

Effect of storage and processing on bioactive compounds and antioxidant activity in whole wheat flour of Indian wheat cultivars

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Abstract

Wheat (*Triticum aestivum* L.) is among the most extensively cultivated cereal crops worldwide serving as a staple food consumed by one-third of the world's population. In addition to providing basic nutrients, wheat also comprehends substantial levels of bioactive substances. These days, polyphenolic compounds are receiving fair attention due to various reports of associated health benefits and nutraceutical effects. Thus, a study was carried out with whole wheat flour of three Indian wheat cultivars (PBW 550, DBW 187 and DBW 222), grown at Karnal location for their soluble phenolic, anthocyanin content and antioxidant potential during storage as well as while processing/baking. During baking, the decrease in soluble phenolic content ranged from 43.2 (DBW 222) to 51.0% (PBW 550) while there was substantial decrease in ABTS radical scavenging activity to the tune of 70.3 (DBW 187) to 81.3% (PBW 550). The baking resulted decrease in anthocyanin content from 34.0 (PBW 550) to 55.9% (DBW 187). During storage of whole wheat flour in paper bags under dark conditions at room temperature, soluble phenolic content (9.5-12.2%) decreased slowly compared to anthocyanins (43.8-60.3%) and antioxidant activity (34.3-54.2%) after 60 days of storage. Overall data showed that anthocyanins, soluble phenolic content, and antioxidant activity decreased in whole wheat flour during storage as well as processing; irrespective of the wheat cultivar under study.

Key words: Soluble phenolic content, Antioxidant activity, Chapatti, Whole wheat flour, Storage

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1. Introduction

In addition to providing calories and protein to humans, wheat also contains substantial levels of bioactive dietary substances viz., carotenoids, flavonoids, and phenolic acids (Adom *et al.*, 2005; Dong-yun *et al.*, 2014; Zieliński and Kozłowska, 2000). The consistent intake of these phytochemicals is associated with lesser risks of cancer (Arts and Hollman, 2005), cardiovascular disease (Mellen *et al.*, 2008) and type 2 diabetes (Lutsey *et al.*, 2007). Antioxidants are comprehensively required via the diet to supplement the insufficient level of endogenous

antioxidants for maintaining body's redox status with increasing age and varied external factors (Narwal *et al.*, 2012) thus, leading to various degenerative diseases. The antioxidant potential and bioavailability depend on the species and variety of grains, fraction of grain and processing conditions. Storage, different forms of processing, formulations and product making can greatly influence the levels of antioxidants in the final product (Narwal *et al.*, 2012). Most of the studies on antioxidants have reported their content in the raw foods, but it is



the antioxidant content of the final product that is more important.

Food antioxidants are lost in significant amounts as a result of food processing, storage, home handling and cooking (Hirawan *et al.*, 2010). Wheat grain and the milled products for human consumption are invariably subjected to heat processing before being consumed. This may include baking, frying, extrusion, puffing and flaking. Invariably, storage of whole wheat flour (WWF) leads to loss of various antioxidants and other bioactive components with storage period. The effect of storage period and heat processing on foods' phytochemical content and antioxidant activity should be well investigated (Narwal *et al.*, 2012). During storage and processing, interactions among nutrients and/or antioxidants/oxidants, may modify the antioxidant activity of foods. Various researchers have reported the antioxidant potential of different wheat types and their fractions (Adom *et al.*, 2003; Kumar *et al.*, 2022; Liyana-Pathirana and Shahidi, 2007; Okarter *et al.*, 2010; Sedaj *et al.*, 2011), as well as the antioxidant activity of wheat-based food products (Angioloni and Collar, 2011; Baublis *et al.*, 2000; Hirawan *et al.*, 2010).

In Indian context, about three-fourth of the bread wheat grown is used for the preparation of flat unleavened bread (chapatti) from whole wheat flour, while the remainder is destined to the preparation of bread, biscuits, cakes *etc.* (Kumari *et al.*, 2020). The current investigation reports about a study carried out with WWF of three cultivars (PBW 550, DBW 187 and DBW 222) grown at Karnal location for their soluble phenolic content, antioxidant potential and anthocyanin content during storage as well as processing (chapatti making).

2. Material and Methods

2.1 Materials and chemicals

Three amber wheat cultivars (DBW 187, DBW 222 and PBW 550) were evaluated for their soluble phenolic content, antioxidant potential and anthocyanins during baking (chapatti making) as well as storage (up to 60 days). For storage, the WWF was stored in paper bags at room temperature in dark. All the cultivars were grown at Karnal location during the cropping season 2019-20 with the recommended package of practice. The chemicals, kuromanin chloride, ABTS, potassium persulfate, Trolox, and gallic acid were purchased from Sigma chemical

company. All other chemicals and solvents were of the highest analytical grade.

2.2 Sample preparation and extraction

The experiments were performed on whole-wheat flour (WWF) prepared in the Cyclotec-1093 mill (Tecator) using a 0.5 mm screen. The methanolic extraction of WWF was done as per Beta *et al.* (2005) with minor modifications. Methanol extract was prepared by adding 1.0 ml of 80% methanol to 0.1 g of each flour sample and mixing followed by constant shaking for 2 h in order to release the soluble phenolics. After shaking, the contents were centrifuged at 5000 g for 10 min (Hermele Z383K, India). The supernatants obtained were collected as methanolic extracts and used to estimate the phenols and total antioxidant activity. For extraction of anthocyanin, procedure of Abdel-Aal and Hucl (1999) was employed with slight modifications. To 200 mg of WWF, 1.6 ml of acidified ethanol (85 mL of 95% ethanol + 15 mL of 1N HCl) was added, mixed well, and allowed to extraction for 30 min with constant shaking. The contents were centrifuged at 8000 g for 25 min. The supernatants obtained were collected as ethanolic extracts and used to estimate the anthocyanin content. Unless stated otherwise, all the samples were taken in duplicates and analyzed further in two replicates.

2.3 Baking/Chapatti preparation

The chapattis were prepared according to AAAC method. The WWF was mixed with known amount of water and the ingredients were hand-kneaded to make the dough and left for 30 min at room temperature (25 ± 1 °C). Forty g dough was rolled up manually in round sheets. Chapattis were baked, allowed to cool down, and stored in sealed container for 1 h. For each replicate, chapattis were freshly prepared and separately stored. Subsequently, 2 chapattis/replicate of each cultivar were used in each experimental analysis.

2.4 Soluble phenolic content

The soluble phenolic content of 80% methanolic extracts was assessed as described by Singleton *et al.* (1999). Briefly, 50 µL of the extracts were mixed with 1.55 mL of water and oxidized with 100 µL of the Folin-Ciocalteu reagent (1 N). After 5 min, the reaction was stopped by adding 300 µL of 20% sodium carbonate solution followed by incubation at 40°C in a water bath for 30 min. The absorbance was



recorded at 765 nm and the concentration of the soluble phenolic content was determined against the gallic acid standard and the content was expressed as μg gallic acid equivalent (GAE)/g d.wt. of WWF.

2.5 Total antioxidant activity (ABTS assay)

The radical cation ABTS⁺ scavenging activity was determined by following Re *et al.* (1999). For the ABTS reagent, 7 mM ABTS was dissolved in water (stock solution), added 2.45 mM potassium persulphate, and allowed the reaction to occur by keeping the mixture in the dark for nearly 16 h. The reagent was diluted with methanol just before experiment till its OD value reached 0.70 at 734 nm against blank. The methanol extract (10 μL) was allowed to react with 1000 μL of ABTS reagent. The absorbance was recorded after 6 min using 80% methanol as blank at 734 nm. The ABTS radical scavenging activity was calculated as %discoloration of sample, expressed as Trolox equivalent antioxidant capacity (TEAC) and defined as nmols of TEAC/g d.wt. of WWF.

2.6 Anthocyanin content

The anthocyanin content was estimated by recording absorbance at 535 nm (Systronics UV-Vis, 2202, India) against acidified ethanol blank as per the procedure of Abdel-Aal and Hucl (1999) with minor modifications. Total anthocyanin content was determined against the kuromanin chloride standard and the content was expressed as **μg kuromanin chloride equivalent/g d.wt.** of WWF as per the equation below:

$$C = (A/\text{Slope}) \times (\text{Total volume}) \times (1/\text{sample wt})$$

Where, C is concentration of total anthocyanin ($\mu\text{g/g}$), A is absorbance reading

2.7 Statistical analysis

The data was reported as the mean $\pm$ standard error of the mean for each sample. The ANOVA was performed to find honest significant difference (HSD) using <http://opstat.pythonanywhere.com/#> statistical software.

3. Results and Discussion

3.1 Effect of baking on soluble phenolic and anthocyanin contents and antioxidant activity

Whole wheat flours of 3 amber cultivars grown at Karnal location were evaluated for their soluble phenolic, antioxidant potential, and anthocyanin content during baking (chapatti making). Soluble phenolic content in

freshly extracted WWF of tested cultivars ranged between 1361.3 (DBW 187) to 1421.3 (DBW 222) μg GAE/g d.wt. while the corresponding range for antioxidant activity was reported between 3782.3 (PBW 550) to 4708.9 (DBW 222) nmols TEAC /g d.wt. (Table 1). A large proportion of wheat is consumed in the form of chapatti in Asian countries which requires baking before consumption. The baking involves high temperature which is all set to affect the nutritional status of commodity being baked. Baking significantly reduced soluble phenolic content, antioxidant activity as well as anthocyanin content in all the cultivars tested. Maximum % decrease in soluble phenolic content was reported with PBW 550 (51.0%) while it did decrease less in case of DBW 222 (43.2%) after baking. The corresponding % maximum and minimum decrease in antioxidant activity during baking was recorded with PBW 550 (81.3%) and DBW 187 (70.3%), respectively (Table 1). Likewise, %decrease in anthocyanin content was found between 34.0-55.9% post-baking (Table 1). Very high temperature during chapatti making (baking) thus leads to significant losses of soluble phenolic and anthocyanin contents and antioxidant activity in WWF of the cultivars tested. Alternatively, it can also be stated that the antioxidants might have protected other nutrients of WWF. A 30% reduction in the antioxidant activity by chapatti making of whole meal flour has been reported by Narwal *et al.* (2012). Similar reduction in antioxidant activity by chapatti making was testified by Kumari *et al.* (2020). The concentration of cereal phenolic compounds is influenced by types, varieties, and grain part where these are concentrated (Žilić *et al.*, 2011, Žilić *et al.*, 2012). While Zong *et al.* (2006) reported lower antioxidant capacity for white or red wheat grains, conversely, using six Canadian wheat genotypes, Beta *et al.* (2005) and Mpofu *et al.* (2006) showed that there was no link between wheat color (red vs. white) with antioxidant activity and total phenolic content.

3.2 Effect of storage on soluble phenolic and anthocyanin contents and antioxidant activity of whole wheat flour

The soluble phenolic and anthocyanin contents and antioxidant activity in WWF of above three cultivars were recorded up to 60 days of storage at room temperature under dark conditions in paper bags. The data of various parameters tested has been given in Table 2. Overall data suggested that soluble phenolic and anthocyanin contents and antioxidant activity decreased continuously during





Table 1 Effect of baking on whole wheat flour of amber wheat cultivars

Cultivar	Solublephenolic content (µg gallic acid equivalent/g d. wt. basis)			ABTS radical scavenging activity (n molstrolox equivalent/g d. wt. basis)			Anthocyanin content (µg/g kuromanin chloride equivalent d. wt. basis)		
	Fresh flour	After baking	%age decrease	Fresh flour	After baking	%age decrease	Fresh flour	After baking	%age decrease
PBW550	1375.8±10.3 ^a	674.2±5.7 ^b	51.0	3782.3±133.6 ^a	709.1±41.0 ^b	81.3	6.7±0.5 ^b	4.4±0.3 ^a	34.0
DBW187	1361.3±6.4 ^a	735.4±4.6 ^a	46.0	4018.2±44.3 ^a	1192.4±38.8 ^a	70.3	8.2±0.2 ^a	3.6±0.3 ^a	55.9
DBW222	1421.3±9.5	806.9±8.8	43.2	4708.9±115.4	1381.1±65.5 ^a	70.7	8.1±0.2 ^a	4.5±0.3 ^a	44.7

(Mean±SEm); n=4; HSD (Honest significant difference)

Table 2 Effect of storage of on whole wheat flour of amber wheat cultivars

Cultivar	Solublephenolic content (µg gallic acid equivalent/g d. wt. basis)			ABTS radical scavenging activity (n mols trolox equivalent/g d. wt. basis)			Anthocyanin content (µg/g kuromanin chloride equivalent d. wt. basis)		
	0 d	15 d	30 d	60 d	0 d	15 d	30 d	60 d	0 d
PBW550	1375.8 ±10.3 ^a	1319.9 ±5.7 ^a	1275.4 ±11.6 ^a	1239.1 ±2.6 ^a	3782.3 ±133.6 ^a	2923.1 ±70.9 ^a	2004.9 ±30.0 ^b	1731.6 ±40.3 ^b	6.7±0.5 ^b
DBW187	1361.3 ±6.4 ^a	1299.2 ±15.1 ^b	1217.4 ±18.0 ^b	1194.6 ±5.5 ^b	4018.2 ±44.3 ^a	2859.1 ±33.0 ^a	2563.6 ±13.4 ^a	2553.1 ±79.3 ^a	8.2±0.2 ^a
DBW222	1421.3 ±9.5	13478 ±12.4 ^a	1291.9 ±5.3 ^a	1285.7 ±4.3	4708.9 ±115.4	3483.7 ±82.1	3492.2 ±54.3	3092.7 ±29.4	8.1±0.2 ^a

(Mean±SEm); n=4; HSD (Honest significant difference)

storage period. There was less decrease in soluble phenolic content (9.5-12.2%) compared to antioxidant activity (34.3-54.2%) and anthocyanin content (43.8-60.3%) after 60 days of storage (Fig. 1). Narwal *et al.* (2012) reported about 25% reduction in the antioxidant activity after 60 days of storage at -20°C, 4°C and room temperature. Anthocyanins get rapidly degraded during food processing

and storage (Kokkaew *et al.*, 2015) as the former have been reported to be less stable being affected by the factors such as light, oxygen, sugar, temperature, and pH (Sadilova *et al.*, 2006; Žilić *et al.*, 2019). Our results are in conformity to various researchers with regard to decrease in antioxidant capacity and anthocyanin content during storage.

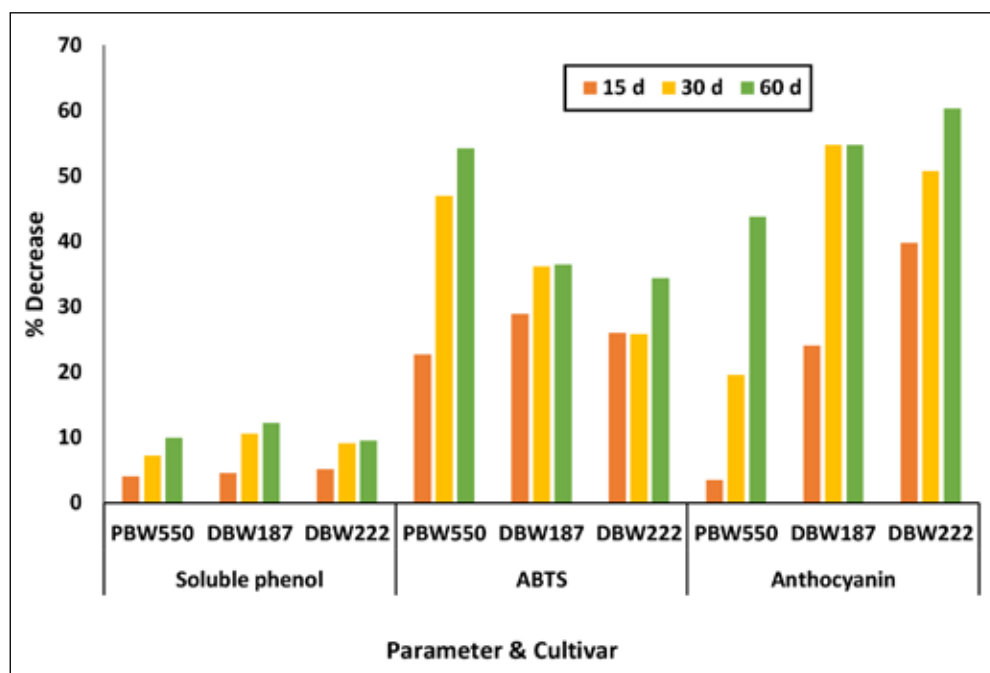


Fig. 1 Percent decrease in various parameters during storage of whole wheat flours of amber cultivars

Conclusion

Overall data inferred that soluble phenolic content, antioxidant activity and anthocyanin content decreased continuously during storage period. The similar trend of decrease was recorded during baking. However, there was less decrease in soluble phenolic content compared to antioxidant activity and anthocyanin content during baking as well as storage.

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Author contributions

All authors contributed equally for preparing the final version of the manuscript.

Conflict of Interest

Authors declare no conflict of interest.

Ethical Approval

The article doesn't contain any study involving ethical approval.

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