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Influence of lacto-fermented traditional and colored wheat grain wholemeal flour on wheat biscuit quality

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ABSTRACT

In this study, the non-treated and lacto-fermented (with *Lacticaseibacillus paracasei* LUHS244) wholemeal of new breed lines of colored wheat (blue 8558-1 and black 8472-5) and traditional wheat "Silva", was evaluated for the lactic acid bacteria (LAB) viable count, acidity, color parameters, amino acid (AA) profile, gamma-aminobutyric acid (GABA), and biogenic amine content. The addition of 50, 100, 150, 200, and 250 g of tested wheat wholemeal was used for biscuit preparation. The chromaticity characteristics of the dough and biscuits, dough pH, acrylamide concentration, overall acceptability, and volatile compounds (VC) were examined. In most cases, fermentation increased the AA and GABA contents in wheat wholemeal. The main biogenic amines in tested wholemeal were putrescine and spermidine. Wheat wholemeal, fermented for 48 h, was selected for biscuit preparation due to its lower pH and higher LAB viable counts. The acrylamide concentration showed correlations with individual VCs. In conclusion, wheat biscuits prepared with 200 and 250 g of non-fermented "Silva" wheat wholemeal, 50 and 100 g of non-fermented and fermented blue wheat wholemeal, as well as 50, 100, and 150 g of non-fermented black wheat wholemeal can be recommended for achieving appropriate acrylamide concentration reduction without impairing the sensory acceptability of the product.

1. Introduction

Consumer preference for a broader variety of food products that include functional nutrients has been on the rise. In addition to berries, fruits, vegetables, and other plant-based sources, colored wheat cereals have become attractive natural ingredients owing to their high contents of functional compounds, including anthocyanins and carotenoids, which are associated with numerous health benefits (Sharma et al., 2020). Colored-grain wheat genotypes (blue, purple, and black) differ from common wheat genotypes (red or white) in that they contain different types of anthocyanins in the aleurone layer and pericarp and possess a higher antioxidant activity than the traditional wheat varieties (Sharma et al., 2018; Xia et al., 2023). As the majority of cereal grain functional compounds are located in the outer aleurone layer of the grain, the incorporation of the grain wholemeal in current food formulas offers a promising strategy to improve the nutritional benefits of wheat

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cereal food products.

In addition to the general health benefits of colored wheat, the high antioxidant properties of their components could also help to control the Maillard reaction during the thermal treatment of cereal products (e.g., biscuits), resulting in a reduction in acrylamide formation of the end products. Alongside the production of toxic and carcinogenic acrylamide, volatile compounds (VC) are also formed during the Maillard reaction, which are associated with the sensory acceptability of the end product (European Food Safety Authority, 2012). However, to date, no study has reported the influence of colored wheat wholemeal on acrylamide formation in biscuits. Considering that cereal products, including biscuits, significantly contribute to the dietary intake of acrylamide, finding natural solutions to reduce the formation of this compound in these products has become a very important issue.

In addition to the use of antioxidants to achieve Maillard reaction control, the fermentation process has also been widely discussed as a strategy toward the reduction of acrylamide formation in cereal products (Gunduz and Pelin, 2023). During fermentation, changes in protein and fat profiles occur and non-desirable compounds may be formed, such as biogenic amines, which are synthesized through the decarboxylation of amino acids (AAs) by exogenous decarboxylases released from the microbial population in the raw cereal material. Taking into consideration these possible non-desirable changes, it is also important to assess the changes in AA profiles and biogenic amine formation in fermented colored wheat grain wholemeal.

For this study, newly created black and blue wheat grain varieties (No. 8472-5 and 8558-1) were chosen because the content of anthocyanins in them is higher compared to traditional ones. For the first time, these new varieties were fermented and used for biscuit production. The main hypotheses of this study were: (I) the colored wheat wholemeal, as an additional source of compounds possessing antioxidant properties, can lead to acrylamide concentration reduction in wheat biscuits; and (II) fermentation of colored wheat wholemeal can lead to an additional effect of reducing the acrylamide content and improving the sensory acceptability of the improved manufactured biscuits.

To test these hypotheses, we evaluated the changes of the wholemeal characteristics of the non-treated and lacto-fermented (with a *Lacticaseibacillus paracasei* LUHS244) wheat variety "Silva" (traditional wheat) and colored wheat grain (the new breeds blue 8558-1 and black 8472-5 wheat), including the LAB viable count, pH, total titratable acidity (TTA), color parameters, AA profile, gamma-aminobutyric acid (GABA) concentration, and biogenic amine content. We further analyzed the influence of these changes on the quality and safety parameters of prepared wheat biscuits, including the chromaticity characteristics of the dough and biscuits, dough pH, acrylamide concentration in biscuits, their overall acceptability, and VC profile.

2. Materials and methods

2.1. Wheat grain varieties and LAB strain

The principal scheme of the first stage of experiment is shown in Fig. 1. The grains of the wheat variety "Silva" (traditional wheat) and the new breeds 8558-1 (blue wheat) and 8472-5 (black wheat) were provided by the Institute of Agriculture, Lithuanian Research Center for Agriculture and Forestry (Akademija, Kedainiai district, Lithuania). The field trials and wheat grain growing conditions are described in detail in **Supplementary File S1** (Field trials and wheat grain growing

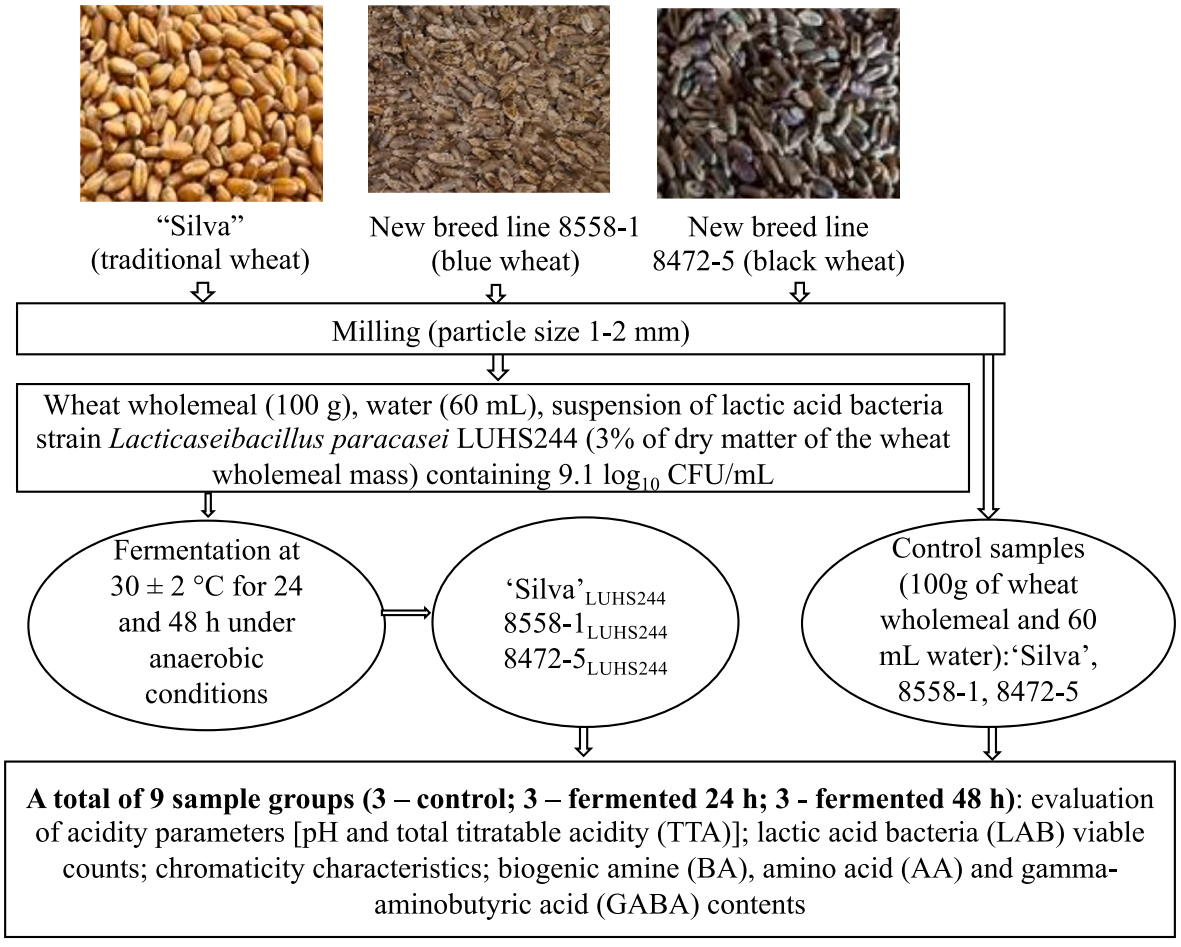

Fig. 1. Principal scheme of preparation and analysis of wheat wholemeal (WW) samples (first stage of the experiment).

conditions). Wheat wholemeal was prepared by milling the wheat grains (moisture content of 14%) with Laboratory Mill 120 (Perten Instruments AB, Stockholm, Sweden) until the particles size was 1–2 mm. The moisture content of the grain was determined using Infratec™ 1241 Grain Analyzer (FOSS, Hilleroed, Denmark).

The LAB strain used for wheat grain wholemeal fermentation (*Lacticaseibacillus paracasei* LUHS244) was obtained from the Lithuanian University of Health Sciences (Kaunas, Lithuania). Our previous studies showed that the LUHS244 strain fermented 27 out of 47 tested carbohydrates; showed tolerance to 30 °C and 37 °C; and high survival in low pH values [the concentration of viable cells after 2 h incubation at pH 2.5 was 9.29 ± 0.1 $\log_{10}$ colony-forming units (CFU)/mL] (Bartkiene et al., 2020). Before the experiments, the LUHS244 strain was stored at −80 °C (Microbank system, Pro-Lab Diagnostics, Birkenhead, UK) and was multiplied in de Man, Rogosa and Sharpe broth (Oxoid Ltd., Hampshire, UK) at 30 ± 2 °C for 24 h, before it was used in wheat wholemeal treatment. The wheat wholemeal, water, and a suspension of the multiplied LAB strain (3% of the dry matter of the wheat wholemeal mass) containing 9.1 $\log_{10}$ CFU/mL were fermented at 30 ± 2 °C for 24 and 48 h in a chamber incubator (Memmert GmbH + Co. KG, Schwabach, Germany). For 100 g of wheat wholemeal, 60 mL of water were used. Non-fermented wheat wholemeal samples (100 g of wheat wholemeal mixed with 60 mL of water) served as the control.

2.2. Biscuit formulation and technological scheme for biscuit preparation

The main formula for biscuits preparation consisted of 200 g of wheat flour (type 550D; 26% gluten, 68% carbohydrate content, 3.9% fiber content, 11.9% protein content, 1.7% fat content, and 0.55–0.62% ash), 100 g of butter (82.0% fat content, 0.80% carbohydrate content, 0.5% protein content, 0.03% salt content), 50 g of saccharose, 3 g of vanilla sugar, 40 g of eggs, 1.5 g of salt, and 2.0 g of baking powder (sodium bicarbonate). The sugar, vanilla sugar, and butter were creamed in a mixer (Guangzhou R & M Machinery, Guangdong, China). Egg mass was added to this cream and mixed for 30 s to obtain a homogeneous mass. Finally, flour was added and mixed for 1 min to obtain a homogeneous dough. Biscuit samples were prepared by the addition of non-fermented and LUHS244-fermented wheat wholemeal to the main biscuit dough formula at levels of 50, 100, 150, 200, and 250 g. Biscuits were formed manually by rolling the dough (at a thickness of 3.0 mm) and stamping. Biscuits were baked in a deck oven (MIWE, Michael Wenz, Germany) at 240 °C for 6 min.

The scheme of preparation and analysis of biscuit sample groups and images of the biscuits are shown in Fig. S4.1. and Fig. S4.2 (**Supplementary File S4**).

2.3. Wheat grain wholemeal characterization

The pH of the wheat wholemeal and water mixture was measured using a pH electrode (PP-15; Sartorius, Goettingen, Germany). TTA was evaluated for a 10 g wheat wholemeal–water mixture sample mixed with 90 mL of water, and the results are expressed in milliliters of a 0.1 mol/L NaOH solution required to achieve a pH value of 8.2 (in Neiman degrees,°N). LAB viable counts were determined according to the method described by Bartkiene et al. (2020). The chromaticity characteristics were evaluated on the sample surface using a CIE L*a*b* system (CromaMeter CR-400, Konica Minolta, Tokyo, Japan). The results are expressed as the CIE color values L* (brightness/darkness), a* (redness/greenness), and b* (yellowness/blueness). Determination of the biogenic amine content was performed according to procedures developed by Ben-Gigirey et al. (1999) with some modifications (**Supplementary File S2**, S.2.1. Determination of the biogenic amine content in wheat wholemeal samples). For analysis of the AA and GABA contents, sample preparation and dansylation were performed according to the method of Cai et al. (2010) with some modifications (**Supplementary File S2,** S.2.2. Determination of gamma-aminobutyric acid content in wheat wholemeal samples).

2.4. Analysis of dough and biscuit samples

The pH of the biscuit's dough was measured using a pH electrode (PP-15; Sartorius, Goettingen, Germany) by inserting it directly into the dough mass.

Before biscuit color measurement, acrylamide, and VC determination, 10 biscuits from each group were milled until the particles size reached 1–2 mm diameter and three milled samples from each group were analyzed. Biscuit color parameters were evaluated using the CIE L*a*b* system (CromaMeter CR-400) on the surface of milled sample as described above. The acrylamide concentration was determined according to the method of Zhang et al. (2006) with some modifications (**Supplementary File S2,** S.2.3. Determination of acrylamide content in biscuits).

The VCs of biscuit samples were analyzed by gas chromatography–mass spectrometry. All procedures are described in detail in **Supplementary File S2** (S.2.4. Determination of biscuit volatile compound profile).

The overall acceptability of biscuits was tested by a panel formed by 10 trained judges using a 10-point Likert scale ranging from 0 (extremely disliked) to 10 (extremely liked).

2.5. Statistical analysis

For wheat wholemeal and biscuit data interpretation, results are expressed as mean values ± standard error of the mean. Six wheats wholemeal or biscuit samples from each group were analyzed for assessment of microbiological and physicochemical parameters. Biscuit overall acceptability was based on a sample size of $n = 10$. Two parallel fermentations of wheat wholemeal were performed with three replicates analyzed from each group. The biscuit preparation was repeated two times and three samples from each group were taken for analysis. To evaluate the effects of fermentation and different amounts of wheat wholemeal on biscuit parameters, data were analyzed by tests of between-subjects effects using the statistical package IBM SPSS Statistics [28.0.1.0(142), Chicago, IL, USA]. In addition, Pearson correlation coefficients were calculated between various parameters. Interpretation of the Pearson's correlation coefficients was done according to Ratner (2009). The results were recognized as statistically significant at $p \leq 0.05$.

3. Results and discussion

3.1. LAB viable counts, acidity, and chromaticity parameters of the wheat wholemeal

The LAB viable counts, pH and acidity (TTA), and chromaticity (L*, brightness; a*, redness; and b*, yellowness) parameters of the wheat wholemeal are shown in Table 1. There were no significant differences in LAB viable counts among different wheat wholemeal samples with the same treatment. The average LAB viable counts in non-fermented wheat grain wholemeal was 5.29 $\log_{10}$ CFU/g, whereas that in the 24-h and 48-h fermented samples was 8.25 $\log_{10}$ CFU/g and 9.33 $\log_{10}$ CFU/g, respectively. Among the non-fermented wheat grain wholemeal samples, the lowest pH values were obtained in 8472-5 black wheat (5.59); after 24 h of fermentation, the pH values of these samples were an average of 2.55% lower than those of the 24-h fermented "Silva" and 8558-1 blue wheat grain wholemeal samples. However, after 48 h of fermentation, the lowest pH was found in "Silva" wholemeal (3.66). After 48 h of fermentation, the pH of all samples was lower than those of the 24-h fermented samples, and the fermentation duration proved to be a significant factor influencing the samples' pH ($p < 0.001$). Comparison of TTA values among the different wheat grain wholemeal samples with the same treatment (i.e., non-fermented, fermented for 24 h, or

Table 1
Lactic acid bacteria (LAB) viable counts, acidity and chromaticity parameters (average ± standard error) of the wheat wholemeal (WW).

Wheat wholemeal samples	LAB viable counts, $\log_{10}$ CFU/g	Acidity parameters		Chromaticity parameters, NBS		
		pH	TTA, °N	L*	a*	b*
'Silva'$_{NF}$	5.17 ± 0.22^{a,A}	5.72 ± 0.02^{c,C}	1.60 ± 0.11^{a,A}	49.1 ± 0.32^{c,A}	7.42 ± 0.22^{c,B}	16.2 ± 0.19c,B
'Silva'$_{F24h}$	8.18 ± 0.14^{a,B}	3.90 ± 0.03^{b,B}	8.60 ± 0.15^{a,B}	48.9 ± 0.25^{b,A}	7.75 ± 0.18^{a,B}	11.8 ± 0.22c, A
'Silva'$_{F48h}$	9.33 ± 0.25^{a,C}	3.66 ± 0.02^{a,A}	12.9 ± 0.18^{a,C}	48.8 ± 0.21^{b,A}	6.82 ± 0.21^{a,A}	15.9 ± 0.16 b, B
8558-1 (blue wheat)$_{NF}$	5.47 ± 0.19^{a,A}	5.68 ± 0.01^{b,C}	1.91 ± 0.14^{b,A}	40.1 ± 0.19^{b,A}	2.62 ± 0.11^{a,A}	8.81 ± 0.24 b, A
8558-1 (blue wheat)$_{F24h}$	8.12 ± 0.28^{a,B}	3.94 ± 0.03^{b,B}	10.8 ± 0.13^{b,B}	49.5 ± 0.23^{c,B}	10.6 ± 0.16^{c,B}	10.3 ± 0.19 b, B
8558-1 (blue wheat)$_{F48h}$	9.18 ± 0.16^{a,C}	3.79 ± 0.02^{c,A}	14.7 ± 0.09^{b,C}	51.2 ± 0.19^{c,C}	11.7 ± 0.21^{b,C}	11.5 ± 0.13a, C
8472-5 (black wheat)$_{NF}$	5.22 ± 0.13^{a,A}	5.59 ± 0.03^{a,C}	2.42 ± 0.11^{c,A}	35.8 ± 0.27^{a,A}	5.29 ± 0.13^{b,A}	8.01 ± 0.09a, A
8472-5 (black wheat)$_{F24h}$	8.45 ± 0.18^{a,B}	3.82 ± 0.01^{a,B}	12.9 ± 0.16^{c,B}	35.4 ± 0.35^{a,A}	8.59 ± 0.14^{b,B}	8.51 ± 0.11a, B
8472-5 (black wheat)$_{F48h}$	9.49 ± 0.17^{a,C}	3.73 ± 0.02^{b,A}	18.6 ± 0.17^{c,C}	45.1 ± 0.29^{a,B}	12.0 ± 0.09^{b,C}	11.4 ± 0.17a, C

NF – non-fermented; F24h – fermented 24 h; F48h – fermented 48 h; LAB – lactic acid bacteria; CFU – colony-forming units; TTA – total titratable acidity; L* – brightness or (−) darkness; a* - redness or (−) greenness; b* - yellowness or (−) blueness; NBS – National Bureau of Standards units.
Data are represented as means (n = 6) ± standard errors (SE). $^{a–c}$ Mean values denoted with different letters indicate significantly different values between the different wheat wholemeal (WW) samples with the same treatment ($_{NF}$, $_{F24}$, and $_{F48}$); $^{A–C}$ Mean values denoted with different letters indicate significantly different values between the same variety of wheat with different treatment ($_{NF}$, $_{F24}$ and $_{F48}$) ($p \leq 0.05$).

fermented for 48 h) showed that the lowest TTA was attained in "Silva" sample groups (both non-fermented and fermented samples) and the highest TTA was obtained in 8472-5 black wheat wholemeal. In all sample groups, the highest TTA was found after 48 h of fermentation, and a positive moderate correlation was detected between LAB viable counts and TTA of the samples ($r = 0.403$, $p = 0.037$). Fermentation duration was a significant factor influencing the TTA of the samples ($p = 0.012$).

With respect to the chromaticity characteristics of samples, the L* coordinates of colored wheat grain wholemeal increased with a prolonged fermentation duration; after 48 h of fermentation, the average L* values of 8558-1 blue and 8472-5 black wheat wholemeal samples were 27.7% and 26.0% higher than those of the corresponding non-fermented samples, respectively. However, opposite trends were found for the a* values, with a decrease in a* values found when increasing duration of fermentation (48 h) by 4.47 times, 2.27 times, and 8.09% for the 8558-1 blue, 8472-5 black, and "Silva" wheat samples, respectively, compared with those of the corresponding non-fermented samples. Fermentation increased the b* values of the colored wheat grain wholemeal, whereas opposite tendencies were found for "Silva" wholemeal b* values during fermentation.

The metabolic activity of LAB, including organic acids production and proteolytic enzymes excretion, influences the acidification of fermentable substrate and is related to various factors (e.g., the presence of dietary fiber, mineral content, composition of carbohydrates, and the duration of the process and temperature) (De Vuyst et al., 2014). It was reported that cereal grain outer layer contains more desirable micronutrients since they are involved in catalytic reactions, which are thus more effective for facilitating LAB multiplication (Chavan and Chavan, 2011). In addition, the ash present in the outer layer of the grains has a major influence on the buffering capacity of the matrix, which leads to higher TTA values of the fermentable substrate that confers better resistance to acidity changes. We found strong positive correlations between the TTA and L* as well as a* values of the samples ($r = 0.655$, $p < 0.001$ and $r = 0.746$, $p < 0.001$, respectively). However, only a moderate correlation was established between the samples' TTA and b* coordinate values ($r = 0.454$, $p = 0.017$). Correlations between other analyzed parameters were not statistically significant.

Anthocyanins, which can impart a blue or purple color to cereal grains, are sensitive to certain environmental conditions and their concentration can vary in fermentable substrate due to activity of such microbial enzymes as chitinase and amylase (Awe et al., 2023). During fermentation, and depending on the microbial strain under use, the carotenoid concentration in a substrate can be increased due to the increased excretion of membrane-associated lipophilic compounds from the cereal grains (Antognoni et al., 2019). The changes in lutein and zeaxanthin, and their concentration during lacto-fermentation can be associated with the formation of volatile carotenoid cleavage derivatives and higher lipid oxidation involving the endogenous lipoxygenase/linoleate system (Antognoni et al., 2019). Additionally, LAB metabolism can lead to higher antioxidative activity of the fermentable substrate, which can conversely reduce oxidation (by inhibiting the enzymes) or increase oxidation (by compounds possessing antioxidant properties) of color compounds (Đorđević et al., 2010). Overall, the results of this study showed that fermentation with *Lc. Paracasei* strain is effective for the treatment of tested wholemeal samples, and the most appropriate characteristics (lower pH, higher TTA, and LAB viable counts) of the wholemeal can be obtained after 48 h of fermentation. Although we found that the main factor influencing changes of the color characteristics of the wholemeal is TTA, further research is needed to evaluate the changes of cereal color compounds in detail.

3.2. AA profile and GABA concentration in wheat wholemeal

AA profiles of non-fermented and fermented wheat grain wholemeal samples are depicted in Table 2. Among the essential AAs, methionine and phenylalanine were not detected in any of the non-fermented cereal grain wholemeal samples. However, fermentation increased methionine and phenylalanine content in "Silva" and colored wheat wholemeal samples. The similar tendencies were noticed for histidine, lysine, and valine in 24 h fermented wholemeal of all types. However, after 48 h of fermentation, the content of these AA decreased in "Silva" (except histidine) and black wheat wholemeal. Among all, the highest threonine concentration was established in 48-h fermented "Silva" samples. For the colored wheat, the highest threonine concentration was found in 24-h fermented samples.

All analyzed factors and their interactions had a significant impact on the methionine, histidine, valine, and threonine content in samples ($p < 0.001$), whereas the duration of fermentation was the only significant factor influencing the phenylalanine content ($p < 0.001$) (Supplementary Table S3.1). No statistically significant association of the analyzed factors or their interactions were found for the leucine and isoleucine concentrations in samples. Moderate positive correlations were found between LAB viable counts and methionine as well as histidine, threonine, leucine and isoleucine contents ($r = 0.582$, $p = 0.001$; $r = 0.549$, $p = 0.003$; $r = 0.430$, $p = 0.025$; $r = 0.489$, $p = 0.010$, respectively) (Supplementary Table S3.2). Moderate positive correlations were found between TTA and histidine as well as valine contents ($r = 0.437$, $p = 0.023$; $r = 0.471$, $p = 0.013$, respectively), whereas a moderate negative correlation was established between the

Table 2
Amino acid and gamma-aminobutyric contents (GABA) (μmol/g) (average ± standard error) in the wheat wholemeal (WW) samples.

Wheat wholemeal samples	'Silva'$_{NF}$	'Silva'$_{F24h}$	'Silva'$_{F48h}$	8558-1 (blue wheat)$_{NF}$	8558-1 (blue wheat)$_{F24h}$	8558-1 (blue wheat)$_{F48h}$	8472-5 (black wheat)$_{NF}$	8472-5 (black wheat)$_{F24h}$	8472-5 (black wheat)$_{F48h}$
Essential amino acids, μmol/g									
Histidine	nd	0.140 ± 0.012a,A	0.335 ± 0.023c,B	0.043 ± 0.003 b,A	0.147 ± 0.012a,B	0.148 ± 0.013 b,B	0.023 ± 0.002a,A	0.186 ± 0.015 b,C	0.097 ± 0.008a,B
Leucine and isoleucine	0.128 ± 0.011a,A	1.42 ± 0.12a,B	4.06 ± 0.26c,C	0.162 ± 0.012 b,A	1.56 ± 0.14a,B	1.64 ± 0.13 b, B	0.110 ± 0.007a,A	2.06 ± 0.15 b,C	1.33 ± 0.11a,B
Lysine	0.081 ± 0.007a,A	0.516 ± 0.031a,B	1.36 ± 0.10c,C	0.128 ± 0.009 b,A	0.565 ± 0.029a,B	0.520 ± 0.028 b,B	0.081 ± 0.005a,A	0.673 ± 0.049 b,C	0.361 ± 0.026a,B
Methionine	nd	0.233 ± 0.019a,A	0.637 ± 0.028 b,B	nd	0.202 ± 0.009a,A	0.215 ± 0.018a,A	nd	0.334 ± 0.029 b,B	0.229 ± 0.021a,A
Phenylalanine	nd	0.498 ± 0.029a,A	1.34 ± 0.11c,B	nd	0.549 ± 0.027a,A	0.573 ± 0.027 b,A	nd	0.689 ± 0.031 b,B	0.478 ± 0.039a,A
Threonine	0.068 ± 0.005a,A	0.208 ± 0.019a,B	0.677 ± 0.032c,C	0.102 ± 0.009c,A	0.333 ± 0.022c,C	0.253 ± 0.019 b,B	0.082 ± 0.007 b,A	0.277 ± 0.021 b,C	0.209 ± 0.019a,B
Valine	0.075 ± 0.007a,A	0.649 ± 0.032a,B	1.53 ± 0.14 b,C	0.124 ± 0.009 b,A	0.638 ± 0.031a,B	0.610 ± 0.031a,B	0.109 ± 0.008 b,A	0.935 ± 0.027 b,C	0.561 ± 0.036a,B
Non-essential amino acids, μmol/g									
Arginine	0.274 ± 0.021 b,A	1.19 ± 0.09a,B	2.34 ± 0.20c,C	0.306 ± 0.025c,A	1.08 ± 0.07a,B	0.999 ± 0.041a,B	0.213 ± 0.018a,A	1.68 ± 0.13 b,C	1.22 ± 0.11 b,B
Glutamine	3.07 ± 0.29 b,A	5.28 ± 0.21 b,B	16.9 ± 0.15c,C	1.29 ± 0.11a, A	3.42 ± 0.21a,C	2.80 ± 0.23a,B	1.13 ± 0.09a, A	9.27 ± 0.76c,B	8.28 ± 0.46 b,B
Asparagine	0.531 ± 0.032a,A	0.841 ± 0.026a,B	1.59 ± 0.13c,C	0.678 ± 0.031 b,B	0.907 ± 0.023 b,C	0.583 ± 0.039a,A	0.483 ± 0.035a,A	1.36 ± 0.11c,C	0.671 ± 0.049 b,B
Glutamic acid	0.108 ± 0.009a,A	0.461 ± 0.033 b,B	1.37 ± 0.11c,C	0.202 ± 0.015 b,A	0.387 ± 0.031a,B	0.354 ± 0.027a,B	0.250 ± 0.021c,A	0.788 ± 0.035c,C	0.570 ± 0.041 b,B
Serine	nd	0.399 ± 0.019a,A	1.07 ± 0.08c,B	0.147 ± 0.011a,A	0.435 ± 0.029a,C	0.308 ± 0.025a,B	0.140 ± 0.012a,A	0.553 ± 0.031 b,C	0.360 ± 0.023 b,B
Aspartic acid	0.323 ± 0.022a,b,A	0.820 ± 0.032 b,B	2.17 ± 0.18c,C	0.293 ± 0.013a,A	0.759 ± 0.024a,C	0.555 ± 0.022a,B	0.349 ± 0.029 b,A	1.48 ± 0.12c,C	0.999 ± 0.043 b,B
Glycine	0.170 ± 0.014 b,A	0.642 ± 0.036a,B	1.62 ± 0.14c,C	0.139 ± 0.011a,A	0.604 ± 0.019a,B	0.664 ± 0.031a,C	0.122 ± 0.008a,A	1.22 ± 0.11 b,C	0.770 ± 0.032 b,B
Alanine	0.216 ± 0.019a,A	1.36 ± 0.11a,B	3.63 ± 0.26c,C	0.399 ± 0.020c,A	1.54 ± 0.13a,B	1.59 ± 0.14 b, B	0.262 ± 0.022 b,A	2.18 ± 0.15 b,C	1.32 ± 0.11a,B
Proline	nd	0.549 ± 0.046a,A	1.28 ± 0.11 b,B	0.086 ± 0.005 b,A	0.526 ± 0.036a,B	0.609 ± 0.023a,C	0.053 ± 0.003a,A	0.954 ± 0.031 b,C	0.652 ± 0.049a,B
Tyrosine	nd	0.122 ± 0.009a,A	0.632 ± 0.029c,B	0.042 ± 0.003a,A	0.231 ± 0.018 b,B	0.326 ± 0.024 b,C	0.043 ± 0.003a,A	0.376 ± 0.029c,C	0.208 ± 0.018a,B
Gamma-aminobutyric acid, μmol/g									
GABA	0.369 ± 0.031c,A	1.64 ± 0.11 b,B	2.81 ± 0.22 b,C	0.253 ± 0.017 b,A	1.23 ± 0.11a,B	1.16 ± 0.09a,B	0.204 ± 0.018a,A	2.88 ± 0.24c,C	1.37 ± 0.12a,B

NF – non-fermented; F24h – fermented 24 h; F48h – fermented 48 h; GABA - gamma-aminobutyric acid; nd – not detected.
Data are represented as means (n = 6) ± standard errors. $^{a–c}$ Mean values denoted with different letters indicate significantly different values between the different wheat wholemeal (WW) samples with the same treatment ($_{NF}$, $_{F24}$, and $_{F48}$); $^{A–C}$ Mean values denoted with different letters indicate significantly different values between the same variety of wheat with different treatment ($_{NF}$, $_{F24}$, and $_{F48}$) ($p \leq 0.05$).

phenylalanine content and pH ($r = -0.409$, $p = 0.034$).

In all cases, fermentation increased the contents of all non-essential amino acids in "Silva" samples and the highest content was found after 48 h of fermentation. In all cases, fermentation increased non-essential amino acid concentrations in colored wheat with different tendencies seen after 24 and 48 h of fermentation. The highest contents of glutamine, asparagine, serine, and aspartic acid were found after 24 h of fermentation of 8558-1 blue wheat wholemeal samples, and the highest contents of glycine, proline, and tyrosine were established in the 48-h fermented sample groups. No significant differences were detected in arginine, glutamic acid, and alanine contents after 24 and 48 h of 8558-1 blue wheat wholemeal fermentation. However, 48-h fermentation reduced the asparagine content in 8558-1 blue wheat wholemeal samples by an average of 1.16 times in comparison to that of non-fermented samples. In all cases, higher contents of non-essential amino acids were found in the fermented 8472-5 black wheat wholemeal samples; however, higher contents of all non-essential amino acids were found in 24-h fermented samples than in the 48-h fermented samples (except for glutamine, with an average content of 8.78 μmol/g in both the 24-h and 48-h fermented samples). Moderate positive correlations were found between LAB viable counts and proline as well as tyrosine concentrations in wholemeal ($r = 0.477$, $p = 0.013$ and $r = 0.471$, $p = 0.013$, respectively); a weak negative correlation was established between the pH and glycine content ($r = -0.398$, $p = 0.040$); and moderate positive correlations were found between wholemeal TTA and glutamic acid, serine, glycine, and proline contents ($r = 0.460$, $p = 0.016$; $r = 0.417$, $p = 0.031$; $r = 0.509$, $p = 0.007$; and $r = 0.433$, $p = 0.024$, respectively) (Supplementary Table S3.2).

The results of this study are in agreement with previous studies reporting that the microbial fermentation of wheat grain wholemeal can result in higher concentrations of both essential and non-essential AAs (Ezemba et al., 2016). It is known that LAB have highly developed versatile proteolytic systems, which could be linked to the strain-dependent AA biosynthesis pathway and the hydrolysis of complex proteins to generate free amino acids (Lim et al., 2019). However, it should be mentioned that these changes, in most of cases, varied between 24 h and 48 h fermentation, with the concentrations of some AAs starting to decrease after 48 h because of further metabolic processes resulting in the production of lower-molecular-weight compounds (e.g., biogenic amines). Additionally, if fermented cereal wholemeal is planned to be used for the manufacture of bread, biscuits, and other products for which preparation thermal treatment is applied, lower quantities of AAs can be associated with a lower intensity of the Maillard reaction, and consequently the formation of non-desirable compounds (e.g., acrylamide). Finally, in addition to the main most appropriate fermented material traits (low pH, high TTA, and LAB viable counts), we recommend the use of 48 h of fermentation of wholemeal samples for biscuits preparation, because most of these samples (blue and black

wheat wholemeal) showed lower AA concentrations in comparison with those of the 24 h samples.

Fermented sample groups had higher GABA concentrations than non-fermented sample groups in every case (Table 2). In "Silva" samples, the highest GABA concentration was found after 48 h of fermentation, The GABA content in fermented 8558-1 blue wheat wholemeal samples was 4.74 times higher compared to the non-fermented. In black wheat wholemeal samples, the highest GABA content was obtained after 24 h of fermentation.

GABA, which can be produced during lacto-fermentation, is a desirable compound in food because of its many established health benefits, including the treatment of neurological disorders, diuretic effect and anti-carcinogenic characteristics (Lee et al., 2023). It was reported that *Lactiplantibacillus plantarum* has the ability to synthesize GABA, and its production is strain-dependent and increased with the addition of the wheat outer layer fraction (Verni et al., 2022). Finally, it is important to emphasize that by using wheat grain wholemeal as a source of dietary fiber to increase the functional value of biscuits, other valuable molecules such as GABA can also contribute to achieving a higher functional and nutritional value of cereal-based products.

3.3. Biogenic amine formation in wheat wholemeal samples during fermentation

The biogenic amine content in wheat cereal wholemeal samples is given in Table S4.1. (**Supplementary File S4**). Tryptamine, phenylethylamine, cadaverine, histamine, tyramine, and spermine were absent in non-fermented and fermented wholemeal. However, putrescine and spermidine were the only ones found in tested wholemeal samples. Putrescine was absent in non-fermented "Silva" samples, but fermentation increased its content. Different tendencies were found for the spermidine content in "Silva" samples, with an average decrease of 5.00 and 1.38 times compared to that of the non-fermented samples.

After 24 h of fermentation, the putrescine content of the 8558-1 blue wheat samples was reduced by an average of 1.87 times in comparison to that of the non-fermented samples, and putrescine was not detected in these samples after 48 h of fermentation. Opposite trends were found for spermidine in 8558-1 blue wheat samples in which fermentation increased the spermidine content by an average of 1.78 and 1.68 times after 24 and 48 h of fermentation, respectively. However, the lowest total biogenic amine content in 8558-1 blue wheat samples was attained after 48 h of fermentation (30.3 mg/kg).

Fermentation reduced the putrescine content by 1.59 times and 1.37 times after 24 h and 48 h, respectively, in 8472-5 black wheat group samples. However, a slight increase in the spermidine concentration was detected after 24 and 48 h of fermentation (on average, 9.86%). The lowest total biogenic amine content was found in 24-h fermented 8472-5 black wheat samples (37.1 mg/kg). Despite some differences in biogenic amine concentrations between cereal wholemeal sample groups, there were no significant effects of the analyzed factors and their interactions (Supplementary Table S3.3). Analysis of correlations between biogenic amine concentrations and AAs, GABA, LAB viable count, and acidity parameters showed a moderate negative correlation between the total biogenic amine content and glutamine concentration in wheat wholemeal ($r = -0.517$, $p = 0.006$), whereas other correlations between these parameters were not significant (Supplementary Table S3.4).

LAB are involved in biogenic amine synthesis and the optimal pH for biogenic amine-forming enzyme activities is in the range of 4.0–5.5 (Jairath et al., 2015). Putrescine is formed from arginine, whereas spermidine is formed from arginine and methionine, and their toxicity depends on synergistic effects (Özogul and Özogul, 2019). Consumption of products containing a high content of biogenic amines can lead to different types of foodborne diseases (Özogul and Özogul, 2019). The increase in the levels of putrescine and spermidine found under prolonged fermentation in this study further highlights the importance of conducting in-depth research on biogenic amines in fermented products.

3.4. Characteristics of the dough and biscuits

Dough and biscuits chromaticity characteristics and dough pH values are shown in Table 3. The control group dough showed higher brightness (L*), and lower redness (a*) and similar yellowness b* when comparing to those of the biscuit samples. In most cases, the L* and b* values of dough, prepared with "Silva" and colored wheat wholemeal, were lower than those of the biscuits. In the case of the "Silva" wholemeal, doughs had lower a* values than biscuits, mostly. Generally, doughs had greater a* values than biscuits in the case of the colored wheat wholemeal.

We found that the a* values of dough and biscuits were significantly influenced by the amount of wholemeal used in the main biscuit recipe ($p < 0.001$) (Supplementary Table S3.5). Although no correlations were found between other chromaticity characteristics of the dough and biscuits with the dough pH, some tendencies in the change of dough pH were unveiled; as expected, in most cases, the pH of the dough was reduced when increasing the fermented wholemeal content. The differences in doughs chromaticity are usually related to the dough composition and its preparation process. As the main biscuit formula and preparation method was the same for all samples in our study, dough color was markedly influenced by the addition of colored wheat wholemeal. Fermentation also induces changes in the color characteristics of the colored wheat wholemeal due to changes in medium pH and glycosylation level of anthocyanins. Observed changes in biscuit color characteristics following thermal treatment could be explained by differences in dough composition, which induce variations in Maillard reaction intensities. The synthesis of brown polymers known as melanoidins (browning) during final stage of the Maillard reaction, is primarily responsible for the color development of thermally treated foods (Starowicz and Zieliński, 2019). The changes in biscuit browning could be attributed to a decrease in pH, which lowers the rate of the Maillard reaction, and the thermal degradation of anthocyanins (Žilić et al., 2016). It was reported that the rate of degradation of anthocyanins increases as the temperature rises, while the presence of saccharose, ascorbic acid, reducing sugars, and Maillard reaction, as well as phenolics also affect anthocyanin degradation (Žilić et al., 2016). Although we detected clear changes in the color compounds of the analyzed matrices, due to the changes in the color characteristics of baking dough and biscuits during their processing, deeper research is required to identify the changes in concrete anthocyanins in raw food materials and end products.

3.5. Acrylamide concentration and overall acceptability of biscuits

The acrylamide concentrations in the biscuit samples are presented in Fig. 2. Acrylamide was absent in biscuit samples prepared with 50, 100, 150, and 200 g of non-fermented 8558-1 blue wheat grain wholemeal. The acrylamide content in biscuits prepared with the 50, 100, 150, and 200 g of fermented 8558-1 blue wheat grain wholemeal was, on average, from 24.7% to 41.9% lower than that in control biscuits. The lowest acrylamide content among biscuits prepared with 8472-5 black wheat grain wholemeal was found in the group with 50 g (2.83 μg/kg), and the highest content was found in the biscuits prepared with 150 g of fermented wholemeal (11.0 μg/kg), although there was no significant difference from the content of the control biscuits.

Most of the biscuit groups prepared with non-fermented and fermented "Silva" wholemeal showed similar or lower concentration of acrylamide in comparison with those of the control biscuits, except for the group prepared with 250 g of fermented "Silva" wholemeal. The lowest concentration of acrylamide was attained in biscuits prepared with 100 g of fermented and 200 g of non-fermented "Silva" wholemeal. Weak positive correlations were found between acrylamide content in biscuits and the L* values of the dough and biscuits ($r = 0.240$, $p = 0.021$; $r = 0.266$, $p = 0.010$, respectively) (Supplementary File S4. Table S4.2). A weak positive correlation was found between the

Table 3
Dough and biscuit chromaticity characteristics and dough pH (average ± standard error).

Dough and biscuit samples	Dough chromaticity characteristics			Biscuit chromaticity characteristics			Dough pH
	L*	a*	b*	L*	a*	b*	
	NBS						
C–B	76.0 ± 0.41^{b}	3.24 ± 0.25^{a}	30.1 ± 0.29^{a}	73.1 ± 0.32^{a}	6.17 ± 0.14^{b}	29.8 ± 0.31^{a}	6.62 ± 0.03
BS-50	71.8 ± 0.28^{a,H}	3.66 ± 0.24^{b,A}	26.6 ± 0.25^{a,F}	73.7 ± 0.41^{b,F}	2.87 ± 0.17^{a,D}	26.2 ± 0.26^{a,I}	6.65 ± 0.02^{F}
BS-100	69.5 ± 0.19^{b,E}	4.84 ± 0.21^{b,C}	24.7 ± 0.21^{b,D,E}	68.3 ± 0.39^{a,D}	2.55 ± 0.11^{a,C}	21.7 ± 0.28^{a,D}	6.62 ± 0.02^{F}
BS-150	66.9 ± 0.23^{a,C}	5.58 ± 0.18^{b,D}	23.3 ± 0.22^{b,C}	68.0 ± 0.28^{b,D}	1.79 ± 0.12^{a,A}	20.9 ± 0.23^{a,C}	6.67 ± 0.04^{F}
BS-200	66.4 ± 0.21^{a,B}	5.56 ± 0.22^{b,D}	22.2 ± 0.19^{b,B}	66.4 ± 0.35^{a,B}	2.16 ± 0.14^{a,B}	19.6 ± 0.29^{a,B}	6.62 ± 0.03^{F}
BS-250	64.5 ± 0.18^{a,A}	5.50 ± 0.19^{b,D}	21.1 ± 0.15^{b,A}	65.8 ± 0.37^{b,B,C}	2.71 ± 0.21^{a,C,D}	18.4 ± 0.15^{a,A}	6.65 ± 0.01^{F}
BS-F50	73.1 ± 0.32^{b,I}	3.68 ± 0.16^{a,A}	26.9 ± 0.16^{a,F}	71.8 ± 0.26^{a,E}	4.17 ± 0.22^{b,E}	26.8 ± 0.22^{a,J}	6.13 ± 0.02^{E}
BS-F100	70.0 ± 0.25^{a,F}	4.33 ± 0.27^{b,B}	25.0 ± 0.19^{b,E}	75.2 ± 0.32^{b,G}	1.61 ± 0.11^{a,A}	24.3 ± 0.18^{a,G}	5.54 ± 0.04^{D}
BS-F150	67.6 ± 0.27^{a,D}	4.71 ± 0.16^{a,B,C}	24.3 ± 0.21^{a,D}	67.1 ± 0.41^{a,C}	5.09 ± 0.25^{b,G}	25.1 ± 0.19^{b,H}	5.04 ± 0.05^{C}
BS-F200	69.8 ± 0.19^{b,E,F}	5.59 ± 0.33^{a,D}	24.2 ± 0.19^{a,D}	63.5 ± 0.33^{a,A}	6.05 ± 0.23^{a,H}	23.9 ± 0.14^{a,F}	4.65 ± 0.04^{B}
BS-F250	71.3 ± 0.16^{b,G}	5.54 ± 0.29^{b,D}	24.4 ± 0.20^{b,D}	63.4 ± 0.29^{a,A}	4.84 ± 0.20^{a,F}	23.1 ± 0.16^{a,E}	4.29 ± 0.03^{A}
Bblue-50	68.6 ± 0.22^{a,G}	0.340 ± 0.022^{b,A}	21.6 ± 0.19^{a,D}	74.2 ± 0.22^{b,H}	0.267 ± 0.019^{a,B}	22.7 ± 0.19^{b,F}	7.06 ± 0.02^{J}
Bblue-100	64.0 ± 0.34^{a,E}	0.447 ± 0.031^{b,B}	18.4 ± 0.11^{a,C}	72.1 ± 0.30^{b,G}	0.117 ± 0.009^{a,A}	20.0 ± 0.15^{b,D}	6.93 ± 0.03^{I}
Bblue-150	65.5 ± 0.28^{a,F}	0.310 ± 0.026^{b,A}	18.4 ± 0.23^{a,C}	70.0 ± 0.28^{b,E}	0.270 ± 0.014^{a,B}	18.7 ± 0.16^{a,B}	6.65 ± 0.02^{H}
Bblue-200	61.1 ± 0.17^{a,D}	0.513 ± 0.025^{a,C}	16.2 ± 0.14^{a,B}	64.2 ± 0.32^{b,B}	2.90 ± 0.15^{b,D}	19.6 ± 0.17^{b,C}	6.71 ± 0.03^{G}
Bblue-250	59.7 ± 0.29^{a,C}	0.627 ± 0.018^{a,D}	15.2 ± 0.10^{a,A}	60.9 ± 0.31^{b,A}	5.35 ± 0.22^{b,H}	20.5 ± 0.31^{b,E}	6.85 ± 0.04^{F}
Bblue-F50	73.0 ± 0.25^{b,H}	2.74 ± 0.14^{a,E}	23.9 ± 0.19^{a,E}	72.1 ± 0.27^{a,G}	3.30 ± 0.19^{b,E}	24.8 ± 0.28^{b,G}	6.00 ± 0.02^{E}
Bblue-F100	65.1 ±	4.26 ± 0.13^{b,F}	18.6 ±	71.0 ±	1.79 ± 0.11^{a,C}	20.8 ±	5.74 ± 0.03^{D}

Table 3 (*continued*)

Dough and biscuit samples	Dough chromaticity characteristics			Biscuit chromaticity characteristics			Dough pH
	L*	a*	b*	L*	a*	b*	
	NBS						
	0.14^{a,F}		0.17^{a,C}	0.41^{b,F}		0.25^{b,E}	
Bblue-F150	59.0 ± 0.22^{a,B}	5.71 ± 0.11^{b,G}	16.4 ± 0.15^{a,B}	65.5 ± 0.35^{b,C}	4.09 ± 0.23^{a,G}	20.9 ± 0.31^{b,E}	5.12 ± 0.01^{C}
Bblue-F200	61.1 ± 0.19^{a,D}	8.62 ± 0.20^{b,H}	16.3 ± 0.13^{a,B}	67.1 ± 0.37^{b,D}	3.97 ± 0.24^{a,F}	16.4 ± 0.14^{a,A}	4.67 ± 0.02^{B}
Bblue-F250	56.6 ± 0.33^{a,A}	8.67 ± 0.24^{b,H}	15.0 ± 0.18^{a,A}	60.4 ± 0.39^{b,A}	6.83 ± 0.17^{a,I}	20.9 ± 0.18^{b,E}	4.44 ± 0.04^{A}
Bblack-50	49.0 ± 0.25^{a,B}	1.29 ± 0.08^{b,A}	22.8 ± 0.21^{b,E}	73.5 ± 0.36^{b,H}	0.207 ± 0.015^{a,A}	21.6 ± 0.19^{a,H}	6.61 ± 0.03^{H}
Bblack-100	59.8 ± 0.21^{a,F}	3.28 ± 0.15^{b,B}	16.9 ± 0.15^{a,D}	67.6 ± 0.41^{b,F}	1.25 ± 0.09^{a,B}	19.3 ± 0.16^{b,E}	6.55 ± 0.04^{H}
Bblack-150	56.6 ± 0.13^{a,D}	3.07 ± 0.22^{b,B}	15.0 ± 0.13^{a,C}	60.5 ± 0.45^{b,C,D}	2.30 ± 0.10^{a,C}	14.8 ± 0.11^{a,A}	6.33 ± 0.03^{G}
Bblack-200	59.1 ± 0.18^{a,E}	3.18 ± 0.14^{b,B}	14.6 ± 0.09^{a,B}	62.7 ± 0.38^{b,E}	2.17 ± 0.09^{a,C}	15.3 ± 0.13^{b,B}	6.44 ± 0.02^{F}
Bblack-250	59.0 ± 0.11^{a,E}	3.96 ± 0.18^{b,C}	15.0 ± 0.14^{a,C}	61.0 ± 0.29^{b,D}	2.16 ± 0.07^{a,C}	14.9 ± 0.12^{a,A}	6.49 ± 0.03^{F}
Bblack-F50	59.2 ± 0.32^{b,E}	4.89 ± 0.21^{b,D}	23.6 ± 0.19^{b,F}	68.9 ± 0.30^{a,G}	4.40 ± 0.21^{a,E}	18.2 ± 0.17^{a,D}	6.30 ± 0.05^{E}
Bblack-F100	59.1 ± 0.26^{a,E}	7.71 ± 0.31^{b,E}	17.2 ± 0.15^{a,D}	67.6 ± 0.27^{b,F}	3.50 ± 0.14^{a,D}	20.6 ± 0.19^{b,F}	5.55 ± 0.04^{D}
Bback-F150	59.1 ± 0.17^{a,E}	7.54 ± 0.16^{b,E}	17.0 ± 0.15^{a,D}	60.1 ± 0.41^{b,C}	4.79 ± 0.12^{a,E}	17.3 ± 0.16^{a,C}	4.94 ± 0.02^{C}
Bblack-F200	54.8 ± 0.21^{b,C}	9.17 ± 0.11^{a,F}	15.0 ± 0.22^{a,C}	53.7 ± 0.58^{a,B}	9.22 ± 0.11^{a,G}	21.1 ± 0.20^{b,G}	4.45 ± 0.03^{B}
Bblack-F250	46.2 ± 0.15^{a,A}	7.20 ± 0.22^{a,E}	11.4 ± 0.16^{a,A}	52.8 ± 0.46^{b,A}	6.83 ± 0.21^{a,F}	18.5 ± 0.16^{b,D}	4.10 ± 0.02^{A}

C–B—control biscuit samples, without addition of wheat wholemeal (WW); BS – 'Silva' grain wholemeal; Bblue – 8558-1 (blue wheat) grain wholemeal; Bblack – 8472-5 (black wheat) grain wholemeal (WW); F – fermented; 50, 100, 150, 200 and 250 g quantity of wholemeal (WW) used for biscuits preparation. L* - brightness or (−) darkness; a* - redness or (−) greenness; b* yellowness or (−) blueness; Data are represented as means (n = 6) ± standard errors (SE). $^{a–b}$ Mean values denoted with different letters indicate significantly different values between the same chromaticity parameter of the dough and biscuit ($p \leq 0.05$). $^{A–J}$ Mean values denoted with different letters indicate significantly different values between the same chromaticity parameter of the dough and biscuit samples and between pH of the dough with different quantities of wholemeal (WW) in the same group ($p \leq 0.05$).

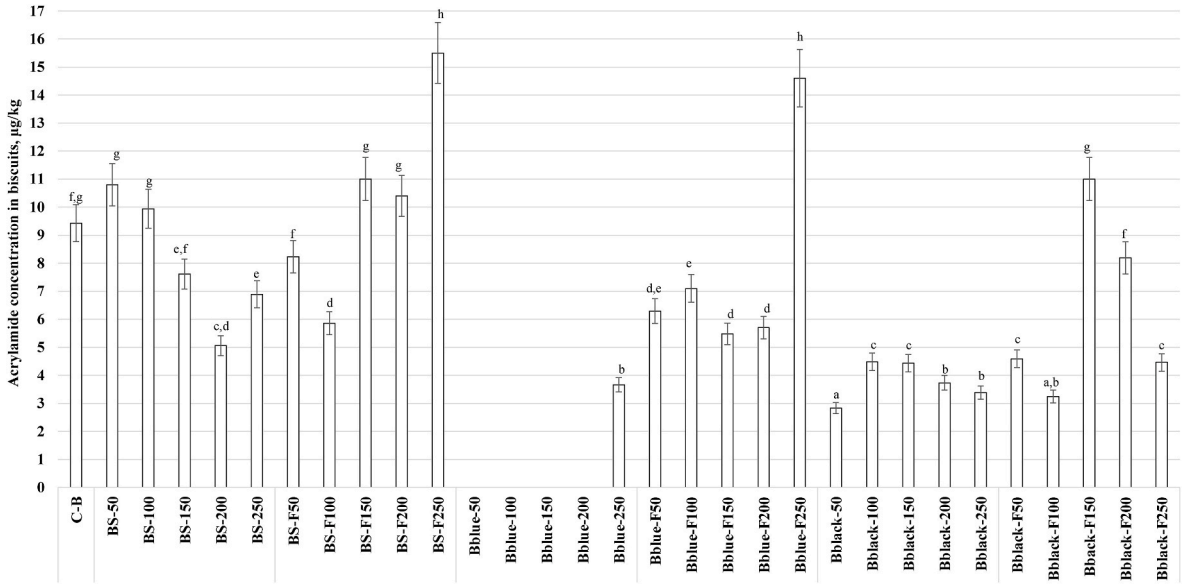

Fig. 2. Acrylamide concentration (μg/kg) in biscuits (C–B—control biscuit samples, without addition of wheat wholemeal; BS – 'Silva' grain wholemeal; Bblue – 8558-1 (blue wheat) grain wholemeal; Bblack – 8472-5 (black wheat) grain wholemeal; F – fermented; 50, 100, 150, 200 and 250 g quantity of wholemeal used for biscuits preparation. Data are represented as means (n = 6) ± standard errors. [a–h] Mean values denoted with different letters indicate significantly different values between acrylamide concentration in biscuit samples ($p \leq 0.05$)).

acrylamide content and b* values of the biscuits ($r = 0.266$, $p = 0.010$). The test of between-subject effects showed that cereal variety is a statistically significant factor influencing the acrylamide content in biscuits ($p < 0.001$).

In this study, acrylamide levels in biscuits were much lower than the limit regulated by the European Commission of 500 mg/kg (European Food Safety Authority, 2012). It is well known that a higher asparagine concentration is present in the outer layer of cereal grain; additionally, the ash content in flour is directly proportional to the reducing saccharides, and all of these compounds influence the effectiveness of the Maillard reaction (Claus et al., 2008). In addition to the reactions occurring between asparagine and carbonyl sources, another alternative route for acrylamide formation is through the oxidation process of fats. Previous studies also showed that the low pH values of baking dough obtained by the addition of organic acids significantly reduces the concentration of acrylamide in bakery products ("FDE - Food Drink Europe Acrylamide Toolbox," 2019). Application of fermentation or the addition of fermented ingredients to the main biscuit formula leads to more complex changes in comparison with the inclusion of separate organic acids. The use of fermentation with LAB has reported to effectively reduce the acrylamide content in bread (Albedwawi et al., 2021). However, this reduction was explained by the decrease in dough pH and not by the microbial activity related with the consumption of acrylamide precursors (Wang et al., 2017). In our study, when comparing all biscuits prepared with different varieties of wheat grain wholemeal, the lowest concentration of asparagine was found in non-fermented 8472-5 black wheat grain wholemeal, whereas the lowest concentration of acrylamide found in biscuits prepared with non-fermented 8558-1 blue wheat grain wholemeal (which had the highest concentration of asparagine compared with that of the non-fermented "Silva" and 8472-5 black wheat grain wholemeal samples) (Table 2). Although the highest content of asparagine was found in "Silva" wholemeal after fermentation, increasing the amount of fermented "Silva" in the main biscuit formula did not effectively increase the acrylamide concentration. These results lead us to conclude that more factors, in addition to asparagine content and pH, are involved in the mechanisms of acrylamide formation in biscuits. The presence of different types of anthocyanins in colored wholemeal could also be related to the lower levels of acrylamide because the usage of compounds possessing antioxidant properties is considered to reduce acrylamide formation due to carbonyl trapping, reduction of saccharide degradation through the Maillard reaction, and radical scavenging activities (Tesby et al., 2018). Indeed, more in-depth research is needed to identify these factors as well as to determine the potential antioxidant capacity of the colored wheat compounds, their changes during their processing, and their overall influence on acrylamide formation.

Results of the overall acceptability of biscuit samples are depicted in Supplementary File Fig. S4.3. In most cases, addition of wheat cereal wholemeal reduced the overall acceptability of the biscuits. However, samples prepared with 50, 100, 150, 200, and 250 g of non-fermented "Silva" wholemeal; samples prepared with 50 and 100 g of non-fermented and fermented 8558-1 blue wheat grain wholemeal; and samples prepared with 50, 100, 150, and 250 g of non-fermented 8472-5 black wheat grain wholemeal showed similar overall acceptability as found for the control samples. The amount of the wholemeal used in the biscuit formula, as well as interaction of the quantity of the wholemeal and variety of cereal grain were statistically significant factors influencing the overall acceptability of the biscuits ($p = 0.017$ and $p = 0.008$, respectively) (Supplementary Table S3.6).

Taking into consideration that both safety and acceptability are very important food characteristics, based on our sensory panel results and detailed characterization, biscuits prepared with 200 and 250 g of non-fermented "Silva" wholemeal; biscuits prepared with 50 and 100 g of non-fermented and fermented 8558-1 blue wheat grain wholemeal; as well as biscuits prepared with 50, 100, and 150 g of non-fermented 8472-5 black wheat grain wholemeal can be recommended for acrylamide reduction in biscuits without impairing the sensory acceptability of the end product.

3.6. VC profile of the biscuits

The profiles of VCs in the biscuits are shown in Fig. 3. Correlations between each VC and acrylamide, as well as overall acceptability are given in Table S4.3 (Supplementary File S4), while significance of the analyzed factors (quantity and variety of cereal grain) and their interaction on VC is given in Table S3.7 (Supplementary File 3). The main VCs in biscuits comprised vanillin, D-limonene, heptyl methyl ketone, benzeneacetaldehyde, hexanoic acid, and hexanal. None of the analyzed

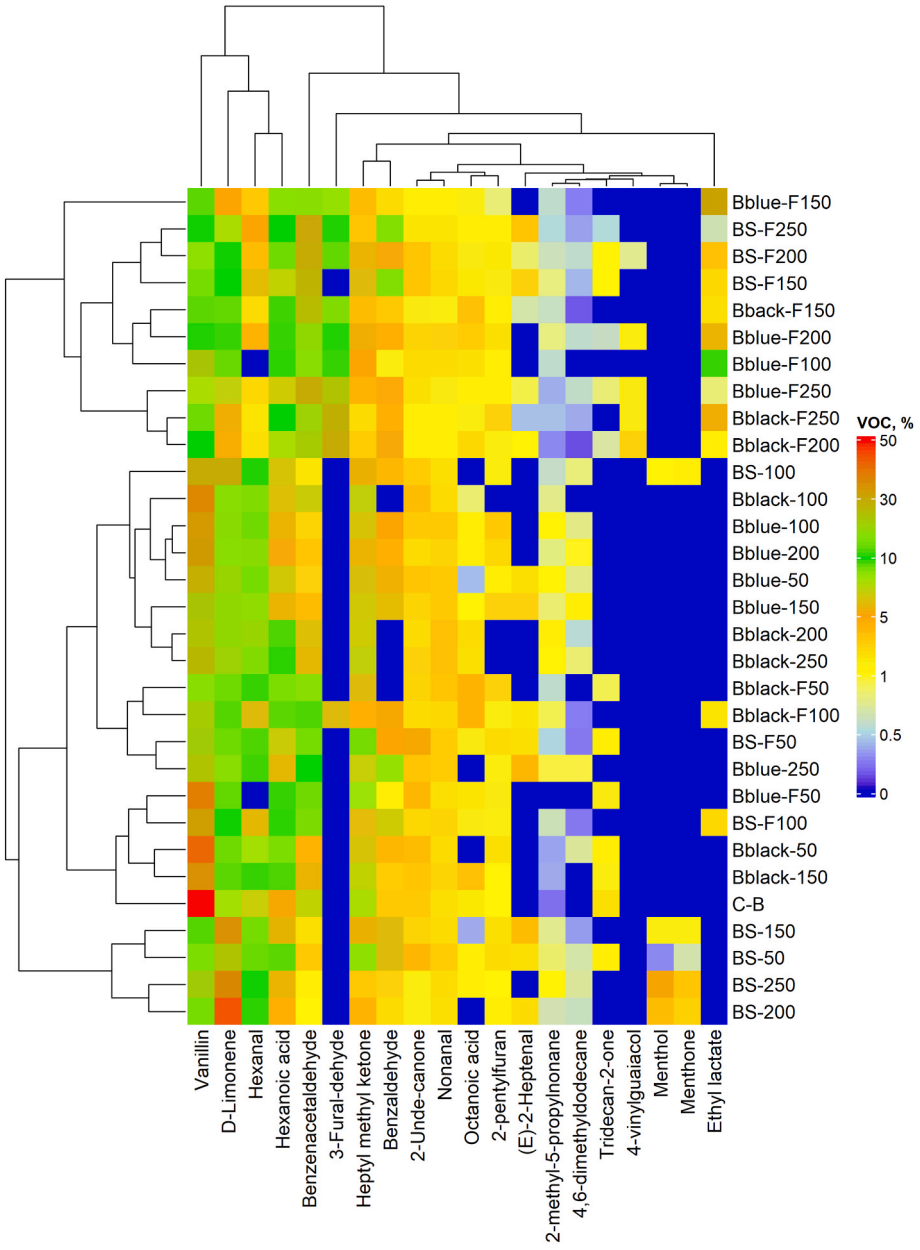

Fig. 3. Volatile compound (VC) of the biscuit samples (% from the total VC content) (C–B - control biscuit samples, without addition of wheat wholemeal; BS - 'Silva' grain wholemeal; Bblue – 8558-1 (blue wheat) grain wholemeal; Bblack – 8472-5 (black wheat) grain wholemeal; F – fermented; 50, 100, 150, 200 and 250 g quantity of wholemeal used for biscuits preparation). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

factors had a statistically significant effect on the vanillin and hexanal content in biscuits. However, the amount of wholemeal in the biscuit recipe was a significant factor influencing the heptyl methyl ketone concentration ($p < 0.001$). Additionally, the variety of cereal grain was a significant factor influencing the hexanoic acid, D-limonene contents, and 3-furaldehyde ($p = 0.049$, $p = 0.019$, $p = 0.026$, respectively) in biscuits. A moderate positive correlation was established between benzeneacetaldehyde content and acrylamide concentration in biscuit samples ($r = 0.393$, $p < 0.001$). Benzeneacetaldehyde is formed during the thermal treatment as a product of the Maillard reaction (Cui et al., 2017). 3-Furaldehyde, a flavor and quality parameter with undesirable effects, was found in most biscuits with fermented black and blue wholemeal. 3-Furaldehyde showed a weak positive correlation with the acrylamide content in biscuits ($r = 0.238$, $p = 0.021$) (Supplementary File S4. Table S4.3). As a.

Almost all samples contained (E)-2-heptenal, benzaldehyde, 2-pentylfuran, nonanal, octanoic acid, and 2-undecanone. Some of these compounds were influenced by the quantity and variety of cereal grain, while others showed significant correlations with acrylamide and overall acceptability (Table S3.7 and S4.3, Supplementary Files S3 and S4). Other VCs found in lower amounts in biscuit samples were ethyl lactate, menthone, menthol, 2-methyl-5-propylnonane, 4,6-dimethyldodecane, 4-vinylguaiacol, and tridecan-2-one (Fig. 3). Although the latter VC concentrations in biscuits were lower, some of them showed significant correlations with the acrylamide content as well as with overall acceptability of the biscuits (Supplementary File S4. Table S4.3).

4. Conclusions

Lower pH, higher TTA, and LAB viable counts in wheat wholemeal were obtained after 48 h of fermentation when compared with those of 24-h fermented samples. However, strong positive correlations were detected between sample TTA and brightness as well as redness values, along with moderate correlations between sample TTA and yellowness values, suggesting the degradation of the cereal wholemeal colored compounds. In most cases, fermentation increased AA and GABA contents in wheat grain wholemeal; however, after 48 h of fermentation, the AA content decreased in some of the wholemeal samples in comparison with that detected at 24 h of fermentation. Degradation of AAs leads to biogenic amine formation, and the main biogenic amines detected in wheat wholemeal were putrescine and spermidine. We found different effects of fermentation and wheat variety on dough and biscuit chromaticity characteristics; however, their correlations with dough pH were not statistically significant, and there was no correlation between the dough pH and acrylamide content in biscuits. Nevertheless, the acrylamide concentration showed correlations with individual VCs (weak negative correlation with nonanal and weak positive correlations with tridecan-2-one, benzeneacet-aldehyde, (E)-2-heptenal, and 3-furaldehyde). Finally, biscuits prepared with 200 and 250 g of non-fermented "Silva" wholemeal, 50 and 100 g of non-fermented and fermented 8558-1 blue wheat wholemeal, as well as 50, 100, and 150 g of non-fermented 8472-5 black wheat wholemeal can be recommended for reduction of acrylamide in biscuits without impairing the sensory acceptability of the final product. Further research is required to identify the specific color compounds in wheat, as well as to analyze their changes during processing, including their possible participation in the Maillard reaction.

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CRediT authorship contribution statement

Elena Bartkiene: Conceptualization, Formal analysis, Methodology, Resources, Supervision, Writing – original draft. **Aiste Valionyte:** Formal analysis, Investigation. **Vytaute Starkute:** Data curation, Formal analysis, Investigation. **Dovile Klupsaite:** Formal analysis, Investigation, Visualization, Writing – review & editing. **Ernestas Mockus:** Data curation, Formal analysis, Investigation, Methodology. **Egle Zokaityte:** Formal analysis, Investigation. **Darius Cernauskas:** Data curation, Formal analysis, Investigation. **João Miguel Rocha:** Supervision, Writing – review & editing. **Fatih Özogul:** Supervision, Writing – review & editing. **Romas Ruibys:** Investigation, Writing – review & editing. **Zilvinas Liatukas:** Data curation, Investigation, Resources. **Vytautas Ruzgas:** Investigation, Resources, Writing – review & editing.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcs.2023.103831.

References

Albedwawi, A.S., Turner, M.S., Olaimat, A.N., Osaili, T.M., Al-Nabulsi, A.A., Liu, S.-Q., Shah, N.P., Ayyash, M.M., 2021. An overview of microbial mitigation strategies for acrylamide: lactic acid bacteria, yeast, and cell-free extracts. Lebensm. Wiss. Technol. 143, 111159 https://doi.org/10.1016/j.lwt.2021.111159.

Antognoni, F., Mandrioli, R., Potente, G., Taneyo Saa, D.L., Gianotti, A., 2019. Changes in carotenoids, phenolic acids and antioxidant capacity in bread wheat doughs fermented with different lactic acid bacteria strains. Food Chem. 292, 211–216. https://doi.org/10.1016/j.foodchem.2019.04.061.

Awe, S., Aransiola, D.M., Irondi, E.A., 2023. Microbial succession and anthocyanin concentration during sorghum fermentation. Meas. Food 12, 100109. https://doi.org/10.1016/j.meafoo.2023.100109.

Bartkiene, E., Lele, V., Ruzauskas, M., Domig, K.J., Starkute, V., Zavistanaviciute, P., Bartkevics, V., Pugajeva, I., Klupsaite, D., Juodeikiene, G., Mickiene, R., Rocha, J.M., 2020. Lactic acid bacteria isolation from spontaneous sourdough and their characterization including antimicrobial and antifungal properties evaluation. Microorganisms 8, 64. https://doi.org/10.3390/microorganisms8010064.

Ben-Gigirey, B., Vieites Baaptista de Sousa, J.M., Villa, T.G., Barros-Velazquez, J., 1999. Histamine and cadaverine production by bacteria isolated from fresh and frozen albacore (Thunnus alalunga). J. Food Protect. 62, 933–939. https://doi.org/10.4315/0362-028x-62.8.933.

Cai, H.-L., Zhu, R.-H., Li, H.-D., 2010. Determination of dansylated monoamine and amino acid neurotransmitters and their metabolites in human plasma by liquid chromatography–electrospray ionization tandem mass spectrometry. Anal. Biochem. 396, 103–111. https://doi.org/10.1016/j.ab.2009.09.015.

Chavan, R.S., Chavan, S.R., 2011. Sourdough technology—a traditional way for wholesome foods: a review. Compr. Rev. Food Sci. Food Saf. 10, 169–182. https://doi.org/10.1111/j.1541-4337.2011.00148.x.

Claus, A., Carle, R., Schieber, A., 2008. Acrylamide in cereal products: a review. J. Cereal. Sci. 47, 118–133. https://doi.org/10.1016/j.jcs.2007.06.016.

Cui, H., Jia, C., Hayat, K., Yu, J., Deng, S., Karangwa, E., Duhoranimana, E., Xia, S., Zhang, X., 2017. Controlled formation of flavor compounds by preparation and application of Maillard reaction intermediate (MRI) derived from xylose and phenylalanine. RSC Adv. 7, 45442–45451. https://doi.org/10.1039/C7RA09355A.

De Vuyst, L., Van Kerrebroeck, S., Harth, H., Huys, G., Daniel, H.-M., Weckx, S., 2014. Microbial ecology of sourdough fermentations: diverse or uniform? Food Microbiol. https://doi.org/10.1016/j.fm.2013.06.002. V International Symposium on Sourdough - Cereal Fermentation for Future Foods, Helsinki 10-12 October 2012 37, 11–29.

Đorđević, T.M., Šiler-Marinković, S.S., Dimitrijević-Branković, S.I., 2010. Effect of fermentation on antioxidant properties of some cereals and pseudo cereals. Food Chem. 119, 957–963. https://doi.org/10.1016/j.foodchem.2009.07.049.

European Food Safety Authority, 2012. Update on acrylamide levels in food from monitoring years 2007 to 2010. EFSA J. 10 https://doi.org/10.2903/j.efsa.2012.2938.

Ezemba, C.C., Anakwenze, V.N., Archibong, E.J., Anaukwu, C.G., Obi, Z.C., Ekwealor, C. C., Ekwealor, I.A., 2016. Methionine production using native starches and proteins in submerged fermentation by Bacillus cereus S8. World J. Pharm. Pharmaceut. Sci. 5 (4), 2056–2067. ISSN 2278–4357.

FDE - Food Drink Europe Acrylamide Toolbox, 2019. https://www.fooddrinkeurope.eu/uploads/publications_documents/FoodDrinkEurope_Acrylamide_Toolbox_2019.pdf. August 29.

Gunduz, B., Pelin, C., 2023. Formulation and processing strategies to reduce acrylamide in thermally processed cereal-based foods. Int. J. Environ. Res. Publ. Health 20, 6272. https://doi.org/10.3390/ijerph20136272.

Jairath, G., Singh, P.K., Dabur, R.S., Rani, M., Chaudhari, M., 2015. Biogenic amines in meat and meat products and its public health significance: a review. J. Food Sci. Technol. 52, 6835–6846. https://doi.org/10.1007/s13197-015-1860-x.

Lee, X.Y., Tan, J.S., Cheng, L.H., 2023. Gamma aminobutyric acid (GABA) enrichment in plant-based food – a mini review. Food Rev. Int. 39, 5864–5885. https://doi.org/10.1080/87559129.2022.2097257.

Lim, Y.H., Foo, H.L., Loh, T.C., Mohamad, R., Abdullah, N., 2019. Comparative studies of versatile extracellular proteolytic activities of lactic acid bacteria and their potential for extracellular amino acid productions as feed supplements. J. Anim. Sci. Biotechnol. 10, 15. https://doi.org/10.1186/s40104-019-0323-z.

Özogul, Y., Özogul, F., 2019. Biogenic amines formation, toxicity, regulations in food. In: Saad, B., Tofalo, R. (Eds.), Biogenic Amines in Food. Analysis, Occurrence and Toxicity. Royal Society of Chemistry, London, UK, pp. 1–13. https://doi.org/10.1039/9781788015813-00001.

Ratner, B., 2009. The correlation coefficient: its values range between +1/−1, or do they? J. Target Meas. Anal. Market. 17, 139–142. https://doi.org/10.1057/jt.2009.5.

Sharma, N., Tiwari, V., Vats, S., Kumari, A., Chunduri, V., Kaur, S., Kapoor, P., Garg, M., 2020. Evaluation of anthocyanin content, antioxidant potential and antimicrobial activity of black, purple and blue colored wheat flour and wheat-grass juice against common human pathogens. Molecules 25, 5785. https://doi.org/10.3390/molecules25245785.

Sharma, S., Chunduri, V., Kumar, A., Kumar, R., Khare, P., Kondepudi, K.K., Bishnoi, M., Garg, M., 2018. Anthocyanin bio-fortified colored wheat: nutritional and functional characterization. PLoS One 13, e0194367. https://doi.org/10.1371/journal.pone.0194367.

Starowicz, M., Zieliński, H., 2019. How maillard reaction influences sensorial properties (color, flavor and texture) of food products? Food Rev. Int. 35, 707–725. https://doi.org/10.1080/87559129.2019.1600538.

Tesby, M.R.L., Neveen, F.A., Neveen, A.E.-W., Nashwa, M.Y., 2018. Effect of thyme, cumin and anise on the formation of acrylamide in some bakery products. Alex. J. Agric. Sci. 63, 183–192. https://doi.org/10.21608/alexja.2018.81839.

Verni, M., Vekka, A., Immonen, M., Katina, K., Rizzello, C.G., Coda, R., 2022. Biosynthesis of γ-aminobutyric acid by lactic acid bacteria in surplus bread and its use in bread making. J. Appl. Microbiol. 133, 76–90. https://doi.org/10.1111/jam.15332.

Wang, Shujun, Yu, J., Xin, Q., Wang, Shuo, Copeland, L., 2017. Effects of starch damage and yeast fermentation on acrylamide formation in bread. Food Control 73, 230–236. https://doi.org/10.1016/j.foodcont.2016.08.002.

Xia, Q., Shui, Y., Zhi, H., Ali, A., Yang, Z., Gao, Z., 2023. Exogeneous selenium enhances anthocyanin synthesis during grain development of colored-grain wheat. Plant Physiol. Biochem. 200, 107742 https://doi.org/10.1016/j.plaphy.2023.107742.

Zhang, Yu, Dong, Y., Ren, Y., Zhang, Ying, 2006. Rapid determination of acrylamide contaminant in conventional fried foods by gas chromatography with electron capture detector. J. Chromatogr. A 1116, 209–216. https://doi.org/10.1016/j.chroma.2006.03.042.

Žilić, S., Kocadağlı, T., Vančetović, J., Gökmen, V., 2016. Effects of baking conditions and dough formulations on phenolic compound stability, antioxidant capacity and color of cookies made from anthocyanin-rich corn flour. LWT–Food Sci. Technol. 65, 597–603. https://doi.org/10.1016/j.lwt.2015.08.057.