JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

EFFECT OF COLOURED WHEAT BRAN ADDED TO BISCUITS ON PHYSICAL AND CHEMICAL CHANGES DURING STORAGE

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https://doi.org/10.55251/jmbfs.13821

ARTICLE INFO

Received 14. 1. 2026
Revised 27. 5. 2026
Accepted 12. 6. 2026
Published 1. 8. 2026

Regular article

OPEN ACCESS

ABSTRACT

Increasing the fibre content in food has various forms, one option is to increase the content by adding fibre (bran) to bakery products such as biscuits. The aim of this study is to investigate the impact of bran from coloured wheat flour on the storage time and the physical, chemical, and sensory properties of biscuits. Bran of coloured wheats (AF Jumiko, AF Oxana, Citrus x Bona Dea N-34 and AF Zora) was added in an amount of 20% to the standard wheat flour. Physical, chemical, and sensory characteristics of the biscuits produced were monitored, namely the dry matter, FRAP, polyphenols, DPPH, colour, and texture. These parameters were measured on day 1, after 14 days and after 28 days of storage. Supporting characterization of the biscuits was performed by determining the fibre, fat, protein, and ash content on day 1. Compared to the control the results confirmed a change in colour depending on the colour variety of wheat used and an increase in antioxidant properties and for some varieties also increasing of polyphenol content. As expected, there was also an increase in the fibre content of biscuits with the addition of bran.

Keywords: Fracturability, fibre, polyphenols, sensory analysis, titratable acidity, anthocyanin

INTRODUCTION

Biscuits are cereal products with rapidly increasing consumption and production in recent years. Various biscuit formulations aim to develop functional products with improved nutritional profiles. With the change in people's dietary habits in recent years, there is a growing number of studies related to the development of functional foods that have positive effects on human health **(Aksoylu *et al.*, 2015).** Many other studies also prove that antioxidants are good for our health and general well-being. They improve the quality of life and fight diseases **(Franco and Martínez-Pinilla, 2017)**.
However, only about 10% of commercial wheat bran is used as a source of insoluble dietary fibre for breakfast cereals and bakeries. Furthermore, wheat bran has higher vitamin and mineral contents than endosperm **(Esposito *et al.*, 2005)** as well as high antioxidant content **(Li *et al.*, 2007)**. The bran plays an important role in the antioxidant activity of wheat hence consumption of whole wheat grain may offer potential physiological benefits **(Liyana-Pathirana and Shahidi, 2007)**. These characteristics give wheat bran very interesting nutritional properties, thus the addition of bran into different types of foods has been studied extensively **(Song *et al.*, 2013)**.
Dietary fibre plays a very important role in human diet. Dietary fibre, consisting of indigestible cellulose, hemicellulose, lignin, gums, and mucilage provides a variety of health benefits. Soluble fibre is known for its hypocholesterolemic effect and insoluble fibre is known for decreasing the risk of colon cancer. β-glucan is known for reducing both, the risk of colon cancer as well as the absorption of glucose in the digestive system **(Sudha *et al.*, 2007)**.
Coloured wheats contain anthocyanin pigments in the outer layers of the grain, which give the grain a red, purple or blue colour **(Aoun and Boukid, 2023)**. Anthocyanins are secondary metabolites of plants that have antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic properties. Anthocyanins are formed and accumulate in the grain during ripening and are not present in the early stage of grain development **(Xia *et al.*, 2023)**.
The objective of this study expands upon previous research by providing comprehensive assessment of how diverse coloured wheat bran varieties influence the physical, chemical, and sensory stability of fortified biscuits throughout a 28-day storage period.

MATERIAL AND METHODS

Cereal Grains

The bran was used from coloured and non-coloured varieties of whole wheat and added it to conventional plain white flour from a store in order to prepare biscuits. The bran was used of the following coloured wheat varieties: AF Jumiko with purple pericarp – GRIN: **01C0108211**, AF Oxana with blue aleurone – GRIN: **01C0108141**, AF Zora with black grain – not listed in GRIN, N-34= Citrus x Bona Dea with yellow endosperm – will not be assigned to GRIN. From non-coloured varieties, we used Vanessa with standard colour – GRIN: **01C0107410**. For control, we used non-whole uncoloured wheat flour of Triticum aestivum L. bought in a store. For the second control, the same flour was used with the addition of synthetic antioxidant of BHT (Butylhydroxytoluen).
AF Jumiko, AF Oxana, AF Zora, Citrus x Bona Dea (N-34), and Vanessa were obtained from the Mendel University in Brno (harvested in 2021), conventional white flour was bought in a store. Whole grains were milled to wholemeal flour using the LM 3100 laboratory mill (Perten Instruments AB, SWE).

Preparation of Biscuits

Ingredients were mixed in order to make the dough needed to bake the biscuits. Main ingredients were purchased in the Czech market: caster sugar (Tesco Stores ČR a.s., CZE), Hera – shortening (UNILEVER ČR, spol. s.r.o., CZE), baking powder (Dr. Oetker, spol. s.r.o., CZE), tap water, and salt (K+S Czech Republic a.s., CZE). Wheat flour bought in the Czech market was used (Babiččina volba, GoodMills Česko, s.r.o, CZE) without bran to produce the control samples of

biscuits and the same flour with the addition of 0.2 g of BHT synthetic antioxidant (Merck KGaA, GER) was used to prepare biscuit samples with BHT.
The biscuits were prepared according to the following formula: wheat flour (100 g), bran (20 g), shortening (30 g), sugar (30 g), baking powder (3 g), salt (0.5 g) and water (40 g).
Ingredients were mixed for 5 min until reaching uniform consistency. After that, square shapes were formed, put on a baking tray lined with baking paper and baked for 10 min in Elena convection oven (UNOX, ITA) preheated to 180 °C.

Storage

Biscuits were packed in quick-closing zipper bags 180 x 250 mm (foil thickness 50 µm) which protect against dust and moisture. Then the biscuits were stored in a temperature-controlled warehouse in the Department of Plant Origin Food Sciences. The samples were stored during a 1-month period and measurements were performed after baking (day 1) and after 14 and 28 days. The temperature in the warehouse was 18–20 °C and humidity 65%.

Dry Matter

The water/dry matter content was measured according to AOAC official method 950.46 – air drying in 100-102 °C, portion 2 g in oven reports loss in weight as moisture [g] or % **(AOAC, 1990b)**. Data were measured in 4 replicates.

Fat Content

The fat content was measured according to AOAC official method – portion of ca 3–4 g (without sand) air drying in oven for ca 6 h in 100-102 °C; then distillation in petroleum ether (AOAC official method 1990, 945.16 oil in cereal adjuncts), evaporation solvent and dry vessel with fat (dried in oven below 1000 was weighted and reported as % in 100 g of sample) **(AOAC, 1990a)**. This quality indicator was measured in 4 repetitions.

Protein Content

The protein content was determined using the Dumas method (TruSpec, Leco, USA) according to EN ISO 16634-1:2008 (nitrogen to protein conversion factors: 6.2); **(AOAC, 1990c)**. The parameter was measured in 4 repetitions.

Ash Content

The ash content was determined by burning in a muffle furnace (Nabertherm model LE6/11/B150) according to ISO 2171:2007. The parameter was measured in 4 repetitions.

Dietary Fibre Content

The content of dietary fibre was measured using the Megazyme total dietary fibre analysis kit (Megazyme International Ireland Ltd, Wicklow, Ireland) according to Mes-Tris AOAC method 991.4321 **(AOAC, 1990d)**. The principle of the method was based on the use of three enzymes (heat stable α-amylase, protease, and amyloglucosidase) under different incubation conditions in order to remove starch and protein components. Dietary fibre fractions were obtained as indigestible residues after enzymatic digestion of non-dietary fibre components. The insoluble residues were isolated by filtration and soluble fibre was precipitated with ethanol. Dried residues correspond to insoluble dietary fibre (IDF) and soluble dietary fibre (SDF), respectively. Determination of residual ash and proteins (as Kjeldahl N 6.25) was carried out on the residues for corresponding corrections. Total dietary fibre (TDF) was calculated as sum of IDF and SDF. Dietary fibre contents were evaluated in 100 g of fresh matter **(AOAC, 1990d)**. The parameter was measured in 4 repetitions.

Sensory Evaluation

The biscuit samples were subjected to sensory evaluation about 24 hours after baking by a panel composed of 10 tasters (staff members from the University of Veterinary Sciences Brno, CZE). Participants in the sensory analysis provided written confirmation of their consent to the publication of the research results and GDPR. The samples (squares 40 × 40 mm and approximately 5 mm thick) coded with 3-digit numbers were served on white plates. Water was also served to the panellists to rinse their mouths before tasting each sample. The sensory characteristics of the biscuits were evaluated according to the following criteria: biscuit colour, taste, texture (crispness), rancid taste, and general acceptability of the biscuit sample. The panellists rated their acceptability of the product on a 10-point hedonic scale. The lowest intensity was on the left side of the scale (0) while the highest was on the right side (10).

Textural Analysis

Hardness (kg.sec^{-1}) and Fracturability (kg.sec^{-1}) were measurements by TA.XT.PLUS (Stable Micro Systems, Godalming, UK). The whole biscuit was measured by Circular Support Rig (A/CS) and a 5.0 mm penetration probe (SMS P/5). The probe penetrated the samples 2.0 mm deep, the penetration rate before the test was set to 1.5 mm/s. The parameter was measured in 4 repetitions.

Colour Analysis

The scanning and measuring of colour characteristics were carried out according to the procedure described in the work by **Král *et al.*(2021)**. Samples were placed in a dark chamber on blue paper. Digital images of all samples were acquired by a computer vision system. Imaging was performed under standard lighting conditions provided by 2 Osram Delux L–1 x 18 W lamps (Osram, München, GER). Individual images were taken with a Canon EOS 600D camera (Canon, Tokyo, JPN) placed on a tripod (Fomei CS 920, CZE). The shooting mode was in manual mode: exposure time 1/40, aperture F 5.6, sensitivity ISO 200, format RAW, resolution 5184 × 3456; 24-bit sRGB. Each sample was scanned 20 times. Subsequently, the images were processed with the NIS-Elements BR 5.20 programme (Laboratory Imaging s.r.o., CZE). The entire surface area of the biscuit was selected for the NIS-Elements evaluation. Subsequently, the colour characteristics of MeanRed, MeanGreen, and MeanBlue were measured and then converted to the CIEL*a*b* system, where L* means lightness, a* indicates the position on the red-green axis and b* on the yellow-blue axis.
The difference between samples and control (ΔE) was monitored. This parameter was calculated using the CIE ΔE2000 equation **(Luo *et al.*, 2001; Sharma, 2017)**.

Determination of Total Polyphenol Content (TPC)

The total polyphenol content was measured using the Folin-Ciocalteu assay and was quantified as mg GAE (gallic acid equivalents) ·g^{-1} **(Talcott *et al.*, 2005)**. The amount of GAE was measured using a UV-VIS spectrophotometer of INFINITE 200 PRO (Tecan Austria GmbH). The parameter was measured in 4 repetitions.

Determination of Antioxidant Activity

In the FRAP (ferric reducing-antioxidant power) assay, reductants ("antioxidants") present in the sample reduce Fe (III)/tripyridyltriazine complex, (with the stoichiometric rule) to the blue ferrous form. The concentration of the blue ferrous form was measured using a UV-VIS spectrophotometer of INFINITE 200 PRO (Tecan Austria GmbH). The final results were expressed as mmol of Trolox equivalents (TE) per kilogram on a dried basis (mmol TE·kg^{-1}) based on**Ou *et al.* (2002)**. The parameter was measured in 4 repetitions.

DPPH Free Radical Scavenging Activity Assay

The total antioxidant capacity of different biscuit samples was determined spectrophotometrically and assessed using 1,1-diphenyl 2-picryl hydrazyl (DPPH) as followed by **Shimada *et al.* (1992)**. The INFINITE 200 PRO (Tecan Austria GmbH) spectrophotometer was used. The parameter was measured in 4 repetitions.

Titratable Acidity

Titratable acidity was determined for all seven samples. 1 g of biscuit was mixed with 10 mL of distilled water. After 30 minutes, 1 drop of phenolphthalein (1 % w/v in ethanol) was added to the sample and this sample was titrated with 0.01 M NaOH until a stable pink colour. The titratable acidity was evaluated according to the next formula:

$$titratable\ acidity = \frac{1000 * volume\ of\ NaOH * molarity}{wight\ of\ flour\ [g]} * \frac{100}{100 - volume\ of\ water}$$

Titratable acidity is expressed in units of mmol·kg^{-1}. The parameter was measured in 4 repetitions.

Statistical Analyses

Two series of each type of biscuits were produced. Analyses were performed in four replicates within series. Factors were the type of biscuits (control, control with BHT, Vanessa, AF Jumiko, AF Oxana, Citrus x Bona Dea N-34 and AF Zora) and storage duration (1, 14, 28 days). Statistical analyses of data were performed using XLSTAT 2021 (Adinsoft FR). Data were statistically expressed using the non-parametric test compared with the Kruskal-Wallis test (Comparison of K samples).

RESULTS AND DISCUSSION

Chemical Composition of Biscuits

The basic chemical composition of biscuits is shown in Table 1. All samples containing bran showed a significant increase ($p < 0.05$) in ash, protein and total dietary fibre content compared to the control. The ash content was highest in the whole grain AF Jumiko biscuit sample. The lowest amount of ash was found in sample of non-wholemeal biscuit (control with BHT).

Table 1 Chemical composition of biscuits (Day 1)

Sample	Ash [%]	Protein [%]	Fat [%]	SDF Fibre [%]	IDF Fibre [%]	TDF [%]
C	1.18±0.03^{ab}	8.29±0.03^{a}	14.78±0.14^{b}	2.65±0.09^{abc}	1.55±0.01^{a}	4.20±0.08^{ab}
C BHT	1.11±0.02^{a}	8.45±0.11^{a}	12.46±3.01^{ab}	2.16±0.06^{a}	1.54±0.01^{a}	3.70±0.05^{a}
V	1.48±0.08^{bc}	9.33±0.04^{b}	13.50 ±0.01^{ab}	3.31±0.17^{c}	4.98±0.16^{b}	8.29±0.01^{c}
J	1.53±0.09^{c}	8.96±0.11^{ab}	13.96±0.09^{ab}	2.49±0.02^{ab}	3.93±0.01^{ab}	6.41±0.01^{abc}
O	1.34±0.07^{abc}	9.08±0.00^{ab}	13.90±0.39^{ab}	3.01±0.05^{abc}	4.78±0.01^{b}	7.78±0.04^{bc}
N-34	1.45±0.12^{abc}	9.11±0.12^{ab}	13.34±0.08^{a}	3.16±0.03^{bc}	4.05±0.11^{ab}	7.21±0.13^{abc}
Z	1.38±0.02^{abc}	9.11±0.15^{ab}	13.29±0.58^{a}	3.15±0.15^{bc}	4.16±0.09^{ab}	7.30±0.6^{abc}

Samples: Control (C), Control with BHT (C BHT), Vanessa (V), AF Jumiko (J), AF Oxana (O), AF Zora (Z), Citrus x Bona Dea (N-34); soluble dietary fibre (SDF), insoluble dietary fibre (IDF); mean values within a column with different superscripts are significantly different ($p < 0.05$); data are mean value ± standard deviation of four replicates.

The addition of bran increased the amount of fibre (Table 1) compared to samples without added bran (control, control with BHT), as confirmed by **Sudha *et al.* (2007)**. The highest TDF contents were observed in biscuits made from the non-coloured wheat variety Vanessa (8.29%) and the coloured varieties AF Oxana (7.78%) and AF Zora (7.3%). The lowest amount of soluble as well as insoluble fibre was in the control biscuits.

The amount of protein ranged from 8.29% (control) to 9.3% (Vanessa). Wheat bran contains 12-20% protein **(Li *et al.*, 2023)**. According to **Dhua *et al.* (2021)**, the protein content of black wheat is generally higher than that of normal wheat (which contains 10-15% protein) and also depends on the wheat variety. Our results confirm this, as biscuits with AF Zora black wheat bran reached one of the highest protein levels at 9.11%.

Antioxidant Activity (DPPH, FRAP) and Total Polyphenol Content (TPC)

Biscuits containing coloured bran have a higher amount of antioxidants and show higher in vitro chemical antioxidant activity and total polyphenol content, thereby enhancing their nutritional profile compared to the control. In the literature, these bioactive compounds are associated with potential health-promoting effects, including the mitigation of oxidative stress and associated chronic conditions **(Ficco *et al.*, 2016)**. The antioxidant parameters of the biscuits are shown in Table 2. The DPPH was highest in the control with BHT and the lowest in the standard control. Among the coloured varieties, DPPH activity was highest for Citrus x Bona Dea (N-34) and lowest for AF Zora. Generally, DPPH activity decreased during storage, with the BHT control showing a statistically significant difference ($p < 0.05$) compared to all other samples throughout the study. After 28 days of storage, there was a statistically significant difference ($p < 0.05$) between the AF Jumiko and Vanessa biscuits. Our findings regarding purple wheat (AF Jumiko) showed higher DPPH values than blue wheat (AF Oxana) on days 1 and 14, which differs from some literature **(Li *et al.*, 2007; Liu *et al.*, 2010)**. After 28 days of storage, blue wheat (AF Oxana) had a higher DPPH than the purple one (AF Jumiko) ($p < 0.05$).

Gunenc *et al.* (2013) compared the antioxidant activity measured by DPPH in soft and durum red wheat bran from different regions of Canada. In the laboratory, DPPH was measured in the range from 5 to 68% depending on the variety and region. Compared to our results, the biscuit samples contained 17% bran and the results matched the range of the study from Canada.

After the addition of the synthetic antioxidant BHT, the biscuits showed the highest antioxidant activity measured by the FRAP method (4.56 mmol TE·kg^{-1}). The value of biscuits baked from the AF Zora black wheat variety (4.39 mmol TE·kg^{-1}) reached the closest to this result. The lowest antioxidant activity after the control sample was for Citrus x Bona Dea (N-34) biscuits. The control sample differed statistically significantly ($p < 0.05$) from biscuits made with the Vanessa and AF Zora varieties, which showed the highest activity according to the FRAP method. In terms of storage time, FRAP increased slightly after 14 days and then decreased after 28 storage days. After 14 days of storage, there is a statistically significant difference ($p < 0.05$) in colour varieties between AF Jumiko and the other varieties, because AF Jumiko had the lowest FRAP value (3.17 mmol TE·kg^{-1}). After 28 days, there is a statistically significant difference ($p < 0.05$) in Vanessa, AF Zora, and control with BHT against control, AF Jumiko, AF Oxana, and Citrus x Bona Dea (N-34). Vanessa, as a non-coloured but whole wheat, surprisingly had the highest amount of antioxidant activity (FRAP) after control with BHT (4.14 mmol TE·kg^{-1}).

In terms of total polyphenol content (TPC method) (Table 2), the highest values were found in Vanessa (16.73 mg GAE·g^{-1}) and AF Zora (15.08 mg GAE·g^{-1}) varieties, rather than the BHT control. Overall, the results indicate that while polyphenol levels may temporarily increase after 14 days, prolonged storage (28 days) generally leads to a reduction in polyphenol content.

Table 2 Effects of fortification agents and storage on antioxidants parameters of biscuits

Storage	Sample	DPPH [%]	FRAP (mmol TE·kg^{-1})	TPC (mg GAE·g^{-1})
Day 1	**C**	7.16±0.63^{abc}	2.59±0.03^{a}	12.83±0.53^{abcd}
	C BHT	21.21±1.35^{g}	4.56±0.04^{i}	13.24±0.64^{abcde}
	V	8.95±0.49^{cdefg}	3.47±0.05^{bcdefgh}	16.73±1.73^{g}
	J	9.47±1.52^{cdefg}	3.29±0.04^{abcdefg}	12.35±0.96^{g}
	O	9.17±2.43^{bcdefg}	3.21±0.05^{abcde}	13.70±1.09^{abcdef}
	N-34	9.63±1.16^{defg}	2.68±0.02^{ab}	14.60±1.00^{bcdefg}
	Z	7.76±0.63^{abcde}	4.39±0.12^{hi}	15.08±1.18^{g}
Day 14	**C**	7.73±1.27^{abcde}	3.27±0.05^{abcdef}	12.45±0.64^{a}
	C BHT	21.29±1.38^{g}	5.08±0.40^{i}	13.48±0.47^{abcdef}
	V	9.84±0.71^{efg}	3.97±0.08^{defghi}	16.47±1.13^{g}
	J	6.47±0.99^{ab}	3.17±0.08^{abcd}	20.85±4.58^{a}
	O	6.21±0.57^{a}	4.11±0.07^{fghi}	15.56±0.41^{fg}
	N-34	8.35±0.73^{bcdefg}	4.00±0.13^{efghi}	16.23±0.29^{g}
	Z	7.73±0.58^{abcde}	4.24±0.07^{hi}	16.96±0.44^{ef}
Day 28	**C**	6.31±1.40^{ab}	2.99±0.03^{abc}	12.87±0.96^{abc}
	C BHT	20.45±2.44^{fg}	4.45±0.02^{hi}	13.17±0.44^{abcde}
	V	9.04±0.90^{cdefg}	4.14±0.06^{ghi}	15.55±0.73^{fg}
	J	6.52±0.65^{ab}	3.26±0.07^{abcdef}	12.54±0.37^{a}
	O	7.28±1.38^{abcd}	3.52±0.05^{cdefgh}	14.64±0.51^{cdefg}
	N-34	8.32±0.39^{bcdef}	3.29±0.03^{abcdefg}	14.78±0.36^{cdefg}
	Z	6.38±1.23^{ab}	4.04±0.02^{efghi}	15.15±0.97^{efg}

Samples: Control (C), Control BHT (C BHT), Vanessa (V), AF Jumiko (J), AF Oxana (O), Citrus x Bona Dea (N-34), AF Zora (Z); ANOVA – different superscripts in the row means a statistically significant difference (Kruskal-Wallis test, $p < 0.05$); values are means ± SD (n = 4). Total polyphenol content (TPC)

Wang *et al.* (2020) compared the black wheat variety with the purple and conventional varieties. It was observed that the amount of polyphenols (free phenols) of black wheat (45.21 mg GAE·100 g^{-1}) were 6 times higher than that of purple wheat and standard wheat. The amount of polyphenols (bound form) in black was 1.6 times higher than in other types of wheat. Similarly, in our study, the highest polyphenol content was confirmed in the black variety of sample AF Zora after 14 and 28 days. A statistically significant difference with other colour varieties except sample AF Jumiko was not confirmed ($p > 0.05$). On the first day of storage, the highest content was confirmed in sample Vanessa, but as on days 14 and 28, it did not differ statistically from other colour varieties except sample AF Jumiko. **Pasqualone *et al.* (2015)** reported polyphenol content in purple wheat biscuits higher (2.58 mg GAE·g^{-1}) compared to control (1.37 mg GAE·g^{-1}). These results are consistent with our study.

Colour Analysis

The addition of coloured wheat bran significantly influenced the colour profile of biscuits (Table 3 and Figure 1; $p < 0.05$). Compared to the control, the AF Zora variety was found to have the lowest lightness (L*), while Citrus x Bona Dea (N-34) showed the highest values of parameter a* due to its specific pigment composition ($p < 0.05$).

The lowest values were reached by the control and the control with BHT samples. In the case of parameter b*, the highest values were reached by control and control

with BHT biscuits. On the contrary, the lowest b* was measured in biscuits AF Zora in all three measurements (the colour of AF Zora wheat bran is black).
When comparing the samples by inspection (ΔE), the lowest values were found for BHT biscuits, while the largest differences were found for AF Zora and Citrus x Bona Dea (N-34) biscuits.
One of the factors influencing the colour of biscuits is the baking time. During heat treatment, carbohydrate / amino acid browning reactions occur, which cause colour and taste to develop. The colour change of the biscuits on the surface is the result of Maillard's reactions **(Lara *et al.*, 2011)**. In their study, **Lu and Zheng (2012)** verified that the amount of acrylamide that is formed during baking has a great influence on the colour of biscuits. To eliminate this factor, the biscuits were baked under the same conditions. Thus, the colour differences are not due to the products of the Maillard's reaction, but to the differences between the analysed samples.

Table 3 Effects of fortification agents and storage on colour of biscuits

Storage	Sample	L*	a*	b*	ΔE
Day 1	C	72.87±0.37^{efgh}	-1.80±0.09^{abc}	20.89±0.20^{defghi}	-
	C BHT	74.38±0.42^{fgh}	-1.65±0.09^{abcd}	21.96±0.08^{fghi}	1.44±0.40^{a}
	V	63.78±0.24^{abc}	1.80±0.05^{gh}	19.05±0.07^{abcde}	8.25±0.25^{bcdef}
	J	63.34±0.27^{abc}	1.12±0.15^{defgh}	18.60±0.08^{abc}	8.30±0.33^{bcdef}
	O	62.78±0.16^{ab}	0.86±0.04^{bcdef}	19.42±0.17^{abcdef}	8.55±0.27^{def}
	N-34	62.53±0.36^{ab}	2.00±0.13^{h}	21.02±0.35^{defghi}	9.17±0.23^{ef}
	Z	60.17±0.22^{a}	0.36±0.09^{abcde}	16.86±0.47^{ab}	10.79±0.10^{f}
Day 14	C	80.50±0.19^{h}	-1.92±0.07^{ab}	23.10±0.42^{i}	-
	C BHT	80.29±0.24^{h}	-2.19±0.10^{a}	22.40±0.14^{hi}	1.38±0.10^{a}
	V	71.66±0.33^{defgh}	1.60±0.05^{fgh}	19.64±0.15^{abcdefg}	7.64±0.33^{abcd}
	J	71.55±0.37^{defgh}	1.16±0.06^{defgh}	19.48±0.12^{abcdefg}	7.58±0.29^{abcd}
	O	71.62±0.28^{defgh}	0.91±0.08^{cdefg}	22.04±0.21^{ghi}	7.09±0.24^{ac}
	N-34	70.92±0.55^{cdefg}	1.49±0.09^{efgh}	20.87±0.12^{defghi}	7.93±0.33^{abcde}
	Z	68.92±0.37^{abcde}	-0.36±0.06^{abcd}	15.83±0.24^{ab}	9.53±0.27^{ef}
Day 28	C	79.08±0.20^{gh}	-1.81±0.07^{abc}	21.19±0.22^{efghi}	-
	C BHT	77.70±0.23^{fgh}	-1.86±0.08^{abc}	22.38±0.06^{hi}	1.21±0.17^{a}
	V	70.81±0.12^{cdefg}	1.52±0.03^{efgh}	17.88±0.13^{abc}	7.30±0.19^{abc}
	J	68.90±0.16^{abcde}	1.11±0.06^{defgh}	18.92±0.11^{abcd}	8.25±0.24^{bcdef}
	O	68.53±0.31^{abcd}	0.96±0.15^{cdefg}	20.18±0.32^{cdefgh}	8.38±0.19^{cdef}
	N-34	70.28±0.13^{bcdef}	1.83±0.08^{gh}	19.47±0.17^{abcdefg}	7.61±0.16^{abcd}
	Z	67.33±0.27^{abcd}	0.10±0.06^{abcd}	15.06±0.08^{a}	9.61±0.30^{f}

Samples: Control (C), Control BHT (C BHT), Vanessa (V), AF Jumiko (J), AF Oxana (O), Citrus x Bona Dea (N-34), AF Zora (Z); mean values within a row with different superscripts are significantly different ($p < 0.05$); data are mean value ± standard deviation

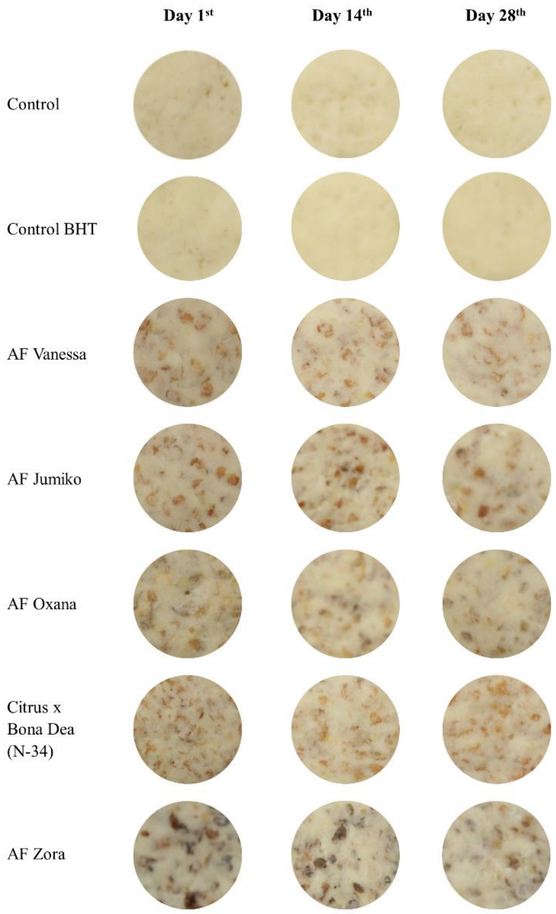

Figure 1 Pictures of biscuits during colour analysis

Dry Matter, Titratable Acidity and Textural Parameters

The evaluation of dry matter, titratable acidity, and textural parameters provides information about the physical and chemical stability of fortified biscuits during storage (Table 4). **Filipčev *et al.* (2017)** state that in the production of bran-containing biscuits, the shelf life is reduced. The Dry matter content remained relatively stable, though a minor decrease observed on day 14 is attributed to the hygroscopic nature of the biscuit matrix and the absorption of ambient moisture. This confirms the importance of selecting appropriate packaging materials capable of protecting the products from drying, wetting, and the absorption of aromatic components **(Hozova *et al.*, 2002).**
On the other hand, bran enrichment significantly increased titratable acidity ($p < 0.05$) compared to the control on days 1 and 14 with the highest values recorded in Vanessa, AF Oxana and AF Zora samples. The higher titratable acidity in biscuits with bran is caused by the presence of acidic substances (folic acid) **(Fardet, 2010; Stevenson *et al.*, 2012; Ma *et al.*, 2021)**. Acidity can also be increased by storing flour due to the release of fatty acids and phosphates. Not only the storage time, but also the storage temperature has an effect on the increase in titratable acidity.

Table 4 Effects of fortification agents and storage on dry matter, titratable acidity and textural parameters

Storage	Sample	Dry Matter [%]	Titratable Acidity (mmol·kg^{-1})	Fracturability (kg·sec^{-1})	Hardness (kg·sec^{-1})
Day 1	C	92.27±0.05^{abcde}	23.12±0.80^{abc}	1.88±0.14^{a}	4.17±1.10^{ab}
	C BHT	93.71±2.04^{de}	22.41±1.53^{ab}	3.01±0.18^{abcd}	5.77±2.85^{abcde}
	V	91.12±0.45^{abc}	31.72±1.60^{f}	3.55±0.49^{bcde}	9.09±2.34^{def}
	J	90.61±0.65^{a}	25.84±0.78^{abcdef}	2.58±0.35^{ab}	6.98±1.30^{bcdef}
	O	92.56±0.22^{bcde}	28.54±0.86^{def}	3.92±0.40^{cde}	7.26±2.65^{abcdef}
	N-34	91.27±0.54^{abcd}	25.03±0.01^{abcde}	3.45±0.82^{bcde}	6.62±2.28^{abcdef}
	Z	90.72±054^{ab}	28.05±2.32^{def}	2.83±0.63^{abc}	9.15±3.92^{cdef}
Day 14	C	91.97±0.52^{abcde}	19.64±0.06^{a}	1.96±1.20^{abcd}	2.53±2.88^{a}
	C BHT	91.78±0.40^{abcde}	23.17±0.70^{abc}	4.04±0.58^{e}	5.88±3.54^{abcde}
	V	91.23±0.25^{abcd}	28.41±0.08^{ef}	3.72±0.63^{bcde}	5.01±1.87^{abc}
	J	90.34±0.02^{a}	25.30±1.36^{abcdef}	3.76±.042^{cde}	6.98±1.92^{abcdef}
	O	91.94±0.23^{abcde}	27.97±1.68^{def}	4.04±0.99^{cde}	6.69±2.67^{abcde}
	N-34	91.00±0.40^{abc}	24.12±0.49^{abcde}	4.06±0.67^{e}	5.06±1.99^{abcd}
	Z	91.11±0.02^{abcd}	29.22±1.71^{ef}	2.73±1.87^{abcde}	8.50±7.87^{abcdef}
Day 28	C	92.990.11^{cde}	21.71±2.04^{ab}	3.62±0.61^{bcde}	6.11±2.87^{abcde}
	C BHT	93.76±0.51^{de}	25.78±0.30^{abcdef}	3.94±0.78^{de}	6.94±3.27^{abcdef}
	V	92.78±0.59^{bcde}	26.33±0.10^{bcdef}	4.20±0.38^{e}	7.18±3.75^{bcdef}
	J	91.31±0.33^{abcd}	23.93±1.30^{abcd}	3.91±0.68^{cde}	9.42±2.72^{ef}
	O	93.23±0.21^{de}	25.39±0.13^{abcdef}	3.51±2.09^{e}	4.38±3.18^{abc}
	N-34	92.88±0.40^{cde}	25.95±1.00^{abcdef}	4.16±.032^{e}	6.13±0.68^{abcde}
	Z	90.70±0.30^{a}	27.45±1.26^{cdef}	3.39±0.36^{abcde}	13.56±3.53^{f}

Samples: Control (C), Control BHT (C BHT), Vanessa (V), AF Jumiko (J), AF Oxana (O), Citrus x Bona Dea (N-34), AF Zora (Z); values are means ± SD ; mean values within a column with different superscripts are significantly different ($p < 0.05$); data are mean value ± standard deviation of four replicates

Textural analysis of fracturability showed that control biscuits differed from fortified samples on days 1 and 14, but on day 28, the differences between the samples were not statistically significant ($p > 0.05$). The highest values, leading to complete disintegration were found in AF Oxana followed by Vanessa. After 14 days, the fracturability in most samples was balanced, while the most distinct difference on day 28 remained between the control and AF Zora.

For hardness, a statistically significant difference was found for control biscuits compared to Vanessa and AF Zora ($p < 0.05$) biscuits on the 1st day of measurement. The statistical difference from the control was also confirmed on day 28 in comparison to AF Jumiko and AF Zora biscuits. Statistical differences were not confirmed for hardness on days 14 and 28 ($p > 0.05$).

Sensory Analyses

Sensory evaluation (Table 5) confirmed that AF Zora biscuits were consistently rated as the darkest, while control samples were the lightest throughout storage ($p < 0.05$).

Table 5 Sensory properties of different biscuit samples during storage

Storage	Sample	Colour	Taste	Crispness	Rancid Taste	Overall Acceptability
Day 1	C	1.45±1.01^{ab}	7.20±1.01^{h}	4.35±2.47^{bcde}	0^{a}	8.00±1.35^{i}
	C BHT	1.25±0.98^{a}	5.50±1.96^{efgh}	4.10±218^{abcd}	0^{a}	6.60±2.13^{efghi}
	V	4.60±1.49^{efgh}	4.20±2.04^{abcdef}	3.00±1.56^{ab}	0^{a}	6.20±1.40^{defghi}
	J	5.00±1.39^{fgh}	4.50±2.01^{bcdefg}	3.40±1.49^{abc}	0^{a}	5.90±1.75^{cdefg}
	O	5.35±0.91^{fgh}	4.75±2.48^{cdefg}	3.50±2.12^{abc}	0^{a}	6.75±1.70^{fghi}
	N-34	4.80±1.16^{fgh}	5.10±1.39^{cdefg}	2.85±1.40^{ab}	0^{a}	6.25±1.36^{defghi}
	Z	5.95±1.69^{h}	5.70±1.48^{fgh}	2.70±0.72^{ab}	0^{a}	7.60±0.81^{hi}
Day 14	C	0.90±0.46^{a}	3.45±1.88^{abc}	2.85±1.25^{ab}	0^{a}	4.20±1.80^{abc}
	C BHT	1.05±1.09^{a}	4.15±1.86^{abcdef}	2.55±1.38^{a}	0^{a}	4.65±1.86^{abcd}
	V	3.30±1.49^{cdef}	2.90±1.43^{ab}	3.05±1.96^{ab}	0^{a}	3.60±1.79^{ab}
	J	3.85±1.42^{efg}	2.60±1.66^{a}	2.95±1.24^{ab}	0^{a}	3.60±1.76^{a}
	O	3.25±1.28^{bcde}	3.40±1.65^{abcd}	3.00±1.05^{ab}	0^{a}	3.90±1.68^{ab}
	N-34	3.50±1.53^{def}	3.80±1.70^{abcde}	3.20±1.27^{ab}	0^{a}	5.00±1.94^{abcde}
	Z	3.70±1.68^{def}	4.00±2.06^{abcdef}	3.05±1.48^{ab}	0^{a}	5.10±1.54^{abcdef}
Day 28	C	2.00±1.16^{abcd}	6.40±1.78^{gh}	5.10±1.91^{cde}	1.00±0.47^{b}	6.90±1.60^{ghi}
	C BHT	1.70±1.34^{abc}	5.40±1.71^{efgh}	5.40±2.37^{cde}	1.40±0.70^{b}	6.30±2.11^{defgh}
	V	4.90±1.37^{fgh}	5.20±1.40^{efg}	5.40±2.12^{de}	1.10±0.57^{b}	5.80±1.75^{cdefg}
	J	4.50±1.27^{efgh}	5.00±1.94^{cdefg}	3.80±1.14^{abcd}	1.10±0.32^{b}	5.70±1.34^{cdefg}
	O	4.80±1.14^{fgh}	5.10±1.20^{defg}	5.80±1.55^{e}	1.20±0.63^{b}	5.80±1.81^{cdefg}
	N-34	4.20±1.55^{efgh}	5.30±2.36^{efg}	5.30±1.77^{de}	1.60±0.70^{b}	5.70±1.89^{bcdefg}
	Z	4.90±1.66^{efgh}	5.00±1.83^{cdefg}	4.90±1.79^{cde}	1.30±0.68^{b}	6.30±1.57^{defghi}

Samples: Control (C), Control BHT (C BHT), Vanessa (V), AF Jumiko (J), AF Oxana (O), Citrus x Bona Dea (N-34), AF Zora (Z); ANOVA = different letter in the column means a statistically significant difference (Kruskal-Wallis test, $p < 0.05$)

On day 1, the control and AF Zora biscuits achieved the highest taste values, differing significantly from the other varieties ($p < 0.05$). However, by day 14, a general decline in sensory quality was observed - taste values decreased by 57% for the control sample, 37% for AF Zora, and 33% for BHT control. There was a statistically significant difference ($p < 0.05$) between samples AF Jumiko, Vanessa and the other samples. We attribute the reduction in the rating of control biscuits to the lower stability of the control samples due to the low antioxidant stability, and thus the subsequent oxidation of fats, which also worsened the palatability from the original 7.20 to 3.45.

Surprisingly, after 28 days, the biscuits tasted better to the sensory panel and the values have increased for most samples. The sensory panel preferred the most AF Zora biscuits, which differed statistically significantly ($p < 0.05$) from the other samples coloured wheat variety.

Crispness was the highest in control and control with BHT control biscuits and the lowest in AF Zora and Citrus x Bona Dea (N-34) biscuits on the 1st day of measurement. In day 1 overall acceptability, however, were the best, for control followed by sample AF Zora. The statistically significant difference ($p < 0.05$) was found between control and Vanessa, AF Jumico, and Citrus x Bona Dea (N-34). After 14 days, the values changed minimally, only for samples control and control with BHT there was a reduction of up to 45%. In contrast, on day 28, the crispness values increased and most samples had higher values than after 14 days.

The rancid taste was not observed on the 1st or 14th day of storage. It was recorded to a lesser extent after 28 days of storage, both with standard wheat biscuits and with coloured wheat bran. A rancid taste can be expected in bran wheat because it contains all the lipids and enzymes of the cereal fractions, making it more prone to rancidity and therefore shorter shelf life **(Pareyt *et al.*, 2011)**.

In our research, we added 20% bran to the biscuits and found that in the overall evaluation, the sensory panel liked both, biscuits with and without bran, the most. The positive overall evaluation was obtained by control (8.0) with non-whole grain, followed by AF Zora biscuits (7.6) with the addition of black wheat bran, which means that consumers prefer both types of biscuits. There was a statistically significant difference ($p < 0.05$) between samples control, control with BHT, AF Zora and other samples.

The scientific team of **Šottníková *et al.* (2019)** also performed a sensory analysis, and samples of wheat biscuits enriched with 6% bamboo fibre gave the best impression. In comparison with our study, our biscuits with coloured wheat bran contained 6.5–8.2% fibre and the panel liked the most (taste evaluation) control biscuits which contained 4.2% fibre. The second preferred were control with BHT control biscuits which contained 3.7% fibre, but also biscuits with black wheat bran which contained 7.3% fibre. From the results it can be deduced that consumers liked biscuits with both, coloured wheat bran as well as standard non-wholemeal flour.

CONCLUSION

The fortification of biscuits with 20% coloured wheat bran represents an effective strategy to significantly enhance their nutritional profile, particularly by increasing dietary fiber, protein, and mineral content. This study confirmed that fortified biscuits possess substantially higher antioxidant activity (FRAP and DPPH) and total polyphenol content compared to standard formulations, with the black wheat variety AF Zora and the yellow variety Citrus x Bona Dea N-34 yielding the most prominent results. Furthermore, while specific antioxidant parameters (DPPH and FRAP) generally decreased over a 28-day storage period, the overall antioxidant capacity and total polyphenol content remained significantly higher than the controls throughout the study. These findings provide suggest that coloured wheat bran is a valuable functional ingredient for the development of bakery products with improved nutritional profiles and high consumer appeal.

Acknowledgements: Special thanks to ing. Petr Martinek CSc. from Agrotest fyto s.r.o. Kroměříž for providing cereal samples.

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