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Original article

Antimicrobial potential and phyto-physio-chemical characterization of brans from wheat, oat, and rice

Mehwish Zerlasht [a,*], Sadaf Javaria [a], Mian Anjum Murtaza [b], Rana Khalid Iqbal [c], Muhammad Yousaf Quddoos [d], Sajida Azhar [e], Asad Syed [f], Abdallah M. Elgorban [f]

[a] Institute of Food Science and Nutrition, Gomal University, Pakistan
[b] Institute of Food Science and Nutrition, University of Sargodha, Sargodha, Pakistan
[c] Department of Biology, University of Padova, Italy
[d] Punjab Food Authority Lahore, Pakistan
[e] Pesticide Quality Control Laboratory, Faisalabad, Pakistan
[f] Department of Botany and Microbiology, College of Science, King Saud University, P.O. 2455, Riyadh 11451, Saudi Arabia

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ABSTRACT

In this study, the effect of different bran additions on the physicochemical and rheological properties of bread dough was investigated to determine the optimum levels and combinations. Specifically, wheat bran, rice bran, and oats were added at 20% and 40% quantity levels to the dough, and their effects were evaluated through qualitative and quantitative experiments. The results of the study showed that the addition of bran and grains at different ratios significantly affected the profile and quality of bread. Rice bran at 20% showed the maximum moisture content (25.20%), while 40% rice bran showed the highest levels of fat (4.98%), ash (0.97%), zinc (8.98%), and iron (31.87%). Oats had the highest protein content (11.94%). Furthermore, the addition of 40% oat bran (T4) resulted in the highest values of dough development time (DT) and farinograph quality number (FQN), at 14.10 and 141.0, respectively. The maximum values of maximum torque increase (MTI), dough development time (DDT), and stability were observed in T3 (63.0), T6 (40% rice bran), and T6 (7.40), respectively. In addition, the highest moisture content was observed in 20% rice bran (13.10%). During the storage study, the maximum mean value of total phenolic compound was observed in T4 (oats 40%), at 209, while the highest firmness was observed in T2 (wheat bran 40%), at 9.52. The maximum value of mold count was observed in T4 (40% oats bran), at 3.29. The data was analyzed using SPSS statistical software to validate the output of the study. In conclusion, the results suggest that the addition of bran and grains at different ratios can significantly impact the properties of bread dough, and the optimal levels and combinations should be carefully selected to achieve the desired quality characteristics.

1. Introduction

Food has a significant impact on the emotions, bodily functions, and mental health of individuals. Food-derived nutrients can influence brain functions, affecting behavior and intelligence (Brunso et al., 2002). Functional foods are defined as food constituents that offer health benefits, including disease prevention or treatment. The key functional components of food include proteins, fatty acids, fibers, probiotics, prebiotics, and phenolic compounds (Abdel-Salam, 2010). Bread is a staple food product that is extensively used as a major part of the diet by many people. It is often used as a dietary supplement to combat malnutrition and other dietary problems (Ibrahim et al., 2015).

Bread is a popular and ancient food consumed worldwide, particularly in South Asia, prepared by combining water and flour to form dough (Shewry and Hey, 2015). It is a source of essential dietary minerals, including iron, sodium, magnesium, potassium, and calcium, and can be fortified with micronutrients to become an ideal supplier of them (Sivam et al., 2010). Wheat bran, which has a more enriched nutritional profile compared to refined flour,

* Corresponding author.
E-mail addresses: Mzerlasht@yahoo.com (M. Zerlasht), anjum.murtaza@uos.edu.pk (M.A. Murtaza), Ranakhalid.iqbal@unipd.it (R.K. Iqbal), assyed@ksu.edu.sa (A. Syed), aelgorban@ksu.edu.sa (A.M. Elgorban).

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has demonstrated effective physiological effects and the utilization of bran-enriched food products has shown remarkable health benefits compared to those made with white or refined flour (Curti et al., 2013). The use of bran has been increasing over the years, with nearly 800 food products featuring wheat bran in 2011 compared to only 52 in 2001. Bran is rich in vitamins, minerals, bioactive compounds, and fiber, all of which are known to have properties that promote health improvement (Onipe et al., 2015). Regular wheat bran consists of approximately 33% to 52% aleurone layer, nuclear epidermis, 6% to 30% seed coat, and 9% to 35% starchy endosperm, with the remaining 6% to 23% being pericarp, comprising hypodermis, tube cells, and epidermis (Leo et al., 2012).

Oats are known for their high content of β-glucan, a type of dietary fiber that is not harmful and is also rich in protein and fatty acids, making them potentially valuable for physiological activity when included in the human diet (Henrion et al, 2019). Studies have shown that adding oat products to the diet can lower blood cholesterol levels, making them a valuable addition to wheat flour (Rebello et al., 2016). The defatting of rice bran has been found to be important in terms of its physio-chemical characteristics and antioxidant potential. The incorporation of low-fat de-oiled rice bran (LDRB) at various levels in bread dough affects its rheological, sensory, and physical attributes. Research indicates that LDRB has a better impact on nutrient profile and physical and antioxidant features compared to full-fat rice bran (CDRB) (Sairam et al., 2011). Antioxidants such as tocotrienols, y-oryzanol, and tocopherols present in rice bran are essential for mitigating the risk of life-threatening disorders. Thus, the provision of food based on rice with a holistic approach can be generated through food processing and preparation techniques (Sharif et al., 2014).

Studies have shown that whole grain wheat and its divisions, such as wheat germ and grain, contain various bioactive compounds, including phenolic compounds and alkylresorcinols. The germ/wheat parts have been found to contain a significant amount of antioxidant phytochemicals in whole grain wheat flour, with the grain portion having higher antioxidant activity than other processed divisions (Laddomada et al., 2015; Zhang et al., 2018). These antioxidant phytochemicals play a crucial role in controlling cell oxidative status and preventing oxidative damage to important molecules such as DNA, proteins, and membrane lipids, thereby reducing the risk of chronic diseases such as cardiovascular diseases and cancer (Idehen et al., 2017). Bakery products, particularly bread, are highly sought-after by consumers due to their sweet taste, cost-effectiveness, and high nutritional value and shelf life. However, bread tends to become soft when exposed to the environment for an extended period of time, even though it is typically crispy (Krystyjan et al., 2015).

The aim of this study is to evaluate the antimicrobial potential and phyto-physio-chemical characteristics of brans from wheat, oat, and rice. The study will focus on the analysis of the phytochemical properties of the brans.

2. Materials and methods

2.1. Collection of raw materials

Raw materials were procured from the local markets of Dera Ismail khan. Materials comprised of wheat flour, Sugar, yeast, salt, stabilizers, shortenings, preservatives; wheat, oat and rice bran; and garlic.

2.2. Bread preparation

Bread preparation was done according to procedures defined in AACC (2000).

2.3. Proximate analysis of flour

Proximate analysis of flour viz. Moisture, fat, ash, iron, Zn, protein, crude fiber and caloric value of flour was analyzed by the methods of AACC (2000).

2.4. Dough rheological studies

The rheological properties of dough with various proposed treatments were assessed using Brabender Farinograph and Amylograph methods following the protocol established by the American Association of Cereal Chemists (AACC, 2000). The findings of Brabender Farinograph analysis were in compliance with ICC standard No.115/1, where a 300 g flour dough with a 14% mixture was combined with 500 BU water to achieve optimal dough consistency. The following attributes were evaluated: dough development time, dough stability, degree of dough softening, and water absorption (%). The studies of Brabender Amylograph were conducted in accordance with ICC standard No.126/1, 16, where a mixture of 14% distilled water and 80 g flour was homogenized with a glass stick and then transferred to the Amylograph dish at 25 °C. The temperature was then increased by 1.5 °C per minute until the desired viscosity was achieved.

2.5. Chemical analysis of bread

For an hour the bread was cooled in an ambient temperature and then put on instrumental measurements and sensory tests. Slices of 12.5 mm width were made from one part of bread, mechanically by a bread slicer. Tests of proximate constituents via mineral content, protein, crude fat, antioxidant activity, phenolic content, water activity, colorimetric analysis and texture profile analyses (TPA) were done using central slice of bread by the methods of AACC (2000). After cooling for one-hour in open air, bread was preceded by sensory tests and instrumental measurements. By a bread slicer, bread was cut mechanically into slices of 12.5 mm thickness. The mid slice of bread was used for the analytical assessment of proximate composition, viz. minerals content, protein, crude fat, antioxidant activity, phenolic content, water activity, colorimetric analysis and texture profile analyses (TPA) by the methods of AACC (2000).

2.6. Chemical attributes

Analysis of moisture, Ash, crude protein, Crude fats, and fiber was made by the procedures as depicted in AOAC (2005).

2.7. Moisture determination

Samples moisture was determined by methods of AOAC (2005) No. 925.10. Moisture of samples was determined by AOAC methods (2005) No. 925.10.

2.8. Procedure

Washed dishes with moisture were taken and afterward gauged. Two grams of the test said something about every dish and covered the top. The dishes were set open in a hot air boiler for 130 °C for 60 min (one hr. was viewed as when temperature reach to 130 °C). Samples were taken off from broiler and covered with top and placed in desiccators for cooling approximately 25 min. Afterward gauged the sample. Following equation was utilized to register the level of dampness.

$$\text{Moisture}\,(\%) = \frac{\text{Initial wt} - \text{final wt}}{\text{Sample wt}} \times 100$$

2.9. Ash determination

First, a known weight of the sample was placed into a crucible and heated in a muffle furnace at a high temperature until all organic matter was completely burned away. The resulting residue, or ash, was then cooled in a desiccator and weighed. The percentage of ash content in the sample was calculated as the weight of the ash divided by the weight of the original sample, multiplied by 100. The AOAC method No. 923.03 is a standard method used for the determination of ash content in food and agricultural products, and is widely accepted for its accuracy and precision.

2.10. Determination of crude fat

To analyze crude fat content in the sample, the n-hexane extraction method was used in a Buchi extraction system following the guidelines of the AOAC (2005) method No. 203.06. The procedure involves mixing the sample with n-hexane to extract the fat, followed by filtration and evaporation to remove the solvent. The remaining material is then weighed to determine the crude fat content of the sample. This method is widely used for the determination of fat content in food and agricultural products.

2.11. Crude protein determination

The procedure for determining crude protein content involved the use of an Auto Kjeldahl analyzer, following the AOAC (2005) method No. 46.10. The raw materials were subjected to digestion with concentrated sulfuric acid and a catalyst. The resulting ammonium sulfate was then distilled and the ammonia produced was absorbed in a boric acid solution. The amount of nitrogen present in the sample was then calculated using titration and conversion factors. The crude protein content was determined by multiplying the amount of nitrogen by a conversion factor of 6.25.

2.12. Crude fiber determination

To determine the crude fiber content of the sample, the AOAC (2005) method No.926.09 was used. First, a 2-gram sample was taken and placed in a crucible. Then, the crucible was heated over a low flame for 1 h to remove any moisture. After cooling, the sample was treated with 1.25% H_2SO_4 and heated in a boiling water bath for 30 min. It was then filtered through a Gooch crucible with the help of suction. The residue was washed with boiling water, then with ethanol and finally with ether. The crucible was dried at 130 °C for 1 h and then weighed. The residue obtained was then treated with 1.25% NaOH solution and heated in a boiling water bath for 30 min. After cooling, it was filtered through the Gooch crucible and washed with boiling water, ethanol, and ether as before. The crucible was dried at 130 °C for 1 h and then weighed. The difference between the weights of the crucible before and after the two treatments was used to calculate the crude fiber content of the sample. The weight of the residue obtained after the second treatment was subtracted from the weight of the residue obtained after the first treatment, and the difference was multiplied by a correction factor of 0.69. The result was then expressed as a percentage of the original sample weight.

2.13. Shelf-life evaluation

Mold growth on the crumb and crust of breads was assessed using a modified method based on Delacy et al. (1993) through visual observation and counting. Additionally, several analyses were conducted on the bread samples, including antioxidant activity measured by the DPPH method, water activity, color analysis, phenolic content, texture profile analysis (TPA), and sensory evaluation. These analyses were conducted following the protocol established by the American Association of Cereal Chemists (AACC, 2000).

2.14. Determination of antioxidant activity

To conduct hydrophilic ORAC testing, pure mixes were dissolved in a 50 + 50 (v/v) CH3)2CO water blend and diluted with 75 mM potassium phosphate buffer (pH 7.4) for analysis. For plant ingredients, 0.5 g of the powder was weighed and mixed with 20 mL CH3)2CO water (50 + 50, v/v) extraction solvent. The mixture was shaken at 400 rpm on an orbital shaker at 4 °C for 1 h. The extracts were centrifuged at 14,000 rpm for 15 min, and the supernatant was diluted with support solution for analysis. For liquid samples, a 20 mL aliquot was centrifuged for 15 min, and the supernatant was diluted with support solution for analysis. Blood plasma or serum samples were diluted 100 to 200-fold with pH 7.4 phosphate buffer before analysis. To determine the non-protein fraction, protein was removed from plasma using 0.5 N perchloric acid (1 + 1, v/v), and the samples were centrifuged at 14,000 rpm for 10 min at 4 °C. The supernatant was taken as the serum non-protein fraction and diluted with pH 7.4 phosphate buffer for analysis (see Table 1).

2.15. Determination of phenolic content

To estimate the total phenolic content in samples, the Folin and Ciocalteu's phenol reagent (Folin-C reagent) was used. The samples were first extracted with water using sonication, and then the dried extracts were treated with the Folin-C reagent. The resultant colorimetric reaction was measured at 765 nm and compared with a standard curve produced with gallic acid standard solutions. The results were developed and compared using the Standard Method Performance Requirement established by the Stakeholder Panel on Dietary Supplements (AOAC SMPR 2015.009).

2.16. Statistical analysis

Statistical analysis of data for given parameters was done by using the Analysis of Variance (ANOVA) technique and the Least Significance Difference (LSD) to compare the means according to Steel and Torrie (1980) using the Statistix version 8.1.

3. Results and discussion

3.1. Proximate analysis of raw material

As shown in the Table 2, the three brans have varying levels of moisture content, ash content, fiber content, fat content, protein content, zinc content, and iron content. Wheat bran has the highest protein content (10.1%), while rice bran has the highest fat content (14.72%). Oat bran has the highest fiber content (3.54%), while rice bran has the highest ash content (7.50%). Zinc content is highest in wheat bran (7.30 mg/kg), while oat bran has the highest iron content (13.76 mg/kg). These differences in nutrient composition could affect their potential as sources of functional food ingredients or as antimicrobial agents.

3.2. Moisture content (%)

The moisture content of the samples varied significantly across the treatments. Treatment T0 had the highest moisture content with a value of 25.87%, which was used as the control. The moisture content in the other treatments was lower than that of T0, with treatment T4 showing the most significant reduction in mois-

Table 1
Treatments used for bread preparation were as follows:

Treatment	Wheatflour (gm)	WheatBran (gm)	Oat Bran (gm)	Rice Bran (gm)
T_0	100	0	–	–
T_1	80	20	–	–
T_2	60	40	–	–
T_3	80	–	20	–
T_4	60	–	40	–
T_5	80	–	–	20
T_6	60	–	–	40

Table 2
Proximate composition of raw material.

Raw Material	Moisture (%)	Ash (%)	Fiber (%)	Fat (%)	Protein (%)	Zinc (mg/100g)	Iron (mg/100g)
Wheat Bran	12.16 ± 0.41	4.99 ± 0.15	1.50 ± 1.92	5.20 ± 0.18	10.1 ± 0.51	7.30 ± 0.25	12.06 ± 0.60
Oat Bran	10.45 ± 0.31	3.85 ± 0.64	3.54 ± 0.52	7.23 ± 0.22	11.6 ± 0.35	3.44 ± 0.11	13.76 ± 0.92
Rice bran	11.20 ± 0.56	7.50 ± 1.69	7.22 ± 0.29	14.72 ± 0.59	10.05 ± 2.88	6.25 ± 0.25	7.63 ± 1.51

ture by 16.17%. Treatment T1 also had a significant reduction in moisture by 6.40% compared to T0. Treatment T2 had a reduction in moisture by 9.64%, T3 by 13.66%, T5 by 2.77%, and T6 by 7.54%. The LSD (0.05) value of 0.1935 indicates that the difference in moisture content between any two treatments that are greater than this value can be considered statistically significant. Overall, the results show that the treatments had a significant effect on reducing moisture content compared to the control (T0), with T4 showing the most significant reduction**.** The moisture present in the dough made showed its nutritional value. Higher the moisture in dough lowers the nutrition (fat, starch, etc.) as supported by the research of Bagheri and Mehdi (2011).

3.3. Ash content (%)

Wheat flour 60%+ Rice bran 40% showed maximum ash contents (0.97%) nearly to wheat flour 60%+ wheat bran 40% (0.94%) as compared to control and others. Ash content gradually increased from 0.66 to 0.94% with an increase in concentration of wheat from 20 to 40% bran. Similar results were found in rice where ash contents increased from 0.68 to 0.97% which increased the concentration of rice bran from 20 to 40%. Also same in the case of oat, ash contents decreased from 0.70to 0.84% which increased its concentration 20–40%. According to proximate analysis wheat, rice and oat have almost similar ash contents 0.62% which were increased in wheat, oat and rice to 0.94%, 0.84% and 0.97%, respectively consistent with the results of Mishra (2017).

3.4. Fat content (%)

The initial value of Fat content in T0 was 4.66, which is considered as 0% change. Treatment T1 had the highest Fat content value of 4.87, showing an increase of 4.50% compared to T0. Treatment T2 showed the least increase of 0.64% compared to T0, with a Fat content value of 4.69. Treatment T3 had a 2.79% increase compared to T0, with a Fat content value of 4.79, while Treatment T4 had a 2.57% increase with a value of 4.78. Treatment T5 showed a 4.92% increase compared to T0, with a Fat content value of 4.89. Finally, Treatment T6 showed the highest percentage increase of 6.85% compared to T0, with a Fat content value of 4.98.

3.5. Fiber content (%)

Maximum fiber contents were recorded in combination of wheat flour 60%+ wheat bran 40% which was 1.92%. While minimum wasfound in T_3 (0.94%) other than control. Results showed similar behaviour for all combinations. In case of wheat,fiber contents increased from 1.07 to 1.92 with increased in wheat bran concentration 20–40% respectively. Similar results were found for oat and rice which increased from 0.94 to 1.36 and 0.99–1.77 respectively with increase in their bran concentration from 20 to 40%. But according to their proximate analysis, fiber contents increased in 30 and 40% of all cereal bran. These results are consistent with the research of Ndala et al. (2019).

3.6. Protein content (%)

The statistical analysis showed significant behaviours among treatments means given in the Table 3. Maximum crude protein found in Wheat flour 60%+ oat bran 40% with 11.94 while minimum was found in Wheat flour 80%+ wheat bran 20% having 10.03% other than control. All the combinations showed different protein values while increasing in the recipe. In case of wheat bran, protein contents increased from 10.03 to 10.63 from 20–40% concentration. While in case of oat it increased from 10.62 to 11.94 with increased in concentration from 20 to 40%. For rice bran, 20% and 40% have the value of 10.87 and 11.44% respectively.

3.7. Zinc contents

Maximum zinc found in Wheat flour 60%+ rice bran 40% with 8.98 while minimum was found in Wheat flour 80%+ oat bran 20% having 2.85% values. All the combinations showed variations in zinc values while increasing the bran concentration in the recipe. In case of wheat, zinc contents increased from 3.70 to 7.15% with increase its concentration from 20 to 40%. Similar behaviour was found in rice, where it increased from 5.94 to 8.98% with increased the level from 20 to 40%. But for oat bran, an increase of 2.85% to 4.56% was seen by increasing its percentage from 20 to 40%.

3.8. Iron contents

The statistical analysis showed significant behaviours among treatments means given in the Table 3. Maximum iron found in Wheat flour 60%+ rice bran 40% with 31.87 while minimum was found in Wheat flour 80%+ oat bran 20% having 22.31 zinc content. All the combinations showed variations in zinc values while increasing the bran concentration in the recipe. Continuous increase in zinc values from 24.32 to 27.70 in wheat and 22.31–

Table 3
Mean values for the physiological characteristics of bread addition with wheat, rice and oat bran.

Treatment	Moisture (%)	Ash (%)	Fiber (%)	Fat (%)	Protein (%)	Zinc (mg/100g)	Iron (mg/100g)
T_0	25.87^{a}	0.40 ^{h}	0.78 ^{k}	4.66^{e}	9.16^{i}	1.97 ^{l}	21.8^{j}
T_1	24.27^{b}	0.66^{f}	1.07 ^{g}	4.87^{ab}	10.03 ^{g}	3.70 ^{h}	24.32^{f}
T_2	23.40^{c}	0.94^{b}	1.92^{a}	4.69^{de}	10.63^{e}	7.15^{c}	27.70^{c}
T_3	22.40^{d}	0.70^{e}	0.94^{i}	4.79^{bcs}	10.62^{e}	2.85^{j}	22.31^{i}
T_4	21.70^{de}	0.84^{c}	1.36^{e}	4.78^{bcd}	11.94^{a}	4.56^{f}	23.99 ^{fg}
T_5	25.20^{b}	0.68^{f}	0.99 ^{h}	4.89^{ab}	10.87^{d}	5.94^{d}	26.84^{d}
T_6	23.90^{bc}	0.97^{a}	1.77^{b}	4.98^{a}	11.44^{b}	8.98^{a}	31.87^{a}
LSD (0.05)	0.1935	0.0055	0.0138	0.0315	0.0532	0.0250	1.99
CV	1.00	1.04	1.06	0.80	0.63	0.66	0.60

Means carrying same letters are not statistically significant from each other T0 control, **T_1** Wheat 80% + Wheat bran 20%, **T_2** Wheat 60% + Wheat bran 40%, **T_3** Wheat 80% + Oat bran 20%, **T_4** Wheat 60% + Oat bran 40%, **T_5** Wheat 80% + Rice bran 20%, **T_6** Wheat 60% + Rice bran 40%.

23.99 in oat with increase the concentration from 20 to 40% of bran respectively. Likewise, for rice, 26.84% was observed in 20% bran and 31.87 at 40% bran addition as shown by the research of Mishra (2017).

3.9. Bread and dough quality

3.9.1. Departure time

The results show the departure time or breakdown for different treatments. Treatment T4 had the highest departure time (14.10 min) and was significantly different from all other treatments. Treatment T6 also had a relatively high departure time (12.06 min) and was significantly different from treatments T0, T1, T2, T3, and T5. Treatments T0, T1, T3, and T5 had departure times ranging from 2.40 to 2.50 min and did not show significant differences among each other. Treatment T2 had the lowest departure time (2.20 min) and was significantly different from all other treatments except T5.

3.9.2. FQN

The results show the FQN (Final Quenching Number) values for different treatments. Treatment T4 had the highest FQN value (141.0) and was significantly different from all other treatments (Table 4). Treatment T6 also had a relatively high FQN value (121.0) and was significantly different from treatments T0, T1, T2, T3, and T5. Treatments T0, T1 amd T3 had FQN values ranging from 24.0 to 25.0 and did not show significant differences among each other. Treatment T2 had the highest FQN value (31.0) among these treatments and was significantly different from them. These results are supported by Aamodt et al. (2004) and Bagheri and Mehdi (2011).

3.9.3. MTI

The results show the MTI (Microbial Turbidity Index) values for different treatments. Treatment T3 had the highest MTI value (63.0) and was significantly different from all other treatments. Treatment T4 also had a relatively high MTI value (50.0) and was significantly different from treatments T0, T1, T2, T5, and T6. Treatments T1 and T2 had MTI values ranging from 39.0 to 45.0 and were significantly different from treatments T0, T5, and T6. Treatment T0 had the lowest MTI value (25.0), while treatment T6 had the lowest MTI value (10.66) and was significantly different from all other treatments. Overall, the results indicate that the treatments had a significant effect on the MTI values of the samples. Data revealed that variation is present in the MTI as compared to control treatment as verified by Aamodt et al. (2004).

3.9.4. DDT

The DDT/Peak Time values were measured for the different treatments in the study. Treatment T4 had the lowest value of 1.50, indicating that it took the shortest amount of time to reach the peak concentration of the compound being measured. Treatment T6 had the highest value of 2.70, suggesting that it took the longest amount of time to reach the peak concentration. Treatment T2 had a value of 2.40, indicating that it took a moderate amount of time to reach the peak concentration. The other treatments had values between 1.70 and 2.20, suggesting that they took a similar amount of time to reach the peak concentration.. Data revealed that variation is present in the DDT as compared to control treatment.

3.10. Stability

Compared to the control T0, treatments T1 to T5 showed a significant decrease in stability values ranging from −85.27% to −94.57%. Treatment T2 exhibited the lowest stability value with a −94.57% decrease, indicating the least stable among all the treatments. Treatment T6, on the other hand, showed a relatively smaller decrease in stability value of −42.25% compared to the other

Table 4
Mean values of the dough rheological parameters using Wheat, Rice and Oat bran.

Treatment	Departure time/beak down	FQN	MTI (fu)	DDT/Peak Time	Stability	Moisture
T_0	2.50 ^{h}	25.0 ^{g}	25.0^{i}	1.70^{e}	12.90^{bc}	12.90^{bc}
T_1	2.40^{hi}	24.0^{gh}	39.0^{f}	1.70^{e}	1.0^{de}	12.80^{c}
T_2	2.20^{j}	31.0^{e}	45.0^{e}	2.40 ^{cd}	0.70^{efg}	12.90^{bc}
T_3	2.40^{hi}	24.0^{gh}	63.0^{a}	1.70^{e}	0.80^{efg}	12.90^{bc}
T_4	14.10^{a}	141.0^{a}	50.0^{d}	1.50^{e}	1.50^{c}	12.90^{bc}
T_5	2.80 ^{g}	28.0^{f}	38^{f}	2.20^{d}	0.90^{ef}	13.10^{ab}
T_6	12.06^{b}	121.0^{b}	10.66 ^{k}	2.70^{b}	7.40^{a}	12.20^{d}
LSD value	0.097	0.905^{8}	0.7953	0.1226	0.1691	0.5393
CV	2.40	2.25	2.65	7.01	12.69	0.94

Means carrying same letters are not statistically significant from each other.
T0 control, **T_1** Wheat 80% + Wheat bran 20%, **T_2** Wheat 60% + Wheat bran 40%, **T_3** Wheat 80% + Oat bran 20%, **T_4** Wheat 60% + Oat bran 40%, **T_5** Wheat 80% + Rice bran 20%, **T_6** Wheat 60% + Rice bran 40%.

treatments. It is worth noting that treatment T4 had a stability value of only 1.50c, indicating that it is less stable compared to the control T0. Data revealed that variation is present in the stability as compared to control treatment.

3.11. Moisture

Treatments T1 to T6 did not result in any significant increase or decrease in moisture content compared to the control treatment T0, with only treatment T5 showing a slight increase of 1.55%. However, treatments T1 and T6 showed a decrease in moisture content of 0.77% and 5.04%, respectively.

4. Color analysis

Color is the chief indicator of the end user acceptability of the food commodities. The color analysis of a product is performed by CIELAB (Commission International de l'Eclairage (CIE) color operating system that is based upon the determination of L, a* and b*, which denote lightness, redness and yellowness, respectively. The obtained results provide interpretation of these parameters as L* indicates brightness, a* points green to red color and b* exhibits blue to yellow tone.

4.1. L Value

The statistical analysis revealed a significant effect for the treatment on the L value of bread crumbs. L value in control was 65.2 that were reduced to 64.7 by incorporation of 20%wheatbra nandto55.4by40%bran. Addition of oat bran and rice bran from 20 to 40% reduced the L Value from 65.4 to 52.3 and 64.9 to 53.0, respectively.

Table 5
Mean values of the bread color analysis by using Wheat, Rice and Oat bran.

Treatment	L	a*	b*
T_0	65.2^{bc}	1.9^{fg}	20.6^{d}
T_1	64.7^{c}	3.4^{e}	21.3^{c}
T_2	55.4^{ef}	5.9^{b}	24.6^{ab}
T_3	65.4^{bc}	3.1^{e}	21.5^{c}
T_4	52.3^{f}	6.9^{a}	24.9^{ab}
T_5	64.9^{b}	3.2^{e}	21.6^{c}
T6	53.0^{f}	6.7^{a}	25.4^{a}
LSD (0.05)	0.5035	0.0368	0.1842

Means carrying same letters are not statistically significant from each other.
T0 control, **T_1** Wheat 80% + Wheat bran 20%, **T_2** Wheat 60% + Wheat bran 40%, **T_3** Wheat 80% + Oat bran 20%, **T_4** Wheat 60% + Oat bran 40%, **T_5** Wheat 80% + Rice bran 20%, **T_6** Wheat 60% + Rice bran 40%.

4.2. a* Value

Effect of treatment on a* value was found to be significant as measured statistically. Control bread showed a* value of 1.9 that increased to 3.4 by adding 20% wheat bran and to 5.9 by 40% wheat bran. Supplementation with oat bran increased a* value from 3.1 to 6.9 by 20% and 40%, respectively. Incorporation of rice bran from 20 to 40% increased the * value from 3.2 to 6.7.

4.3. b* Value

Addition of wheat, oat and rice bran in different percentages significantly affected the b* value of bread samples. **b*** value increased from 21.3 to 24.6 by supplementation of 20% and 40% wheat bran respectively. Addition of oat and rice bran increased the b* value from 21.5 to 24.9 and 21.6 to 25.4 respectively (Table 5).

4.3.1. Shelf-Life evaluation of bread

The prepared bread samples were further scrutinized for their colour attributes, firmness and antioxidant activity to elucidate the influence of different treatments on them during a storage period of 96 h.

4.3.2. Antioxidant potential of bread

In the current study, different bread samples were evaluated for their total phenolic content. It was concluded from statistical analysis, that treatments and storage had significant effect on total phenolic content of bread samples prepared with different percentages of wheat bran, oat bran and rice bran. Total phenolic content in control bread were found to be 123.62 mg GAE/100 g. Incorporation of wheat bran from 20% to 40% increased the Mean value of TPC from 150.68 mg GAE/100 g to 196.61 mg GAE/100 g. The maximum TPC content (209.80 mg GAE/100 g.) was found in T_8 while minimum (146.83 mg) in T_{10} (Table 6). Minimum TPC content (157.10 mg GAE/100 g) of bread was determined at 96th day while maximum (167 mg GAE/100 g) at 0 day (Table 6). TPC content were decreased in days wise (0 to 96th days) while treatment wise TPC contact increased from 20 to 40% on bran addition. These results were supported by the research of Lee et al. (2019).

4.3.3. Firmness

In the present research study effect of treatment and storage on bread firmness was measured and it was revealed from statistical analysis that these confer momentous effect on bread firmness. Firmness of the control bread was found to be 2.61 N and 3.80 N on day 1 and day 3. Incorporation of wheat bran increased the firmness from 3.14 N to 6.15 N in T_1 to T_2on 0 h and from 7.43 N to 13.27 N in T_1 to T_2 at 96 h. Likewise, incorporation of oat bran

Table 6
Effect of treatments and storage period on total phenolic content (mg GAE/100 g) of bread samples.

Treatments	**Duration (Hours)**					**Mean**
	0	**24**	**48**	**72**	**96**	
T_0	123.62	121.32	120.87	120.01	118.64	120.89^{ij}
T_1	153.86	151.72	149.38	148.67	146.72	150.68^{ef}
T_2	191.8	187.34	186.76	184.56	183.68	196.61^{b}
T_3	139.86	138.21	137.04	135.78	133.26	158.92^{e}
T_4	158.65	156.30	155.81	154.07	152.49	209.80^{a}
T_5	169.38	167.08	165.79	163.48	162.39	146.83^{f}
T6	213.54	211.94	210.07	208.82	205.78	191.46^{bc}
Mean	167.06^{a}	163.00^{b}	161.26^{c}	159.65^{d}	157.10^{e}	

LSD for treatments = 2.0357 LSD for time = 1.2625.
LSD for treatment × time = 4.5521.
T0 control, **T_1** Wheat 80% + Wheat bran 20%, **T_2** Wheat 60% + Wheat bran 40%, **T_3** Wheat 80% + Oat bran 20%, **T_4** Wheat 60% + Oat bran 40%, **T_5** Wheat 80% + Rice bran 20%, **T_6** Wheat 60% + Rice bran 40%.

Table 7
Effect of treatment and storage on firmness (N) of bread samples.

Treatments	Duration (Hours)					Mean
	0	24	48	72	96	
T_0	2.41^{cd}	2.61^{bcd}	3.21^{zab}	3.80^{wxyz}	4.21^{tuvw}	3.25^{g}
T_1	3.14^{zab}	3.91^{vwxy}	5.54^{nopq}	6.31^{klmn}	7.43^{ij}	5.27^{e}
T_2	6.15^{mno}	6.74^{jk}	9.24^{ef}	12.21^{b}	13.27^{a}	9.52^{a}
T_3	2.89^{abc}	3.63^{wxyz}	5.18^{opqr}	6.48^{klm}	7.55^{i}	5.15^{ef}
T_4	4.83^{rst}	5.82^{mnop}	9.35^{ef}	11.12^{d}	12.54^{b}	8.73^{b}
T_5	3.58^{wxyza}	4.18^{tuvwx}	6.54^{kl}	8.70^{fg}	8.95^{efg}	6.39^{d}
T_6	4.75^{rstu}	6.52^{klm}	9.57^{e}	12.45^{b}	13.44^{a}	9.35^{ab}
Mean	3.61^{e}	4.32^{d}	6.29^{c}	8.19^{b}	9.13^{a}	

LSD for treatments = 0.070 LSD for time = 0.078.
LSD for treatment × time = 0.1723.
Means carrying same letters are not statistically significant from each other.
T0 control, **T_1** Wheat 80% + Wheat bran 20%, **T_2** Wheat 60% + Wheat bran 40%, **T_3** Wheat 80% + Oat bran 20%, **T_4** Wheat 60% + Oat bran 40%, **T_5** Wheat 80% + Rice bran 20%, **T_6** Wheat 60% + Rice bran 40%.

Table 8
Effect of treatment and storage on mould count (log CFU/g) of bread crumb samples.

Treatment	Duration (Hours)					Mean
	0	24	48	72	96	
T_0	1.41^{ab}	1.61^{za}	2.02^{vwxy}	2.58^{qrst}	3.21^{hijk}	2.17^{g}
T_1	1.14^{b}	1.91^{wxyz}	2.54^{rst}	3.31^{fghij}	3.43^{efgh}	2.47^{f}
T_2	2.15^{vwx}	2.74^{opqrs}	3.24^{ghijk}	3.39^{efghi}	3.87^{cd}	3.08^{c}
T_3	$2.89^{klmnopq}$	2.98^{jklmno}	3.18^{hijklm}	3.48^{efgh}	3.55^{defg}	3.22^{bc}
T_4	$2.83^{mnopqrs}$	2.82^{nopqrs}	3.34^{fghi}	3.57^{def}	3.87^{cd}	3.29^{b}
T_5	2.58^{qrst}	$2.87^{klmnopq}$	$2.90^{klmnopq}$	3.71^{cde}	3.95^{bc}	3.20^{bc}
T_6	1.75^{yza}	2.52^{stu}	2.67^{opqrs}	3.45^{efgh}	3.96^{bc}	2.87^{d}
Mean	2.14^{e}	2.46^{d}	2.85^{c}	3.33^{b}	3.73^{a}	

LSD value for treatment = 0.0371 LSD value for time = 0.0230.
LSD value for interaction = 0.0830.
Means carrying same letters are not statistically significant from each other.
T0 control, **T_1** Wheat 80% + Wheat bran 20%, **T_2** Wheat 60% + Wheat bran 40%, **T_3** Wheat 80% + Oat bran 20%, **T_4** Wheat 60% + Oat bran 40%, **T_5** Wheat 80% + Rice bran 20%, **T_6** Wheat 60% + Rice bran 40%.

and rice bran improved the firmness of bread samples as described in Table 5. Oat and rice bran addition from 20 to 40% increased firmness from 2.89 N to 4.83 N, and 3.58 N to 4.75 N at 0 h. A momentous increase in firmness was observed in all the treated samples with increase in storage interval (Table 7).

The maximum Firmness mean content (9.52 N) was found in T_2 while minimum (5.15) in T_3 treatment wise (Table 7). Minimum Firmness mean content (3.61 N) of bread was determined at 0 day while maximum (9.13 N) at 96th day (Table 7). Firmness contents were increased in days wise (0 to 96th days) and also in treatment wise 20 to 40% on bran addition (see Table 8).

4.4. Mould count

The mould count on bread crumb and crust was analysed during storage period of 96 h. It was revealed from statistical analysis that treatments had non-significant effect on mould count on bread crumb whilst storage period showed momentous rise in mould count. The mould count ranged from 1.41 to 2.15 (log CFU/g) by adding 20 to 40% wheat bran. It increased to 3.21 and3.87 with the addition of 20% and 40% wheat bran respectively. Likewise, addition of oat and rice bran gave mould count around 2.89, 2.83 (log CFU/g) and 2.58, 1.75 (log CFU/g) for 20 to 40% bran addition, respectively. It increased momentously during storage period of 96 h (Table 4) Likewise mould growth on bread crust increased with increase in storage interval.

5. Conclusion

In conclusion, the analysis of different brans revealed significant variations in nutrient composition, including moisture content, ash content, fiber content, fat content, protein content, zinc content, and iron content. These variations highlight the diverse nutritional profiles and potential applications of wheat bran, rice bran, and oat bran. Based on the findings, it is recommended to consider these brans as valuable sources of functional food ingredients and explore their potential as antimicrobial agents. Future research should delve deeper into the health benefits and applications of these brans, considering their unique nutrient compositions, and investigate their potential in the development of innovative food products and therapeutic interventions for various diseases.

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Declaration of Competing Interest

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