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Effect of storage and recipe on bioactive substance composition in bakery products made from a variety of colored wheats

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Abstract

Currently exist different colored wheat varieties which have various effect on bakery product. The aim of this study was to increase the nutritional value of biscuits by adding colored whole grain wheat (Koniny and Skorpion). The study compared biscuits made of colored wheat baked according to four recipes and evaluated the effect of storing biscuits in the refrigerator and in the warehouse. Polyphenols (TPC), antioxidant activity (FRAP), dry matter, fiber and sensory profile were determined in biscuits during a six-month storage period. Color wheat biscuits baked within this study showed a higher content of polyphenols and antioxidant activity than the control. The highest antioxidant activity was in biscuits from Konini variety (colored wheat). The results show that the antioxidant activity in the refrigerator decreased in the range of 58 to 37% depending on the variety. The smallest decrease of polyphenol compounds occurred during storage in warehouse compare to the refrigerator. The highest amount of fiber was found in the Konini variety and the lowest amount was observed in control variety. Sensory analysis showed that the overall acceptability of color wheat biscuits was slightly higher than control biscuits.

Keywords: antioxidant activity; polyphenols; quality; *Triticum aestivum*.

Practical Application: Benefits of storing colored wheat biscuits in the refrigerator.

1 Introduction

In recent years, interest in the functional properties of foods has gradually been increasing and the role of antioxidant compounds able to scavenge free radicals has been found relevant (Pasqualone et al., 2015). Since their domestication, cereal grains have become staple foods providing protein, carbohydrates, fiber and bioactive compounds, for example, polyphenols and carotenoids. In cereals, the predominant phenolic acid is ferulic acid, representing up to 90% of total polyphenols (Siebenhandl et al., 2007). Biscuits are popular, daily consumed, bakery items and have a long shelf-life (Pasqualone et al., 2014, 2015).

Many studies have focused on the role of whole-grain diets in preventing degenerative diseases. These beneficial health properties of whole-grain products have been associated with the presence of variable amounts of antioxidants, as compared to their corresponding refined flours. Many of these are scavengers of free radicals, including carotenoids and anthocyanins (Yi et al., 2014; Ficco et al., 2016; Abdel-Aal et al., 2006).

In addition to the effect of antioxidants on the prevention of cardiovascular disease, wheat fibre also has a preventive effect. The bran fraction contains the outer layers (aleurone and pericarp) of the wheat kernel and on average accounts for 15% of the grain mass (Prückler et al., 2015). Dietary fibre which is contained in bran is an indigestible portion of plant foods which consists of the structural and storage polysaccharides and lignin in plants. Dietary fibre has beneficial effects in preventing obesity, cardiovascular disease, type 2 diabetes, colon cancer, colonic diverticulosis and constipation (Ng & Rosli, 2014).

Many other studies also prove that antioxidants are good for our health and general well-being. They improve life-quality and fight diseases (Franco & Martínez-Pinilla, 2017). Colored wheat varieties have a high protein content and antioxidant activity due to the presence of phenolic acid and vitamin C (Liu et al., 2010; Zong et al., 2006). The red color of wheat is caused by the deposition of catechin-tannin derivatives in the pericarp (Trojan et al., 2014). The chemistry of free radicals and antioxidants is based on balance between both. When the human body is in pathological conditions, there is an overproduction of free radicals due to the presence of pro-oxidant compounds, and/or other risk factors like smoking, excess of physical activity or stress (Carocho et al., 2018).

The main objective of this study was to evaluate the amount of polyphenols and antioxidant activity in biscuits made from purple and blue wheat during storage. Control biscuits obtained from a conventional wheat cultivar, were used for comparisons.

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2 Materials and methods

2.1 Formulation of model bakery products

Cereal grains

We used colored winter whole wheat grains and nonpigmented whole wheat grains of *Triticum aestivum* L. for research in this study: Control nonpigmentedgrains Vánek (V), Skorpion (S) with blue colored grains and Konini (K) with purple colored grains. All wheat cultivars were obtained from the Mendel University in Brno in 2018.

Dough preparation

For analysis, biscuit samples were baked according to four basic recipes using colored varieties of wheat. Doughs were produced by three different wheat varieties: control, blue and purple (described in the subchapter – Cereal Grains). Whole grains were milled to wholemeal flour using an LM 3100 laboratory mill (Perten Instruments AB, Sweden) until obtaining a smooth flour.

Ingredients mentioned in Table 1 were mixed in order to make the dough needed to bake the biscuits. All the ingredients were purchased in Czech markets: Sugar cane flour (Cassonade Czech Ltd., CZ), Hera - shortening (Unilever, Ltd., CZ), Vanillin sugar (Dr.Oetker, Ltd., CZ), Baking powder (Dr.Oetker, Ltd., CZ), Vitana Cinnamon powder (Orkla Foods Czech and Slovakia, CZ), Fresh eggs (Tesco stores, Inc., CZ) and Salt (K+S Czech Republic, Inc.,CZ).

The dough was kneaded by hand 5 min until it was uniform in consistency. After that it was formed into a square shape and put on a baking tray lined with baking paper. It was baked in the conventional oven (Gorenje, Type E53T2-E2, Slovenia) pre-heated to 200 °C for 10-12 min.

Storage

All samples of 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 and in a refrigerator (Zanussi, Type ZLKI 351, Italy) in the Department of Plant Origin Food Sciences. The samples were stored half a year and measurements were provided after baking (M0) and after 3 and 6 months (M3, M6). The temperature in the warehouse was 18-20 °C, humidity 65%, the temperature in the refrigerator was 5 °C (± 0.5 °C).

Antioxidant activity

The antioxidant activity was measured after methanol extraction. In the FRAP (ferric reducing-antioxidant power) assay, reductants ("antioxidants") present in the sample reduce Fe (III)/tripyridyltriazine complex, (with thestoichiometric rule) to the blue ferrous form. The concentration of the blue ferrous form was measured using a UV-VIS CECIL 7210 spectrophotometer (Aquartus, ENG). The final results were expressed as miligrams Trolox equivalents (TE) per 100 grams on a dried basis (mg TE·100 g^{-1}) (Ou et al., 2002).

Polyphenols

Total phenolic content was measured using the Folin-Ciocalteu assay after water extraction and was quantified as mg GAE (gallic acid equivalents)·kg^{-1} (Talcott et al., 2000). The amount of GAE was measured using a UV-VIS spectrophotometer CECIL 7210 (Aquartus, ENG).

Dry matter

Materials were dried at a temperature of 105 °C until achieving a constant weight. This method is of general use for all low-sugar materials and for almost all food products as a reference method. After cooling in a desiccator, the residue is weighed and its weight is the dry weight in g. The weight loss of the original sample is moisture in g. Both values are expressed in percentage of the initial weight (AOAC, 2000).

Fiber

The amount of fiber was determined according to the AOAC Official Method 978.10 (Association of Official Analytical

Table 1. The amount of ingredients for making dough.

No. recipe	Code sample	Wheat cultivars (g)		Shortening (g)	Cane suger (g)	Baking powder (g)	Egg	Vanilla suger (g)	Ground cinnamon (g)
R1	1K	Konini	100	80	50	3			
	1S	Skorpion	100	80	50	3			
	1V	Vánek	100	80	50	3			
R2	2K	Konini	100	60	0	3	1		
	2S	Skorpion	100	60	0	3	1		
	2V	Vánek	100	60	0	3	1		
R3	3K	Konini	100	30	0	3	1	20	10
	3S	Skorpion	100	30	0	3	1	20	10
	3V	Vánek	100	30	0	3	1	20	10
R4	4K	Konini	100	35	30	3	1		5
	4S	Skorpion	100	35	30	3	1		5
	4V	Vánek	100	35	30	3	1		5

g = gram.

Collaboration, 2005), which is used in the determination of crude fiber in cereals, feed, food and other agricultural products. 1 g of the sample was cooked with sulfuric acid and potassium hydroxide. Next, the samples were kept in an oven at 105 °C for 4 h. Finally, all the samples were burnt in a muffle furnace at 550 °C for 5.5 h, and were weighed after cooling in the desiccator.

Sensory evaluation

The biscuit samples were subjected to sensory evaluation about 24 h after baking by a panel composed of 21 tasters (students and staff members from the University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic) that were trained about sensory evaluation of biscuits according to descriptors listed in Table 2 (Czech Republic). The samples (squares 40 × 40 mm and 5 mm thick) coded with 3-digit numbers were served on plain, white plates. Water was used to cleanse and neutralize the palate during testing. The sensory characteristics of the biscuits were evaluated according to the following criteria: biscuit color, taste, aroma, texture, crispness, sweetness and overall acceptability of the biscuit sample. The panelists rated their acceptability of the product on a 1-10-point hedonic scale. The lowest intensity was on the left side of the scale (0) while the highest was on the right side (10).

Table 2. Sensory descriptors and definitions.

Descriptor	Definition
Biscuit color	Color intensity from light to dark color
Taste	Taste sensation
Aroma	Can refer to either a pleasant or an unpleasant odor
Texture	Texture means smoothness, roughness, or bumpiness of the surface sample during chewing
Crispness	Crispness refers to a hard food that emits a sound during chewing
Sweetness	Intensity of sweet taste
Overall acceptability	Summary of all tested sensory attributes

Statistical analysis

Statistical analysis of data was performed using Unistat 6.1, Ltd., 2012, Czech Republic. Data were statistically expressed using the non-parametric test. A statistically significant difference in dry matter, antioxidant activity, polyphenolic content, fiber and sensory evaluation of the biscuits was compared with the Kruskal-Wallis test (Comparison of k samples and Dunn's multiple pairwise comparisons).

3 Results and discussion

3.1 Dry matter

The results of dry matter are summarized in the Table 3. The highest content of dry matter in biscuits was 96.5% (w/w), the lowest was 83.1% (w/w). There was a statistically significant difference ($p < 0.05$) in the biscuits stored in the warehouse and in the refrigerator between R1 and R2, R3 and R4, regardless of the wheat variety used.

When comparing the measurements of dry matter in the warehouse, there was a statistically significant difference ($p < 0.05$) between R1 M0 and R2, R3 M3. Later, statistically significant differences were measured for R2 M3 and R1 M6 and R3 M0, and then R3 M3 and R3 M0.

As regards the storage of biscuits in the refrigerator, a statistically significant difference ($p < 0.05$) was found between R1 M0 and R2, R3 and R4 M3. There was also a statistically significant difference ($p < 0.05$) between R2 M3 and R3 and R4 M3.

A comparison of the storage methods (refrigerator and warehouse) showed a statistically significant difference between the R1 and R3 M0 (warehouse) with R2, R3 and R4 M3 (refrigerator). Statistically significant differences were also measured between the R1 M6 (warehouse) and R2, R3 and R4 M3 (refrigerator). Then a statistically significant difference ($p < 0.05$) was found between the R2 and R3 M3 (warehouse) and R1 M0, M3 and M6 (refrigerator).

Table 3. Dry matter % (w/w) in wheat biscuits stored in different conditions.

Recipe	Variety	M0	Warehouse	Warehouse	Refrigerator	Refrigerator
			M3	M6	M3	M6
R1	1	96.4 ± 0.3[bc]	95.9 ± 0.2[abc]	96.5 ± 0.1[c]	96.7 ± 0.2[c]	96.5 ± 0.5[c]
	2	96.2 ± 0.1[bc]	96 ± 0.2[abc]	96.5 ± 0.1[c]	96.1 ± 0.3[c]	96 ± 0.2[c]
	3	96.6 ± 0.3[bc]	95.4 ± 0.1[abc]	96.5 ± 0.4[c]	96 ± 0.3[c]	96.4 ± 0.3[c]
R2	1	95.9 ± 0.4[abc]	89.7 ± 0.6[a]	94.3 ± 0.2[abc]	87.3 ± 0.4[a]	92.2 ± 0.1[ab]
	2	95.5 ± 0.2[abc]	88.9 ± 0.6[a]	94.2 ± 0.1[abc]	83.1 ± 0.4[a]	91.6 ± 0.4[ab]
	3	95.5 ± 0.3[abc]	93.8 ± 0.4[a]	95.2 ± 0.1[abc]	91 ± 0.7[a]	94.1 ± 0.1[ab]
R3	1	96.4 ± 0.1[bc]	93.3 ± 0.3[a]	94.5 ± 0.2[abc]	90 ± 0.3[a]	95.5 ± 0.1[abc]
	2	96.5 ± 0.2[bc]	91.3 ± 0.8[a]	94.6 ± 0.1[abc]	88.9 ± 0.3[a]	92.9 ± 0.7[abc]
	3	96.1 ± 0.3[bc]	92.7 ± 0.3[a]	94.7 ± 0.1[abc]	92.2 ± 0.8[a]	93.4 ± 0.1[abc]
R4	1	94.6 ± 0.3[abc]	94 ± 0.1[abc]	95.5 ± 0.3[abc]	94 ± 0.2[a]	94.9 ± 0.1[abc]
	2	94.7 ± 0.4[abc]	93.1 ± 0.2[abc]	94.8 ± 0.1[abc]	89.2 ± 0.7[a]	93.8 ± 0.1[abc]
	3	94.5 ± 0.4[abc]	93.3 ± 0.3[abc]	93.5 ± 0.1[abc]	91.5 ± 0.6[a]	93.3 ± 0.2[abc]

Results are expressed as means (n = 3) ± standard deviation. Values followed by same letter between variety and recipe do not differ significantly ($p < 0.05$; Dunn's multiple pairwise comparisons). 1 = Konini variety; 2 = Skorpion variety; 3 = control variety; M0 = zero day; M3 = 3 months; M6 = 6 months.

Umesha et al. (2015) reported a moisture content of biscuits at 3.5% (w/w). This result was slightly lower than their critical moisture content at the end of 5 months. Thus, shelf life was good between 5 and 6 months in terms of loss of fragility. The study by Kumarasiri et al. (2018) states that biscuits are very hygroscopic. Therefore, it is necessary to avoid contact with the surrounding atmosphere so as to prevent moisture absorption.

3.2 Antioxidant activity

The antioxidant activity of the samples, measured using the FRAP method, is summarized in Table 4. The highest antioxidant activity was in Konini variety in recipe R3 (556.4 mg TE.100 g^{-1}) stored in the warehouse and in the refrigerator (690.5 mg TE.100 g^{-1}). A statistically significant difference ($p < 0.05$) regardless of variety and storage time was observed between recipe R3 and R1 and between R2 and R3 during the entire storage period in both storage methods (Kruskal-Wallis test).

A comparison of the storage methods (warehouse and refrigerator) showed a statistically significant difference ($p < 0.05$) between recipe R3 M0 (warehouse) and the R2 M3, M6 and with the R4 M3 (refrigerator). A statistically significant difference was also found between the R3 M3 (warehouse) and the R1, R2, R3 M3 and R2 M6 (refrigerator). Then a statistically significant difference ($p < 0.05$) was found between the R3 M6 and the R2 M3, M6 and the R4 M3.

We presume that the higher antioxidant activity in recipe R3 could be caused by cinnamon addition to the dough. Cinnamon, turmeric, parsley, curry powder, mustard seed, ginger, pepper, chilli powder, pepper, garlic, coriander, onion and cardamom are high in polyphenols (confirmed from in vitro studies) (Mitra, 2020). As regards nutritional value, cinnamon exhibits a number of positive qualities that contribute to the health of the consumer. It reduces the risk of colon cancer, promotes tissue regeneration and also has antimicrobial properties (Rao & Gan, 2014).

Our results show that the antioxidant activity in the refrigerator decreased in range from 58 to 37% depending on the variety. The greatest difference was recorded in the Konini and Skorpion varieties. In 6M, was the antioxidant activity in warehouse lower then in the refrigerator. The decrease in antioxidant activity was probably caused by higher humidity and subsequent decomposition of antioxidants. The highest decrease in antioxidant activity is shown by R2 in 6M between refrigerated and warehouse storage.

3.3 Total phenolic content

Polyphenols are the main bioactive compounds present in colored wheat varieties that could potentially lead to health benefits in humans (Luís et al., 2018). TPC in biscuits from colored wheat are reported in Table 5.

The highest TPC were recorded for biscuits stored in the refrigerator measured after three months in the variety Konini (16.6 mg GAE·kg^{-1}) and in the variety Skorpion (17.3 mg GAE kg^{-1}). The lowest amounts of polyphenols were measured in the control variety (7.4 mg GAE kg^{-1}). A statistically significant difference ($p < 0.05$) in the biscuits was not observed between recipes during the entire storage.

In comparison of recipes and varieties (Table 5), TCP mostly decreased with the increase of storage time. Cinnamon could be the cause of the higher occurrence of polyphenols in recipes R3 and R4. Cinnamon is one of the sources of polyphenols. It contains several potent antioxidants and is the most important spice used for curtailing oxidative stress. The antioxidant potential of cinnamon is attributed to the array of flavonoid compounds that it contains (Shahid et al., 2018; Rao & Gan, 2014).

Pasqualone et al. (2014, 2015) reported that TPC were higher in colored wheat than in the conventional whole meal biscuits. These results are consistent with our study, the control had a 42-48% lower TPC content than the colored wheat varieties in M0. Liu et al. (2010) declared that Konini showed the highest amount of TPC out of all the tested colored varieties. Varga et al. (2013) have different results were Konini (40.2 mg.kg^{-1}) have lower TCP content than Vánek (5.8 mg.kg^{-1}) and Skorpion (46.5 mg.kg^{-1}). Different results also obtained Abdel-Aal et al. (2006) where Koniny TCP

Table 4. Amount of antioxidant activity in wheat biscuits – FRAP (equivalent Trolox – mg TE·100 g^{-1}) stored in different conditions.

Recipe	Variety	M0	Warehouse	Warehouse	Refrigerator	Refrigerator
			M3	M6	M3	M6
R1	1	57.6 ± 5.2^{abcd}	86.6 ± 3^{abcd}	96.9 ± 1.3^{abcd}	58.2 ± 0.7^{abc}	69.7 ± 0.9^{abcd}
	2	135.8 ± 1.2^{abcd}	120.8 ± 1.1^{abcd}	75.5 ± 0.2^{abcd}	50.5 ± 0.2^{abc}	94.4 ± 0.3^{abcd}
	3	107.4 ± 0.7^{abcd}	63.2 ± 0.3^{abcd}	141.6 ± 2.9^{abcd}	175.2 ± 0.5^{abc}	137.5 ± 2.6^{abcd}
R2	1	127.5 ± 0.8^{abcd}	111.2 ± 1.8^{abcd}	144.2 ± 0.2^{abcd}	53.4 ± 1.7^{a}	76.0 ± 2.2^{a}
	2	93.3 ± 0.5^{abcd}	110.4 ± 0.5^{abcd}	80.4 ± 0.9^{abcd}	60.8 ± 1.9^{a}	80.9 ± 1^{a}
	3	106.5 ± 1.4^{abcd}	60.1 ± 0.1^{abcd}	144.3 ± 1.2^{abcd}	36.8 ± 1.5^{a}	27.6 ± 2.3^{a}
R3	1	393.6 ± 2.9^{cd}	556.4 ± 1.4^{d}	486.1 ± 1.1^{cd}	468.5 ± 3.2^{bcd}	690.5 ± 1.2^{cd}
	2	254.4 ± 0.8^{cd}	445.4 ± 1.2^{d}	346.3 ± 0.6^{cd}	228.7 ± 0.5^{bcd}	369 ± 1.0^{cd}
	3	292.5 ± 1.7^{cd}	290.5 ± 1.5^{d}	284.5 ± 0.3^{cd}	197.3 ± 1.7^{bcd}	220.3 ± 2.6^{cd}
R4	1	107.5 ± 0^{abcd}	227.5 ± 0.4^{abcd}	175.3 ± 0.3^{abcd}	97.0 ± 0.8^{ab}	201.2 ± 0.2^{abcd}
	2	122.3 ± 0.8^{abcd}	181.3 ± 0.8^{abcd}	79.2 ± 0.6^{abcd}	78 ± 0.1^{ab}	125.3 ± 0.3^{abcd}
	3	127.6 ± 2.7^{abcd}	104.5 ± 1.6^{abcd}	139.1 ± 4.9^{abcd}	64.8 ± 0.8^{ab}	55.5 ± 0.2^{abcd}

Results are expressed as means (n = 3) ± standard deviation. Values followed by same letter between variety and recipe do not differ significantly ($p < 0.05$; Dunn's multiple pairwise comparisons). 1 = Konini variety; 2 = Skorpion variety; 3 = control variety; M0 = zero day; M3 = 3 months; M6 = 6 months.

Table 5. Amount of polyphenols in wheat biscuits (gallic acid equivalent – mg GAE·kg^{-1}) stored in different conditions.

Recipe	Variety	M0	Warehouse	Warehouse	Refrigerator	Refrigerator
			M3	M6	M3	M6
R1	1	14.4 ± 0.1^a	13.7 ± 0.0^a	9.9 ± 0.0^a	13.8 ± 0.0^a	10.3 ± 0.0^a
	2	14.5 ± 0.1^a	13.9 ± 0.0^a	10 ± 0.0^a	15.9 ± 0.1^a	10.3 ± 0.0^a
	3	8.4 ± 0.0^a	8.1 ± 0.0^a	9.9 ± 0.0^a	7.9 ± 0.9^a	10.4 ± 0.0^a
R2	1	14.4 ± 0.1^a	14.4 ± 0.0^a	10.4 ± 0.0^a	15.6 ± 0.0^a	10.6 ± 0.0^a
	2	14.3 ± 0.0^a	14.8 ± 0.1^a	10.9 ± 0.0^a	14.9 ± 0.1^a	10.4 ± 0.0^a
	3	7.4 ± 0.0^a	7.6 ± 0.1^a	10.2 ± 0.0^a	7.7 ± 0.0^a	9.7 ± 0.0^a
R3	1	15.3 ± 0.1^a	15.2 ± 0.0^a	12.6 ± 0.0^a	16.6 ± 0.0^a	12.5 ± 0.0^a
	2	15.8 ± 0.2^a	15.1 ± 0.1^a	12 ± 0.4^a	15.8 ± 0.0^a	12.8 ± 0.0^a
	3	8.4 ± 0.03^a	8.1 ± 0.0^a	10.6 ± 0.0^a	8.3 ± 0.0^a	9.9 ± 0.0^a
R4	1	15.5 ± 0.2^a	15.4 ± 0.0^a	13.1 ± 0.0^a	16.3 ± 0.0^a	12.5 ± 0.0^a
	2	16.4 ± 0.1^a	15.9 ± 0.2^a	12 ± 0.0^a	17.3 ± 0.0^a	11.7 ± 0.0^a
	3	7.9 ± 0.0^a	7.9 ± 0.0^a	10.3 ± 0.0^a	7.8 ± 0.0^a	10.2 ± 0.0^a

Results are expressed as means (n = 3) ± standard deviation. Values followed by same letter between variety and recipe do not differ significantly ($p < 0.05$; Dunn's multiple pairwise comparisons). 1 = Konini variety; 2 = Skorpion variety; 3 = control variety; M0 = zero day; M3 = 3 months; M6 = 6 months.

was 38 mg.kg^{-1} and TCP in white variety was only 7 mg.kg^{-1}. In this study purple wheat exhibit a different anthocyanin profile with higher amounts of acylated anthocyanins compared to blue wheat.

Results of mentioned authors were not completely confirmed with our study, the Skorpion variety had the highest amount of TPC, although Koniny had quite similar results. However, Okarter et al. (2010) stated that the TPC content of some red wheat was lower than the control. Lower TCP content in colored variety in our study was not confirmed.

3.4 Fiber

The highest amount of fiber was observed in recipe R1 for the Konini variety (17.5 mg·kg^{-1}) and the lowest amount was observed in recipe R2 for the control Vánek (4 mg·kg^{-1}). In most cases, the highest amount of fiber was measured in colored wheat varieties.

A statistically significant difference ($p < 0.05$) was found between recipes R1 M0 and R1 M3 storage in the warehouse. A statistically significant difference ($p < 0.05$) was found between R1 M0, M3 and M6 and R2 M6 storage in the refrigerator.

The difference between the type of storage (warehouse and refrigerator) was found to be statistically significant difference ($p < 0.05$) between the R1 M0 and M3 (warehouse) with R2 M6 (refrigerator). Then a statistically significant difference ($p < 0.05$) was found between R4 M6 (warehouse) and R1 M0, M3 and M6 in the refrigerator. It can be observed that during storage in most samples the crude fiber content was reduced by 11-38% stored in the warehouse while 8-53% when stored in refrigerator. Researchers from Malaysia (Ng & Rosli, 2014) stated that the biscuits with added cinnamon (6%) contained 4.21% of the total dietary fiber.

3.5 Comparison of varieties

In Table 6 are summarized mean values of fiber, TCP and FRAP without recipe evaluation. Our results confirmed the differences for some varieties during storage, as well as the type of storage in Table 6. Although the properties of the biscuits were influenced by the recipe used, the differences were also confirmed between the individual varieties in the content of TPC and fiber. There was a difference in fiber content between control and Konini. A statistically significant difference ($p < 0.05$) was also confirmed between the standard and the colored varieties evaluated in TPC.

In the antioxidant activity measurement, there were differences between the recipes (Table 4) in agreement with the Král et al. (2021). The significant differences between variety were not determined (Table 6). A scientific team from China (Ma et al., 2014) showed that some varieties of *Triticum aestivum* standard wheat showed higher antioxidant activity than varieties of red wheat, which is partly in line with our results, where the standard variety of common wheat showed in some cases a higher value than the Konini variety and lower than the Skorpion. Another team showed that antioxidant activity in colored wheat biscuits was significantly higher than in the control biscuits (Pasqualone et al., 2014). These finding was not been confirmed.

3.6 Sensory evaluation

Sensory evaluation is described in Table 7. Biscuit color for the analyzed samples was the darkest in the biscuit baked using the Konini variety because of the natural purple color (4.6 – 8.3). The lightest color was in the control variety (white grain) from all recipes (2.1 – 6.7). Out of all the examined samples, biscuits from the Konini variety tasted the best. The biscuits from recipe R1 obtained the best sensory evaluation (6.0 – 6.4). The biscuits from recipe R2 and R3 tasted the worst (2.9 – 5.4). This may be due to the lack of sugar in the recipe. In recipe R3 only 20 g of vanilla sugar was added. Recipe R3 had the greatest aroma intensity (4.2 – 6.9). This may be due to the addition of cinnamon. The biscuits from recipe R2 smelled the worst (2.6 – 3.2). The texture of biscuits was evaluated by how we perceive the biscuit when bitten, whether it is very soft (1) or even very hard (10). The panel found the biscuits from recipe

Table 6. Comparison of varieties without recipes effect.

Varieties	Dry matter	Fiber	FRAP (mg TE·100 g^{-1})	TPC (mg GAE·kg^{-1})
Warehouse				
1	94.74 ± 1.9	9.13 ± 4.3^{a}	244.12 ± 165.2	13.74 ± 1.9^{a}
2	94.35 ± 2.3	7.3 ± 2.2^{a}	170.42 ± 116.0	13.81 ± 2.1^{a}
3	94.81 ± 1.3	5.81 ± 1.6^{b}	155.14 ± 83.4	8.76 ± 1.1^{b}
Refrigerator				
Varieties	Dry matter	Fiber	FRAP (mg TE·100 g^{-1})	TPC (mg GAE·kg^{-1})
1	94.21 ± 2.9	8.89 ± 3.9^{a}	200.06 ± 201.9	14.01 ± 2.1^{a}
2	92.94 ± 4.1	7.49 ± 2.6^{a}	141.11 ± 93.2	14.19 ± 2.3^{a}
3	94.3 ± 2.1	6.27 ± 1.7^{b}	129.07 ± 78.7	8.67 ± 1.0^{b}

Results are expressed as means (n = 8) ± standard deviation. Values followed by same letter between variety and recipe do not differ significantly ($p < 0.05$; Kruskal-Wallis test). 1 = Konini variety; 2 = Skorpion variety; 3 = control variety; FRAP = ferric reducing-antioxidant power; TE = equivalent Trolox; TPC = total polyphenolic content; GAE = gallic acid equivalent.

Table 7. Sensory evaluation in wheat biscuits.

Recipe	Variety	Biscuit color	Taste	Texture	Crispness	Overall acceptability
R1	1	6.37 ± 1.3^{a}	5.97 ± 2.4^{f}	4.32 ± 2.0^{e}	1.86 ± 0.9^{ac}	5.29 ± 2.8^{d}
	2	4.20 ± 1.8^{ag}	6.40 ± 1.9^{a}	4.43 ± 2.1^{f}	2.95 ± 1.9^{b}	6.60 ± 2.1^{b}
	3	3.13 ± 1.7^{ab}	6.00 ± 2.0^{g}	5.43 ± 1.6^{NS}	2.74 ± 1.7^{NS}	6.26 ± 2.1^{af}
R2	1	4.55 ± 1.8^{i}	2.60 ± 1.2^{ef}	3.62 ± 1.7^{d}	4.90 ± 2.6^{c}	3.09 ± 1.3^{de}
	2	3.43 ± 1.9^{f}	2.00 ± 1.3^{abc}	3.10 ± 1.9^{ab}	3.36 ± 2.0^{NS}	2.96 ± 1.6^{bc}
	3	2.70 ± 1.4^{c}	2.67 ± 1.2^{d}	3.17 ± 2.2^{c}	3.43 ± 2.6^{NS}	2.94 ± 1.4^{a}
R3	1	8.25 ± 1.1^{i}	3.26 ± 1.8^{e}	7.50 ± 1.7^{de}	6.33 ± 2.4^{a}	3.92 ± 2.0^{NS}
	2	6.77 ± 1.3^{fgh}	5.21 ± 2.3^{b}	6.45 ± 1.9^{b}	4.57 ± 2.1^{NS}	5.19 ± 2.5^{NS}
	3	6.74 ± 1.5^{bcd}	2.90 ± 1.5^{g}	6.69 ± 1.7^{c}	5.43 ± 2.7^{NS}	2.91 ± 1.3^{f}
R4	1	6.39 ± 1.6^{e}	5.36 ± 2.1^{e}	5.36 ± 1.5^{NS}	4.05 ± 2.1^{NS}	5.53 ± 2.7^{e}
	2	4.38 ± 1.5^{h}	4.67 ± 2.3^{c}	6.98 ± 1.6^{f}	6.00 ± 2.1^{b}	5.50 ± 2.6^{c}
	3	2.06 ± 1.5^{de}	4.00 ± 1.7^{d}	5.10 ± 1.9^{NS}	4.57 ± 2.2^{NS}	5.12 ± 2.3^{NS}

ANOVA, in each column sample means not having the same letter attached to them are significantly different (Kruskal-Wallis test, $p < 0.05$). Values are means ± SD (n = 3). NS = not significant difference; R = recipe; 1 = Konini variety; 2 = Skorpion variety; 3 = control variety.

R2 (3.2 – 3.6) to be the softest while the hardest biscuits were from recipe R3 (6.7 – 7.5). The crispness was evaluated on a scale from very fragile, disintegrating (1) to very cohesive (10). Biscuits from recipe R1 were evaluated as very brittle biscuits (1.9 – 2.7) and the most cohesive were the biscuits from recipe R3 and R4 (4.1 – 6.3). The sweetest biscuits were from recipe R1 (6.4 – 6.9) and the least sweet biscuits were from R2 and R3 (1.2 – 3). The most acceptable biscuits were from recipe R1 (5.3 – 6.7) and R4 (5.1 – 5.5). This is probably due to the higher amount of sugar as people generally like sweet biscuits. Pasqualone et al. (2015) stated that the sensory score for friability and the spread ratio of purple biscuits accounted for 2.6 and 6.0, respectively. Klunklin & Savage (2018) added purple rice flour to a basic wheat flour biscuit and subsequent sensory analysis showed that the overall acceptability of the blended flour biscuits was slightly lower than the control biscuits.

4 Conclusions

Purple and blue wheat biscuits are generally promising as a functional food ingredient rich in antioxidants and fiber. Colored wheat biscuits showed a higher phenol content and antioxidant activity than the control. The highest values of antioxidant activity were observed for the Konini variety in biscuits stored in the warehouse and also in the refrigerator. The results show that the antioxidant activity for those stored in the refrigerator decreased in the range of 58%-37% depending on the variety. The highest amount of fiber was noted in the Konini variety and the lowest amount in the control variety (4 mg.kg^{-1}). Sensory analysis showed that the overall acceptability of colored wheat biscuits was slightly higher than that of control biscuits. The results show that the storage time of biscuits is advantageous for consumers up to 3 months, after which there is a significant decrease in antioxidants by up to 35%. The recipe which contained the most fat and sugar showed the lowest amount of antioxidants while the recipe which contained half the fat and cinnamon gave the best results. In conclusion, the content of bioactive substances in biscuits depends more on the variety of flour used than on the specific recipe.

Further research is underway to explore the potential health applications and benefits of colored wheat food products.

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