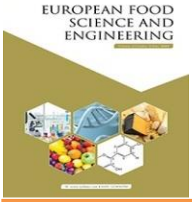

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Development of functional bran-enriched bread with acceptable physical and sensory properties: combination of selected high fiber and polyphenol brans with strong flour wheat genotypes

Çiğdem Mecitoğlu Güçbilmez[1*], Mehmet Şahin[1], Aysun Göçmen Akçacık[1], Seydi Aydoğan[1], Sümeyra Hamzaoğlu[1], Berat Demir[1], Sadi Gür[1], Musa Türköz[1], Enes Yakışır[1] and Ahmet Yemenicioğlu[2]

[1] Bahri Dağdaş International Agricultural Research Institute, General Directorate of Agricultural Research and Policy, Ministry of Agriculture and Forestry, Konya, Türkiye
[2] Department of Food Engineering, Faculty of Engineering, İzmir Institute of Technology, İzmir, Türkiye

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ABSTRACT

The aim of this study was to obtain high-polyphenol and -fiber bread with acceptable physical and sensory properties by using brans and flours of selected red and white wheat genotypes. For this purpose, breads obtained from bran-enriched flours (% bran:wheat ratios: 10:90, 20:80, 30:70) of different red or white wheat were characterized for their polyphenol content, antioxidant activity (ABTS and DPPH methods), dietary fiber content, and physical (volume and weight, symmetry and crumb pore structure, crust and crumb color) and sensory properties for two succeeding harvesting years. The increase of bran content of flours up to 30% caused significant increases in total dietary fiber (3-fold), and phenolic content (1.6-fold) and antioxidant activity (2-fold) of breads. The antioxidant parameters and dietary fiber content of bread improved when flour and bran of red wheat genotypes were used instead of those of white wheat genotypes. The bran-enrichment reduced the physical quality parameters such as specific volume, pore structure and symmetry of obtained breads, but sensory properties (color, taste, odor, appearance, overall quality) of breads were acceptable even at 30% bran content. Combination of selected high-fiber and -polyphenol brans with strong flour wheat cultivars gives highly functional bread with acceptable quality.

1. Introduction

Wheat is the most important globally produced agricultural raw material since it is the number one source of flour, the most critical food ingredient important for human nutrition (Sarfaraz et al., 2017). Wheat bran is the main by-product formed during production of flour, but majority of this grinding fraction cannot be valorized sufficiently and used heavily as an animal feed ingredient (Rosa et al., 2013; Sarfaraz et al., 2017). The bran has a great potential as an ingredient of functional foods since it contains not only nutrients such as proteins, vitamins, and minerals, but also dietary fiber and bioactive phenolic compounds important for human health (Rosa et al., 2013; Sarfaraz et al., 2017). However, extensive efforts are needed to develop innovative methods of valorizing bran and exploiting its nutritional and functional components in development of functional foods.

The recent studies have showed that the intake of wheat bran could provide the highly functional dietary fiber that could make a great contribution to human health (Zhao et al., 2019; Ma et al., 2022). The fibers cannot be digested in the small intestine, but some of them (prebiotics) might be fermented fully or partially in the large intestine by the probiotic bacteria that could produce bioactive short chained fatty acids affecting immunity and cancer by mediating cytokine production and cell growth rate (Yemenicioğlu et al., 2020). Different studies in the literature have suggested that wheat bran dietary fiber might play essential roles in the prevention of colorectal cancer and some other diseases such as diabetes, cardiovascular diseases, and obesity (Zhao et al., 2019; Rudrapal et al., 2022). The bran is also very rich in antioxidant polyphenols, especially phenolic acids. In fact, it is the bran phenolic acids responsible for the antioxidant activity in different cereal products (Li et al., 2021). The main phenolic acid in wheat bran is ferulic acid (FA), but small amounts of vanillic, p-coumaric (p-CA), and caffeic acids (CA) also exist in this grinding fraction (Rosa et al. 2013). The frequent intake of natural antioxidants has been attracting a huge interest since these bioactive compounds might show different health benefits such as antimicrobial, antioxidant, and anti-inflammatory activity as well as preventive effects on

*Corresponding author
E-mail address: cigdemmecitoglu@hotmail.com

major diseases such as cancer, obesity and diabetes (Rudrapal et al., 2022). It is thought that the bran phenolic acids might play an important role in the anticarcinogenic activity of wheat products (Challacombe et al., 2012). Bouzaiene et al. (2015) noted that CA, p-CA or FA reduced cell adhesion and migration in critical processes involved in tumor metastasis. Besides potential health benefits, the enrichment of food with natural antioxidants also helps controlling of lipid oxidation and reducing need for synthetic antioxidants that cause great health concerns in the consumers (Li et al., 2021).

The enrichment of flour with bran is an effective way to increase dietary fiber and phenolic intake since such enriched flours could easily be involved in human diet in many different ways (e.g., consumption of bread, bun, cake, muffin, desserts, soups etc.) (Ma et al., 2022). The fortification of bread with bran is a very popular application since daily amount of bread consumed by many people could meet a significant portion of minimum recommended dietary fiber intake of 25 g/day (Dziki et al., 2014; Guiné et al., 2016). However, it is a well-known truth that the use of bran-enriched flours in bread production leads to significant sensory and organoleptic quality losses in breads (Hemdane et al., 2015). Therefore, it has been suggested that the bran-enriched bread manufacturers should use strong wheat flours to counteract the negative impacts of bran on bread quality (Hemdane et al., 2016).

Recently, our research group have performed a screening study to identify the outstanding wheat cultivars having brans with the richest fiber and polyphenol contents and flours with the best bread-making quality. In the current study, identified wheat cultivars having brans with the highest fiber and polyphenol contents, and flours with the strongest bread-making quality were combined (flours with 0, 10, 20, 30% bran were obtained) to develop functional bran-enriched breads. The bread samples obtained from wheat of 2 subsequent harvesting seasons were characterized for their dietary fiber, antioxidant capacity and polyphenol contents as well as physical and sensory properties to prove applicability of the developed strategy. This work is original firstly in that it is the first study showing the potential of local Turkish wheat cultivars for development of functional bran-enriched breads. Secondly, this is one of the rare studies showing the possibility of limiting negative impacts of added bran on physical properties of bread by using strong flour wheat genotypes.

2. Materials and Methods

2.1. Materials

The red-grained (Taner and Bezostaja 1) and white-grained (Tosunbey and Aliağa) registered commercial varieties widely produced in Turkey were cultivated at Bahri Dağdaş International Agricultural Research Institute during the 2018-2019 and 2019-2020 growing periods. Wheat varieties, having strong flours with good bread-making quality and brans with high phenolic content, antioxidant activity, and dietary fiber content were selected by a screening study made in the project funded by General Directorate of Agricultural Research and Policy, Turkey (TAGEM). In order to obtain bread with the best functional and physical properties, from the white wheat, Tosunbey was selected for its flour while Aliağa was selected for its bran. From red wheat, Taner was selected for its flour while Bezostaja 1 was selected for its bran. The flours and brans of specified varieties were mixed at different ratios given below and used in bread-making. 2,2-Difenil-1-pikrilhidrazil (DPPH), 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ferulic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA) while Folin-Ciocalteu was obtained from Merck (Darmstadt, Germany). Megazyme Total Dietary Fiber Assay kit, Celite® 545 was purchased from Megazyme (Wicklow, Ireland). All other solvents and chemicals were of reagent grade.

2.2. Preparation of flours, brans and bran-enriched flours

To obtain wheat flour and bran, wheat samples were tempered for 12 h to 14.5% moisture content according to the AACC 26-95 method (AACC, 2000) and milled to 82 mesh powder by using Brabender Quadrumat Junior (model 880101, Brabender Ohg Duisburg, Germany) according to the AACC 26-50 method (AACC, 2000). As part of our strategy (combination of selected high fiber and polyphenol brans with strong flour wheat genotypes) the bran-enriched white wheat flours ($WWF_{10:90}$, $WWF_{20:80}$, $WWF_{30:70}$) were obtained by mixing different amounts of bran from Aliağa white wheat genotype with flours of Tosunbey white wheat genotype (% bran:wheat ratios: 10:90, 20:80, 30:70). The flour of Tosunbey was used as control white wheat flour (WWF_{ctrl}). On the other hand, the bran-enriched red wheat flours ($RWF_{10:90}$, $RWF_{20:80}$, $RWF_{30:70}$) were prepared by mixing different amounts (similar bran:wheat ratios with white wheat) of bran from Bezostaja-1 red wheat genotype with flours of Taner red wheat genotype. The flour of Taner was used as control red wheat flour (RWF_{ctrl}).

2.3. Preparation of doughs and bread making

The dough preparation and baking were performed according to AACC method 10-10B (AACC, 2000) using obtained flours with slight modifications. For this purpose, on the basis of 100 g flour, 1.5% salt, 3.0% yeast, and then amount of water determined by farinograph (cc) were added and kneaded with a mixer (KitchenAid, model 5K45SS, USA) until a mature dough was formed. The dough bulk fermentation was carried out twice, for 30 min, in a fermentation cabinet at 30 ºC and 70-80% relative humidity. Doughs were punched and molded after these periods. After fermentation at 55 °C for another 30 min, baking was conducted in an air-convection oven (Enkomak, MD 45 FC, Konya, Türkiye) at 230 ºC for 15-20 min. The breads of flours defined in section 2.2 were named with the same principle only by modifying WWF or RWF as WWB and RWB, respectively (WWB_{ctrl}, $WWB_{10:90}$, $WWB_{20:80}$, $WWB_{30:70}$, RWB_{ctrl}, $RWB_{10:90}$, $RWB_{20:80}$, $RWB_{30:70}$).

2.4. Gluten content and water binding capacity of bran-enriched wheat flour mixtures

Different bran-enriched wheat flours (0, 10, 20, 30% bran in flour, w/w) used ratios for bread making were analyzed for wet gluten content, dry gluten content, and water binding capacity according to the standard AACC method 38-12A (AACC, 2000) using Glutomatic (Bastak, Ankara, Turkey).

2.5. Antioxidant activity of doughs and breads

The extracts of dough and bread used in analysis of antioxidant activity were prepared as follows: The slices of bread and thin-layered dough pieces kept at room temperature after baking were ground before analysis using a grain grinder (model HC-200, P.R.C) and mill (Retsch, model ZM200, Retsch GmbH Haan, Germany). 5 grams of bread or dough sample was extracted with 20 mL of 70% ethanol (v/v) at ambient temperature for 15 h using Orbital Shaker (model OS-20, Germany). The extract was then filtered through Whatman no:1 filter paper (Abozed et al., 2014).

DPPH· scavenging activity

The DPPH· radical scavenging activity of dough and bread extracts was measured using a modified version of the Brand-Williams et al. (1995) method. The extracts (0.4 mL) were mixed with DPPH solution (4 mL) prepared in 6x10^{-5} mol/L methanol. The mixtures were kept in the dark for 30 min at ambient conditions, centrifuged at 6000 rpm for 5 min, and their absorbance were read at 515 nm. FA was used as a standard and results were given as the concentration of extracts that quench 50% of DPPH radicals in the reaction mixture (IC_{50} value). These analyses were performed in duplicate for two replications.

ABTS·+ scavenging activity

The Trolox equivalent antioxidant capacity (TEAC) of dough and bread extracts was also determined spectrophotometrically according to the method of Re et al. (1999). 7 mM ABTS solution containing 2.45 mM potassium persulfate was prepared and kept in the dark for 16 h to form ABTS radical cation ($ABTS^{\cdot+}$). The $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.700 at 734 nm. The extracts of samples (25 µL) were mixed with ABTS radical solution (2 mL) and their absorbances were measured at 734 nm after 10-minute reaction. The % inhibitions of $ABTS^{\cdot+}$ radical solution were expressed as µmol Trolox equivalents per g of bread (or dough) dry weight. The analyses were performed in duplicate for two replications.

2.6. Total phenolic content of breads

The total phenolic content (TPC) in bread extracts was determined spectrophotometrically (Shimadzu, Model UV-1601, Japan) according to the Folin-Ciocaltaeu method given by Singleton & Rossi (1965) using gallic as a standard. The TPC was calculated and expressed as mg GA equivalents per kg of bread dry weight. The analyses were conducted in duplicate for two replications.

2.7. Total dietary fiber contents of breads

The Megazyme Total Dietary Fiber Assay Kit, which was developed based on the AACC 32-05.01 (AACC, 2000) and AOAC 985.29 (AOAC, 1986) methods, was used to determine the total dietary fiber content of bread samples. The samples were prepared for the analysis and measured according to the product manual.

2.8. Physical analysis of breads

The volume and weight measurements of the breads were carried out to calculate their specific volume. Bread volume was determined by the rapeseed displacement method, according to AACC 10-05.01 (AACC, 2000). After one hour from baking, the breads were sealed in polyethylene bags. After 24 h, symmetry and crumb pore structure were evaluated by scoring (0-10) (Elgün et al., 2014). The crust and crumb color of the breads were determined in terms of L (for lightness), a (for redness), and b (for yellowness) values using the Hunter Lab Color device (MiniScan XE Plus, Model 45/0-L, USA).

2.9. Sensory evaluation of breads

The sensory assessment was conducted with bread samples within 24 h after baking. Bread quality was evaluated with a hedonic scale of five-point in terms of taste, odor, color, appearance, and general appreciate (5 Points: Very Good, 4 Points: Good, 3 Points: Acceptable, 2 Points: Not Sufficient, 1 Point: Bad) (Chen et al., 1996). Fifteen assessors composed of experienced people aged between 18 and 57 years participated in the organoleptic evaluation.

2.10. Statistical analysis

Statistical analyses were performed with Student's t-test in JMP11 (2014) program (SAS Institute, ISBN: 978-1-62959-560-3), and differences were considered significant if $P<0.01$.

3. Results and Discussion

3.1. Antioxidant capacity and dietary fiber content of selected brans

The brans of white and red wheat genotypes, Aliağa and Bezostaja-1, were employed in the current work since they were among the brans having the highest antioxidant potential and dietary fiber contents in our recent screening project (TAGEM 2020). The average antioxidant parameters (IC_{50}, TEAC, TPC) and dietary fiber contents of brans from Aliağa and Bezostaja-1 genotypes grown at two succeeding seasons are seen in Table 1. The antioxidant parameters and dietary fiber contents of each bran did not show a significant variation originating from seasonal differences. Nocente et al. (2019) found no significant effect of cropping year for total phenols in durum wheat. No significant differences were also determined between TPC, IC_{50}, and TEAC values of red and white wheat bran used in this study. Babu et al. (2018) reported that there was no statistically significant variation in total phenolic content or antioxidant activity between red and white wheat varieties. The average dietary fiber contents of red and white wheat brans were also not considerably different, but brans of red wheat genotype contained slightly higher dietary fiber contents than brans of white wheat genotype at both seasons.

3.2. Effect of bran-enrichment on gluten content and water binding capacity of flours

The flours of red and white wheat genotypes, Taner and Tosunbey, known for their good bread-making quality were employed in the current study. The effects of enriching these flours with different amounts of bran on wet and dry gluten content and water binding capacity of obtained bran-enriched flours are seen in Table 2. In both red and white wheat flours, addition of bran at different ratios (% bran:wheat ratios: 10:90, 20:80, 30:70) caused a concentration dependent significant reduction in wet and dry gluten content of bran-enriched flours. This finding is compatible with previous studies that also reported decrease of wet gluten content of bran-enriched flours (Kaprelyants et al., 2013; Lee et al., 2020).

Table 1. Functional and physical properties of brans used in breads.

Genotype	Bran						
	Phenolic content (mg GA/kg bran dm)	IC_{50} (mg bran dm/mL)	TEAC (µmol Trolox/g bran dm)	Dietary fiber (% dm)	L	a	b
Bezostaja 1	1647±205	5.46±1.46	10.38±2.59	52.56±4.79	60.85±2.07^{b}	7.62±0.58^{a}	15.00±1.03^{b}
Aliağa	1549±126	6.47±1.33	10.96±0.83	49.06±3.03	69.15±0.71^{a}	5.98±0.53^{b}	17.01±1.26^{a}
LSD	300.01	3.49	3.55	13.33	4.88	1.14	1.00
CV (%)	5.72	9.97	18.13	7.99	2.29	5.13	1.89
Significance	ns	ns	ns	ns	**	**	**

a-b: Values in the same column with different superscripts indicate a statistically significant difference.
IC_{50}: is the concentration of bran extracts to quench 50% of DPPH radicals in the reaction mixture (IC_{50} ferulic acid: 0.00784 mg/mL); TEAC: Trolox equivalent antioxidant capacity; GA: Gallic acid; dm: Dry matter; L: lightness; a: redness; b: yellowness; CV: coefficient of variation; LSD: least significant differences; **: indicate significance at the level of 0.01; ns: not significant

Table 2. Average properties of white (Tosunbey, Aliağa) and red (Taner, Bezostaja 1) wheat flour blends for 2 succeeding years.

Genotypes		Bran ratio (%)	Flour blend		
			Wet gluten content (%)	Dry gluten content (%)	Water binding capacity (%)
Flour	Bran				
Taner	Bezostaja 1	0	37.13±1.25^{a}	12.73±0.37^{a}	24.40±1.57^{a}
Taner	Bezostaja 1	10	33.35±2.81^{b}	11.43±1.29^{bc}	21.92±1.57^{b}
Taner	Bezostaja 1	20	29.83±3.07^{c}	9.71±0.50^{d}	20.12±2.65^{c}
Taner	Bezostaja 1	30	23.70±0.80^{e}	7.48±0.46^{e}	16.22±0.67^{d}
Tosunbey	Aliağa	0	34.10±2.16^{b}	12.04±1.06^{ab}	22.06±1.18^{b}
Tosunbey	Aliağa	10	31.00±1.06^{c}	10.8±0.42^{c}	20.20±0.71^{c}
Tosunbey	Aliağa	20	25.38±1.70^{d}	9.10±1.24^{d}	16.28±0.50^{d}
Tosunbey	Aliağa	30	21.55±1.66^{f}	7.28±0.56^{e}	14.27±1.21^{e}
MV (Taner-Bezostaja 1)			31.00	10.33	20.67
MV (Tosunbey-Aliağa)			28.00	9.81	18.20
LSD			1.23	0.72	0.97
CV %			2.76	4.76	2.34
Significance			**	**	**

a-f: Values in the same column with different superscripts indicate a statistically significant difference.
MV: mean value; CV: coefficient of variation; LSD: least significant differences; **: indicate significance at the level of 0.01

In flours of red and white wheat genotypes, the bran-enrichment at 20 and 30% caused almost 1.2-1.3 and 1.6-1.8-fold reduction in wet and dry gluten contents, respectively. There were no significant differences between the dry gluten contents of bran-enriched red and white wheat flours at similar bran contents (P>0.01). However, the final wet gluten contents of bran-enriched flours of red wheat genotype are significantly higher than that of bran-enriched flours of white wheat genotype at similar bran ratios (P<0.01). Moreover, the bran-enrichment also caused a concentration dependent reduction in water binding capacity of red and white flours.

3.3. Effect of bran-enrichment on antioxidant capacity of doughs

The effect of bran-enrichment on IC_{50} and TEAC based antioxidant capacity of obtained doughs are seen in Figure 1a and Figure 1b. Although doughs of both control (non-enriched by bran) and bran-enriched red wheat flours showed significantly lower IC_{50} value than doughs of control and bran-enriched white wheat flours, both doughs of different wheat types showed similar TEAC values. The IC_{50} values previously determined for red and white wheat brans against DPPH radical were similar. Thus, it appears that the different IC_{50} of doughs obtained from bran-enriched flours could be originated mainly from variations in phenolic profiles of red and white wheat flours. The different responses of DPPH and ABTS in various extracts are originated mostly from different polyphenol compositions of samples. For example, Platzer et al. (2021) reported that some dihydrochalcones and flavanones show much better reactivity against ABTS than DPPH free radical. However, in the current study, the doughs of bran-enriched red flours contained some polyphenols that are more reactive against DPPH than polyphenols in bran-enriched white wheat doughs.

On the other hand, the addition of bran increased the TEAC, but reduced the IC_{50} of both red and white wheat doughs at a concentration dependent manner. This finding clearly proved the possibility of increasing antioxidant capacity of doughs by bran-enrichment. The bran-enrichment of red and white wheat flours at 10, 20 or 30% caused almost 1.4, 1.9 and 2.8-fold, and 1.3, 1.8 and 2-fold reduction in IC_{50} of resulting red and white wheat doughs, respectively. In contrast, the similar specified changes in bran ratios of red and white wheat flours caused a more limited (1.2 to 1.3-fold) increase in TEAC than IC_{50} of resulting bran-enriched doughs. These findings clearly showed the better reactivity of DPPH free radical with bran and flour antioxidants in dough than ABTS free radical.

3.4. Effect of bran-enrichment on antioxidant capacity of breads

The effect of bran-enrichment of flours on IC_{50} and TEAC, and TPC of white and red wheat breads are seen in Figure 1c and Figure 1d, and Figure 2a respectively. The WWB_{ctrl} and

RWB_{ctrl}, and $WWB_{10:90}$ and $RWB_{10:90}$ showed similar TPC values. However, $RWB_{20:80}$ and $RWB_{30:70}$ showed significantly higher TPCs than $WWB_{20:80}$, and $WWB_{30:70}$, respectively. It is also important to note that all red wheat breads obtained with or without bran enrichment showed significantly higher TEAC and lower IC_{50} than respective white wheat breads. The results also clearly showed that the increase of bran ratio in flours caused a concentration dependent increase in TPC and resulting antioxidant parameters of both red and white breads. This finding is in agreement with those of Benítez et al. (2018) who also reported improved polyphenol content and antioxidant activity of breads by enriching flours with wheat bran and wheat fiber. In the current study, the enrichment of flours with 10 or 20% bran caused only 1.2 to 1.4-fold improvement in antioxidant parameters (reduction in IC_{50}, and increase in TPC and TEAC) of red and white wheat breads. However, bran-enrichment of flours at 30% caused the largest improvements in antioxidant parameters and lead to almost 1.6-fold increase in TPCs, 2.0 and 2.2-fold reductions in IC_{50} values, and 1.7- and 1.5-fold increases in TEACs of red and white wheat breads, respectively. Lee et al. (2020) stated that antioxidant activity in bread samples increased significantly with a higher bran substitution rate. Also, Benítez et al. (2018) reported that replacing refined wheat flour with wheat bran and wheat fiber improved antioxidant polyphenol contents and antioxidant activity in breads. These findings clearly show the importance of reaching the bran ratio of 30% to obtain a substantial increase in polyphenol content and resulting antioxidant activity in breads. Moreover, it is also clear that the use of bran-enriched flours and flours of red wheat genotypes in bread making are important tools to boost polyphenol content and antioxidant capacity of breads.

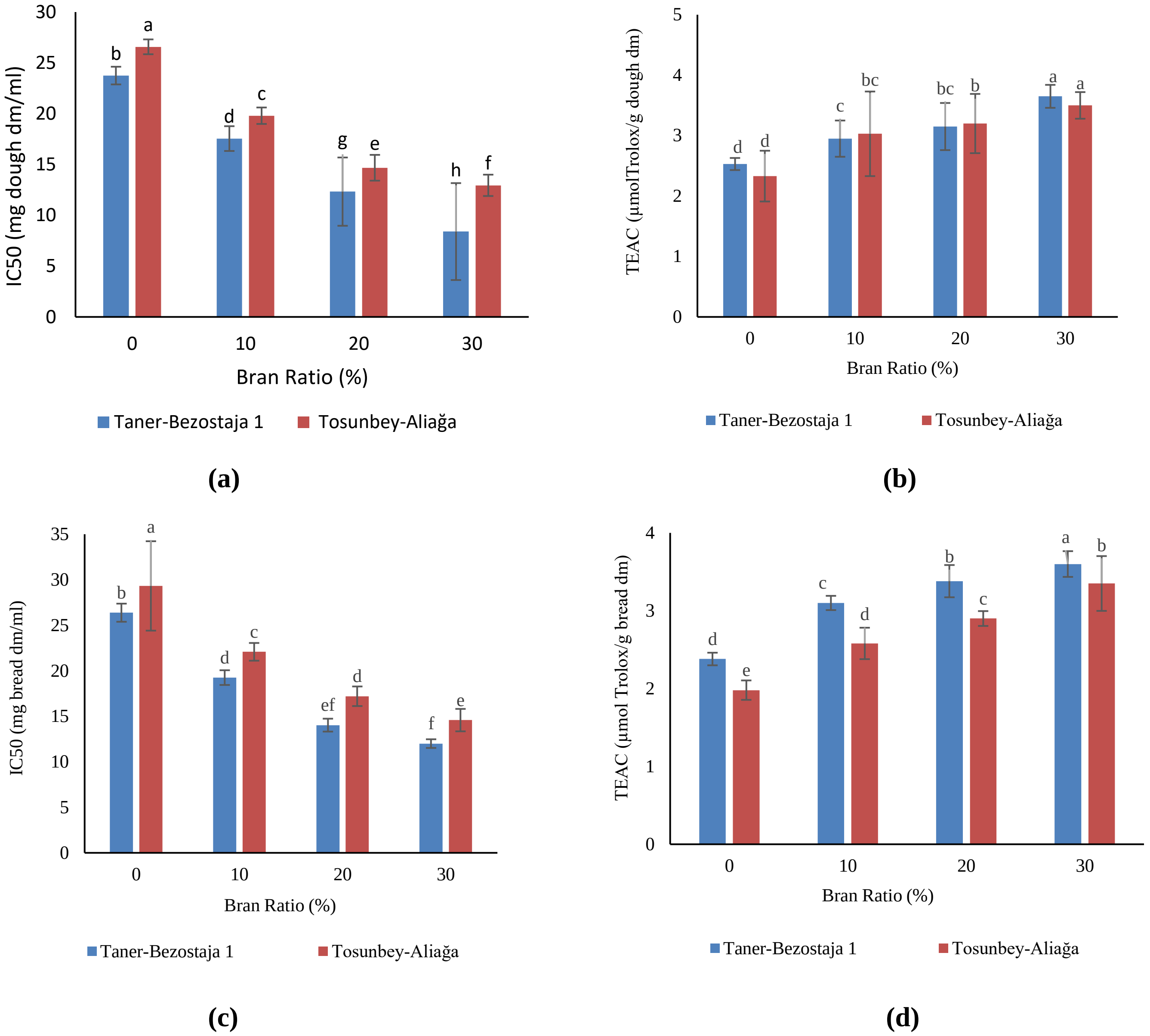

Figure 1. The effect of bran-enrichment of flours on IC_{50} and TEAC based antioxidant capacity of white and red wheat doughs (a, b) and breads (c, d). (Red wheat flour-bran: Taner-Bezostaja 1; White wheat flour-bran: Tosunbey-Aliağa.) Bars with different characters indicate a significant difference at $P<0.01$.

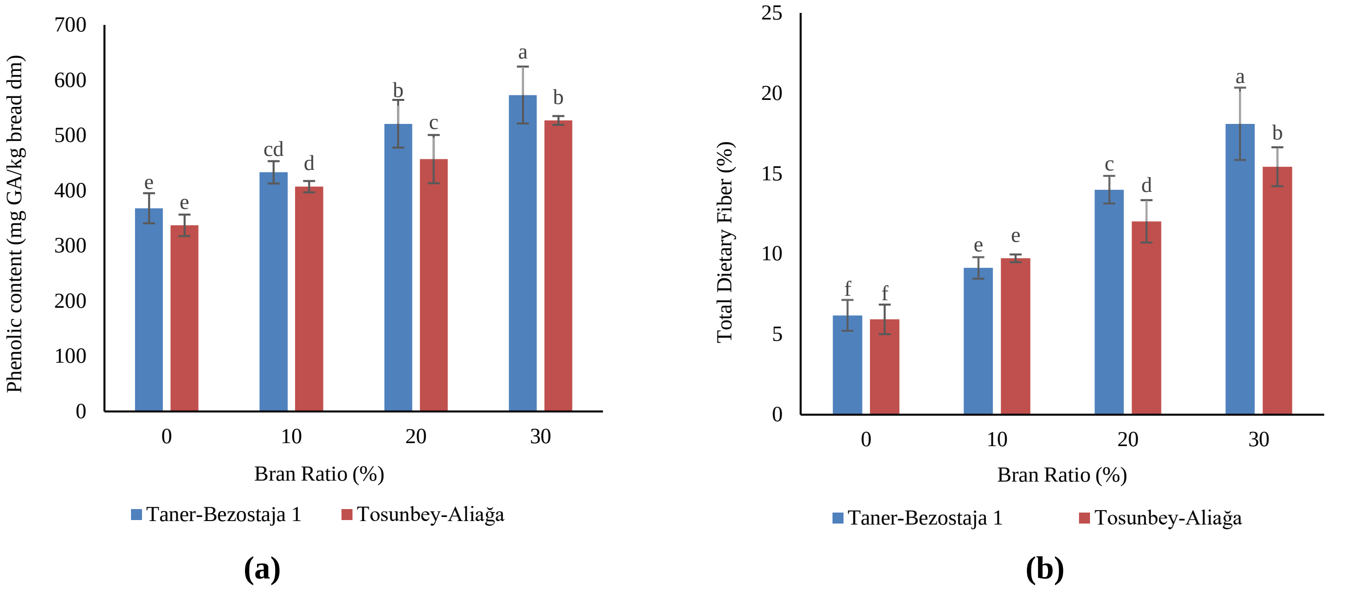

Figure 2. The effect of bran-enrichment of flours on (a) TPC and (b) total dietary fiber content of white and red wheat breads. Bars with different characters indicate a significant difference at P<0.01

3.5. Effect of bran-enrichment on dietary fiber content of breads

The effect of bran-enrichment on dietary fiber content of breads are presented on Figure 2b. The dietary fiber contents of red or white breads increased significantly at a concentration dependent manner as bran ratio of flours was increased. It is important to note that the dietary fiber content of red and white breads increased by 1.5 and 1.6, 2.3 and 2, and 2.9 and 2.6-fold by addition of 10, 20 and 30% bran in their flours, respectively. The WWB_{ctrl} and RWB_{ctrl}, and $WWB_{10:90}$ and $RWB_{10:90}$ showed similar dietary fiber contents. In contrast, $RWB_{20:80}$ and $RWB_{30:70}$ showed significantly higher dietary fiber content than $WWB_{20:80}$ and $WWB_{30:70}$, respectively. Thus, it appeared that a significant benefit of red wheat bran-enrichment on dietary fiber content of bread observed only at high bran ratios. These findings are in line with those of Messia et al. (2016) and Pavlovich-Abril et al. (2015). However, Messia et al. (2016) observed a higher (almost 3.4-fold) increase in total dietary fiber content of bran-enriched breads than that in the current study by using flour enriched with 20% of bran (w/w). In contrast, Pavlovich-Abril et al. (2015) obtained a slightly lower (almost 2.4-fold) increase in total dietary fiber content of breads than that in the current work by enriching flour with 30% of bran (w/w).

3.6. Physical properties of bran-enriched breads

The physical properties of the obtained bran-enriched breads were also evaluated for two succeeding seasons, and the overall average parameters were provided to show applicable limits of bran enrichment (Table 3). As expected, the bran-enrichment of flours caused increase of bread weight and reduction of bread volume (Figure 3a and Figure 3b). Thus, the specific volume of both red and white wheat breads reduced significantly as the bran concentration in flour was increased. However, it is important to note that bran-enriched red wheat breads showed significantly higher specific volume than bran-enriched white wheat breads at any given bran ratio. The control red and white wheat breads lacking bran showed similar pore structure. The addition of 10% bran in wheat flour did not affect the pore structure of red wheat breads while this caused a significant reduction in pore structure of white wheat breads. However, further increase in bran ratio of flours did not affect the pore structure of both red and white wheat breads significantly. The addition of 10% bran in red wheat flours, and 10 or 20% bran in white wheat flours did not affect the symmetry of resulting breads, but further increase in bran ratios caused significant reduction of bread symmetry. This finding fits to the current knowledge that breads made from white wheat are more resistant than those form red wheat against loss of physical quality parameters caused by bran-enrichment. It is well-known that the dietary fibers in bran reduce gas retention and weaken dough development which in turn leads to low bread volume, and poor bread structure (Gómez et al., 2011). Especially the aleurone layer, some bran fraction rich in phenolic acid FA affects bread quality negatively. The FA monomers bind onto insoluble cell wall polysaccharides may interfere with the formation of the gluten network and diminishes desired physical properties of bread (Piber & Koehler, 2005; Hemdane et al., 2016; Lee et al., 2020).

The bran-enrichment did not significantly affect the crust lightness (L) of red and white wheat breads, but it reduced their crumb L value significantly depending on the bran ratio of flours. In general, the crust L values for all white wheat breads were slightly higher than those of red wheat breads, but significantly different crust L values were observed between WWB_{ctrl} and RWB_{ctrl}, and $WWB_{30:70}$ and $RWB_{30:70}$. Although the RWB_{ctrl} showed significantly higher crumb L value than WWB_{ctrl}, all bran-enriched white wheat breads showed significantly higher crumb L values than those of red wheat breads. The bran-enrichment did not correlate well with crust redness (a) of breads, possibly due to the interference and masking effect of brown colored Maillard reaction products formed during baking. However, crumb a value increased significantly as bran ratio was increased between 0 and 20%, and 0 and 30% for red and white wheat breads, respectively. The RWB_{ctrl} and WWB_{ctrl} showed similar crumb a value, but all bran enriched red wheat breads showed significantly higher crumb a value than white wheat breads at same bran ratios. No significant effect of bran-enrichment on crust yellowness (b) of red and white wheat breads was observed.

Table 3 Average results of some physical analysis for bran-enriched breads obtained from white (Tosunbey, Aliağa) and red (Taner, Bezostaja 1) wheat flour-bran mixtures for two succeeding years**.**

Genotypes: Flour	Genotypes: Bran	Bran ratio (%)	Bread Weight (g)	Bread Volume (cm^3)	Specific Volume (cm^3/g)	Pore Structure	Symmetry	L (crust)	a (crust)	b (crust)	L (crumb)	a (crumb)	b (crumb)
Taner	Bezostaja 1	0	138±3.68^{d}	576±31a	4.18±0.31^{a}	9.0±0.81^{a}	9.00±0.00^{a}	41.15±2.56^{c}	11.65±1.74^{a}	17.15±1.41	67.08±1.58^{a}	1.78±0.16^{f}	17.25±0.43^{a}
Taner	Bezostaja 1	10	140±4.13^{bc}	568±8a	4.03±0.14^{b}	8.5±1.00^{a}	8.75±0.50^{ab}	42.48±5.03^{bc}	9.60±0.64^{bcd}	16.48±1.48	56.20±1.20^{c}	4.83±0.35^{d}	15.65±0.48^{b}
Taner	Bezostaja 1	20	142±5.31^{b}	526±17b	3.73±0.24^{c}	7.25±0.50^{b}	8.25±0.50^{b}	40.65±3.64^{c}	10.63±3.22^{ab}	16.50±2.73	47.78±1.55^{e}	6.63±0.16^{a}	14.25±0.12^{c}
Taner	Bezostaja 1	30	144±4.23^{a}	468±21c	3.25±0.20^{d}	7.25±0.95^{b}	7.5±1.29^{c}	40.93±2.61^{c}	8.50±0.94^{d}	15.18±1.00	44.13±1.88^{f}	6.03±0.28^{b}	13.80±0.30^{c}
Tosunbey	Aliağa	0	138±5.04^{d}	524±6b	3.80±0.12^{c}	8.5±0.57^{a}	9.25±0.50^{a}	47.08±1.34^{a}	9.83±4.05^{bcd}	18.63±2.30	62.2±3.55^{b}	1.68±0.33^{f}	15.80±3.24^{b}
Tosunbey	Aliağa	10	139±2.64^{cd}	514±28b	3.73±0.26^{c}	7.5±0.57^{b}	9.00±0.00^{a}	45.6±4.12^{ab}	8.40±2.01^{d}	16.90±1.57	57.18±3.21^{c}	3.08±0.34^{e}	16.13±2.45^{b}
Tosunbey	Aliağa	20	141±4.07^{bc}	473±15c	3.35±0.19^{d}	7.5±0.57^{b}	8.75±0.50^{ab}	43.95±1.40^{abc}	10.13±3.38^{abc}	17.13±1.76	50.55±2.76^{d}	4.55±0.45^{d}	16.23±1.93^{b}
Tosunbey	Aliağa	30	144±3.32^{a}	431±23d	3.00±0.23^{e}	7.5±0.57^{b}	8.25±0.95^{b}	47.53±3.93^{a}	8.68±1.81^{cd}	17.45±1.94	48.43±2.96^{e}	5.33±0.40^{c}	16.25±1.62^{b}
MV (Taner-Bezostaja 1)			141	535	3.69	8.0	8.38	41.30	10.09	16.32	53.79	4.81	15.23
MV (Tosunbey-Aliağa)			141	486	3.47	7.88	8.81	46.04	9.26	17.52	54.59	3.66	16.10
LSD			1.69	16.04	0.12	0.94	0.64	4.05	1.60	1.94	1.88	0.30	0.78
CV %			0.80	2.09	2.23	7.95	4.95	6.14	10.95	7.61	2.30	4.64	3.28
Significance			**	**	**	**	**	**	**	ns	**	**	**

a-f: Values in the same column with different superscripts indicate a statistically significant difference.
L: lightness; a: redness; b: yellowness
MV: mean value; CV: coefficient of variation; LSD: least significant differences; **: indicate significance at the level of 0.01; ns: not significant

(a)

(b)

Figure 3. Bread slices produced with different flour and bran combinations. On the left, bread samples containing flour from the Tosunbey variety and bran from the Aliağa variety at substitution levels of 0%, 10%, 20%, and 30% (from top to bottom). On the right, bread samples containing flour from the Taner variety and bran from the Bezostaja 1 variety at the same substitution levels. Images represent (a) the 2018-2019 growing season and (b) the 2019-2020 growing season.

The bran-enrichment reduced the crumb b values of red wheat bread slightly to moderately, but no significant effect of bran-enrichment on crumb b value was determined for white wheat breads. This information indicates the importance of bran type on bread color.

3.7. Sensory properties of bran-enriched breads

The results of sensory tests of breads obtained from white and red wheat flours with or without addition of bran are presented in Table 4. The WWB_{ctrl} showed the highest sensory scores including color, taste, odor, appearance and overall quality. It is interesting to report that the RWB_{ctrl} and $WWB_{10:90}$ showed almost similar sensory properties. This result proved the superior sensory properties of white wheat breads than the red wheat breads. As expected, the increase of bran ratio in flours caused parallel reductions in sensory properties of both white and red wheat breads. However, all bran-enriched breads showed acceptable sensory scores even at the highest bran enrichment ratio of 30%. The overall quality scores of $WWB_{20:80}$ and $RWB_{20:80}$, and $WWB_{30:70}$ and $RWB_{30:70}$ were similar. The $RWB_{20:80}$ and $RWB_{30:70}$ also showed similar taste scores, but $WWB_{20:80}$ showed a better taste score than $RWB_{20:80}$. The odor scores of $WWB_{20:80}$ and $WWB_{30:70}$ were also superior than those of $RWB_{20:80}$ and $RWB_{30:70}$, respectively. However, $RWB_{30:70}$ received higher color and appearance scores than $WWB_{30:70}$.

It is clear that use of strong wheat variety flours to obtain bran-enriched breads can be achieved with acceptable sensory and bread physical characteristics. This finding is in agreement with Hemdane et al. (2016) who emphasized that it is a must to use flours of wheat with strong bread-making quality in manufacturing of high-quality bran-enriched-breads.

Table 4 Average sensory scores of breads obtained from white (Tosunbey, Aliağa) and red (Taner, Bezostaja 1) bread wheat flour-bran mixtures for 2 succeeding years.

Genotypes		Bran Ratio (%)	Color	Taste	Odor	Appearance	Overall quality
Flour	Bran						
Taner	Bezostaja 1	0	4.53±0.26[a]	4.45±0.10[a]	4.23±0.38[a]	4.43±0.12[a]	4.48±0.11[a]
Taner	Bezostaja 1	10	4.05±0.27[b]	4.18±0.27[b]	4.00±0.57[b]	4.15±0.40[b]	4.13±0.36[b]
Taner	Bezostaja 1	20	3.38±0.12[d]	3.28±0.22[d]	3.4±0.28[de]	3.68±0.16[c]	3.48±0.26[cd]
Taner	Bezostaja 1	30	3.43±0.05[d]	3.3±0.16[d]	3.3±0.18[e]	3.4±0.13[d]	3.5±0.18[cd]
Tosunbey	Aliağa	0	4.13±0.37[b]	4.1±0.36[b]	4.05±0.40[b]	4.25±0.28[ab]	4.23±0.35[b]
Tosunbey	Aliağa	10	3.63±0.04[c]	3.68±0.24[c]	3.5±0.17[cd]	3.78±0.16[c]	3.68±0.08[c]
Tosunbey	Aliağa	20	3.23±0.32[e]	3.58±0.44[c]	3.58±0.38[c]	3.65±0.49[c]	3.6±0.42[c]
Tosunbey	Aliağa	30	2.89±0.33[f]	3.28±0.44[d]	3.48±0.49[cd]	3.18±0.26[e]	3.3±0.29[d]
MV (Taner-Bezostaja 1)			3.72	3.80	3.73	3.92	3.89
MV (Tosunbey-Aliağa)			3.59	3.66	3.60	3.72	3.70
LSD			0.14	0.18	0.15	0.21	0.20
CV %			2.62	3.19	2.68	3.71	3.58
Significance			**	**	**	**	**

a-f: Values in the same column with different superscripts indicate a statistically significant difference.
MV: mean value; CV: coefficient of variation; LSD: least significant differences; **: indicate significance at the level of 0.01

4. Conclusions

Although the bran-enrichment interferes with optimal physical and sensory properties of breads, the increased consumer demands to healthier food having higher dietary fiber and antioxidant bioactive compounds have become the hottest research topic in the development of functional foods. In the current study, we offered an applicable solution to this challenging problem by combining recently selected high-fiber and high-polyphenol brans with strong flour wheat genotypes having good bread-making quality. The results obtained clearly showed possibility of improving dietary fiber content and antioxidant status of white and red breads considerably by increasing bran content of flours up to 30%. As expected, the addition of bran caused reduction of bread quality at a concentration dependent manner, but the breads obtained showed acceptable overall quality scores even at the highest bran ratio tested. The flours of white wheat gave superior bread quality than at low bran ratios, but differences between overall quality parameters of breads disappeared as bran ratio increased to obtain the desired functional properties in breads. Finally, this work showed benefits of using red wheat flour and bran instead of white wheat flour and bran in improving dietary fiber and antioxidant capacity of breads. The results of the current work are a step forward to show importance of collaboration between agronomists and food scientists in creating alternative breeding and processing strategies that serve development of functional bakery products.

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Conflicts of Interest

The authors state that they have no conflicts of interest.

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