DOI: 10.29050/harranziraat.1399957
Harran Tarım ve Gıda Bilimleri Derg. 2025, 29(1): 105-117

Research Article/Araştırma Makalesi

A comprehensive assessment of the antioxidant capacity and sensory properties of wheat-based cereal breads produced in different formulations

Farklı formülasyonlarda üretilen buğday bazlı tahıl ekmeklerinin antioksidan kapasitelerinin ve duyusal özelliklerinin kapsamlı bir değerlendirmesi

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To cite this article:
Arslan Burnaz, N., Ertop, M. & Baltacı, C. (2025). A comprehensive assessment of the antioxidant capacity and sensory properties of wheat-based cereal breads produced in different formulations. Harran Tarım ve Gıda Bilimleri Dergisi, 29(1): 105-117

DOI: 10.29050/harranziraat.1399957

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Received Date:
05.12.2023
Accepted Date:
12.01.2025

ABSTRACT

In this study, it was aimed to investigate the physicochemical, sensory and antioxidant properties of some wheat-based cereal bread types that are offered for sale and frequently consumed in the markets in Turkey. Bread types were produced with the help of a home-type bread-making machine, and 100% white wheat flour bread (WB) was used as a control. Sensory evaluations were determined by using the nine-point hedonic scale method. Total phenolic contents were determined by using Folin-Ciocalteau's method. Antioxidant capacities were determined by using ABTS, DPPH, and FRAP assays. Besides, all data were evaluated by using principal component analysis (PCA) to discriminate the properties of bread types. Whole-wheat bread showed the highest antioxidant potentials (200.00µM TEAC for ABTS, 147.50µM TEAC for DPPH, 116.94µM TEAC for FRAP) and the highest amount of phenolic content (64.30mg GAE/100gDW), but a low overall acceptability score (6.00/9.00). As a result, the addition of wheat-based cereal flours affected the antioxidant, sensory, and physical properties of the produced breads ($p<0.05$). Different cereal flours added to the bread formulation positively affected the antioxidant properties of all breads, but only wheat bran and oat flour addition positively affected the overall sensory acceptability of the bread types. Hereby this study, individuals will be made aware of consuming bread types enriched with different cereal flours including important micronutrients instead of WB which is so poor in micronutrients. The study also provides convenience to individuals in the production of these breads in a home-type bread-making machine. This study reveals the various data for functional properties of bread types that are consumed less than WB.

Key Words: Antioxidant activity, Cereals, Bread, Sensory evaluation, PCA

ÖZ

Bu çalışmada, Türkiye'de marketlerde satışa sunulan ve sıklıkla tüketilen bazı buğday bazlı tahıllı ekmek türlerinin fizikokimyasal, duyusal ve antioksidan özelliklerinin araştırılması amaçlamıştır. Ev tipi ekmek yapma makinesi yardımıyla ekmek çeşitleri üretilmiş olup, kontrol olarak %100 beyaz buğday unu ekmeği (WB) kullanılmıştır. Duyusal değerlendirmeler dokuz noktalı hedonik ölçek yöntemi kullanılarak belirlenmiştir. Toplam fenolik madde içerikleri Folin-Ciocalteau yöntemi kullanılarak belirlenmiştir. Antioksidan kapasiteleri ABTS, DPPH ve FRAP testleri kullanılarak belirlendi. Ayrıca, ekmek çeşitlerinin özelliklerini ayırt etmek için tüm veriler temel bileşenler analizi (PCA) kullanılarak değerlendirilmiştir. Tam buğday ekmeği en yüksek antioksidan potansiyelleri (ABTS için 200.00µM TEAC, DPPH için 147.50µM TEAC, FRAP için 116.94µM TEAC) ve en yüksek fenolik içeriği (64.30mg GAE/100g DW) göstermiş, ancak genel olarak düşük kabul edilebilirlik puanına (6.00/9.00) sahip olduğu saptanmıştır. Sonuç olarak, farklı tahıl unlarının ilavesi üretilen ekmeklerin antioksidan, duyusal ve fiziksel özelliklerini etkilemiştir ($p<0.05$). Ekmek formülasyonuna eklenen farklı tahıl unları, tüm ekmeklerin antioksidan özelliklerini olumlu yönde etkilemiş; ancak yalnızca buğday kepeği ve yulaf unu ilavesi ekmek türlerinin genel duyusal kabul edilebilirliğini olumlu yönde etkilemiştir. Böylelikle bu çalışma ile bireyler, mikrobesinler açısından çok fakir olan WB yerine önemli mikrobesinler içeren farklı tahıl unlarıyla zenginleştirilmiş ekmek çeşitlerini tüketme konusunda bilinçlendirilmiş olacaktır. Çalışma ayrıca bu ekmeklerin ev tipi ekmek yapma makinesinde üretilmesi konusunda da bireylere kolaylık sağlamaktadır. Bu çalışma, WB'den daha az tüketilen ekmek türlerinin fonksiyonel özelliklerine ilişkin çeşitli verileri ortaya koymaktadır.

Anahtar Kelimeler: Antioksidan aktivite, Tahıllar, Ekmek, Duyusal değerlendirme, PCA

Introduction

The fact that consumers are aware that nutrition and health are directly related provided them to expect additional benefits from consumed food besides their nourishing properties (Siró et al. 2008). These developments pioneered the emergence of the "functional food" term as a new approach. A food item can be considered "functional" if it is nutritionally adequate and provides additional benefits to human physiology and metabolic functions (Roberfroid 1999). Functional foods can be obtained by food enrichment and supplement or removing unwanted compounds from the foods in order, to support the diet (Hasler 2002). The Codex Alimentarius defines food enrichment as the addition of micronutrients to foods for the purposes of preventing or correcting a demonstrated deficiency (Dary and Mora 2013). Enriching foods in terms of some nutrients is a public health practice that is made to prevent diseases caused by inadequate consumption of them. In the literature, studies are showing that some food items are effective in reducing the risk of developing type-II diabetes, coronary heart, kidney stones, cardiovascular, gastrointestinal diseases, and cancer (Angulo-López et al. 2023; Younas et al. 2020). It is possible to come across products that have been placed in the food industry. For example, enriched/fortified products such as calcium/iron added to milk, dual-fortified salt with iodine, vitamin-added sugar/margarine/spreadable oils, and micronutrient items added to bread/wheat flour (Akhtar, Anjum, and Anjum 2011; Diosady et al. 2002; Perales et al. 2006; Rosell 2008).

Wheat is the most produced and consumed grain variety in the world and Turkey. It is widely used in human nutrition and especially as a raw material for bread production. Bread is the main part of the daily intake of our diet. In Turkey, bread is the basic ingredient of meals because it is cheaper, and easily accessible compared to other foods. Also, approximately 45% of daily energy is obtained from bread. So, it is a good source of energy and an important part of the daily diet (Wrigley 2009). For these reasons, enrichment studies on bread are becoming even more important. In recent years, researchers are trying to increase the nutritional value of bread with natural additives (Dimov et al. 2018; Prokopov et al. 2018; Torrijos et al. 2021).

Polyphenols are natural substances found in all plants and are called secondary metabolites. They are responsible for the organoleptic properties and health benefits of plant products. They have positive effects on the treatment of many diseases and the immune system (Can et al. 2014; Jiang et al. 2021). The type and amounts of phenolic compounds influence the quality, sensory properties, and acceptability of bread by consumers. (Xu, Wang, and Li 2019).

In Turkish Food Codex, "bread" is defined as the product obtained by respectively kneading, shaping, fermentation, and baking the mixture of wheat flour, water, salt, yeast, sugar (if needed), enzymes, and permitted additives. In addition to these components if the bread includes cereal products and natural ingredients called "bread types". In Codex Alimentarius bread types are called "bread-derived products".

Although people consume large amounts of bread every day, they are not aware of the bioactive effects. Up till today, there isn't any performed work about the phenolic content and antioxidant activities of these "bread and bread types" that are defined in the Turkish Food Codex. Therefore, the purpose of this study was to investigate the phenolic contents and antioxidant activities of the bread and bread types mentioned in the “Turkish Food Codex” to contribute to the consumer's nutritional balance. Also, by using the bread-making machine, each bread could produce in the same conditions; comparable accurately and easily. Besides, with the help of a bread-making machine, and the results of the analysis of the bread types, novel bread formulations can be developed under the name “functional bread”. Thus, in this study, the total phenolic contents and antioxidant activities of the white flour bread called bread (WB, the most being consumed traditional bread in Turkey) were compared to the bread types. Furthermore, the sensory properties and acceptability of the bread and bread types were examined to inform consumers who have never tasted the bread types. Moreover, all data were evaluated by using principal component analysis (PCA) to discriminate the properties of bread types.

Materials and Methods

Experimental setup

Firstly, the breads scoped in the “Bread and Bread Types Communiqué” were researched. (B-1: whole wheat flour bread (100%), B-2: whole wheat flour bread (60%), B-3: white bread (100%), B-4: wheat bran bread (10%), B-5: rye flour bread (30%), B-6: maize flour bread (20%), B-7: oat flour bread (15%), B-8: multi-grain bread (5% rye, 5% maize, 5% oat, 5% barley flour)). The amounts of their ingredients were calculated, and they were adapted to the bread making machine (Table 1). So, eight different commercial breads were prepared and used as samples.

Materials used in bread making

White wheat flour (amount of ash 0.65-1.1%, 14.3% moisture, 11.3% protein, 60% water absorption) and wheat bran were procured from Cesur Mills Company (Trabzon, Turkey). The iodized table salt (Billur Salt Company, İzmir) and instant active dry yeast (Dr. Oetker Company, İzmir) were obtained from a supermarket. Whole wheat flour (max. 1.4% ash, 14.5% moisture, 7.0-12.4% protein, 2.6% lipid, 59.8% carbohydrate, ‰ 0.03 sodium, ‰ 0.32 calcium), oat flour (max. 1.3% ash, 10.5% fiber, 7.0-13.0% protein, 3.6% lipid, 49.6% carbohydrate), rye flour (max. 0.7% ash, 8.15% fiber, 11.65% protein, 0.27% lipid, 36.06% carbohydrate, ‰ 2.82 sodium), maize flour (9.6% fiber, 9.3% protein, 3.8% lipid, 63.1% carbohydrate) and barley flour (max. 1.2% ash, 4.80% fiber, 7.0-12.0% protein, 1.3% lipid, 56.05% carbohydrate, ‰ 2.82 sodium) were obtained from Doğalsan Company (Yenimahalle, Ankara).

Materials used in chemical analyzes

The chemicals used in the analyzes were either analytical grade purity and were imported by Merck and Sigma-Aldrich.

Instruments used in analyzes

A home type bread making machine (Arçelik K-2715) was used (Figure 1). Laboratory equipments such as homogenizer (Daihan Sci., WiseTis HG-15A), vortex (IKA®, USA), analytical balance (Ohaus, Balances, USA), centrifuge (NF 1200R, Turkey), spectrophotometer (Epoch BioTek Ins., USA), multi-heating magnetic stirrer (Wisestir, Daihan) were used for the preparation of bread samples extracts and analyzes.

Figure 1. (a) A home type bread making machine with double-compartment mold (Arçelik K-2715) and (b) the breads obtained on preliminary testing during program optimization in terms of volume and texture in the machine.

Preparation of breads

The steps fermentation and baking are both essential for the flavor of the wheat bread. So, the bread processing program is important for sensory analysis (Heiniö et al. 2016).

The special program number 11 was selected in the bread-making machine and the method previously optimized was adjusted. Program steps in order: knead 1 (14 min), rise 1 (32 min), knead 2 (8 min), rise 2 (31 min), last fermentation (50 min), bake (62 min, 180°C); total time 3 h 17 min (Burnaz, Ertop, and Karataş 2018). The quantities of water, table salt, yeast, and cereal flours used in making the bread dough were added according to the formulations in Table 1. These amounts are calculated by considering the percentages in the "Bread and Bread Types Communiqué" (in Turkish Food Codex).

Table 1. Bread ingredients (Turkish Food Codex Communiqué on Bread and Bread Types, 04 January 2012- 28163).

Ingredients (g)	Bread Ingredients (codes*)							
	B-1	B-2	B-3	B-4	B-5	B-6	B-7	B-8
White wheat flour	–	120	300	270	210	240	255	240
Whole wheat flour	300	180	–	–	–	–	–	–
Wheat bran	–	–	–	30	–	–	–	–
Rye flour	–	–	–	–	90	–	–	15
Maize flour	–	–	–	–	–	60	–	15
Oat flour	–	–	–	–	–	–	45	15
Barley flour	–	–	–	–	–	–	–	15
Salt	3.87	3.87	3.87	3.87	3.87	3.87	3.87	3.87
Yeast	3	3	3	3	3	3	3	3
Water	180	180	180	180	180	180	180	180

*B-1: Whole wheat flour (100%) bread, B-2: Whole wheat flour (60%) bread, B-3: White bread (control), B-4: Wheat bran (10%) bread, B-5: Rye flour (30%) bread, B-6: Maize flour (20%) bread, B-7: Oat flour (15%) bread, B-8: Multi-grain bread (5% rye flour, 5% maize flour, 5% oat flour, 5% barley flour).

Determination of specific volumes of breads

After baking, the breads were taken out of the mold and kept at room temperature. At the end of the waiting period, the bread weights (g) and bread volumes (cm^3, by the technique of displacement with rapeseed) were measured (International 2001). In bread, the "specific volume" that is used as a quality parameter was measured. The specific volume is calculated as the ratio of bread volume to bread weight and expressed as cm^3/g (Dogan and Yıldız, 2009). With the help of double-compartment mold, the breads were prepared in duplicate.

Preparation of bread extracts

Five grams of bread samples were weighed, and 50 ml of 80% methanol was added on, then homogenized. Subsequently, the external surfaces of erlens were covered with aluminum foil and the mixtures were stirred at 750 rpm for 2 h in a multi-heating magnetic stirrer at 37°C. Finally, the extracts were transferred to conical centrifuge tubes and centrifuged at 5000 rpm and 20°C for 15 min, filtered through black banded filter paper and the volumes were completed to 50 ml with 80% methanol solution (Burnaz et al. 2018). The extracts were stored at -18°C until use in analyses. In the study, the bread samples were prepared to be four repeats. In analyses, the samples were prepared in triplicate.

Total phenolic contents (TPC)

TPC values of the bread extracts were detected by using the Folin–Ciocalteu method (Burnaz 2021; Singleton and J A Rossi 1965). Gallic acid (GAE) and ferulic acid (FAE) were used as standards. TPC values were calculated as mg “ferulic acid or gallic acid”/100 g bread dry weight (DW).

Ferric reducing antioxidant power (FRAP)

FRAP values, as determined by the previous method (Benzie and Strain 1996) with some modifications was applied and the results were expressed as TEAC (Trolox® equivalent antioxidant capacity) by reference to standard curves (Ilyasoɫlu and Burnaz 2015). Trolox® was used as a standard. Primely, 1450 µl fresh FRAP solution was added to each 50 µl of standard/extracts and vortexed. After 20 min incubation at room temperature, the absorbance was read at 595 nm. The results were calculated as µM TEAC.

DPPH and ABTS radical scavenging activity

The stable $DPPH^{\bullet}$ (2,2 diphenyl-1-picrylhydrazyl) radical was prepared according to a previously described method (Brand-Williams, Cuvelier, and Berset 1995). The stable $ABTS^{\bullet+}$ (2,29-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) radical was prepared according to a previously described method (Re et al., 1999). The results were calculated as µM TEAC and radical scavenging percentages (%) by the following formula:

$$Scavenging\ \% = [(A_B - A_A)/A_B\ ]x100$$

A_B = absorption of blank Radical solution (t=0 min); A_A = absorption of extract (t=60 min for DPPH, t=20 min for ABTS).

Sensory analysis

Sensory analyses of breads were carried out by 30 semi-trained volunteer panelists from faculty members. Before the study, necessary permissions and approvals were obtained from the ethics committee of our university. Before tasting, a form was established to determine the like/dislike status about the bread samples (Altuğ Onoğur and Elmacı 2011). The panelists evaluated the breads according to shell/inner color, crumbling, texture, appearance, brittleness, brightness, odor, and flavor properties and given scores for overall acceptability. For overall acceptability nine-point hedonic scale sensory analysis form was used, ranging from “1-dislike extremely to 9-like extremely (Lawless and Heymamn 2010). Results were calculated as average scores. If the “acceptability average score” is above 5, the bread is “acceptable” (Torbica, Hadnadev, and Dapčević 2010).

Statistical analysis

A statistical program (SPSS®, Version 20) was used. Significance P values and standard deviations (SD) were calculated by using the related program. The bread sample results were compared by using One-Way ANOVA-Duncan’s test (Tables 2 and 3).
In order to visualize the relationship between the results of the analyzes in bread varieties, the data were statistically transformed using PCA with the correlation matrix method (Figure 2). Another statistical software package program (XLSTAT Addinsoft SARL 2019) was used to perform PCA. All analyses were carried out in triplicates and results were given as mean ± standard deviation (SD). ANOVA was used to compare the significant

differences in the mean values at p<0.05. Different letters in the same column are significantly different (p<0.05).

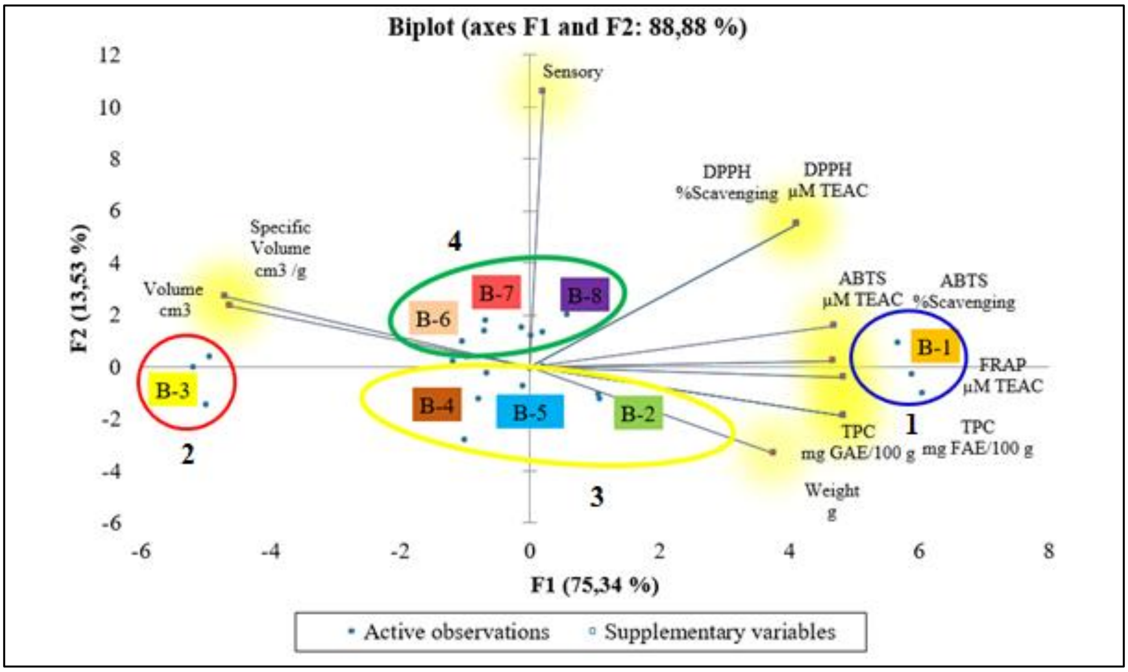

Figure 2. Principal component analysis (PCA) of descriptive sensory, antioxidant and physical analyses of breads

Results and Discussion

Bread quality properties

By using weight and volume measurements, specific volumes (cm^3/g) of breads were calculated. The results of specific volumes of breads are listed as follows: B-3> B-7>B-8>B-6>B-5>B-4>B-2>B-1 (Table 2). The specific volume of the WB (B-3) was found to be the highest (11.07 cm^3/g) and the whole wheat (100%) bread (B-1) was found to be the lowest (26.46 cm^3/g). There was no significant difference between B-6 and B-8 breads in terms of specific volume, but there was a significant difference between the other breads. White flour provides gluten being adequate and high quality, obtaining a strong and elastic dough, excellent gas holding ability, increased bread volume, improved texturization, small and homogeneous pores (Kundakcı and Göçmen 1992). Therefore, since bread made with 100% whole wheat flour without white flour (B-1) cannot form a porous structure, its volume and specific volume are the lowest. While the increase in the proportion of white flour in the formulation positively affects the specific volume, the addition of wheat bran (B-4) has a negative effect. Also, it was seen that different cereal flours (rye, oat, maize, and barley) except the white wheat flour participating in the formulation during the preparation of the bread types affected the amount of gluten in the negative direction. The specific volume, which is the bread quality parameter, and the inner texturing structure of the bread was also adversely affected. The significant reduction in the volume of whole grain-rich bread is primarily due to the dilution of gluten in the flour blends. Similarly, Ragaee et al. (2011) reported a drop in the loaf volume for rye, barley, oat, and cellulose-enriched breads, respectively. In contrary to our work, they found that there were no significant differences in the loaf or specific volume between control white bread and wheat whole grain-enriched bread (Ragaee et al. 2011).

Total phenolic contents

Cereal grains are a good source of natural antioxidant compounds. Phenolic acids are the major antioxidants in cereal grains and have a huge potential to be beneficial for health (Dziki et al. 2014). Whole grains are good sources of dietary fibers, minerals, vitamins, and phytochemicals (Okarter and Liu 2010). The TPC of the WB (B-3) was found the lowest (15.68 mg GAE/100 g and 12.06 mg FAE/100 g). On the contrary, the TPC of the whole wheat flour bread (B-1) was found the highest (64.31 mg GAE/100 g and 56.27 mg FAE/100 g). It has nearly 4 times higher TPC value than the WB. The TPC values of other bread types were found close to each other. The results of TPC values of breads are listed as follows: B-1>B-2>B-5>B-8>B-4>B-6>B-7>B-3 (Table 2). Zieliński and Kozłowska (2000) have investigated the antioxidant properties of aqueous and methanolic (80%) extracts of raw cereal grains and reported that the highest amounts of phenolics extracted by water from barley, wheat, and rye, respectively. As compared to barley, the oat and buckwheat grain were included about five folds lower total phenolics if extracted by water. In a study on free and bound phenolic fractions of various fiber-enriched breads, compared to the control white bread, oat-grain added bread included the highest level of free phenolics followed by rye-, wheat-, and barley grain added breads, respectively.

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Antioxidant Activities

It is scientifically acknowledged that free radicals and reactive oxygen species formed during cellular metabolism adversely affect the pathogenesis of chronic diseases. Dietary antioxidants fight against free radicals to help reduce the risk of these diseases. In this study, the antioxidant capacities of breads were evaluated based on three different in-vitro antioxidant methods.

The FRAP values of bread extracts are given in Table 2, in terms of µM TEAC values. The FRAP value of the WB was found the lowest (23.06 µM TEAC), and the whole wheat flour bread was found the highest (116.95 µM TEAC), likewise TPC values. The results of FRAP values of breads are listed as follows: B-1>B-2>B-5>B-7>B-6>B-8>B-4>B-3 (Table 2).

In literature, cereal products have important antioxidant potentials. The DPPH results showed that the antioxidant activities of cereals or cereal products are higher than widespread fruits and vegetables but lower than berries (Fogarasi et al. 2015).

Table 2. Changes in the antioxidant capacities and physical properties of the wheat-based homemade cereal breads

Sample no.*	Antioxidant capacities							Physical properties		
	TPC mg GAE/100 g	**TPC mg FAE/100 g**	**DPPH µM TEAC**	**DPPH %Scavenging**	**FRAP µM TEAC**	**ABTS µM TEAC**	**ABTS %Scavenging**	**Weight g**	**Volume cm³**	**Specific Volume cm³ /g**
B-1	64.31[a] ±2.45	56.27[a] ±2.23	147.50[a] ±2.52	15.97[a] ±0.27	116.95[a] ±5.02	200.00[a] ±3.57	26.46[a] ±0.47	444.71[a] ±6.29	876.33[f] ±11.02	1.97[g] ±0.01
B-2	44.71[b] ±1.63	38.46[b] ±1.49	85.16[c] ±1.53	9.22[c] ±0.16	84.44[b] ±3.94	150.74[b] ±1.70	19.94[b] ±0.22	423.64[b] ±7.33	983.33[e] ±22.30	2.32[f] ±0.02
B-3	15.68[f] ±1.48	12.06[f] ±1.34	52.83[e] ±3.46	5.72[e] ±0.38	23.06[f] ±1.27	70.74[e] ±4.49	11.07[c] ±3.11	410.31[d] ±3.69	1276.33[a] ±18.50	3.11[a] ±0.02
B-4	35.98[cd] ±2.84	30.52[cd] ±2.58	70.83[d] ±3.46	7.66[d] ±0.38	49.17[e] ±1.67	112.96[d] ±2.80	17.25[b] ±3.70	421.72[bc] ±4.44	1048.33[d] ±10.60	2.49[e] ±0.01
B-5	41.88[b] ±1.08	35.88[b] ±0.99	85.50[c] ±3.06	9.26[c] ±0.33	73.05[c] ±2.92	158.52[b] ±3.57	18.48[b] ±1.60	410.65[cd] ±7.58	1069.00[cd] ±27.22	2.60[d] ±0.02
B-6	32.44[de] ±2.84	27.30[de] ±2.58	82.83[c] ±4.58	8.97[c] ±0.50	64.17[d] ±3.82	136.30[c] ±0.64	16.68[b] ±0.09	416.83[bcd] ±4.94	1102.00[c] ±11.53	2.64[c] ±0.01
B-7	29.37[e] ±2.04	24.51[e] ±1.85	117.83[b] ±6.24	12.75[b] ±0.68	65.56[d] ±3.37	140.74[c] ±4.20	17.28[b] ±0.57	419.77[bcd] ±4.61	1157.33[b] ±15.63	2.76[b] ±0.01
B-8	37.6 3[c] ±2.49	32.02[c] ±2.26	120.16[b] ±8.14	13.01[b] ±0.88	63.33[d] ±2.20	151.85[b] ±11.88	19.32[b] ±0.65	411.19[cd] ±6.65	1096.00[c] ±24.56	2.67[c] ±0.02

Note: Results are presented as means; ± standard deviations (n=3). Different superscript letters (a-g) in the same column are significantly different (Duncan's test, p<0.05).

* B-1: Whole wheat (100%) bread, B-2: Whole wheat flour (60%) bread, B-3: White bread (control), B-4: Wheat bran (10%) bread, B-5: Rye flour (30%) bread, B-6: Maize flour (20%) bread, B-7: Oat flour (15%) bread, B-8: Multi-grain bread (5% rye flour, 5% maize flour, 5% oat flour, 5% barley flour).

GAE: Gallic acid equivalent, FAE: Ferulic acid equivalent, TEAC: Trolox® equivalent antioxidant capacity.

In current study, DPPH activities of bread types' extracts were calculated as TEAC values and scavenging percentages (%). The DPPH scavenging percentage of WB was found the lowest (5.72%), in common with TPC and FRAP. Besides, the DPPH scavenging percentage of whole wheat bread was found the highest (15.97%). The results of DPPH scavenging percentages of breads are listed as follows: B-1>B-8>B-7>B-5>B-2>B-6>B-4>B-3 (Table 2). It has been determined that the diversification of white bread with different cereal flour/bran, also causes an increase in the DPPH scavenging percentage of breads (Table 2). In a study likewise, Ragaee et al. (2011) reported that the DPPH radical scavenging activity of bread enriched with 30% wholegrain flours was two-fold higher when compared with the control bread. In similar studies, the antioxidant capacities of muffins were measured with the help of DPPH and ABTS scavenging assays. Soong et al. (2014) reported that the whole, barley muffin exhibited the highest free radicals scavenging ability, while rice muffin was the lowest ($p<0.05$). Also, the ABTS and DPPH radical scavenging activities of the wheat, maize, and oat muffins, comparable ($p>0.05$).

The ABTS scavenging percentages and µM TEAC values of bread types' extracts were given in Table 2. DPPH values were significantly lower than ABTS values for all types of bread samples. The ABTS scavenging percentage of the WB was found the lowest (11.07%), in common with other tests. The results of ABTS scavenging percentages of breads are listed as follows: B-1>B-2>B-8>B-5>B-7>B-4>B-6>B-3 (Table 2). The investigated values as means antioxidant functionality of bread types were found better when compared to WB (Table 2). Although their ordering differed slightly, the whole wheat flour bread placed on the top and the WB control bread placed on the last, in all antioxidant analysis. Especially there is a strong correlation between the ranking of results of TPC and ABTS. Although the amount of oat is added in the formula less than maize, the results of the oat flour bread sample in the antioxidant assays were found higher than that of the maize flour bread. In this study, it has been determined that the diversification of white bread with different cereal flour/bran causes an increase in TPC and antioxidant activities of bread.

Generally, the studies show that the antioxidant capacity and TPC of bread change based on the wheat variety, flour type, and color (Masisi, Beta, and Moghadasian 2016).

Sensory evaluations

The average scores that were given by panelists were shown in Table 3. As a result of sensory analysis, the flavor was the most important factor affecting the acceptability of bread. That is, the flavor factor has a linear relationship with the acceptability values of the bread types. Although the TPCs and antioxidant activities of whole wheat bread (B-1) were found to be quite high, the overall acceptability of the whole wheat bread (6.00/9) was evaluated as "like slightly" by panelists. The phenolic content of oat flour bread (B-7) was low, but the antioxidant activity values were moderate, and the overall acceptability of this bread was highest in sensory evaluation (8.27/9), and the overall acceptability was found to be "like very much". Oat is a distinctive cereal grain because of its amount of low starch and high beta-glucan content. It has positive health effects (Heiniö et al. 2016). In a similar study, the addition of 15% wheat bran to wheat bread increased flavor intensity in sourdough baking. Also, the addition of 5% bran to wheat flour had increased the number of free amino acids, dietary fiber, and antioxidant activities of dough compared to white wheat flour, enhanced the flavors (Coda et al. 2014).

Statistical evaluation

With the help of One-Way ANOVA, post-hoc Duncan's test, sensory evaluation, and overall acceptability (Table 3) of breads, and significant differences among the antioxidant tests (Table 2) were compared. The results were presented in Tables 2 and 3 by adding SD (standard deviation). Statistically, there is no significant difference between the overall acceptability of most bread types and control white bread ($p>0.05$), except B-1, B-2, and B-7. In other respects, there is a significant difference between antioxidant tests ($P<0.05$).

Table 3. Sensory evaluation of bread types.

Bread type*	Shell color†	Inner color†	Crumbling†	Porous texture†	Flavor†	Odor†	Brittle ness†	Bright ness†	Overall acceptability scores†
B-1	8.26^{a}	7.46^{a}	4.20^{c}	3.60^{e}	6.00^{d}	7.26^{a}	7.20^{a}	3.00^{e}	6.00^{de}
B-2	7.20^{bc}	6.86^{b}	5.80^{b}	5.40^{d}	5.73^{d}	6.43^{b}	6.80^{abc}	4.53^{d}	5.80^{e}
B-3	5.06^{e}	5.26^{d}	7.20^{a}	8.60^{a}	6.87^{bc}	6.36^{b}	2.86^{e}	7.80^{a}	7.00^{bc}
B-4	7.60^{b}	7.33^{a}	4.13^{c}	7.33^{b}	7.27^{ab}	6.40^{b}	5.80^{d}	4.40^{d}	7.33^{b}
B-5	6.66^{c}	6.86^{b}	3.53^{d}	6.73^{c}	6.20^{cd}	5.40^{c}	7.13^{ab}	4.13^{d}	6.27^{d}
B-6	5.80^{d}	5.13^{d}	3.73^{cd}	7.20^{be}	7.23^{ab}	7.20^{a}	6.46^{c}	7.60^{a}	7.00^{bc}
B-7	7.13^{bc}	7.46^{a}	5.80^{b}	6.73^{c}	7.83^{a}	6.40^{b}	6.86^{abc}	5.33^{c}	8.27^{a}
B-8	5.5^{de}	5.80^{c}	4.06^{c}	3.53^{e}	6.40^{cd}	6.86^{ab}	6.66^{bc}	6.26^{b}	6.40^{d}

† Mean values, (N=30). Different superscript letters in the same column are significantly different from each other (Duncan's test, $p > 0.05$).

* B-1: Whole wheat flour (100%) bread, B-2: Whole wheat flour (60%) bread, B-3: White bread (control), B-4: Wheat bran (10%) bread, B-5: Rye flour (30%) bread, B-6: Maize flour (20%) bread, B-7: Oat flour (15%) bread, B-8: Multi-grain bread (5% rye flour, 5% maize flour, 5% oat flour, 5% barley flour).

Principal component analysis

In order, to determine possible differences between bread samples prepared from different flours, a PCA model was created by using the results of antioxidant, sensory and physical analysis (Figure 2). PCA analysis was performed on the average values of eleven analysis parameters aiming to determine the similarities and differences between the bread samples. PCA data constituted 75.34% for the first component, 13.34% for the second component, 5.13% for the third component, 3.48% for the fourth component, and 1.19% for the fifth component of the total variation of the data. The top five components account for 98.68% of the variances for all data.

In the disclosure of data in PCA analysis; while the parameters with the highest factor coordinate values for PC1 and contributing to the correlations were TPC, DPPH, FRAP, ABTS, weight, volume and specific volume, the contribution of sensory analysis was not in this section. The contribution of sensory analysis has been significant in PC2. The PCA model created four separate clusters based on differences between samples prepared by adding different flours. These clusters are: 1(B-1), 2(B-3), 3 (B-2, B-4, B-5), and 4 (B-6, B-7, B-8). The correlation loads of the first two PCs showed high correlations of all the parameters examined. The clear distinction between the samples pointed to differences in some of the parameters investigated. As seen in the PCA graph, samples B-6, B-7, B-8 had the highest sensory scores, while samples B-2 and B-4 had low sensory properties. The B-1 sample has the highest value in terms of antioxidant activity, and the B-2 sample ranks second. The B-3 sample has been shown to have the lowest antioxidant capacity. Table 4, which includes loading and score data, gives the rotated loadings and correlations for each analysis. Also in Table 4, the score values for each bread sample and each major ingredient are found. TPC, FRAP, ABTS, DPPH scores and weight results on the first key ingredient were higher in B-1 and B-2 breads than in other breads. These values were found to be lower for B-3 bread. When the second main component was interpreted, bread samples B-7 and B-8 got the highest score in terms of sensory analysis. Sensory results for B-2 and B-4 breads are lower than for other bread types.

Loads are: TPC, FRAP, ABTS, DPPH, and weight in the first component; sensory in the second component; volume and specific volume in the third component.

Table 4. The loadings and the scores of the first five rotated principal components

The loadings	F1	F2	F3	F4	F5
TPC mg GAE/100 g	0.970	-0.160	-0.106	-0.018	-0.051
TPC mg FAE/100 g	0.970	-0.160	-0.106	-0.018	-0.051
DPPH µM TEAC	0.827	0.467	0.287	-0.090	0.025
DPPH %Scavenging	0.827	0.467	0.287	-0.090	0.025
FRAP µM TEAC	0.972	-0.033	-0.016	-0.089	-0.123
ABTS µM TEAC	0.942	0.135	-0.064	-0.220	-0.139
ABTS %Scavenging	0.941	0.020	-0.079	-0.090	0.296
Sensory	0.042	0.900	-0.300	0.310	-0.029
Weight	0.757	-0.284	0.411	0.414	-0.022
Volume	-0.926	0.199	0.285	-0.089	-0.032
Specific Volume	-0.941	0.228	0.164	-0.162	-0.019
The scores	**F1**	**F2**	**F3**	**F4**	**F5**
B-1	5.878	-0.170	0.499	0.362	0.061
B-2	1.184	-1.211	-0.533	0.028	-0.075
B-3	-5.029	-0.402	0.521	0.222	0.070
B-4	-0.929	-0.979	-0.455	0.739	0.301
B-5	0.046	-0.278	-0.717	-0.802	-0.303
B-6	-0.925	0.016	-0.222	0.068	-0.196
B-7	-0.495	1.527	0.878	-0.031	-0.048
B-8	0.268	1.496	0.028	-0.586	0.190

The results show that there is a relationship between the breads produced using different flours in terms of antioxidant, sensory and physical analysis.

Conclusions

In this study, the phenolic contents, antioxidant activities, and sensorial properties of white bread and bread types were investigated. The total phenolic content and antioxidant capacity of the bread are significantly enhanced in the case of different cereal flours used. The ingredients in bread types have contributed to phenolic content and the antioxidant activity. So, bread types can be accepted as functional products in terms of antioxidants.

The results of this research will contribute to the studies on designing and developing bread formulations and production processes for bread production in the bread making machine.

Daclarations

Ethics committee approval: Ethics Board of Gümüşhane University. (date: 05.12.2018; no: 95674917-108.99-E.37457)

Conflict of interest: None.

Author Contributions: N.A.B: Methodology, analysis, investigation, writing original draft, review and editing. M.H.E: Methodology, analysis, review and editing. C.B: Methodology, analysis, review and editing.

Acknowledgement: This research was financially supported by Gümüşhane University Scientific Research Fund [Project code: 13.A0114.02.2].

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