IMPACT OF PARTICLE SIZE REDUCTION AND HYDROTHERMAL TREATMENTS ON THE DIETARY FIBER, FUNCTIONAL AND ANTIOXIDANTS PROPERTIES OF WHEAT BRAN

Effect of Hydrothermal Treatments on Wheat Bran

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Abstract: Milling of wheat generates by products such as which can be used to improve the technological performance with healthy compounds. The aim of this study was to elucidate the impacts of reducing of particle sizes and hydrothermal treatment on different cultivars of wheat bran. All samples were investigated for functional properties, antioxidants properties and dietary fiber content. Hydrothermal treatment (wet steeping in acetate buffer at pH 4.8 at 55°C for 60 min) has given for the enhancement of dietary fiber and antioxidants properties of different sieve size particles of wheat bran particle size along with hydrothermal treatment dietary fiber was increased with increasing of particle size (PBW-154- 24.9% to 60.5%, HD2697-31.7% to 51.4%) and antioxidants and functional properties are also increases with increasing of particle size. Particle size reduction decreased swelling capacity, water holding capacity, and oil holding capacity, while bulk density was decreases. Wheat bran has the highest value of total phenolic content, because it is well know that the phenolic compounds are concentrated in the bran and germ fraction of wheat that are removed during the milling of wheat into white flour.

Index terms- hydrothermal treatment, wheat bran, oil holding capacity, swelling capacity, water holding capacity.

1. INTRODUCTION

Its botanical name is Triticum estivum. Wheat is a grass and it's also cultivated for its seeds and animal foods (straw). Now a day's China is a top one producer of wheat in the whole worlds and India has top two positions in cultivation of wheat. In 2016, world production of wheat was 749 million tons (FAO, 2016). Wheat grain comprises the tree distinct part- bran, germ and endosperm, but when its refined, its loss many valuable nutrients like dietary, antioxidants and other nutrients, which are intact with outer layer of wheat, which are removed from the wheat flour and are discarded or used as a cattle food. Outer layer of wheat are contain beneficial nutrients, which plays important role in our body. Wheat bran considered as a rich sources of dietary fiber with antioxidants potential, which may help to decrease the incidence of noncommunicable disease like- diabetes, hypertension, cardiovascular diseases, cancer etc. It also has the antinutritional properties (phytate, oxalic acid, tannic acid etc.), which are chalets essential multivalent metal and makes them unavailable. Antioxidants properties of wheat bran may help to defense against oxidative stress. Chemically oxidative stress is associated with increased production of oxidizing species or significant decreases in the effectiveness of antioxidants defenses. Antioxidants are mainly two types – endogenous antioxidants ant exogenous antioxidants. Endogenous oxidants are enzymes such as superoxide dismutase, glutathione peroxides and non enzymatic compounds, such as uric acid, bilirubin, albumin, and metallothioneins (Kalcher et al. (2004). Exogenous antioxidants are derived from natural sources (vitamin, flavonoids, anthocynine, flavine, p-qumaric acid, ferrulic acid etc.), but can also be synthetic compounds like butylhyroxyanisole, butylhydroxytoluene, gallates, etc. (Litescu et al. 2011). Efficient reduction of particle size may help to releases valuable components such as antioxidants from the wheat bran matrix and thereby enhancing the nutritional properties, but can also have negative effects, as reduction of particle size results reduce the functional and rheological properties of the developed processed food for example dough making properties, appearance, texture, taste etc.

2. MATERIALS AND METHODS

PBW-154 and HD-2967 variety were procured from local market of Allahabad. Both cultivars of wheat were used to prepare bran samples for which they were passed through different sieve sizes (710, 510, 300, 150 and 53µm.). Sieved bran particle were performed by hydrothermal treatment according to Moharraf et al. (2009) at optimum pH. and temperature.

2.1. **COLOR VALUE**

Color value of the wheat bran was measured using Xrite (Grandville, MI, USA). The color attributes i.e. Hunter lightness (L*), redness (a*), and yellowness (b*) were recorded 3 times for each particle size of wheat bran (n=3) according to chen et al. (1997).

2.2. **FUNCTIONAL PROPERTIES**

2.2.1. BULK DENSITY

Bulk density was determined according to the method given by Chau & Huang (2003) using a graduated cylinder (10 ml) previously weighed and filled with sample to 10 ml by constant dapping until there is no functional change in volume and the contents is weighed. The content was weighed and the blank density of sample was calculated as given per milliliter.

2.2.2. OIL HOLDING CAPACITY (OHC)

OHC was measured according to the method of Chau and Huang (2003) with slight modification one gram of sample was mixed with vegetable oil (1: 10). The mixture was stirred for 30 min at room

temperature. Then all samples were centrifuged (2500 gm, 30 min) and the supernatant were transferred to measuring cylinder of 10 ml and volume was measured. The OHC was expressed as milliliter of vegetable oil hold per gram of sample.

2.2.3. WATER HOLDING CAPACITY (WHC)

The water absorption capacity was determined essentially according to the method of Chau and Huang (2003) one gram of flour sample was mixed with 10 ml of distilled water in a centrifuge tube for 1 min in a vortex and then centrifuged at 3000-5000 rpm for 30-45 min. After separation of the content the volume of supernatant was recorded and used for determination of water absorption. The results are expressed as ml/gm of sample.

2.2.4. SWELLING CAPACITY

The Swelling was determined according the method of Robertson et al. (2001). Flour sample of 0.2g of dry sample were added in calibrated measuring cylinder and makeup the volume up to the mark with distilled water and it placed in incubator at room temp for 18 hrs. Then the initial and final bed volume was recorded and swelling capacity was calculated as ml per gram of dry sample.

SC = V2 - V1/M * 100

2.3. TOTAL DIETARY FIBRE

This assay determines the total dietary fiber content of food using a combination of enzymatic and gravimetric methods (AACC, 2000). Dried and fat-free samples were gelatinized with heat stable α -amylase and then enzymatically digested with proteases and amyloglucosidases. Ethanol was used to precipitate the soluble dietary fiber. The residue was then filtered and washed with ethanol and acetone. After drying the residue was weighed. Total dietary fiber was calculated as weight of the residue minus the weight of the protein and ash in the sample.

2.4. ANTIOXIDANTS PROPERTIES

For the determination of wheat bran antioxidants properties the cultivars of different particle size treated with methanol. For preparation of methanolic extracts one gram of wheat bran was extracted with 5 ml of methanol (50%) in a screw-capped tube in the dark condition at room temperature for 24 hrs. The tubes were centrifuged at 2000 rpm for 5 min. The supernatant was collected and kept in refrigerator for further analysis (Moore et al. 2006). The total phenolic content of the samples was determined by the Folin-Ciocalteu method (Matthaus 2002). Free radical scavenging activity of various extracts was measured from the bleaching of purple colored methanol solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) by the method of Brand-Williams et al.(1995), total flavonoid content was determined by Aluminum chloride method, described by Boetang et al. (2008).

2.5. SCANNING ELECTRON MICROSCOPY (SEM)

Scanning Electron Microscopy of both cultivars of wheat bran with different particle sizes were analyzed by Electron Probe Micro Analyzer (EPMA) according to the method described by Prabhasankar et al. (2003). Jeol J x A 8100, Condition: Å 10.0KVolt, current: 1x10⁻¹⁰Amp. The samples were coated with 20 nm thick carbon layer to make the surface conducting before taking SEM images. The coating was done using Jeol JEE-420 vacuum evaporator.

3. RESULTS AND DISCUSSION

All samples were sieved to determine particle size distribution. (53, 150, 300, 500 and 710 um). Sieving determine in all particle size, 710µm was greater than 53µm particle size of both cultivars of wheat bran. There is two cultivars of wheat were analyzed for their physical properties, color value, dietary fiber and antioxidants properties.

3.1. **COLOR VALUE**

According to the results L, a and b value (lab) decreasing of bran particle size increased the lightness (control-(L- value), while reduced the redness (a-value), and blueness (b-value) of the samples. The inner layers of the bran, which are closer, to the endosperms are generally into smaller particle sizes during milling. These layers contain more starchy materials and hence are lighter in color than the outer layers. The L-value of the control (+36.6 to +56.93) was higher than hydrothermal treated samples of both cultivars of wheat bran (21.94 to 46.31). Hydrothermal treatment were reduced the a-value (1.8 to 5.52) of the samples with particle sizes greater than 53µm compare to contract. The b value of the control (10.94 to 15.68) and hydrothermal treated wheat bran (8.2 to 18.6) were the increased with reducing of particles sizes, because the reduction of particle size increased the germ part, which are starchy and pretenses. That are reduced the lignin and increased the blueness and the redness of the sample. Majzoobi et al. (2014) also reported similar kinds of results. They studied the color value of different particle size and hydrothermal treated bran samples and found that L-values were increased with reduction of particles size, while +a and +b values were decreased with reduction of particle size.

3.2. **Functional Properties**

Interactions of the bran molecules with water described by SC and WHC of the both cultivars of wheat bran are presented in table 3. The present study showed that by decreasing the particle size of bran with hydrothermal treatments, SC (5.8 to 2.2 ml/g) and WHC (5.8 to 1.6 ml/g) were decreased, while bulk density 6.88 to 10.8 g/ml) were also increased, because the porous matrix structure of the insoluble fiber chain can hold large amounts of water through hydrogen bonds (Kethireddipalli et al. 2002). These parameters are related to the amount of hydrocolloids (e.g. cellulose, pentosans and proteins) present in the bran. Noort et al. (2010) reported that the particle size reduction of bran from 1,000 to 75µm increased bulk density, whereas it reduced the WHC, OHC and SC that are similar to our study. According to results of present study, as explained in table 3, bran with smaller particle sizes were found to be less effective in holding water and have lower SC in aqueous media. As a consequence, they may be less effective in promoting rapid transit of digestion through the gut.

3.3. **DIETARY FIBER CONTENT**

Dietary fiber from wheat bran mainly contains cellulose, lignin and hemicellulose, of which the major portion consists of insoluble fiber. Present study showed the fine particle sizes were obtained from inner layers of the bran fraction, which contain less fiber, then the outer layers of wheat. These results showed that total dietary fiber (PBW154-24.9 to 60.5 % and HD-2967-31.7 to 51.4 %), insoluble fiber (PBW-154- 20.3 to 52.1 % & HD-2967-27.2 to 42.7) and soluble fiber content (PBW-154- 8.4 to 4.6 % & HD-2967- 4.5 to 8.7 %) of the bran were increased with the larger particle size in comparison to smaller particle size (710 to 53 µm.). Previous study showed that the reducing the particle size from 710 to 50µm there was about 60.5% reduction in Total, 8.4% soluble and 52.1% in insoluble fiber content of the bran (Majoobi et al. 2014). Insoluble dietary fibers promote human health by supporting the growth of the intestinal microflora, increase the fecal bulk and decrease the intestinal transit (Gomez et al. 2011). Recently the importance of consuming dietary fiber has increased owing to its relation with the reduction of blood cholesterol level, lower inulin demand and improved laxative properties (Gomez et al. 2013). The recommended daily intake of total dietary fiber is ranges from 30 to 38g/day for male and 21to 26g/day for female (Gomez et al. 2013).

3.4. ANTIOXIDANTS PROPERTIES

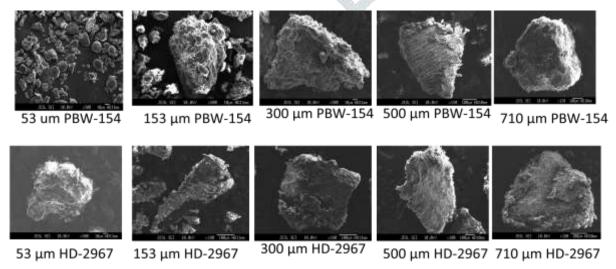
Results of antioxidants compounds and their properties were depicted in table 4. Total phenolic might be the major contributor of wheat antioxidant properties. The highest concentration of total phenolic compound was observed in hydrothermal treated wheat bran (121 to 165 mg GAE/100gm in PBW-154 and 83.7 to 123 mg GAE/100gm in HD-2967) with increased particle size compared to untreated particle size (65.17 to 89.19 mg GAE/100gm in PBW-154 and 39.28 to 85.62 mg GAE/100gm in HD-2967) of both cultivars of wheat bran. In present study, the total phenolic content was similar to previously published results (Liyana- Pathirana & Shahidi 2006).

Total flavonoid content was measured by aluminum chloride method. The highest concentrations of total flavonoid contents (0.083 to 0.754 (mg QE/g) in PBW-154 and 0.07 to 0.68 (mg QE/g) in HD-2967) were observed in hydrothermal treated sample with increasing of particle size (53µm to 710µm.). The results of flavonoid content for different particle size were inconsistent with the previous studies on wheat bran samples (Feng & McDonald, 1989). Similar kinds of study were also determined by Adom et al. (2005) on the increasing flavonoid content of bran with increasing particle size. Flavonoid has been shown to exhibit potent antioxidant and anticancer activity and is a source of antioxidants in the diet (Ou et al. 2002).

The DPPH assay measures single electron through to determine the antioxidants reducing capacity and is highly reproducible (Herald et al. 2012). A freshly prepared DPPH solution was shown in dark blue or purple colour but it was changed when reacts the DPPH solution with alcoholic extracts of wheat bran and it reduces the color from dark blue to light blue as per their concentration of antioxidants in wheat bran at 515 nm by spectrophotometer. Free radicals scavenging activity of reduction particle size and hydrothermal treated bran was shown on table 4. That was increased in hydrothermally treated bran (46.82% to 68.7% in PBW-154, and 41.8% to 71.5% in HD-2967) with increasing particle size (53 μm to 710 µm) compared to untreated bran (34.3% to 65.9% in PBW-154 and 31.5% to 67.5% in HD-2967), because antioxidant compared was scavenged the free radicals that was obtained from outer layer of wheat. Previous study determined the free radicals scavenge activity in wheat bran that was similar to our study (Brower et al. 2014). Similar to our (Zhou et al. 2004) study the free radical scavenging activity of seven cultivars of wheat bran from different countries and found that at 50% acetone extracts have higher value antiradical activity.

Ferrous and cupric ions are the most effective pro-oxidants in food systems (Yamaguchi et al. 1988) and ferrous ions are commonly found in food systems, high chelating activity of investigated extracts would be beneficial in retarding metal catalyzed oxidation (Kehrer, 2000). Ferrous ion chelating activity was also affected by hydrothermal treatment and reduction of particles size. Ferrous ion chelating activity of wheat bran was presented in table 4. Highest ferrous ion chelating activity was obtained in hydrothermal treated bran (39.8% to 65.8% in PBW-154 and 37.9% to 64.5% in HD-2967) with increasing particle size comparison in to untreated bran (33.6% to 56.1% HD-2967) with increasing of particle size.

3.5. Scanning Electron Microscopy (SEM)



This figure shows the SEM micrographs of two cultivars of wheat bran with different particle sizes.

A comparison of images obtained by scanning electron microscope of both cultivars of wheat bran at different fractions. The micrograph observation of cultivars of wheat bran PBW-145 has the smooth surface comparison HD-2967 cultivars of wheat bran. That was similar to that of earlier studies of Aranyi & Hawrylewics (1968, 1969) and Rojas et al. (2000). The micrograph of coarse bran fractions (300 µm to 710 μm) shows the higher aggregates of starch and protein matrix than single starch granules. The micrograph of fine flour fractions (<53and 53–300 μm) showed higher numbers of loosened single starch granules than aggregates of starch and protein matrix as compared to the coarse bran fractions.

4. CONCLUSION

The results of this study showed that hydrothermal treatment with reduction of particle size of both cultivars of wheat have significant effects of these treatments on the functional and nutritional properties. Decreasing bran particle size along with hydrothermal treatment have positive effect on lightness, while WHC, SP, and OHC had negative effect on color value 'L' (lightness) while negative effect on WHC, SC and OHC. Particle size reduction had negative effect on soluble fiber, insoluble fiber content and antioxidants properties also. Applying hydrothermal treatments increased total and soluble fiber contents, while reduced insoluble fiber content. The highest amount of total and soluble fiber content obtained for the hydrothermal bran (24.9-60.5% and 4.6-8.4% respectively) compared to control sample. Further studies are needed to evaluate this in specific food products where claims could be affected by changes in the particle size of wheat bran.

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Table 1. Effect of size reduction and hydrothermal treatment on color characteristics of wheat bran (n=3) PBW-154 and HD-2967

Average Particle sizes	PBW-154		HD-2967	
of the bran (µm.)				
	Control	Hydrothermaled	Control	Hydrothermaled
L-Value				
710	36.60± 2.75 ^b	21.94± 1.40°	41.06± 1.05 ^b	26.76± 1.35 ^a
500	38.16± 3.42 ^a	23.99± 1.23 ^a	49.25± 2.81 ^a	33.51± 1.42 ^b
300	41.54± 1.45 ^d	28.88± 2.31 ^b	53.13± 2.32 ^d	36.59± 1.20°
150	49.78± 4.26 ^b	40.02± 1.6 ^e	55.97± 3.37°	37.47± 2.09 ^d
053	52.59± 4.31°	46.06± 3.14 ^d	56.93± 3.25°	46.31±2.56 ^e
a-value		. 44	DA.	
710	$+3.98\pm0.5^{\circ}$	$+5.51\pm0.4^{\circ}$	$+3.6\pm0.6^{d}$	+ 5.52± 0.5 ^d
500	$+3.85\pm0.26^{d}$	+4.38± 0.1 ^b	$+3.34\pm0.5^{\circ}$	+ 4.94± 0.7°
300	$+3.17\pm0.80^{d}$	+3.71± 0.1a	$+3.08\pm0.4^{e}$	+3.58± 0.1a
150	$+2.81\pm0.30^{b}$	+2.98± 0.3 ^e	$+2.75\pm0.17^{b}$	+ 3.30± 0.3 ^b
053	+ 2.02± 0.10 ^a	+1.8± 0.3 ^d	$+2.12\pm0.3^{a}$	+ 2.66± 0.2 ^e
b-value				
710	$+ 12.4 \pm 0.96^{b}$	$+18.6\pm0.10^{c}$	+ 15.6± 1.25 ^d	+ 16.60± 1.7 ^b
500	+ 12.5± 1.89 ^b	+13.1± 0.3 ^b	+ 14.2± 0.9°	+ 16.89± 2.19°
300	+ 11.6± 0.32°	+10.2± 0.10 ^a	+ 14.0± 0.52 ^e	+ 11.08± 1.05 ^a
150	+ 11.23±0.65 ^b	+9.0± 0.58 ^d	$+ 13.9 \pm 2.86^{b}$	+ 10.84± 1.08 ^d
053	+ 10.94±0.7 ^a	+8.2± 0.5°	+ 12.8± 1.78 ^a	+ 9.9± 0.93e

All data are to mean ±SD of three replicates. For each value means within a row not sharing a common letter differ significantly (p<0.05).

Table 2. Effect of size reduction and hydrothermal treatments on functional properties of wheat bran (n=3) PBW-154 and HD-2967

Average	PBW-154		HD-2967	
Particle				
sizes (µm.)	Control	Hydrothermaled	Control	Hydrothermaled
Bulk Density	y (g/ml)			
710	6.43 ± 0.16^{d}	7.45±0.10 ^a	8.17± 0.02°	6.88 ± 0.60^{a}
500	7.25 ± 0.10^{c}	8.88±0.16 ^b	8.03± 0.01°	7.45 ± 0.10^{a}
300	9.91± 0.10 ^b	9.44±0.13°	7.58± 0.04 ^b	8.43± 0.20 ^b
150	9.29± 0.13 ^a	10.6±0.20 ^d	6.45± 0.13 ^{Ab}	8.50± 0.20 ^b
053	10.15± 0.7 ^a	10.8±0.10 ^d	5.55 ± 0.05^{a}	9.92± 0.51°
Water Holdin	ng Capacity (ml/g)	Le Da	1381	
710	6.3± 0.15 ^d	5.8±0.20e	2± 0.10°	3.0± 0.30 ^d
500	5.6± 0.10 ^{Cd}	5.1± 0.20 ^d	1.2± 0.43 ^b	2.7 ± 0.40^{c}
300	4.2± 0.10 ^b	3.4± 0.20°	1.6 ± 0.10^{b}	2.2± 0.40 ^b
150	2.9± 0.10 ^{Bc}	2.4± 0.20 ^b	1.4± 0.10 ^b	1.8± 0.20 ^a
053	2.2± 0.15 ^a	1.8± 0.20 ^a	0.8± 0.20 ^a	1.6± 0.20 ^a
Oil Holding (Capacity (ml/g)			l
710	1.8± 0.20 ^b	1.4± 0.20 ^d	2.8 ± 0.10^{a}	1.8± 0.20°
500	1.7± 0.10 ^b	1.9 ± 0.20^{c}	2.4 ± 0.05^{a}	1.6± 0.20°
300	1.6± 0.15 ^b	1.8± 0.20 ^b	1.8± 0.10 ^b	1.2± 0.20 ^b
150	1.4± 0.10 ^b	1.5± 0.30 ^b	1.6 ± 0.15^{a}	1.0± 0.20 ^a
053	0.8± 0.20 ^a	1.0±0.20 ^a	0.8± 0.25 ^b	0.8± 0.20 ^a
Swelling Cap	pacity ml/g)			

710	6.1± 0.47 ^e	5.8± 0.30 ^d	4.1± 0.15 ^d	4.0± 0.20°
500	4.9 ± 0.10^{c}	4.5 ± 0.30^{c}	4.0± 0.16 ^d	3.5 ± 0.20^{Bc}
300	4.2± 0.07 ^d	3.8± 0.20°	$3.3 \pm 0.10^{\circ}$	3.1 ± 0.20^{b}
150	3.5 ± 0.10^{b}	3.6 ± 0.30^{b}	2.4 ± 0.11^{b}	2.5 ± 0.20^{a}
053	2.1± 0.25 ^a	2.8± 0.20 ^a	2.1± 0.10 ^a	2.2 ± 0.10^{a}

All data are to mean ±SD of three replicates. For each value means within a row not sharing a common letter differ significantly (p<0.05)

Table 3. Effect of size reduction on Total, soluble, and Insoluble fiber content of wheat bran (n=3) PBW-154 and HD-2967

Average particle size of the	Cultivars of wheat bran	
bran (μm)		
	PBW-154	HD-2967
Total fibor (0/)	46	
Total fiber (%)	A SA	
710	60.5± 2.20e	51.4± 3.20 ^e
500	57.3± 2.20 ^d	48.9± 2.10 ^d
300	52.1± 3.25°	42.9± 2.10°
150	50.9± 1.30 ^b	36.5± 1.20 ^b
53	24.9± 1.20 ^a	31.7± 1.20 ^a
Soluble fiber (%)		
710	8.4± 0.20°	8.7± 0.20°
500	7.2 ± 0.20^{c}	7.9± 0.20 ^d
300	6.4± 0.10 ^b	6.0 ± 0.10^{c}
150	5.8± 0.20 ^b	5.1± 0.20 ^b
53	4.6± 0.20 ^a	4.5± 0.20 ^a
Insoluble fiber (%)	1	

710	$52.1\pm 2.10^{\rm e}$	42.7± 3.20°
500	50.1 ± 2.10^{d}	41.0± 2.20 ^d
300	45.7± 2.20°	36.8± 2.20°
150	45.1± 1.20 ^b	31.4± 1.40 ^b
53	20.3± 1.30 ^a	27.2± 1.20 ^a

All data are to mean \pm SD of three replicates. For each value means within a row not sharing a common letter differ significantly (p<0.05).

Table 4. Effect of size reduction and hydrothermal treatments on Antioxidants properties of wheat bran (n=3) PBW-154 and HD-2967

Average	PBW-154	4 6	HD-2967	7
Particle sizes of	1	ALE A	- 34) I	
the bran (µm.)	Control	Hydrothermaled	Control	Hydrothermaled
Total Phenolic C	Content (mg GAE/100	g)		
710	89.19± 1.89 ^d	165± 9.16°	85.62± 1.38 ^d	123±1.35 ^d
500	80.35± 1.76°	149± 11.25°	70.62± 2.89°	115± 1.62°
300	72.05± 4.24°	142± 10.89bc	62.32± 3.87°	105± 1.41°
150	69.24± 6.31 ^b	135± 12.72ab	48.92± 2.52 ^b	97.8± 4.74 ^b
053	65.17± 2.01 ^a	121± 1.81 ^a	39.28± 4.79 ^a	83.7± 2.41 ^a
Total Flavonoid	Content (mg QE/g)			I
710	0.53 ± 0.07^{c}	0.754 ± 0.01^{b}	0.45 ± 0.013^{c}	0.68 ± 0.20^{d}
500	0.44 ± 0.019^{c}	0.650± 0.01 ^b	0.31 ± 0.012^{b}	0.10 ± 0.05^{d}
300	0.11± 0.03 ^b	0.139±0.02 ^b	0.07 ± 0.006^{a}	0.09 ± 0.04^{c}
150	0.10± 0.04 ^a	0.107 ± 0.09^{a}	0.06± 0.0007 ^a	0.08± 0.03 ^b
053	0.09 ± 0.04^{a}	0.083± 0.13 ^a	0.05 ± 0.004^{a}	0.07 ± 0.07^{a}

DPPH (%)				
710	65.9± 3.21°	68.7±2.3 ^e	67.7± 6.10 ^b	71.5±4.7 ^e
500	59.7± 2.8 ^{bc}	63.9±1.9 ^d	49.5± 6.74 ^{ab}	64.4±3.9 ^d
300	53.4± 2.96 ^{bc}	58.6±3.6°	43.7± 3.01 ^a	56.5±3.8°
150	47.8± 4.3 ^{ab}	52.7±2.1 ^b	33.5± 2.80 ^a	49.7±2.3 ^b
053	34.3± 1.31 ^a	46.8±1.5 ^a	31.5± 2.31a	41.8±2.4ª
Ferrous ion	n chelating activity (%)			
710	56.1± 2.15 ^d	65.8±2.8 ^e	57.3± 2.07 ^d	64.5±2.1e
500	53.9± 1.11 ^d	58.6±3.1 ^d	52.4± 0.96 ^d	61.5±1.7 ^d
300	46.3± 1.11°	51.7±1.8°	46.0± 1.59°	52.6±2.5°
150	38.3± 3.44 ^b	48.6±3.2 ^b	39.7± 0.48 ^b	45.8±2.9 ^b
053	33.6± 0.62 ^a	39.8±2.4ª	33.3± 1.16 ^a	37.9±2.1ª

All data are to mean ±SD of three replicates. For each value means within a row not sharing a common letter differ significantly (p<0.0)