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PHYSICOCHEMICAL AND ANTIOXIDANT PROPERTIES OF DIFFERENT MILLING FRACTIONS OF INDIAN WHEAT CULTIVARS

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ABSTRACT

Wheat cultivars were studied for their physical properties (1000 kernel weight, bulk density, and l/b ratio). They were debranned and milling fractions obtained were bran and refined wheat flour (RWF). Whole wheat flour (WWF) was also obtained by milling the grains without removal of bran portion. These fractions were studied for their hunter color characteristics and antioxidant (total phenolic content, TPC; antioxidant activity, AOA; and total flavonoids content, TFC properties. Total phenolic content, total flavonoids content, and antioxidant activity differed significantly ($p<0.05$) among cultivars for different fractions. Studies revealed that bran fraction had significantly ($p<0.05$) higher bioactive compounds than other fractions for the cultivars studied. TPC, TFC and AOA followed the order: RWF<WWF<bran. Thus wheat bran, may serve as an excellent source of raw material for its potential applications as nutraceuticals and functional food ingredients.

KEY WORDS: **Wheat cultivars; Color; Antioxidant activity; Total phenolic content; Total flavonoids content.**

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INTRODUCTION

Wheat (*Triticum aestivum*) belongs to Poaceae family and occupies about 25% of the total global area under cereal production. India ranks 3rd in the world in wheat production with total worldwide production of 713 million tonnes. In India, wheat occupied an area of nearly 29650000 ha with total production of 935100 tonnes[1]. Wheat endosperm contains mostly starch and protein, whereas bran and germ are rich in dietary fiber, minerals and phytochemicals, which play important roles in nutrition and health benefits for humans[2]. The antioxidants in wheat include carotenoids, tocopherols, flavonoids and phenolic acids. Wheat phenolic acids include ferulic, vanillic, syringic, sinapic, caffeic and p-coumaric acids[3-5] have been demonstrated to be a source of nutritional antioxidants. Whole-grain cereals have received considerable attention in the last several decades due to the presence of unique blend of bioactive components like phytochemicals and antioxidants[6]. The outermost layers of the grains possess a high phenolic content and antioxidant activity[7-9]. The consumption of wheat with bran in the form of whole grain may provide beneficial health effects[4]. The present investigation was undertaken to study the physicochemical and antioxidant properties of different milling fractions of wheat cultivars grown in India.

MATERIALS AND METHODS

Materials

Six wheat cultivars (cv.) (PBW-343, WH-1080, PBW-590, WH-283, WH-896 and WHD-943) were procured from Haryana Agricultural University, Hisar, Hayana. The grains of each variety were cleaned and stored for further evaluation.

Reagents

Standard gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferrozine, catechin, foline-ciocalteu's reagent, ascorbic acid, $ABTS^+$ were procured from Sigma Aldrich (Steinheim, Germany). All chemicals were of analytical grade. Each test was performed in triplicates on dry weight basis.

1000 kernel weight, bulk density and l/b ratio

Wheat grains were randomly selected and 1000 kernels were counted. The counted grains were then weighed and expressed in gms. For measuring the bulk density, wheat grains were gently filled in a 100 ml graduated cylinder, previously tared. The bottom of the cylinder was gently tapped on a laboratory bench, several times, until there was no further diminution of the sample level after filling to the 100 ml mark. Bulk density was calculated as weight of sample per unit volume of sample (g/ml). l/b ratio was determined with the help of vernier-calliper.

Milling and proximate composition

Debranning of wheat grains were carried out using a rice polisher (Khera, India). Wheat grains were placed in the polisher's chamber, which was run till the bran was completely removed. Two milling fractions obtained was bran and wheat grains without bran. These milling fractions were then ground. Flour obtained from the fraction grains with their bran removed was designated as refined wheat flour (RWF). Whole wheat flour (WWF) was prepared by the grinding the whole wheat.

Proximate composition

Wheat flour from different cultivars was tested for moisture, ash, fat, fibre and protein contents by employing the standards methods of analysis[10]. The carbohydrate content was calculated by difference. All the results were recorded on a dry weight basis (dwb).

Color characteristics

Color measurement of flours was carried out using a Hunter Colorimeter fitted with optical sensor (Hunter Associates Laboratory Inc. Restan VA., USA) on the basis of L^*, a^*, b^*colour system. The colour difference (ΔE) was calculated as:

$$\Delta E = \{ (\Delta L^{*}) + (\Delta a^{*}) + (\Delta b^{*}) \}^{1/2}$$

L^* value indicates the lightness, 0-100 representing dark to light. The a^* value gives the degree of the red-green color, with a higher positive a^* value indicating more red. The b^*value indicates the degree of yellow-blue color, with a higher b^* value indicating more yellow.

Total phenolic content (TPC)

The total phenolic content was determined by following the Folin–Ciocalteu method[11]. Wheat flour samples (200 mg) were extracted with 4 ml acidified methanol (HCl/methanol/ water, 1:80:10, v/v/v) at room temperature (25 °C) for 2 h using wrist action shaker (NarangScietific, Delhi, India).The mixture was centrifuged at 3000 rpm for 10 min on a centrifuge (Remi, Mumbai, India). The supernatant was used for determination of total phenolic content. Aliquot of extract (200 µl) was added to 1.5 ml freshly diluted (20-fold) Folin–Ciocalteu reagent. The mixture was allowed to equilibrate for 5 min and then mixed with 1.5 ml of sodium carbonate solution (60 g/l). After incubation at room temperature (25 °C) for 90 min, the absorbance of the mixture was read at 725 nm. Acidified methanol was used as a blank. The results were expressed as µg of gallic acid equivalents (GAE)/g of flour.

Antioxidant activity (AOA)

Antioxidant activity was measured by following a modified version of the method[12]. This involved the use of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution in the methanol. Ground wheat samples (100 mg) were extracted with 1 ml methanol for 2 h and centrifuged at 3000 rpm for 10 min. The supernatant (100 µl) was reacted with 3.9 ml of a 6 $x10^{-5}$mol/l of DPPH solution. Absorbance (A) at 515 nm was read at 0 and 30 min using a methanol as blank. Antioxidant activity was calculated as % discoloration.

% Antioxidant activity =(1 –(A of $sample_{t=30}$/A of $sample_{t=0}$))x100

Total flavonoid content (TFC)

The total flavonoids content was determined by following the method[13]. Wheat extract (250 µl) was diluted with 1.25 ml of distilled water. Sodium nitrite (75µl of 5% solution) was added and the mixture was allowed to stand for 5 min. Further, 150 µl of a 10% aluminium chloride was added and the mixture was allowed to stand for 5 min. After that, 0.5 ml of 1 M sodium hydroxide was added and the solution was mixed well. The absorbance was measured immediately at 510 nm using a spectrophotometer (Systronics, Ahmadabad). Catechin was used as standard and the results were reported as µg of catechin equivalents (CE)/g of flour.

Statistical analysis

The data reported in all the tables are an average of triplicate observations and were subjected to one way analysis of variance (ANOVA) using Minitab statistical software version 14 (Minitab Inc, USA).

Table 1

Physical parameters and proximate composition of wheat cultivars

Wheat cultivars	1000 kernel weight (g)	Bulk density (g/100ml)	l/b ratio	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Crude fiber (%)	Carbohydrate (%)
PBW-343	48.2^{b}	0.746^{a}	3.6^{ab}	8.91^{ab}	1.52^{a}	3.22^{ab}	9.67^{a}	0.89^{a}	75.67^{d}
WH-1080	46.5^{a}	0.788^{ab}	3.7^{ab}	9.03^{ab}	1.69^{b}	2.99^{a}	12.33^{bc}	0.86^{a}	72.88^{bc}
PBW-590	49.5^{c}	0.768^{ab}	3.7^{ab}	8.96^{ab}	1.66^{ab}	3.48^{b}	12.11^{b}	0.91^{a}	73.03^{c}
WH-283	51.2^{d}	0.781^{b}	3.8^{ab}	9.18^{ab}	1.69^{b}	3.34^{b}	12.25^{bc}	0.92^{a}	72.62^{b}
WH-896	47.9^{b}	0.767^{ab}	3.5^{a}	8.56^{a}	1.72^{b}	3.01^{a}	13.19^{d}	0.82^{a}	72.7^{b}
WHD-943	48.3^{b}	0.799^{b}	3.9^{b}	9.78^{b}	1.58^{ab}	3.15^{ab}	12.96^{c}	0.93^{a}	71.6^{a}

Means followed by the similar superscript within the column do not differ significantly (p<0.05)

Table 2

Hunter color characteristics of flours from different wheat cultivars

Wheat cultivars	L*	a*	b*	ΔE
PBW-343	71.23^{b}	1.62^{a}	9.31^{b}	74.56^{b}
WH-896	74.48^{c}	2.57^{d}	13.23^{d}	77.60^{c}
WH-1080	75.22^{d}	2.08^{b}	10.44^{c}	77.35^{c}
PBW-590	78.84^{e}	2.26^{c}	10.69^{c}	80.61^{e}
WH-283	55.92^{a}	1.55^{a}	8.49^{a}	60.40^{a}
WHD-943	75.84^{d}	2.62^{d}	13.30^{d}	78.81^{d}

Means followed by the similar superscript within the column do not differ significantly (p<0.05)

Table 3

Total phenolic content of different fractions of wheat cultivars

Wheat Cultivars	WWF (µg GAE/g)	RWF (µg GAE/g)	Bran (µg GAE/g)
PBW-343	974^{a}	178^{a}	1275^{a}
WH-896	1069^{b}	221^{b}	1385^{b}
WH-1080	1116^{c}	249^{c}	1597^{c}
PBW-590	1155^{d}	299^{d}	1626^{d}
WH-283	1290^{e}	428^{e}	1969^{e}
WHD-943	1399^{f}	436^{e}	2002^{f}

Means followed by the similar superscript within the column do not differ significantly (p<0.05)

Table 4
Antioxidant activity of different fractions of wheat cultivars

Wheat Cultivars	WWF (%)	RWF (%)	Bran (%)
PBW-343	13.2[a]	4.1[a]	25.8[a]
WH-896	16.8[c]	4.8[b]	29.4[c]
WH-1080	15.7[b]	4.7[b]	27.6[b]
PBW-590	19.2[d]	6.2[d]	33.9[d]
WH-283	21.6[f]	6.4[d]	36.4[e]
WHD-943	20.4[e]	5.8[c]	35.9[e]

Means followed by the similar superscript within the column do not differ significantly (p<0.05)

Table 5
Total flavonoids content of different fractions of wheat cultivars

Wheat Cultivars	WWF (µg CE/g)	RWF (µg CE/g)	Bran (µg CE/g)
PBW-343	98[cd]	44[d]	204[ab]
WH-896	87[b]	23[a]	212[b]
WH-1080	75[a]	34[c]	199[a]
PBW-590	91[c]	29[b]	256[c]
WH-283	102[d]	31[bc]	287[d]
WHD-943	86[b]	26[ab]	313[e]

Means followed by the similar superscript within the column do not differ significantly (p<0.05)

RESULTS AND DISCUSSION

Physical parameters and proximate composition of different wheat cultivars

Physical and proximate composition of different wheat cultivars are summarized in Table 1. 1000 kernel weight of different cultivars ranged from 46.5 to 51.2, the highest for cv.PBW-590 and the lowest for cv.WH-896 was observed. The bulk density of grains varied from 0.746 to 0.799 g/ml with cv.WHD-943 showing the highest value. l/b ratio of cultivars ranged from 3.5 to 3.9, cv.WHD-943 had the longest grains. Proximate composition varied significantly ($p<0.05$) among cultivars. The ash and crude fat content of different cultivars ranged between 1.52-1.72% and 2.99-3.48%, respectively. Cv.PBW-590 had the highest whereas cv.WH-1080 showed the lowest fat content among different cultivars. The moisture, fat and ash content of 9.93, 0.86 & 0.45%, respectively for wheat cultivars have been reported[14]. Protein content for wheat cultivars ranged between 9.67 to 13.19%. The highest protein content was observed for cv.WH-896 whereas the lowest value was for cv.PBW-343. The protein content of 14.89% for hard wheat flour and 11.24% for soft wheat flour has been reported[15]. The crude fiber and carbohydrate contents of cultivars varied from 0.82 to 0.93% and 71.6 to 75.67%, respectively. Cv.WH-896 had the lowest fiber and cv.PBW-343 had the highest carbohydrate contents, whereas cv.WHD-943 had the highest fiber and the lowest carbohydrate content.

Color characteristics flours from different wheat cultivars

Hunter color values (L*, a*, b* and ΔE) of flours from different wheat cultivars are shown in Table 2. Varietal differences were observed for various color parameters. The L* value indicates the lightness, 0–100 representing dark to light ranged from 55.92 to 78.84. The highest L* value of flour from cv.PBW-590 indicated its lighter color than other flours studied. The a* value, indicator of degree of the red-green color, ranged between 1.55 to 2.62 with flour from cv.WHD-943 having more red color than its counterparts. The b* value indicates the degree of yellow-blue color, with higher positive b* value indicating more yellow. The b* value for wheat flours ranged from 8.49 to 13.30, the lowest for cv.WH-283 and the highest for cv.WHD-943 flour. ΔE, indicator of total color difference, ranged from 60.40 to 80.61 for different flours. The differences in the color characteristics of wheat flours may be attributed to differences in colored pigments of the flours, which in turn depend on the biological origin of the plant. Hunter color parameters (L*, a* and b* values) of the wheat grains in the range from 56.4 to 63.8, 6.9 to 7.8 and 18.4 to 23.2, respectively have been reported[16].

Total phenolic content (TPC) of different fractions of wheat cultivars

Phenolic compounds are considered as a major group of compounds that contribute to the antioxidant activity

of cereals[17]. The results are expressed as µg of gallic acid equivalents per gram of dry mater. TPC of different milling fractions of wheat cultivars followed the following order: bran>whole wheat flour (WWF) > refined wheat flour (RWF) (Table 3). For bran, RWF and WWF fractions, TPC ranged between 1275 to 2002, 178 to 436 and 974 to1399 µg GAE/g, respectively. The highest TPC for all the fractions were observed for cv. WHD-943 whereas cv.PBW-343 had the lowest values. Most of the antioxidants in grains were located in their bran and germ fractions[18]. TPC of 176-195 µmol of gallic acid equiv/100 g of flour) for the endosperm from different wheat cultivars has been reported[19]. TPC value of 434 µg GAE/mg for Indian wheat cultivar has been reported[20].The bran layers of wheat have the highest content of total phenolics ranging from 1258 to 3157 µg GAE/g[21].

Antioxidant activity (AOA %) different fraction of wheat cultivars

The free-radical scavenging activity of the extracts of milling fractions was evaluated using the DPPH, as DPPH is a stable free radical (Table 4). The antioxidant activity (AOA) of milling fractions differed significantly ($p<0.05$). The DPPH radical scavenging capacity of WWF, RWF and bran from different cultivars ranged from 13.2 to 21.6, 4.1 to 6.4 and 25.8 to 36.4%, respectively. The bran fraction had significantly ($p<0.05$) higher AOA in comparison to WWF and RWF. AOA for different fractions were the highest and the lowest for cv.WH-283 and cv.PBW-343, respectively. The DPPH radical scavenging capacity of whole wheat and bran of soft wheat was 393 and 1309 µl/g has been reported[22]. Bran was significantly higher in antioxidant activity as compared to refined flour[4].

Total flavonoids content (TFC) of different fractions of wheat cultivars

Flavonoids are an important class of phytochemicals in wheat contributing to its health beneficial properties. They are the predominant class described in phenolic food content investigations, because they account for approximately two-thirds of the dietary phenols[22.] The total flavonoids content (TFC) differed significantly ($p<0.05$) among flours from different wheat cultivars (Table 5) and ranged from 75 to 102 and 23 to 44 µg CE/g for WWF and RWF, respectively. TFC in the range from 105 to 148 µmol CE/100 g in wheat varieties has been reported[24]. TFC of flours from bran fraction ranged from 199 to 313 µg CE/g, with cv.WHD-943 and cv.WH-1080 exhibited, the highest and the lowest values. The flavonoids content of bran/germ fractions in wheat varieties (740-940 µmol of catechin equivalent/100 g) was 10-15-fold higher ($p<0.01$) than refined flour fractions (60-80 µmol of catechin equivalent/100 g of flour)[19].

CONCLUSION

This study demonstrated the potential antioxidant properties of different milling fractions of wheat cultivars. The TPC, TFC and antioxidant properties differed significantly ($p<0.05$) among different milling fractions examined. Cv.WH-283 with the lowest hunter color L*, a* and b* values had the highest AOA and TFC values. Bran fraction from all cultivars studied had a higher TPC, TFC and AOA followed by WWF and the lowest was observed for RWF. Thus wheat, particularly wheat bran, may serve as an excellent source of raw material with potential application as nutraceuticals and functional food ingredients that may exert potential health benefits.

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