-Popowska, D.; Nawracała, J.; Perkowski, J. Quantitative Profile of Phenolic Acids and Antioxidant Activity of Wheat Grain Exposed to Stress. *Eur. Food Res. Technol.* 2019, 245(8), 1595–1603. DOI:10.1007/s00217-019-03262-8.

[13] Soycan, G.; Schär, M. Y.; Kristek, A.; Boberska, I.; Alsharif, S. N.; Corona, G.; Spencer, J. P. Composition and Content of Phenolic Acids and Avenanthramides in Commercial Oat Products: Are Oats an Important Polyphenol Source for Consumers? *Food Chem.* 2019, 3, 100047. DOI: 10.1016/j.foodch.2019.100047.

[14] Guan, X.; Jin, S.; Li, S.; Huang, K.; Liu, J. Process Optimization, Characterization and Antioxidant Capacity of Oat (*Avena Sativa L.*) Bran Oil Extracted by Subcritical Butane Extraction. *Molecules.* 2018, 23(7), 1546. DOI: 10.3390/molecules23071546.

[15] Chen, C.; Wang, L.; Wang, R.; Luo, X.; Li, Y.; Li, J.; Chen, Z. Phenolic Contents, Cellular Antioxidant Activity and Anti-proliferative Capacity of Different Varieties of Oats. *Food Chem.* 2018, 239, 260–267. DOI: 10.1016/j.foodchem.2017.06.104.

[16] Nongalleima, K.; Ajungla, T.; Singh, C. B.; Chingakham, C.; Singh, B. Phytochemical, Total Phenolic, Total Flavonoid and Total Flavonoid Content Estimation in *Citrus Macroptera Montrouz.* *J. Med. Plant Stud.* 2017, 5(3), 114–211.

[17] Yodpitak, S.; Mahatheeranont, S.; Boonyawan, D.; Sookwong, P.; Roytrakul, S.; Norkaew, O. Cold Plasma Treatment to Improve Germination and Enhance the Bioactive Phytochemical Content of Germinated Brown Rice. *Food Chem.* 2019, 289, 328–339. DOI: 10.1016/j.foodch.2019.03.061.

[18] Wei, Q.; Wu, Z.; Chen, G. Dynamic Changes in the Phenolic Composition and Antioxidant Activity of Oats during Simultaneous Hydrolysis and Fermentation. *Food Chem.* 2020, 305, 125269. DOI: 10.1016/j.foodchem.2019.125269.

[19] Du, R.; Song, Q.; Zhang, Q.; Zhao, F.; Kim, R. C.; Zhou, Z.; Han, Y. Purification and Characterization of Novel Thermostable and Ca-Independent α-Amylase Produced by *Bacillus Amyloliquefaciens* BH072. *Int. J. Biol. Macromol.* 2018, 115, 1151–1156. DOI: 10.1016/j.ijbiomac.2018.05.004.

[20] Allwood, J. W.; Xu, Y.; Martinez-Martin, P.; Palau, R.; Cowan, A.; Goodacre, R.; Howarth, C. Rapid UHPLC-MS Metabolite Profiling and Phenotypic Assays Reveal Genotypic Impacts of Nitrogen Supplementation in Oats. *Metabolomics.* 2019, 15(3), 1–19. DOI: 10.1007/s11306-019-1501-x.

[21] Irakli, M. N.; Skendi, A.; Papageorgiou, M. D. HPLC-DAD-FLD Method for Simultaneous Determination of Mycotoxins in Wheat Bran. *J. Chromatogr. Sci.* 2017, 55(7), 690–696. DOI: 10.1093/chromsci/bmx022.

[22] Zrzková, M.; Capouchová, I.; Paznocht, L.; Eliášová, M.; Dvořák, P.; Konvalina, P.; Bečková, L. Variation of the Total Content of Polyphenols and Phenolic Acids in Einkorn, Emmer, Spelt and Common Wheat Grain as a Function of Genotype, Wheat Species and Crop Year. *Plant Soil Environ.* 2019, 65(5), 260–266. DOI:10.17221/134/2019-pse.

[23] Smuda, S. S.; Mohsen, S. M.; Olsen, K.; Aly, M. H. Bioactive Compounds and Antioxidant Activities of Some Cereal Milling By-Products. *J. Food Sci. Technol.* 2018, 55(3), 1134–1142. DOI: 10.1007/s13197-017-3029-2.

[24] Shah, L.; Ali, A.; Yahya, M.; Zhu, Y.; Wang, S.; Si, H.; Ma, C. Integrated Control of Fusarium Head Blight and Deoxynivalenol Mycotoxin in Wheat. *Plant Pathol.* 2018, 67(3), 532–548. DOI: 10.1111/ppa.12785.
[25] De Colli, L.; De Ruyck, K.; Abdallah, M. F.; Finnan, J.; Mullins, E.; Kildea, S.; Danaher, M. Natural Co-Occurrence of Multiple Mycotoxins in Unprocessed Oats Grown in Ireland with Various Production Systems. *Toxins*. 2021, 13(3), 188. DOI: 10.3390/toxins13030188.

[26] de Matos, E. M.; Ribeiro, L. C.; Prestes, O. D.; Da Silva, J. A.; de Farias, B. S.; Luiz, A. D. A.; Zanella, R. Multiclass Method for the Determination of Pesticide Residues in Oat Using Modified QuEChERS with Alternative Sorbent and Liquid Chromatography with Tandem Mass Spectrometry. *Food Anal. Method*. 2019, 12(12), 2835–2844. DOI: 10.1007/s12161-019-01641-1.

[27] Gangopadhyay, N.; Hossain, M. B.; Rai, D. K.; Brunton, N. P. A Review of Extraction and Analysis of Bioactives in Oat and Barley and Scope for Use of Novel Food Processing Technologies. *Molecules*. 2015, 20(6), 10884–10909. DOI: 10.3390/molecules200610884.