

- bicolor* L. Moench). Forage Research 29(4): 195-197.
- Singh, A. and Pallavi. 2019. Effect of various sources of nutrients on sorghum fodder. International Journal of Current Microbiology and Applied Sciences 8(2): 890-894.
- Singh, D., Nainwal, R. C. and Tewari, S. K. 2015. Integrated nutrient management in non-traditional crop sorghum (*Sorghum bicolor* L. Moench) under partially Reclaimed soil. Progressive Research-An International Journal 10(5): 2499-2502.
- Tiwana, U. S. and Chaudhary, D. P. 2009. Effect of integrated nutrient management on the yield and quality of forage sorghum. Forage Research 35(1): 56-58.

*J. Agric. Res. Technol., Special Issue (1) : 076-086 (2022)*

DOI: <https://doi.org/10.56228/JART.2022.SP113>

## Physical and Nutritional Aspects of Biscuits Prepared with Purple Wheat

Jyoti Verma<sup>1</sup>, Varsha Rani<sup>2</sup>, Karnika<sup>\*</sup>, Sangeeta Rani<sup>3</sup>, Deepankar<sup>4</sup>, Shrawan Kumar<sup>5</sup>

Department of Food and Nutrition, PAU, Ludhiana - 141 004 (India)

\*Email : [chaudhary.karnika12@gmail.com](mailto:chaudhary.karnika12@gmail.com)

### Abstract

As oxidative stress has been linked to the advancement of a number of degenerative illnesses, bioactive substances including polyphenols, anthocyanins, and carotenoids have sparked the interest of food scientists and manufacturers for their health-promoting and disease-preventive properties. The objective of this study was to develop anthocyanin-rich biscuits using purple wheat (100%) and compare their physical and nutritional content with commonly consumed wheat (100%) biscuits. Biscuits were analyzed for thickness, width and spread ratio. 9-point hedonic scale was used for analysis of organoleptic acceptability. Biscuits were analyzed for nutritional parameters i.e., proximate composition, sugars, total and available (*in vitro*) calcium, iron and zinc, *in vitro* digestibility of protein and starch, phytic acid, total lysine, DPPH radical scavenging activity, total phenolic content (TPC), total flavonoid content (TFC) and total anthocyanin content (TAC). T-test was used to compare the differences between regular wheat and purple wheat. Results of thickness, width and spread ratio of purple wheat prepared biscuits was 0.81 cm, 3.04 cm and 3.75 ( $W T^{-1}$ ) respectively. Biscuits prepared with purple wheat depicted significantly higher ( $P < 0.05$ ) protein, fat, fiber, carbohydrates and mineral content than regular wheat. Total lysine, *in-vitro* digestibility of protein and starch were also observed higher in purple wheat biscuits i.e., 4.24 g  $16^{-1}$  g N, 82.57% and 42.98% respectively. Total antioxidant activity of purple wheat prepared biscuits also had significantly ( $P < 0.01$ ) higher DPPH activity (65.64 mg TE  $100^{-1}$  g), TPC (125.4 mg GAE  $100^{-1}$  g), TFC (66.19 mg RE  $100^{-1}$  g) and TAC (3.01 mg C3GE  $100^{-1}$  g) than regular wheat.

**Key words : Purple wheat, Anthocyanin, Antioxidant, Thickness, Biscuit.**

Purple wheat grain demand increasing dramatically in last 2 years because of the potential of genotypes in developing cultivars having the benefit of human health. Commonly

1. \*Dept. of Food and Nutrition, 2. Dept. of Foods and Nutrition, 3. Dept. of Extension Education and Communication Management, 4. Dept. of Mathematics and Statistics, and 5. Dept. of Statistics, Kirori Mal College, University of Delhi, Delhi.

consumed wheat (*Triticum aestivum*) is tawny in color and has a low amount of anthocyanin whereas purple wheat contains a high amount of anthocyanin along with other phytochemicals that grabbing attention over the world nowadays (Syta *et al.* 2018; Calderaro *et al.* 2019). Anthocyanin possesses strong anti-microbial activity against various Gram-positive and Gram-

negative human pathogens (Burdulis *et al.* 2009; Cisowska *et al.* 2011). Oxidative stress has been implicated in the progression of a number of degenerative diseases, including diabetes, rheumatoid arthritis, osteoporosis, cancer, cystic fibrosis, Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS). These diseases are characterized by extensive oxidative damage to lipids, proteins, and DNA (Arts & Hollman, 2005; Uttara *et al.* 2009; Patel *et al.* 2013).

In recent years, importance of bioactive compounds such as polyphenols, pigments, and carotenoids have attracted more and more interest of both the communities of food researchers and food manufactures due to their health-promoting and disease-preventing effects in both *in vitro* and *in vivo* studies (Havrlentov *et al.* 2014). Purple wheat bran contains high antioxidant capacity determined by 3 assay ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid), DPPH (1,1-diphenyl-2-picrylhydrazyl), and ORAC (oxygen radical absorbance capacity) with 74, 94, and 100 percent inhibition of free radical 2 ion as supported by *in vitro* research evidences (Gamel *et al.* 2020). Purple wheat possesses anti-aging properties and reported that anthocyanin extract from purple wheat extends the life span of wild type and *mev-1* (*hn1*) mutant worms by 9.2 to 10.5 percent as these worms were found to be sensitive to oxidative stress reported in *in vivo* study (Chen *et al.* 2013).

Biofortified crops such as purple wheat have huge market potential. Apart from having health-promoting effects, these grains possessed all the features required for commercial product development, paving way for their industrial utilization (Knievel *et al.* 2009; Sharma *et al.* 2018). Addition of purple wheat in diet can contribute a significant amount of anthocyanin without the fear of increasing blood sugar while having large proportions of blueberries. Purple

wheat contained ash (5.5%), crude protein (8.5%), crude fat (3.03%), crude fiber (7.3%), moisture content (10.76%), utilized carbohydrate (64.85%) and dry gluten (0.283 g). It also contained condensed tannin, total phenol and anthocyanin content (25.6 mg 100<sup>-1</sup> g, 253 mg 100<sup>-1</sup> g and 197.4 mg 100<sup>-1</sup> g), respectively (Kassegn, 2017).

In baking process free phenolic acid increases in bread, cookie, and muffin while bound phenolic acid content decreases in bread but least affected on muffin and cookie products. Colored wheat bran antioxidant properties as affected by thermal processing (Abdel-Aal and Rabaski 2013). Bread and bakery products become part of our diet because they are rich in nutrients and dietary fiber. Common wheat flour has low antioxidant property so there is a need to fortify it with colored wheat flour for improving nutrition and health benefits (Dziki *et al.* 2014). The addition of 2 to 6 percent purple wheat flour bran into common wheat flour during noodles making increased the amount of dietary fiber and antioxidant content of the final product (Song *et al.* 2013). Commonly consumed bakery item biscuit has a longer shelf life and can be used to harbor functional ingredients for human health. Purple wheat biscuit reported high antioxidant activity and total phenolic compounds (Pasqualone *et al.* 2015). Colored wheat is not only rich in antioxidant activity but also contains a higher amount of proteins, essential amino acids, and essential nutrients like zinc, iron, and magnesium than common wheat (Tian *et al.*, 2018). Because of its nutritional characteristics, purple wheat is highly suitable for product making and commercial utilization (Sharma *et al.*, 2018). Keeping these facts in view, this research was undertaken to develop anthocyanins rich pasta using biofortified purple wheat and to evaluate biscuit for physical, sensory and nutritional aspects.

## Materials and Methods

**Collection of samples :** Seed sample of purple wheat was obtained from National Agri-Food Biotechnology Institute (NABI), Punjab and other ingredients required for the preparation of products and packaging materials were purchased from the local market in a single lot. Grain sample was cleaned and freed from broken seeds, dust and other foreign materials.

**Physical Characteristics of biscuits :** Width and thickness of biscuits thus, prepared were measured using vernier calliper. Randomly five biscuits were taken. After measuring the width of biscuit from one side, it was rotated at 90° and remeasured to get the average width (W) and then thickness (T) of biscuits were measured. Spread ratio was calculated according to AACC method 10-50 D (AACC, 1983). Spread ratio was the ratio of biscuits width to the thickness (W/T) and was determined by dividing width (cm) by thickness (cm).

**Sensory analysis :** Cooked biscuit was evaluated in terms of color, taste, aroma, appearance, texture and overall acceptability using 9-point hedonic rating scale by a panel of 30 semi-trained judges. Rating of pasta was expressed on a 9 to 1 point rating scale as liked extremely, liked very much, liked moderately, liked slightly, neither liked nor disliked, disliked slightly, disliked moderately, disliked very much and disliked extremely, respectively. An overall acceptability score of 6 or above was considered acceptable and further evaluated for nutritional parameters.

**Nutritional composition :** The Association of Official Analytical Chemists (AOAC, 2010) methods were used to determine the moisture, carbohydrate, fats, protein, and ash in cooked and dried pasta samples. The micro-Kjeldhal apparatus was used to determine the nitrogen concentration of the samples. To convert it to crude protein, the nitrogen value

was multiplied by 6.25. The moisture and ash contents of pasta were measured using weight difference method, while crude fat was determined using the Socs Plus apparatus using petroleum ether as the solvent, crude fiber was analyzed as acid and alkali resistant.

Samples for total sugars and starch were extracted using 80% ethanol by employing the methodology of Cerning and Guilhot (1973) and the contents were estimated using the methods of Yemm and Willis (1954). Reducing sugar was determined according the methods of Somogyi (1945) and non-reducing sugar was calculated as a difference of total soluble sugar and reducing sugar.

The acid digested (HNO<sub>3</sub>:HClO<sub>4</sub>; 5:1 v/v) samples were estimated for total calcium, iron and zinc by Atomic Absorption Spectrophotometer 240 FS (Australia) using the method earlier mentioned by John *et al.*, (2020). In vitro available calcium and zinc were extracted as per method of Kim and Zemel (1986) and sample for in vitro available iron was extracted as per the methodology of Rao and Prabhavathi (1978) earlier described by John *et al.*, (2020).

*In vitro* protein digestibility (%) was estimated using modified enzymatic method explained by Mertz *et al.* (1983) and *In vitro* starch digestibility (mg maltose released per g of starch) was assessed as per the method given by Singh *et al.* (1982). Total lysine (g 16<sup>-1</sup> gN) was estimated by the method of Balsubramanian and Sadasivam (1987). Phytic acid (mg 100<sup>-1</sup> g) content was analyzed by using the method of Davies and Reid (1979).

**Antioxidant activity :** Total anthocyanin content (TAC) was analysed in a type U-1100 spectrophotometer by following the methodology of Abdel-Aal and Hucl (1999), the acidified MeOH extract was filled in 1 cm thick cavities and measured at 535 nm. An empty microcuvette was used to set the reading to zero,

followed by a cuvette containing just acidified MeOH. Using the calibration curve, the findings were determined and reported as mg cyanidin-3-glucoside equivalents per kg dry matter (ppm). The extinction coefficient was used to compute the total anthocyanin concentrations.

Total phenolic content (TPC) was measured spectrophotometrically using the Folin-Ciocalteu reagent as described by Singleton (1999). Acidified MeOH extract (0.1 ml) was added to the reaction mixture, which was then oxidised using 0.5 ml Folin-Ciocalteu reagent (1:10 Folin-Ciocalteu:water) and 0.8 ml 7.5 percent  $\text{Na}_2\text{CO}_3$ . Instead of extract, 0.1 ml water was used to prepare the blank. The combination was heated in a water bath at  $50^\circ\text{C}$  for 5 minutes and then cooled to room temperature before being measured using a type U-1100 spectrophotometer at 760 nm.

Total flavonoids content was estimated using aluminium chloride colorimetric technique explained by Zhishen *et al.*, (1999). From various sample aliquots, a final amount of 5 ml was prepared using distilled water. Then, in test tubes, added 0.5 ml of 5%  $\text{NaNO}_2$  and, after 5 minutes, added 0.6 ml of 10%  $\text{AlCl}_3$  and mixed again. After 6 minutes, 2 ml of 1N NaOH was added and stirred. With the addition of 2.1 ml distilled water, a volume of 10 ml was made. Pink colour absorption was measured at 510 nm.

The DPPH radical scavenging activity was measured using methodology given by Brand-Williams *et al.* (1995). Different known sample aliquots were collected using methanol, and the volume was built up to 1 ml. It was then filled with 3 ml of DPPH reagent and properly mixed before being incubated at  $37^\circ\text{C}$  for 20 minutes. Absorbance of oxidised solution was read against methanol as a blank at 517nm.

**Statistical analysis :** The data obtained was statistically analyzed in terms of mean,

standard deviation and independent t- test (equal variance t- test and unequal variance t- test) was used to differentiate between normal and purple wheat as well as their products for physical, functional and nutritional composition.

## Results

The data on physical characteristics i.e., thickness, width and spread ratio of biscuit. Thickness and width of wheat flour-based biscuits was found to be 0.81cm and 3.04cm, respectively whereas thickness and width of purple wheat flour-based biscuit was 0.8cm and 3.02cm, respectively. A non-significant difference was observed for thickness and width between the biscuits prepared using normal and purple wheat flour. Spread ratio of wheat flour-based biscuit was higher (3.77) than the biscuits 48 prepared with purple wheat flour (3.75). However, this difference was found to be non-significant.

Purple wheat flour-based biscuits was adjudged as liked very much by obtaining the mean score of 8.86 for overall acceptability. Results indicated that purple wheat-based biscuits attained higher scores for aroma, texture, taste, and overall acceptability whereas regular wheat flour-based biscuits attained higher scores for colour and appearance (Table 2).

The content of moisture, crude protein, ash, fat and crude fiber were found to be 2.61

**Table 1.** Physical characteristics of biscuits

Physical characteristics	Thick-ness (cm)	Width (cm)	Spread ratio (W/T)
<b>Biscuit</b>			
Wheat (control)	0.80 ± 0.01	3.02 ± 0.04	3.77 ± 0.04
Purple Wheat	0.81 ± 0.01	3.04 ± 0.06	3.75 ± 0.06
t- value	2.45NS	0.96NS	1.39NS

NS= non- significant (there was no difference between variables)

percent, 11.04 percent, 1.32 percent, 18.14 percent and 3.03 percent, respectively in the biscuits prepared from normal wheat flour. On the other hand, biscuits prepared using purple wheat flour had 2.07 percent of moisture, 13.17 percent of crude protein, 1.8 percent of ash 18.84 percent of fat and 4.68 percent of crude fiber. Non-significant difference was observed for moisture content of biscuits developed using normal wheat and purple wheat flour. Further, it was observed that biscuits prepared with purple wheat flour had significantly ( $P < 0.05$ ) higher contents of protein, ash, fat and crude fiber than the biscuits prepared using normal wheat flour.

The starch content (34.52%) of biscuits prepared with normal wheat flour was significantly ( $P < 0.01$ ) higher than that of biscuits prepared with purple wheat flour (31.94%). The contents of total soluble sugars (31.12%), reducing sugars (2.28%) and non-reducing sugars (28.84%) were significantly ( $P < 0.01$ ) higher in the biscuits prepared with purple wheat flour than the total soluble sugars (25.57%), reducing sugars (1.08%) and non-reducing sugars (24.49%) contents of biscuits prepared with normal wheat flour.

Total iron, calcium and zinc content of wheat flour-based biscuits were depicted to be 4.39 mg  $100^{-1}$  g, 31.74 mg  $100^{-1}$  g and 3.28 mg  $100^{-1}$  g whereas 6.58 mg  $100^{-1}$  g, 53.18 mg  $100^{-1}$  g and 4.45 mg  $100^{-1}$  g were found in

purple wheat flour-based biscuits. Available iron, calcium and zinc content of wheat flour-based biscuits were observed to be 1.07 mg  $100^{-1}$  g, 13.85 mg per 100g and 0.89 mg  $100^{-1}$  g, whereas 1.78 mg  $100^{-1}$  g, 28.26 mg  $100^{-1}$  g and 1.72 mg  $100^{-1}$  g available iron, calcium and zinc content were found in purple wheat flour-based biscuits (Table 4).

As per the results represented in Table 4, it was observed that biscuits prepared using purple wheat had significantly ( $P < 0.01$ ) higher digestibility of protein (82.57%) and starch (40.98 mg maltose  $g^{-1}$ ) than the normal wheat-based biscuits. Results showed that biscuits prepared with normal wheat flour contained 2.33 g  $16^{-1}$  gN of total lysine whereas biscuits prepared with purple wheat flour had significantly ( $P < 0.05$ ) higher content (4.24 g  $16^{-1}$  gN) of total lysine. The phytic acid content in normal wheat-based biscuits was found to be 129.58 mg  $100^{-1}$  g which was found to be significantly lower in purple wheat-based biscuits i.e., 97.71 g  $100^{-1}$  g.

Results showed that 65.64 mg TE per 100g, 125.4 mg GAE  $100^{-1}$  g, 66.19 mg RE  $100^{-1}$  g and 3.01 mg C3GE  $100^{-1}$  g of DPPH, TPC, TFC and TAC content was observed in purple wheat flour-based biscuits. Purple wheat-based biscuits had significantly ( $P < 0.01$ ) higher DPPH, TPC, TFC and TAC as compared to normal wheat-based biscuits (Table 5).

**Table 2.** Mean scores of organoleptic characteristics of biscuits

Products	Mean Scores					
	Color	Appearance	Aroma	Texture	Taste	Overall acceptability
<b>Biscuit</b>						
Wheat (control)	9 ± 0.52	9 ± 0.52	8.3 ± 0.48	8.2 ± 0.63	8.4 ± 0.48	8.58 ± 0.526
Purple Wheat	8.7 ± 0.45	8.9 ± 0.78	9 ± 0.28	8.74 ± 0.46	8.98 ± 0.48	8.86 ± 0.49
t-value	1.51NS	0.37NS	4.36*	2.40NS	2.96NS	1.37NS

NS= non- significant (there was no difference between variables). \*=significant difference at 5% level of significance

## Discussion

The present study was investigated to develop biscuits with purple wheat. A non-significant difference was observed in present study for physical characteristics (thickness, width and spread ratio) of purple wheat and common wheat-based biscuit (Table 1). According to Pasqualone *et al.*, (2015) spread ratio of purple wheat flour biscuit was lower than control that might be due to high gluten index and low friability (texture). Results of present study are closely related to earlier study done by Pasqualone *et al.*, (2015).

Overall acceptability of purple wheat- based products was recorded higher than normal wheat- based products. Color and appearance were found higher in normal wheat-based products, whereas mean scores of aroma, texture and taste were found higher in purple wheat- based products and adjudged as liked very much by panelists. Pasqualone *et al.*, (2015) reported significance difference in color of purple wheat and convectional wheat but no significance difference found in taste attributes. Chen *et al.* (2013) discovered that bran-enriched flour changed the rheological characteristics of dough, affecting the end-quality products and sensory qualities.

Biscuit was observed to have higher crude protein, fat, crude fiber and ash content in all purple wheat- based products as compared to control however, had lower moisture content than normal wheat- based products. Results of present study are in close proximity of earlier investigations conducted by Pasqualone *et al.*, (2015). According to Pasqualone *et al.*, (2015) moisture content of purple wheat- based biscuits was 7.7 percent while in normal wheat- based biscuit it was reported as 7.9 percent. Purple wheat- based biscuit contained higher protein than conventional biscuit due to the presence of high protein content in purple wheat. Carbohydrate (total soluble sugar, reducing

sugar, non- reducing sugar and starch) content was found higher in all purple wheat- based products except starch (Table 3). Total minerals

**Table 3.** Proximate and carbohydrate composition of biscuits (% on dry matter basis)

Parameters	Wheat (control)	Purple wheat	t-value
Moisture	2.61 ± 0.83	2.07 ± 0.17	1.10NS
Crude Protein	11.04 ± 1.26	13.17 ± 0.22	6.67**
Ash	1.32 ± 0.1	1.80 ± 0.02	8.15*
Fat	18.14 ± 0.56	18.84 ± 0.28	3.87*
Crude fiber	3.03 ± 0.6	4.68 ± 0.6	6.46**
Total Soluble Sugar	25.57 ± 0.59	31.12 ± 0.84	18.73**
Reducing Sugar	1.08 ± 0.27	2.28 ± 0.51	7.2**
Non- Reducing Sugar	24.49 ± 0.82	28.84 ± 1.12	10.86**
Starch	34.52 ± 1.21	31.94 ± 1.13	5.40**

NS= non- significant (there was no difference between variables). \*=significant difference at 5% level of significance. \*\*=significant difference at 1% level of significance. Values are mean ± SD of three independent determinations

**Table 4.** Mineral profile and digestibility of macronutrients of biscuits (on dry matter basis)

Products	Wheat (control)	Purple wheat	t-value
Iron (mg 100 g <sup>-1</sup> )	4.39 ± 1.23	6.58 ± 0.24	6.05**
Calcium (mg 100 <sup>-1</sup> g)	31.74 ± 0.62	53.18 ± 0.93	66.45**
Zinc (mg 100 <sup>-1</sup> g)	3.28 ± 0.19	4.45 ± 0.38	9.54**
Available iron (mg 100 <sup>-1</sup> g)	1.07 ± 0.31 (24.37)	1.78 ± 0.47 (27.05)	4.37*
Available calcium (mg 100 <sup>-1</sup> g)	13.85 ± 0.61 (43.63)	28.26 ± 0.67 (53.14)	55.10**
Available zinc (mg 100 <sup>-1</sup> g)	0.89 ± 0.21 (27.22)	1.72 ± 0.96 (38.70)	2.92*
Protein digestibility %	75.00 ± 2.89	82.57 ± 2.71	6.62**
Starch digestibility (mg maltose released g <sup>-1</sup> )	34.92 ± 2.17	40.98 ± 3.05	5.61**

\*=significant difference at 5% level of significance. \*\*=significant difference at 1% level of significance. Values are mean ± SD of three independent determinations

(iron, calcium and zinc) and available minerals (iron, calcium and zinc) was reported higher in all purple wheat-based products than control (Table 4). In vitro digestibility of protein and total lysine content were recorded higher in all products prepared from purple wheat as compared to normal wheat-based products. Starch digestibility and phytic acid content were noticed lower in all purple wheat-based products as compared to wheat-based products (Table 4-5). Total lysine content higher in all products prepared from purple wheat might be due to high protein content present in purple wheat. Availability of minerals and protein digestion were also high in all purple wheat prepared products due to 79 less phytic acid content present in purple wheat. Carbohydrate (total soluble sugar, reducing sugar and non-reducing sugar and starch) contents were recorded high in all purple wheat products except starch because purple wheat contained less starch amount than normal wheat.

Cooking's influence on the antioxidant activity has been described by many authors. Results of present study are closely related with study of other workers Li *et al.*, (2015), Pasqualone *et al.*, (2015), Yu and Beta (2015) and Parizad *et al.*, (2020). DPPH radical scavenging activity and TPC content reported higher in purple wheatbased biscuit than conventional due to higher bioactive compound present in purple wheat Pasqualone *et al.*, (2015). Antioxidant activity was reported lower in products than flour might be due to loss of antioxidant while mixing and kneading process was done and in this study noodle preparation was done Li *et al.*, (2015). In the present study total flavonoid content in all products was recorded lower than flour. This loss might be occurred due to the breakdown of flavonoid during heating or extraction of glycosides by steam (Li *et al.*, (2015). High anthocyanin content was observed in purple wheat-based products whereas in normal wheat-based

**Table 5.** Phytic acid, total lysine and antioxidant profile of biscuits

Products	Wheat (control)	Purple wheat	t-value
Phytic acid (mg 100 <sup>-1</sup> g)	129.58 ± 1.2	97.71 ± 1.6	55.20**
Total lysine (g 16 <sup>-1</sup> gN)	2.33 ± 0.29	4.24 ± 0.23	8.94*
DPPH (mg TE 100 <sup>-1</sup> g)	25.93 ± 1.13	65.64 ± 1.08	88.00**
TPC (mg GAE 100 <sup>-1</sup> g)	102.04 ± 1.28	125.4 ± 1.36	43.33**
TFC (mg RE 100 <sup>-1</sup> g)	31.07 ± 1.2	66.19 ± 1.41	65.71**
TAC (mg C3GE 100 <sup>-1</sup> g)	0.29± 0.02	3.01 ± 0.09	78.25**

\*\*=significant difference at 1% level of significance. TPC: Total Phenolic Content; TFC: Total Flavonoid Content; TAC: Total anthocyanin content. Values are mean ± SD of three independent determinations

products, lower anthocyanin content was found. Similar results were reported by other workers, Pasqualone *et al.*, (2015). Anthocyanin content is water soluble, high heat, temperature and light sensitive. Anthocyanin content was decreased might be due to higher degree of hydroxylation and methoxylation of aglycon. In biscuit, TAC content was decreased than raw wheat might be due to diluting effect of other ingredients and combined effect of light, air and thermal treatment. TAC might be affected by oxidative enzyme such as polyphenol oxidase Pasqualone *et al.*, (2015). The antioxidant activity of biscuits made from purple wheat was increased by more than 15 percent (Pasqualone *et al.*, (2015). Most of the research studies have shown that, while anthocyanin concentration decreases during heat treatment and product preparation, antioxidant activity increases or decreases far less than anthocyanin content decreases. This might be due to an increase in overall phenolic content, or it could be because the breakdown product of anthocyanins after heating and has stronger antioxidant activity than the coloured and glycosylated forms (Kumari *et al.*, 2020).

## Conclusion

Considering the nutritional and antioxidant profiles of purple wheat, as well as the sensory and physical characteristics of the developed product, it is possible to conclude that purple wheat can be successfully used to develop nutritious and appealing biscuits with the antioxidant power of large quantity of blueberries without sacrificing sensory attributes of final product. Purple wheat biscuits had higher levels of protein, dietary fibre, antioxidant and minerals and also enhanced availability and digestibility of nutrient. Developed biscuits may be beneficial in the treatment of oxidative stress due to its high antioxidant content and it will also add diversity due to its unique colour. Further, food products developed with purple wheat may offer health benefits due to its high antioxidant activity however; further research should be conducted to analyze its retention and bioavailability during high pressure high temperature cooking.

## Acknowledgements

Authors would like to acknowledge the financial help received from CCSHAU, Hisar as PG scholarship throughout the research, we wish to acknowledge laboratory of experiential learning and Food Analysis, Department of Foods and Nutrition, CCS, Haryana Agricultural University, Hisar.

## Conflict of interest

The authors declare that there are no conflicts of interest pertinent to this article.

## References

- AACC. 1984. Approved methods of analysis. The American Association of Cereal Chemists, St. Paul Minnesota.
- AACC. 2000 Approved methods of American Association of Cereal Chemists. St Paul, MN: Am. Assoc. Cereal Chem Inc.
- Abdel-Aal, E. S. M., and Rabalski, I. 2013. Effect of baking on free and bound phenolic acids in wholegrain bakery products. *Journal of Cereal Science*. 57(3): 312-318.
- Abdel-Aal, P., and Hucl, E. S. M. 1999. A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheat. *Cereal Chemistry Journal*, 76, 350– 354.
- Abdel-Aal, M., Hucl, P. and Rabalski, I. 2018. Compositional and antioxidant properties of anthocyanin-rich products prepared from purple wheat. *Food Chemistry*. 1-30.
- Abdel-Aal, Y. C. J. and Rabalski, I. 2006. Anthocyanin composition in black, blue, pink, purple and red cereal grains. *Journal of Agricultural and Food Chemistry*. 54 (13): 4696-4704.
- Akkafu, A. F., Koffi, M. D., Cisse, M. and Niamke, L. S. 2018. Physicochemical and functional properties of flours from three purple maize varieties named Voilet de Katiola in cote d' Ivoire. *Asian Food Science Journal*. 4(4): 1-10.
- Anand, K., Sharma, P. and Chaturvedi, N. 2013. A comparative study of varietal variations on nutritional quality of wheat malt and sensory attributes of prepared non-conventional malted beverages. *Beverage Food World*, 40(2), 34-36.
- AOAC. 2010. Official Methods of Analysis of Association of Official Analytic Chemists. Arlyngton, Virginia, U.S.A.
- Arts, I. C. and Hollman, P. C. 2005. Polyphenols and disease risk in epidemiologic studies. *American Journal of Clinical Nutrition*. 81(1): 317-325.
- Balsubramaniam, T. and Sadasivam, S. 1987. *Plant Foods Hum. Nutr.* 37, p. 41.
- Barczak, B. 2005. Acylated anthocyanins as stable natural food colorants. A review. *Polish Journal of Food and Nutrition Sciences*. 55:107-116.
- Bartl, P., Albrecht, A., Skrt, M., Tremlova, B., Ostadalova, M., Smejkal, K., Vovk, I. and Ulrih, P, N. 2015. Anthocyanin in purple and blue wheat grains and in resulting bread: quantity, composition, and thermal stability. *International journal of Food Science and Nutrition*. 66 (5): 514-519.
- Barrera, G. N., Pérez, G. T., Ribotta, P. D., León, A. L. 2007. Influence of damaged starch on cookie and bread-making quality, *European Food Research Technology*. 225: pp. 1-7.
- Brand-Williams, W. Cuvelier, M. E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology*, 28(1), 25-30.
- Braun, H, J., Atlin, G. and Payne, T. 2010. Multi-location



- testing as tool to identify plant response to global climate change. In *Climate Change and Crop production*. Volume 13, pp. 115-138.
- Burdulis, D., Sarkinas, A., Jasutiene, I., Stackevicene, E., Nikolajevs, L. and Janulis, V. 2009. Comparative study of anthocyanin composition, antimicrobial and antioxidant activity in bilberry (*Vaccinium myrtillus* L.) and blueberry (*Vaccinium corymbosum* L.) fruits. *Acta Pol. Pharm.* 66: 399-408.
- Buresova, I., Trojan, V. and Helis, M. 2019. Characteristics of flour and dough from purple and blue wheat grain. *Slovak Journal of Food Sciences*. Volume. 13, pp. 163-166.
- Cabrita, L., Fossen, T. and Andersen O. M. 2000. Colour and stability of the six common anthocyanin 3-glucosides in aqueous solutions. *Food Chemistry*. 68: 101-107.
- Calderaro, A., Barreca, D., Bellocco, E., Smeriglio, E., Trombetta, D. and Lagana, D. 2019. Colored phytonutrients: Role and applications in the functional foods of anthocyanins. In *Phytonutrients in Food*, 1st ed., pp. 177-195.
- Cerning, J. and Guillhot, J. 1973. Changes in carbohydrate composition during maturation of wheat and barley kernel. *Cereal Chem.* 50: 220-222.
- Chandra, S., Singh, S. and Kumari, D. 2015. Evaluation of functional properties of composite flours and sensorial attributes of composite flour biscuits. *Journal of Food Science and Technology*. 52: 3681-3688.
- Chen, W., Muller, D., Richling, E. and Wink, M. 2013. Anthocyanin-rich purple wheat prolongs the life span of *Caenorhabditis elegans* probably by activating the DAF-16/FOXO transcription factor. *Journal of Agricultural and Food Chemistry*. 61(12): 3047-3053.
- Cisowska, A., Wojnicz, D. and Hendrich, B. A. 2011. Anthocyanin as antimicrobial agents of natural plant origin. *Nat. Prod. Commu.* 6: 149-156.
- Davies, N. T. and Reid, H., 1979. An evaluation of the phytate, zinc, copper, iron and manganese contents of, and Zn availability from, soya-based textured-vegetable-protein meat-substitutes or meat extenders. *British Journal of Nutrition*, (41) 579-589.
- Du, S., Jiang, H., Yu, X. and Jane, J. 2014. Physicochemical and functional properties of whole legume flour. *LWT - Food Science and Technology*. 55: 308-313.
- Dziki, D., Rozylo, R., Gawlik-Dziki, U. and Swieca, M. 2014. Current trends in the enhancement of antioxidant activity of wheat bread by the addition of plant materials rich in phenolic compounds. *Trends in Food Science and Technology*. 40(1): 48-61.
- Eliasova, M., Kotikova, Z., Lachman, J., Orsak, M. and Martinek, P. 2020. Influence of baking on anthocyanin content in colored-grain wheat bread. *Plant, Soil and Environment*, 66(8): 381-386.
- Fang, Y. Z., Yang S., and Wu, G. 2002. Free radicals, antioxidants and nutrition. *Nutrition*. 18: 872-879.
- Ficco, M, B, D., Simone, De, V., Leonardis, De, M, A., Giovanniello, V., Nobile, D, A, M., Padalino, L., Borrelli, M, G. and Vita, De, P. 2016. Use of purple durum wheat to produce naturally functional fresh and dry pasta. *Food Chemistry*. 205: 187-195.
- Gamel, T, H., Wright, J, A., Pickard, M. and Abdel-Aal, M. 2020. Characterization of anthocyanin-containing purple wheat prototype products as functional foods with potential health benefits. *Cereal Chemistry*. 97(1): 34-38.
- Gani, A., Wani, S, M., Masoodi, F, A. and Gousia, H. 2012. Whole-grain cereal bioactive compounds and their health benefits: a review. *Journal of Food Process and Technology*. 3: 146-155.
- Giusti, M. M. and Wrolstad R. E. 2003. Acylated anthocyanins from edible sources and their applications in food systems. *Biochemical Engineering Journal*. 14: 217-225.
- Grausgruber, H., Atzegersdorfer, K., Bohmdorfer, S. 2018. Purple and blue wheat- health- promoting grains with increased antioxidant activity. *Cereal Foods World*. 63: 217-220.
- Guo, Z., Zhang, Z., Xu, P. and Guo, N. Y. 2013. Analysis of nutrient composition of purple wheat. *Cereal Research Communications*. 41(2): 293-303.
- Havrlentova, M., Psenakova, I., Zofajova, A., Ruckschloss, L. and Kraic, J. 2014. Anthocyanins in wheat seed, a mini review. *Nova biotechnological et Chimica*. 13: 1-10.
- Ibanoglu, E. 2002. Kinetic study of colour changes in wheat germ due to heat. *Journal of Food Engineering*. 51: 209-213.
- Ihegwuagu, N. E., Omojola, M. O., Emeje, M. O. and Kunle, O. O. 2009. Isolation and evaluation of some physicochemical properties of *Parkia biglobosa* starch. *Pure and Applied Chemistry*. 81: 97-104.
- Iyer, I. and Singh, U. 1997. Functional properties of wheat and chickpea composite flours. *Food Australia*, 49(1), 27-31.
- Jaafar, S. N. S., Baron, J., Ehx, S. S., Rosenal, T., Bohmdorfer, S. and Grausgruber, H. 2013. Increased anthocyanin content in purple pericarp and blue aleurone wheat crosses. *Plant Breeding*. 132(6): 546-552.

- Janeckova, M., Hrivna, L., Juzl, M., Nedomova, S., Vyhnanek, T., Trojan, V. and Mrkvicova, E. 2014. Possibilities of using purple wheat in producing bakery products. *Mendel Net.* 41: 412-416.
- Kassegn, H. H. 2017. Determination of proximate composition and bioactive compounds of Abyssinian purple wheat. *Cogent Food and Agriculture.* 4(1): 142-145.
- Kent, N. L. and Evers, A. D. 1994. *Technology of cereals*, Elsevier Science Ltd, UK.
- Kim, H. and Zemel, M. B. 1986. In vitro estimation of the potential bioavailability of calcium from sea mustard milk and spinach under stimulated normal and reduced gastric acid conditions. *J. Food Sci.* 51: 957-963.
- Klunklin, W. and Savage, G. 2018. Effect of substituting purple rice flour for wheat flour on physicochemical characteristics, in- vitro digestibilities and sensory evaluation of biscuit. *Journal of Food Quality.* 1-8.
- Knievel, D. C., Abdel-Aal, E. S. M., Rabalski, I., Nakamura, T. and Hucl, P. 2009. Grain colour development the inheritance of high anthocyanin blue aleurone and purple pericarp in spring wheat (*Triticumaestivum* L.). *Journal of Cereal Science.* 50: 113-120.
- Král, M., Pokorná, J., Tremlová, B., Oš ádalová, M., Trojan, V., Vyhnanek, T., Walczycka, M. 2018. Colored Wheat: Anthocyanin Content, Grain Firmness, Dough Properties, Bun Texture Profile. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 67(3): 685-690.
- Kumari, A., Sharma, S., Sharma, N., Chunduri, V., Kapoor, P., Kaur, S., Goyal, A. and Garg, M. 2020. Influence of biofortified colored wheats (purple, blue and black) on physicochemical, antioxidant and sensory characteristics of chapatti (Indian flatbread). *Molecules.* 25 (5071): 1-14.
- Li, Q., Yang, Q. and Beta, T. 2010. Comparison of antioxidant activities of different colored whet grains and analysis of phenolic compounds. *Journal of Agriculktural and Food Chemistry.* 58: 9235-9241.
- Li, Y., Ma, D., Sun, D., Wang, C., Zhang, J., Xie, Y. and Guo, T. 2015. Total phenolic, flavonoid content, and antioxidant activity of flour, noodles, and steamed bread made from different colored grains by three milling methods. *The Crop Journal.* 3: 328-334.
- Lia, D., Wanga, P., Luob, Y., Zhaoa, M. and Chena, F. 2017. Health benefits of anthocyanin and molecular mechanisms: update from recent decade. *Critical Reviews in Food Science and Nutrition.* 57: 1729-1741.
- Lindsey, W. L. and Norwell, M. A. 1969. A new DPTA\_TEA soil test for zinc and iron. *Agron. Abst.*
- Litvyak, V. 2018. Size and morphological features of native starch granules of different botanical origin, *Ukrainian Food Journal*, 7(4): pp. 563-576.
- Lutsey, P. L., Jacobs, D. R., Kori, S., Mayer-Davis, E., Shea, S. and Stefan, L. M. 2007. Whole grain intake and its cross-sectional association with obesity, insulin resistance, inflammation, diabetes and subclinical CVD: The MESA study. *British Journal of Nutrition.* 98: 397-405.
- Ma, D., Zhang, J., Li, Y. and Wang, C. 2018. Quality of noodles made from cloured- grained wheat. *Czech Journal of Food Science.* 36(4): 314-320.
- Mazzaracchio, P., Tozzi, S., Barbiroli, G., Kindl, M. and Pifferi, P. G. 2012. Adsorption behaviour of some anthocyanins by wheat gluten and its fractions in acidic conditions. *International Journal of Food Science Technology.* 47: 390-398.
- Mertz, E. T., Kiresis, A. W. and Sxtell, J. D. 1983. *In vitro* digestibility of protein in major food cereals.
- Morgounov, A., Karaduman, Y., Akin, B., Aydogan, S., Baenziger, S. P., Bhatta, M., Chudinov, V., Dreisigacker, S., Govindan, V., Guler, S., Guzman, C., Nehe, A., Poudel, R., Rose, D., Gordeeva, E., Shamanin, V., Subasi, K., Zelenskiy, Y. and Khlestkina, E. 2020. Yield and quality in purple- grained wheat isogenic lines. *Agronomy.* 10(86): 1-14.
- Nayak, B., Liu, R. H. and Tang, J. 2015. Effect of processing on phenolic antioxidant of fruits, vegetable, and grains- a review. *Critical Reviews in Food Science and Nutrition*, 55, 887-918.
- Padalino, L., Mastromatteo, M., Lecce, L., Cozzolino, F. and Del Nobile, M. A. 2013.
- Parizad, A. P., Marengo, M., Bonomi, F., Scarafoni, A., Cecchini, C., Pagani, A. M., Marti, A. and Iametti, S. 2020. *Foods.* 9 (163): 1-13.
- Pasqualone, A., Bianco, M. A., Paradiso, M. V., Summo, C., Gambacorta, G., Caponio, F. and Blanco, A. 2015. Production and characterization of functional biscuits obtained from purple wheat. *Food Chemistry* 180: 64-70.
- Patel, K. K., Jain, A. and Patel, D. K. 2013. Medicinal significance, pharmacological activities and analytical aspects of anthocyanins 'delphinidin': A concise report. *J. Acute Dis.* 2(3): 169-178.
- Rao, B. S. N. and Prabhavathi, T. 1978. An in vitro method for predicting the bioavailability of iron from foods. *Am. J. Clin. Nutr.* 31:169.
- Rosario, R. R. and Flores, D. M. 1981. Functional properties of four types of mung bean flour. *Journal of Science and Food Agriculture*, 32, 175.

- Sachdeva, R. and Punia, D. 2013. Nutritional evaluation and development of products from wheat. M.Sc. Thesis, CCS Haryana Agricultural University, Hisar, India.
- Saini, P., Kumar, N., Kumar, S., Mwaurah, W. P., Panghal, A., Attkan, K. A., Singh, K. V., Garg, K. M. and Singh, V. 2020. Bioactive compounds, nutritional benefits and food applications of colored wheat: a comprehensive review. *Critical Reviews in Food Science and Nutrition*. 1-14.
- Sathe, S. K. and Salukhe, D. K. 1981. Functional properties of the great northern bean. protein emulsion, foaming, viscosity and gelation properties. *Journal of Food Science*, 46, 71-76.
- Seo, Y., Moon, Y. and Kweon, M. 2021. Effect of purple wheat bran addition on quality and antioxidant property of bread and optimization of bread- making conditions. *Appl. Sci.* 11, 4034: 1-18.
- Sharma, N. S. 2019. Has India found the answer to two of its most pressing lifestyle problems? <https://economictimes.indiatimes.com/news/economy/agriculture/has-india-found-the-answer-to-two-of-its-most-pressing-lifestyle-problems/articleshow/69612827.cms?from=mdr>
- Sharma, N., Tiwari, V., Vats, S., Kumari, A., Chunduri, V., Kaur, S., Kapoor, P. and Garg, M. 2020. Evaluation of anthocyanin content, antioxidant potential and antimicrobial activity of black, purple and blue colored wheat flour and wheat- grass juice against common human pathogens. *Molecules*. 25(5785): 1-19.
- Sharma, S., Chunduri, V., Kumar, A., Kumar, R., Khare, P., Kondepudi, K. K., Bishnoi, M. and Garg, M. 2018. Anthocyanin bio- fortified coloured wheat: Nutritional and functional characterization. *PLoS ONE* 13(4): e0194367.
- Singh, U. and Singh, B. 1991. Functional properties of sorghum-peanut composite flour. *Cereal Chem.* 68(5): 460-463.
- Singh, U., Khedekar, M. S. and Jambunathan, R. 1982. Studies on desi, kabuli, and chickpea cultivars. The level of amylase inhibitors, level of oligosaccharides and in vitro starch digestibility. *J. Food Sci.* 47: 510.
- Singleton, V. L. and Rossi, J. A. 1999. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, 16: 144-158.
- Somogyi, M. 1945. A new reagent for the determination of sugar. *J. Biol. Chem.* 160: 160-161.
- Syter, O., Bosko, P., Zivcak, M., Brestic, M. and Smetanska, I. 2018. Bioactive phytochemicals and antioxidant properties of the grains and sprouts of colored wheat genotypes. *Molecules*. 23 (2282): 1-14.
- Tadhani, M. B., Patel, V. H. and Subash, R. 2009. *In vitro* antioxidant activities of *Stevia rebaudiana*.
- Tian, S. Q., Chen, Z. C. and Qiao, Y. F. 2017. Analysis of main physicochemical parameters in purple wheat with different milling technology. *Journal of Food Processing Preservation*. 13382. <https://doi.org/10.1111/jfpp.13382>.
- Tian, S, Q., Chen, C, Z. and Wei, C, Y. 2018. Measurement of color- grained wheat nutrient compounds and the application of combination technology in dough. *Journal of Cereal Science*. 83: 63-67.
- Usenko, I. N., Khlestkina, K. E., Asavasnti, S., Gordeeva, I. E., Yudina, S. R. and Otmakhova, S. Y. 2018. Possibilities of enriching food products with anthocyanins by using new form of cereals. *Foods and Raw Materials*. Vol.6. no.1: 128-136.
- Uttara, B., Singh, V. A., Zamboni, P. and Mahajan, T. R. 2009. Oxidative stress and neurodegenerative disease: A review of upstream and downstream antioxidant therapeutic options. *Current Neuropharmacology*. 7: 65-74.
- Vetrimani, R. and Rahim, A. 1994. Effect of drying of vermicelli in hot air oven on its cooking quality. *Journal of Food Science and Technology*. 31: 400-403.
- Xiangyu Bai, Shuangpan Yang, Li Zeng, Wei Han and Xu Ran. 2021. Study on physicochemical properties of purple waxy wheat starch, *International Journal of Food Properties*, 24:1, 471-481, DOI: 10.1080/10942912.2021.1901732
- Yemm, E. W. and Willis, A. J. 1954. The estimation of carbohydrates in plant extract by anthrone. *Biochem. J.* 57:508.
- Yu, L. and Beta, T. (2015). Identification and antioxidant properties of phenolic compounds during production of bread from purple wheat grain. *Molecules*. 20: 15525- 15549.
- Zanoletti, M., Parizad, A. P., Lavelli, V., Cecchini, C., Menesatti, P., Marti, A. and Pagani, A. 2016. Debranning of purple wheat: Recovery of anthocyanin-rich fractions and their use in pasta production. *Food Science and Technology*. 1-28.
- Zhishen, J., Mengcheng, T. and Jianming, W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64 (4): 555-559.
- Živan ev, D., Torbica, A., Mastilovi, J., Kneževi, D. and Djukic, N. 2012. Relation among different parameters of damaged starch content, falling number and mechanical damage level, *Ratarstvo i povrtarstvo*, 49: pp. 282-287.