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BIOACTIVE COMPOUNDS, ANTIOXIDANT PROPERTIES AND ALPHA-GLUCOSIDASE INHIBITION OF DICOCCUM WHEAT AND ITS HULL

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Abstract: Dicoccum wheat species is gaining popularity amongst the consumers due to its suggested health benefits. As the reports on the bioactive components on dicoccum wheats are very scanty, especially on Indian dicoccum wheat species, we have determined antioxidant properties and alpha-glucosidase inhibition of dicoccum wheat and its hull, which is not explored for its bioactive compounds. In the present study, we found that protein content in dicoccum varieties, DDK-1029 and DDK-1025 was 12.4% and 13.6%, respectively. In these wheats, polyphenol content was 134 and 181 mg GAE/100g, while flavonoid content was 172 and 195 mg CE/100g. Polyphenol contents and antioxidant potentials were not significantly different in dicoccum and aestivum species. However, dicoccum flour extracts showed 2-fold higher inhibition of α-glucosidase activity. On the other hand, dicoccum wheat had low peroxidase activity, which is desirable for chapati and pasta making. Hull which is a by-product in dicoccum wheat was constituted about 20% of the dicoccum grain. Hull had the highest amount of antioxidant compounds such as polyphenols and flavonoids compared to aestivum and dicoccum wheat flours. Hull extract exhibited potential antioxidant properties and inhibited alpha-glucosidase activity, which indicates its ability to reduce postprandial glucose levels. As the glumes remain tightly closed over the grain, in dicoccum wheat, they cannot be removed by threshing. Hence, hull is removed mechanically before it is milled into flour. In this study, we provide value addition to the hull and for the first time its health benefits were reported.
Keywords: Dicoccum wheat, hull, aestivum wheat, bioactive compounds, antioxidant properties, peroxidase.

1. Introduction

Wheat is unique among the cereals because of its ability to form visco-elastic dough and a variety of products [1]. The visco-elastic property of dough is mainly due to the gluten proteins, viz., gliadins and glutenins [2]. *Triticum aestivum* (bread wheat) is the major wheat grown in the world followed by *Triticum durum* (Macaroni wheat) and *Triticum dicoccum* (emmer wheat). Although common wheat (*Triticum aestivum*) is the major species grown in the world, there has been growing interest in recent years in dicoccum due to it's suggested health benefits. Dicoccum is one of the ancient wheat species and advantage of this wheat is

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that it can grow in regions where common wheat cannot be grown favourably, and its resistance to rust disease and heat stress [3]. Semolina prepared from dicoccum wheat was reported to have better cooking quality compared to aestivum wheat and comparable cooking tolerance to durum wheat [4]. Bhuvaneswari et al., [5] studied the vermicelli and extrusion quality of eight dicoccum wheat varieties and reported that some of these varieties were good and some were poor.

However, reports on bioactive compounds from dicoccum wheat and their antioxidant properties are very limited [6, 7]. Hence, in the present study, we have determined the content of bioactive compounds, antioxidant potential of dicoccum wheat and compared to that of aestivum wheat flours. In addition, inhibition of α-glucosidase by dicoccum extracts, which indicates its potential to alleviate postprandial glucose levels and phenol oxidase activities in wheat, extracts which causes undesirable dough browning were also determined. In hulled wheat like dicoccum, glumes remain tightly closed over the grain, hence, hull cannot be removed by threshing. Thus, hull is an additional by-product during processing of dicoccum into kernel. Therefore, bioactive compounds, antioxidant properties and inhibition of α-glucosidase by dicoccum hull extract were also determined. In order to have comparison, we have also taken two aestivum (common) wheat varieties.

2. Materials and Methods

2.1. Materials

Dicoccum wheat varieties were procured from Agricultural College Seed Centre, University of Agricultural Sciences, Dharwad, Karnataka, India. The hull (Figure 1) was manually removed from DDK-1029 seed and its yield was 20g from 100g of seed.

Figure 1. Hull of dicoccum wheat

2.2. Determination of protein content

The total protein content in the wheat samples was determined by micro-Kjeldhal method [8].

2.3. Determination of total polyphenol content (TPC) and total flavonoid content (TFC)

Sample (1 g) was finely powdered using mortar and pestle and transferred into a conical flask, then added 20 ml of aqueous solvents (80% acetone or 80% methanol) and was kept on a magnetic stirrer for 2 h. The solution was centrifuged at 8000xg for 10 min at 4°C. Supernatant was collected and the residue was re-extracted with 10 ml of aqueous solvents using same procedure. Both the supernatants were pooled and stored at 4°C for further analysis.

The total polyphenol contents in the extracts were estimated using the method described by Chun et al., [9]. The sample containing 200 µl of extract was made up to 3 ml with distilled water, and added 250 µl of Folin-Ciocalteu reagent and 750 µl of Na_2CO_3 solution (7%). The solution was vortexed and incubated for 8 min at room temperature. Then, the solutions were incubated for 2 h in dark at room temperature. Absorbance was taken at 765 nm. The total polyphenol content in the extract was expressed as gallic acid equivalents (GAE).

The total flavonoid content in acetone and methanol extracts of different wheat samples were estimated using the method described by Zou et al., [10]. A 100 µl of extract was made up to 1.25 ml with distilled water and to it, 75 µl of 5% $NaNO_2$ and 150 µl of 10% $AlCl_3$ solutions were added. Then, the solution was mixed and incubated for 5 min at room temperature. To this, 0.5 ml of distilled water was added. The resulting solution was incubated for 2 h in dark at room temperature. Finally, the solution was made up to 2.5 ml with distilled water and immediately absorbance was taken at 510 nm. Catechin was used as a standard. The total flavonoid content in the acetone and methanol extracts of different wheat varieties were expressed as catechin equivalents (CE).

2.4. Determination of anti-oxidant property

The DPPH radical scavenging activity of the extracts of wheat samples were determined according to the method described by Ajila et al., [11]. To 200 µl (4µg) of extract,1 ml of 100 µM DPPH (in methanol) solution was added, the mixture was shaken vigorously and left in dark at room temperature for 20 min. The absorbance of the resulting solution was measured at 517 nm. The capacity to scavenge DPPH radical was calculated by using the equation given by Ajila et al., [11].

The reducing power of the extracts of different wheat samples were determined according to the method described by Yen and Chen [12]. The extracts corresponding to 4µg GAE were made up to 500 µl with 0.2 M phosphate buffer (pH 6.6) and mixed with 1 ml of potassium ferricyanide (0.1%) and the mixture was incubated at 50°C for 20 min. Trichloroacetic acid (TCA) (500 µl, 10%) was added to the reaction mixture and centrifuged at 8,000xg for 10

min. The supernatant obtained was mixed with equal volume of distilled water and 300 µl of 1% ferric chloride was added and the absorbance was measured at 700 nm.

2.5. α-Glucosidase inhibition assay

α-Glucosidase inhibition in the extracts of different wheat samples was determined according to the method described by Girish et al., [13]. Methanol extract (2 µg) samples were mixed with 50 µl *p*-nitrophenyl-α-D-glucopyranoside (PNPG). This volume was made up to 2.8 ml with 50 mM sodium phosphate buffer (pH 6.8) and the reaction was initiated by adding 20 µl of α-glucosidase enzyme. Control was taken without the extract. The reaction was monitored by the increase in absorbance at 405 nm.

2.6. Isolation and quantification of peroxidase and polyphenol oxidase activities

Sample (1g) was ground to fine powder using acid washed sand in a mortar and pestle and homogenized into solution after addition of 10 ml of sodium phosphate buffer (50 mM, pH 7.0) and centrifuged at 8000xg for 10 min at room temperature. The supernatant was collected and stored at 4°C for further analysis. Peroxidase activity in phosphate buffer extracts was determined using H_2O_2 and *o*-dianisidine as substrates and polyphenol oxidase activity was determined using catechol as substrate, as described by John et al., [14].

2.7. Data analysis

All experiments were carried out in triplicates and results are expressed as mean ± SD. GraphPad Prism Software 5 was used for analysis.

3. Results and discussion

3.1. Isolation and characterization of proteins in flours of different wheat varieties

There is a renewed interest on consumption of dicoccum wheat as they are suggested to be rich in bioactives and also due to their organoleptic qualities. However, the studies on bioactive compounds and proposed health benefits are very limited. Therefore, nowadays interest in analysis of bioactive compounds in ancient wheat is growing in different parts of the globe. In India, production of dicoccum wheat is around 10 million tons [15].

The protein content in dicoccum wheats viz., DDK-1029 and DDK-1025, were found to be 13.6% and 12.4%, respectively while in aestivum wheats, DWR-162 and Lok-1, it was 13.7% and 11.5%, respectively (Table 1). Earlier, Bhuvaneswari et al., [6] reported protein content in different dicoccum wheats and it was found to range between 11.84% and 15.26%. The values found for dicoccum wheat in the present study also falls within the range reported by them. Earlier, Srivastava et al., [16] reported the protein content in Lok-1 and DWR-162 as 12.4% and 13.3%, respectively. The values reported in the present study are slightly

different from the earlier reported values. These little variations in protein contents could be attributed to changes in agro-climatic conditions like soil, temperature and rain fall, among others. However, the hull has very low protein content (Table 1).

Table 1. Protein content in different wheat varieties and hull of DDK-1029

Wheat samples	Total protein content (%)
Dicoccum varieties	
DDK-1029 hull	3.36 ± 0.08^{a}
DDK-1029	13.62 ± 0.28^{d}
DDK-1025	12.36 ± 0.27^{c}
Aestivum varieties	
DWR-162	13.73 ± 0.28^{d}
Lok-1	11.45 ± 0.17^{b}

Data expressed as mean±SD; Different superscript letters within the same column are significantly different ($P< 0.05$)

3.2. Phytochemical content in flours of different wheat species and dicoccum hull

Interest in phenolic compounds is increasing as they exhibit antioxidant properties, which are involved in ameliorating different diseases. Between the two solvents used for the extraction, aqueous methanol (80%) extracted higher quantities of polyphenols (1.2 to 3.6 fold higher) compared to aqueous acetone (80%). Flavonoid content was also found to be higher in methanol extracts (data on acetone extracts is not shown). The differences in values found between these solvents for polyphenols and flavonoids may be due to differences in solvent polarity. Thus, the values obtained with methanol extraction were only considered for further discussion. DDK-1029 and DDK-1025 had 180mg and 133mg content of total polyphenols respectively and these values are comparable to aestivum wheats. Flavonoid content was varied from 172 to 241 mg CE/100g in these varieties (Table 2). However, the flavonoid content was found to be higher in aestivum wheats compared to dicoccum wheats. No reports are available with respect to flavonoid contents in dicoccum wheats. On the other hand, dicoccum hull contained more amounts of polyphenols as well as flavonoids compared to dicoccum as well as aestivum whole wheat flours (Table 2).

Table 2. Phytochemical content in different wheat varieties and hull of DDK-1029

Wheat samples	Total phenolic content (mg GAE/100g)	Total flavonoid content (mg CE/100g)
DDK-1029 hull	348.00 ± 8.96^{g}	257.33 ± 14.04^{f}
DDK-1029	180.52 ± 8.30^{e}	194.96 ± 11.77^{c}
DDK-1025	133.61 ± 5.32^{d}	171.85 ± 3.40^{b}
DWR-162	175.15 ± 3.85^{e}	210.51 ± 8.98^{d}
Lok-1	144.28 ± 5.07^{d}	241.11 ± 15.75^{e}

Data expressed as mean±SD; Different superscript letters within the same column are significantly different (P< 0.05)

3.3. Antioxidant properties in flours of different wheat species and dicoccum hull

DPPH radical scavenging activity was comparable in both dicoccum and aestivum wheats species (Figure 2A). On the other hand, reducing power capacity was comparable in all the varieties studied except DWR-162 (Aestivum sp.), which showed lower value (Figure 2B). Hull extract also showed good antioxidant property which is comparable with dicoccum flour extracts.

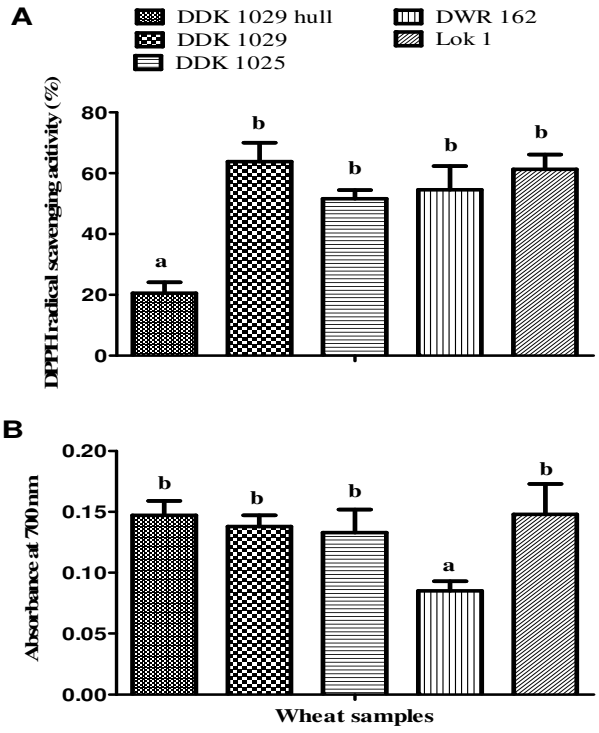

Figure 2. Anti-oxidant properties of different wheat varieties and hull of DDK-1029 A) DPPH radical scavenging activity; B) Reducing power capacity

3.4. α-Glucosidase inhibitory activity of flours in different extracts of wheat samples

α-Glucosidase is involved in the hydrolysis of starch and release of glucose. Inhibition of this enzyme activity decreases postprandial glucose levels observed in Type-2 diabetic patients

[17]. α-Glucosidase inhibitors from plant sources are an attractive strategy to manage postprandial hyperglycemia. Extracts of dicoccum flours as well as hull showed higher alpha-glucosidase inhibition compared to aestivum wheats. At 2µg concentration, dicoccum extracts showed around 40% and hull extract showed 35% inhibition, while aestivum wheats showed less than 20% inhibition α-glucosidase activity (Figure 3). In the present study, dicoccum wheat flours and hulls showed significantly higher α-glucosidase inhibition, although the quantities of polyphenols and flavonoids were not significantly different in these two species. These changes may be due to the differences in individual phenolic and flavonoid composition, which needs to be further, investigated. Earlier, studies indicated that dicoccum wheat was reported to have low glycemic index and its starch has slow digestibility [5, 18]. Thus, dicoccum wheat based products can be used for the management of Type-2 diabetes.

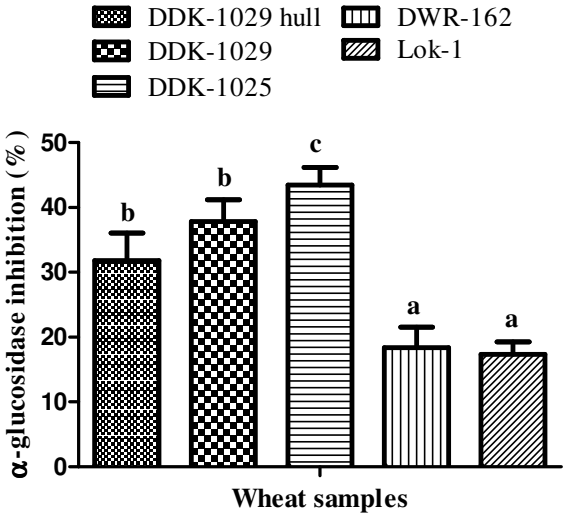

Figure 3. α- Glucosidase inhibition activity in different wheat varieties and DDK-1029 hull

3.5. Peroxidase and polyphenol oxidase activity of flours in different wheat varieties

Dicoccum varieties contain around 100 U/g of peroxidase activity, while in aestivum wheats it was 130-170 U/g flour (Table 3). Hull contained negligible activity of these enzymes. Earlier, Hemalatha et al., [19] reported the POD activities for different aestivum varieties grown in a single location and the POD activities varied from 981 U/g to 5803 U/g flour. The varieties used in their study were grown at a single location, but POD activities showed a 6-fold variation indicating that the enzyme activities are varietal dependent. The varieties used in the present study were different from those used by the earlier authors and were grown in a

different location. Changes in the enzyme activities may be due to agro-climatic conditions. It is to be noted that dicoccum wheat varieties had lower POD activity compared to aestivum wheat varieties. Aestivum wheat bran and soy hull were reported to have higher activity, while dicoccum wheat hull had lesser activity compared to the above two sources. Thus, hull characteristics are different from that of wheat bran [20, 21].

The PPO activity in all the wheat flour extracts ranged from 3 to 5U and the hull extract had 3U (Table 3). Earlier, Hemalatha et al., [19] reported the PPO activity in wheat flours (aestivum) of different varieties and the values reported in the present study are about 10 fold lower than the reported values. As mentioned earlier, the low enzyme values may be due to the differences in the soil, environmental conditions and varietal differences. In contrast to POD activity, the PPO activities were not significantly different between dicoccum and aestivum wheat as well as with hull.

Table 3. Peroxidase (POD) and polyphenol oxidase (PPO) activities of different wheat varieties and hull of DDK-1029

Wheat samples	POD activity (U/g)	PPO activity (U/g)
DDK-1029 hull	1.46 ± 0.25^{a}	2.93 ± 0.4^{a}
DDK-1029	98.64 ± 4.9^{b}	3.73 ± 0.4^{a}
DDK-1025	93.46 ± 2.8^{b}	3.73 ± 0.2^{a}
DWR-162	133.06 ± 2.0^{c}	3.20 ± 0.21^{a}
Lok-1	169.46 ± 1.3^{d}	5.33 ± 0.12^{a}

Data expressed as mean±SD; Different superscript letters within the same column are significantly different ($P< 0.05$)

Peroxidase and polyphenol oxidase exhibit both beneficial as well as deleterious effects in the food industry. Browning in foods like chapati, vegetables like potatoes and fruits like apple, these enzyme activities are not desirable. Having low enzyme activities is another positive point in dicoccum wheats compared to that of aestivum. Thus, dicoccum wheats had significantly lower peroxidase as well as polyphenol oxidase activities. It should be noted that dicoccum wheats are tetraploid variety devoid of D-genome, which encodes for 5+10 or 2+12 subunits and therefore, dicoccum wheat may not be suitable for good bread preparation like hard aestivum wheats. On the other hand, dicoccum wheats may be more suitable for chapati or pasta products as dicoccum is devoid of 5+10 subunits. It has been reported that varieties having high activity of peroxidase yield dough browning, which may yield dark chapati or undesirable coloured pasta products [19, 22].

4. Conclusion

In conclusion, dicoccum wheats exhibited antioxidant properties and potential alpha-glucosidase inhibition. A low peroxidase and polyphenol oxidase activity in dicoccum wheats is a positive indicator for the colour of wheat-based products like chapati and pasta products. Dicoccum wheat hull extract exhibited potential alpha-glucosidase inhibition and antioxidant properties. Thus, the present study indicates that dicoccum wheats exhibit health benefits like control of glycemic index, which may be due to the following properties: i) it's slow digestibility of starch and ii) ability to inhibit alpha-glucosidase activity. Hull being a by-product, can be used as a source of bioactive compounds or it can be ground and its powder can be used in food products. The present study gives further scope for research on identification of flavonoids and polyphenols in hull as well as with dicoccum wheats.

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