



Green lentil fortification of wheat bread: a strategy for quality improvement and acrylamide reduction[☆]

Vytaute Starkute^{a,b}, Elena Bartkiene^{a,b}, Ernestas Mockus^a, João Miguel Rocha^c, Darius Cernauskas^d, Erika Mozuriene^a, Romas Ruibys^e, Gul Ebru Orhun^f, Dovile Klupsaite^{a,*}

^a Institute of Animal Rearing Technologies, Faculty of Animal Sciences, Lithuanian University of Health Sciences, Tilzes Str. 18, Kaunas 47181 and 44307, Lithuania.

^b Department of Food Safety and Quality, Veterinary Academy, Lithuanian University of Health Sciences, Tilzes Str. 18, Kaunas 47181, Lithuania.

^c Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, Porto 4169-005, Portugal.

^d Food Institute, Kaunas University of Technology, Radvilenu Road 19, Kaunas 50254, Lithuania.

^e Institute of Agricultural and Food Sciences, Agriculture Academy, Vytautas Magnus University, K. Donelaičio g. 58, 44248 Kaunas, Lithuania.

^f Canakkale Onsekiz Mart University, Bayramic Vocational College, 17020 Çanakkale, Turkey.

ARTICLE INFO

Keywords:

Lentils
Fermentation
Volatile compounds
Acrylamide
Saccharides
Overall acceptability

ABSTRACT

This study aimed to assess how non-treated (N), milled (M), and with *Lactiplantibacillus plantarum* fermented (F) green lentils affect the quality and safety parameters, including volatile compound (VC) profile and acrylamide (AA) concentration, of wheat bread (WB). The overall acceptability (OA) of WB with 5, 10, 15, and 20% lentils, as well as with 25% the non-M F and N non-F lentils was similar to that of the control. The addition of M lentils resulted in a higher increase in AA concentration in WB, compared to those prepared with non-M lentils. Lentil quantity and type added significantly influenced most of the VC formation in bread. Correlations between AA content in WB and separate VC were found. Finally, it can be suggested to supplement the bread with 5, 10, or 15% fermented non-milled green lentils to provide the safest variant with a low AA level as well as favorable OA.

1. Introduction

Well-rounded profile of amino acids (rich in lysine, low in sulfur-containing amino acids), high nutritional quality, and bioactive compounds, which are crucial for preventing degenerative diseases and enhancing overall health, of lentils makes them a favorable functional food ingredient or even a food product (Ganesan & Xu, 2017). The protein content in lentils mainly consists of storage proteins categorized by their solubility: albumins (16%), globulins (70%), glutelins (11%), and prolamins (3%) (Jarpa-Parra, 2018). They are also low in fat and sodium, while being rich in potassium, with a sodium-to-potassium ratio of 1:30 (Padovani et al., 2007). Furthermore, lentils serve as an excellent plant-based source of iron, provide a variety of essential minerals, and vitamins (B1, B2, B3, B5, B6, and B9), as well as α , β , and γ tocopherols, and phyloquinone (Ryan et al., 2007; Soltan, 2013). Finally, plant-based protein sources present a cost-effective solution for meeting future protein demands while enhancing dietary quality across various income levels (Aggarwal & Drewnowski, 2019). Lentil-based baked

goods and extruded products are gaining popularity worldwide because of their enhanced nutritional benefits (Chelladurai & Erkinbaev, 2020). The rise in lentil production and usage is largely fuelled by the development of innovative lentil-based items, heightened health awareness, demand for organically sourced lentil products, and a greater understanding of healthy diets among consumers.

Bread is among the most widely consumed bakery products globally, leading to extensive research on its properties (Lemos et al., 2024). Various types of bread exist, and white bread is being the most favored due to its appealing sensory qualities (Bakke & Vickers, 2007). However, wheat bread lacks certain essential amino acids and dietary fibers. In this context, adding lentil flour to the main wheat bread recipe could provide significant advantages. On the other hand, adding components high in protein could accelerate up the production of derivatives of the Maillard reaction, such as acrylamide. Acrylamide is recognized as a potential human carcinogen and has been shown to have neurotoxic, genotoxic, mutagenic, and enzyme-inhibitory effects (Shapla et al., 2018; Yan et al., 2023). In food, reducing sugars and asparagine are the

[☆] This article is part of a Special issue entitled: 'Acrylamide Research' published in Food Chemistry: X.

* Corresponding author.

E-mail address: dovile.klupsaite@ismu.lt (D. Klupsaite).

precursors of this compound, which formation takes place at temperatures higher than 120 °C and low water activity (Mollakhalili-Meybodi et al., 2021; Stadler et al., 2002). The *Acrylamide Toolbox* tool, created in 2005, provides valuable advice and suggestions on how to minimize acrylamide formation in food products for industry and home producers (Calabrese et al., 2024; Parker, 2024). The variety of crop, cultivar, and harvest season all influence the presence of acrylamide precursors (Mollakhalili-Meybodi et al., 2021). By applying optimized techniques for managing wheat crops and utilizing biotechnological methods to develop wheat varieties with reduced asparagine content, it may be possible to lower acrylamide formation in bread (Kaur & Halford, 2023). Recipe reformulation can affect acrylamide levels in both beneficial and adverse ways regardless of whether the goal is to reduce acrylamide synthesis (Parker, 2024). In general, the choice of bread ingredients, along with the processing (fermentation, water activity, acidity) and baking conditions (temperature, duration), determines the final acrylamide content in bread (Mollakhalili-Meybodi et al., 2021; Streekstra & Livingston, 2020).

Despite bread being a staple in many diets, there is limited research on the concurrent presence of this contaminant in enriched with lentils bread. This raises significant concerns, especially with the rising demand for nutritionally enhanced bread, underscoring the need to evaluate whether these products are safe and if the actual daily intake of acrylamide is underestimated. Further investigation into new, with lentils enriched, bread formulations and the evaluation of the quality and safety of these products will be essential for expanding lentil application in bread production. This study aimed to assess how non-treated, milled, and *Lactiplantibacillus plantarum*-fermented green lentils affect the quality and safety parameters of wheat bread. Key factors evaluated included overall acceptability, specific volume, shape coefficient, crumb porosity, texture hardness, moisture content, mass loss after baking, crust colour characteristics, volatile compound (VC) profile, and acrylamide concentration.

2. Materials and methods

2.1. Features of green lentils, lactic acid bacteria (LAB) used to ferment lentils, and conditions, under which fermentation was performed

Green lentils from Ltd. "Galinta ir partneriai" (Kaunas, Lithuania) had the following composition per 100 g (based on the label information): total carbs 48.5 g, protein 24.0 g, and fat 1.5 g. Lentils were ground using a Laboratory Mill 120 (Perten Instruments AB, Stockholm, Sweden) until the particles were between 1 and 2 mm in size in order to assess the impact of the lentils' particle size on sourdough properties as well as wheat bread quality and safety criteria. The collection of the Lithuanian University of Health Sciences (Kaunas, Lithuania) provided the *Lactiplantibacillus plantarum* No. 122. Prior to the experiment, this strain was grown for 24 hours at 30 °C in anaerobic circumstances in De Man, Rogosa, and Sharpe (MRS) broth culture medium (Biolife, Milano, Italy). To create a 3% (w/w) multiplied LAB strain per lentil–water mixture, 3 mL of fresh LAB cultured in MRS broth (average cell concentration of 8.6 log₁₀ CFU/mL) were injected in 100 g of lentils (lentils/water ratio was 1:1 w/w). In a chamber incubator (Memmert GmbH Co. KG, Schwabach, Germany), the lentil samples were fermented anaerobically for 24 hours at 30 °C. Non-fermented samples (mixed with water) were analyzed and (or) used for bread preparation as non-fermented control. Nonfermented and fermented green lentils features as acidity, colour, microbiological parameters, free amino acid profile, γ -aminobutyric acid level, and volatile compound (VC) profile are provided in **Supplementary file 1** and in our previous study (Mockus et al., 2023).

2.2. Wheat bread preparation and analysis methods

2.2.1. Wheat bread preparation

1.0 kg of wheat flour, 1.5% salt, 3% instant yeast, and 1.0 L water

comprised the basic bread recipe (control bread samples). To produce the tested bread groups, five, ten, fifteen, twenty, and twenty-five percent of green lentils (based on the weight of wheat flour, as ingredient content in bread recipes is calculated relative to flour according to bread technology rules) were added to the basic recipe in four forms: non-fermented non-milled, non-fermented milled, fermented non-milled, and fermented milled. Following bread samples groups were prepared: control bread (without lentils); 5 groups with 5, 10, 15, 20, and 25% of non-fermented (NF) and non-milled (NM) lentils; 5 groups with 5, 10, 15, 20, and 25% of fermented (F) NM lentils; 5 groups with 5, 10, 15, 20, and 25% of NF milled (M) lentils; 5 groups with 5, 10, 15, 20, and 25% of fermented F milled (M) lentils.

In a dough mixer (KitchenAid Artisan, Ohio, USA), the dough was mixed for three minutes at low speed and then for seven minutes at high speed. The dough was then allowed to relax for 15 minutes at 24 ± 2 °C. The dough was then divided into 375 g loaves and proofed for 60 minutes at 32 ± 2 °C and 80% relative humidity. For 25 minutes, the bread was baked at 220 °C in a deck oven (EKA, Borgoricco PD, Milano, Italy).

2.2.2. Analysis of bread quality parameters, including overall acceptability and crust colour coordinates

Before being examined, bread samples were allowed to cool at room temperature (22 ± 2 °C) for 12 hours. Following parameters were evaluated:

Overall acceptability. Ten skilled judges conducted the analysis using a five-point Likert scale, with 5 representing "extremely like" and 0 representing "extremely dislike," in accordance with ISO 11136 (ISO 11136:2014 *Sensory analysis—Methodology—General guidance for conducting hedonic tests with consumers in a controlled area*, 2014). A randomly generated three-digit code was used to identify each bread group sample, which was then put on a plate one at a time. A quarter of a slice of each of the breads under test was given to each participant. Participants were given water to rinse their palates between sampling.

Total titratable acidity (TTA). After homogenizing 10 g of the sample with 90 mL of distilled water, TTA (in Neiman degrees, °N) was calculated and represented as the volume, in milliliters, of 0.1 mol/L NaOH needed to reach a pH of 8.2;

Specific volume by the rapeseed displacement method American Association of Cereal Chemists (AACC) method (AACC, 2003);

Shape coefficient, calculated by measuring and dividing the height and width of a bread slice;

Crumb porosity, according to LST method 1442 (LST, 1996);

Moisture content, according to the International Association for Cereal Science and Technology (ICC) Standard Method 110/1 1 (ICC Method, 1976);

Mass loss after baking, by comparing the mass of the loaf dough before and after baking;

Texture hardness, determined as the maximum compression force using the Texture Profile Analysis (TPA) (Stevens-LFRA Texture Analyzer, Poland). Slices of 2 cm thickness were compressed to 10 % of their original height at a crosshead speed of 10 mm/s;

Crust colour coordinates, evaluated using a CIE L*a*b* system (CromaMeter CR-400, Konica Minolta, Tokyo, Japan).

2.2.3. Acrylamide and sugars analysis in produced bread

The method of Zhang et al. (2006) was followed to determine the acrylamide concentration, however with some adjustments described in Klupsaite et al. (2023). The target analyte's derivatization using bromination served as the basis for the acrylamide detection. A gas chromatograph–electron capture detector (GC–ECD) (Shimadzu GC-17A, Kyoto, Japan) was used for the analysis, and the analytical standard employed was acrylamide. The limit of detection (LOD) was determined to be 1.75 µg/kg, and the limit of quantification (LOQ) was set at 11.9 µg/kg. Broader description of the method is provided in **Supplementary file 1**

To determine the sugar concentration, sample clarification with

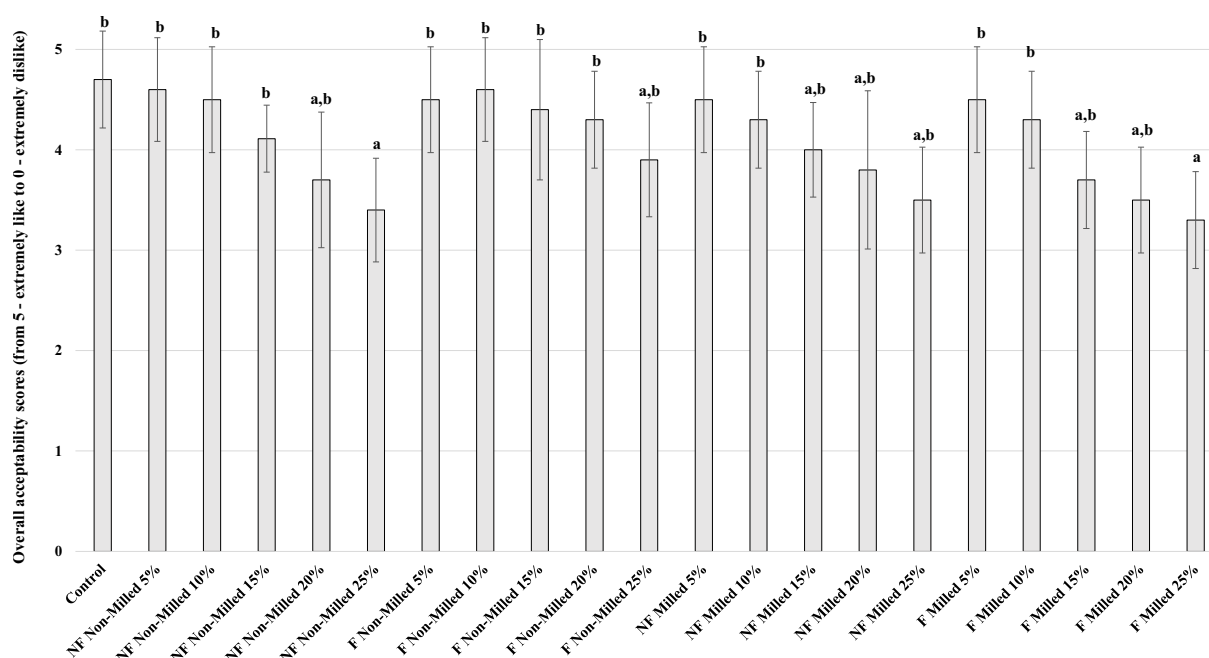


Fig. 1. Overall acceptability of bread samples (NF – non-fermented; F – fermented with *Lactiplantibacillus plantarum* No.122 strain; 5%, 10%, 15%, 20%, 25% – quantities of non-fermented non-milled, non-fermented milled, fermented non-milled, and fermented milled green lentils in bread formula. Data expressed as mean values ($n = 10$) \pm SD; ^{a–b} – Mean values within the columns with different letters are significantly different ($p \leq 0.05$).

Carrez I and Carrez II solutions was performed. High-performance liquid chromatography (HPLC) with Evaporative Light Scattering Detector (ELSD) LTII (Shimadzu Corp., Kyoto, Japan) was used for analysis. Each phase of sugars determination process is described in Klupsaite et al. (2023). The limit of detection (LOD) was determined to be 0.07 g/100g, and the limit of quantification (LOQ) was set at 0.20 g/100g. Broader description of the method is provided in **Supplementary file 1**

2.2.4. Analysis of volatile compound (VC) profile in produced bread

The gas chromatography-mass spectrometry (GC-MS) method was used to analyze the VC of bread samples. Analysis was conducted using a solid-phase microextraction (SPME) device with Stable-flex™ fiber coated with a 50 μ m PDMS-DVB-Carboxen™ layer (Supelco, Bellefonte, Pennsylvania, USA). The procedure was performed as described in Mockus et al. (2024) with the following modifications: 2 g of the sample and 10 mL of 1M phosphate buffer (pH = 3) and 25 % NaCl were used for the headspace extraction of the samples; a low polarity Stabilwax™-DA column (Restek, USA) was used to separate VC. Broader description of the method is provided in **Supplementary file 1**

2.3. Statistical analysis

The results were presented as mean values \pm standard deviations (SD) (overall acceptance, $n = 10 \pm$ SD; acrylamide and sugars in bread, $n = 2 \pm$ SD; physical chemical properties, $n = 3 \pm$ SD). Data were analyzed using multivariate ANOVA and Tukey HSD tests as post-hoc tests (statistical software IBM SPSS Statistics (29.0.0.0.(241), Chicago, Illinois, USA)) to assess the effects of fermentation, milling, and varying amounts of lentils on bread quality characteristics. Additionally, Pearson correlations between different variables were determined. The findings were deemed statistically significant when the p-value was 0.05 or less ($p \leq 0.05$).

3. Results and discussion

3.1. Overall acceptability of wheat bread enriched with green lentils

First of all, the overall acceptability of the bread samples was evaluated (Fig. 1). In general, addition of 25% non-fermented non-milled and fermented milled lentils to bread significantly reduced overall acceptability, compared to control bread. Overall acceptability of the rest breads with lentils was similar to control bread. It was found that quantity of lentils and used form of lentils (milled or non-milled) significantly affected bread overall acceptability ($p \leq 0.001$ and $p = 0.049$, respectively).

Lentils are valued for their nutritional advantages and health benefits, but as an ingredient in food products, they sometimes provide undesirable flavours such as “beany,” “green,” and “grassy.” These off-flavours may hinder consumer acceptance (Vurro et al., 2024). The reduced overall acceptability of breads with 25% green lentils in our study could be affected by this reason too. A variety of VC in lentil flour and derived food products combine to produce the beany flavor, such as (E,E)-2,4-heptadienal, 1-octen-3-ol, 1-octen-3-one, 3-octen-2-one, 2-pentyl furan, 3-methyl-1-butanol, 2-butanone, 2-pentanone, 2-hexanone, 3-isobutyl-2-methoxypyrazine, 1-hexanol, and 2-isopropyl-3-methoxypyrazine, while hexanal, heptanal, and nonanal create “green” and “grassy” flavor (Vurro et al., 2024). In our study, most breads with 25% green lentils contained 1-octen-3-ol, 3-methyl-1-butanol, and nonanal in greater area percentage (of all identified compounds), compared to control breads. Flavour notes in lentils that are noticeable as undesirable ones can result from lipid oxidation (Chigwedere et al., 2022). The occurrence of off-flavours in legumes and products prepared from legumes is a well-established issue (Saffarionpour, 2024). The range of compounds identified as contributing to this diverse and intricate flavour profile can differ across studies, as each VC may alter its sensory impact based on its concentration (Vurro et al., 2024). Therefore, further, in our study separate VC of the bread samples were identified and their relations with overall acceptability of the bread samples were evaluated. Also, oxidation compounds, including nonanal and 2-methyl-1-hexanol, tend to increase

Table 1

Bread quality parameters

Samples	Total titratable acidity, °N	Specific volume, cm ³ /g	Shape coefficient	Porosity, %	Moisture content, %	Mass loss after baking, %
Control	0.8±0.05 ^b	3.55±0.04 ^{e,f,g,h}	2.03±0.08 ^a	63.2±0.87 ^f	19.5±0.24 ^a	10.2±0.74 ^{a,b,c,d,e,f}
5%	0.7±0.01 ^a	3.26±0.15 ^{b,c,d,e}	2.21±0.02 ^{b,c}	66.3±1.50 ^g	20.1±0.17 ^{a,b}	13.0±0.13 ^{h,i,j}
10%	0.7±0.01 ^a	3.14±0.17 ^{a,b,c,d}	2.23±0.02 ^{b,c,d}	61.8±1.13 ^{e,f}	20.2±0.25 ^{a,b}	12.6±0.43 ^{h,i,j}
NF Non-Milled	15% 0.9±0.01 ^c	3.66±0.13 ^{f,g,h}	2.13±0.02 ^{a,b}	56.8±0.58 ^{c,d}	20.8±0.09 ^{b,c,d,e}	12.3±0.58 ^{g,h,i,j}
20%	0.9±0.01 ^c	3.71±0.11 ^{f,g,h}	2.36±0.02 ^{d,e,f}	50.5±0.65 ^b	21.4±0.54 ^{d,e,f,g,h}	11.8±0.20 ^{e,f,g,h,i,j}
25%	1.1±0.01 ^d	3.84±0.06 ^h	2.64±0.05 ^{i,j}	47.3±0.84 ^a	21.8±0.26 ^{f,g,h,i,j}	13.4±0.13 ^{i,j}
5%	1.3±0.01 ^f	3.14±0.05 ^{a,b,c}	2.32±0.08 ^{c,d,e}	74.5±1.24 ⁱ	21.6±0.40 ^{e,f,g,h,i,j}	9.71±0.20 ^{a,b,c,d}
10%	1.5±0.01 ^h	3.42±0.12 ^{c,d,e,f}	2.49±0.03 ^{f,g,h}	80.8±2.23 ^j	21.5±0.13 ^{d,e,f,g,h,i,j}	10.1±0.16 ^{a,b,c,d,e}
F Non-Milled	15% 1.6±0.01 ⁱ	3.64±0.12 ^{f,g,h}	2.41±0.02 ^{e,f,g}	67.9±0.31 ^{g,h}	21.4±0.03 ^{d,e,f,g,h,i,j}	10.2±0.17 ^{a,b,c,d,e,f}
20%	1.7±0.01 ^j	3.79±0.02 ^{g,h}	2.80±0.02 ^j	59.4±0.87 ^{d,e}	21.9±0.20 ^{g,h,i,j}	9.31±0.96 ^{a,b,c}
25%	1.9±0.01 ^l	3.69±0.10 ^{f,g,h}	2.64±0.04 ^{i,j,k}	50.9±1.15 ^b	22.3±0.13 ^{h,i,j,k}	10.3±0.81 ^{a,b,c,d,e,f,g}
5%	1.2±0.01 ^e	2.97±0.04 ^{a,b}	2.47±0.01 ^{f,g,h}	82.5±1.20 ^j	20.2±0.25 ^{a,b}	11.4±0.21 ^{c,d,e,f,g,h,i}
10%	1.2±0.01 ^e	3.04±0.07 ^{a,b}	2.14±0.01 ^{a,b}	69.1±0.60 ^{g,h}	20.4±0.08 ^{a,b,c}	11.6±0.35 ^{d,e,f,g,h,i}
NF Milled	15% 1.3±0.01 ^f	3.16±0.03 ^{b,c,d}	2.41±0.01 ^{e,f,g}	66.4±1.65 ^g	20.9±0.19 ^{b,c,d,e,f}	12.2±0.29 ^{f,g,h,i,j}
20%	1.4±0.01 ^g	3.48±0.17 ^{d,e,f,g}	2.60±0.02 ^{h,i,j}	61.3±0.53 ^{e,f}	22.0±0.06 ^{g,h,i,j}	12.5±0.43 ^{h,i,j}
25%	1.4±0.01 ^g	3.56±0.23 ^{e,f,g,h}	2.78±0.03 ^{k,l}	59.1±0.66 ^{d,e}	22.3±0.31 ^{i,j,k}	13.9±0.29 ⁱ
5%	1.6±0.01 ⁱ	2.82±0.09 ^a	2.16±0.01 ^{a,b}	70.9±0.64 ^h	20.6±0.31 ^{b,c,d}	9.40±0.69 ^{a,b,c}
F	10% 1.8±0.01 ^k	3.24±0.05 ^{b,c,d,e}	2.36±0.14 ^{d,e,f}	68.0±0.42 ^{g,h}	21.3±0.38 ^{c,d,e,f,g}	11.2±1.68 ^{b,c,d,e,f,g,h}
15%	1.9±0.01 ^l	3.26±0.05 ^{b,c,d,e}	2.31±0.01 ^{c,d,e}	62.0±0.34 ^{e,f}	21.9±0.05 ^{g,h,i,j}	8.52±0.41 ^a
Milled	20% 2.1±0.01 ^m	3.56±0.10 ^{e,f,g,h}	2.52±0.02 ^{g,h,i}	57.9±0.52 ^{c,d}	22.4±0.27 ^{h,k}	9.21±1.44 ^{a,b}
25%	2.5±0.01 ⁿ	3.82±0.03 ^h	2.68±0.03 ^{j,k,l}	55.4±0.59 ^c	22.9±0.77 ^k	11.0±0.91 ^{b,c,d,e,f,g,h}

°N – Neiman degrees; NF – non-fermented; F – fermented with *Lactiplantibacillus plantarum* No.122 strain; 5%, 10%, 15%, 20%, 25% – quantities of non-fermented non-milled, non-fermented milled, fermented non-milled, and fermented milled green lentils in bread formula. Data expressed as average (n = 3) ± SD; ^{a-n} Mean values within the lines with different letters are significantly different (p ≤ 0.05).

Table 2

Influence of analysed factors and how they interact with bread quality parameters

Factors	Dependent Variable					
	Specific volume	Shape coefficient	Porosity	Moisture content	Mass loss after baking	Total titratable acidity
	p values					
,quantity of lentils ^a	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
,fermentation ^a	0.049	<0.001	<0.001	<0.001	<0.001	<0.001
,milling ^a	<0.001	0.077	<0.001	0.016	0.341	<0.001
,quantity of lentils ^a * ,fermentation ^a	0.004	<0.001	<0.001	0.062	0.043	<0.001
,quantity of lentils ^a * ,milling ^a	0.002	<0.001	<0.001	<0.001	0.022	<0.001
,fermentation ^a * ,milling ^a	0.117	<0.001	<0.001	0.113	0.424	<0.001
,quantity of lentils ^a * ,fermentation ^a * ,milling ^a	0.056	<0.001	<0.001	0.023	0.014	<0.001

Factor or factors interaction is significant, when p ≤ 0.05.

during the milling process (Trindler et al., 2022). The VC profile, as well as overall acceptability, can change based on the type of treatment applied, influenced by physical and chemical modifications of components such as proteins and lipids, as well as enzymatic activity. Some treatments, e.g., pH adjustments, may reduce lipid oxidation and lower the levels of oxidation compounds. Conversely, other methods like fermentation, can generate novel chemical substances that enhance the VC profile and help mask off-flavours.

The presence of polyphenols and tannins in pulse hulls can give them a bitter taste (Wang et al., 2009). Accordingly, dehulling lentils has been shown to enhance their flavor and palatability (Oduro-Yeboah et al., 2023; Pal et al., 2017). Dehulling and other techniques (cooking, sprouting, milling) can effectively minimize antinutritional factors in lentils (Dewan et al., 2024). It was reported that dehulling reduced tannin levels, slightly reduced phytic acid, but increased stachyose and verbascose in lentils (Dhull et al., 2023).

Moreover, in our study produced breads with a 25% of green lentils may be less well-liked overall, because that could possibly be influenced by their harder texture. It was reported that globulin, which is prevalent in lentils, can make dough stiffer and the texture harder (Osemwota et al., 2022; L.-L. Zhang et al., 2022).

3.2. Quality characteristics of wheat bread enriched with green lentils

The main bread quality parameters are shown in Table 1. It can be

seen that the addition of fermented lentils increased the TTA of bread samples, and, the highest TTA showed bread groups, prepared with fermented milled lentils. Fermentation, milling, and quantity of lentils as well as their interactions significantly affected TTA (p ≤ 0.001) (Table 2). Shape coefficient and moisture content were shown to have moderately to strongly positive correlations with bread TTA (r = 0.526, p ≤ 0.001 and r = 0.736, p ≤ 0.001, respectively). Also, TTA showed moderate negative correlation with bread mass loss after baking (r = -0.512, p ≤ 0.001). The addition of lentils had a diverse effect on the porosity of the bread crumb. Compared to control bread, higher porosity was found in samples with the following lentils addition: 5% non-fermented non-milled; 5, 10, 15%, fermented non-milled; 5, 10, 15% non-fermented milled; 5 and 10% fermented milled. The remaining samples' porosity was either the same as or less than that of the control bread. When comparing samples in each group, an increase in the quantity of added lentils resulted in a reduction in porosity. A strong negative correlation was found between bread porosity and specific volume (r = -0.697, p ≤ 0.001). In most cases, the addition of lentils increased the bread's moisture content. Bread samples with 10% non-fermented non-milled, 5% fermented non-milled, non-fermented milled (5, 10, 15%), and 5% fermented milled lentils had lower specific volume than control bread, while rest of the samples shared similar specific volume with control bread. Bread moisture content and specific volume and shape coefficient were found to be moderately and strongly positively correlated (r = 0.529, p ≤ 0.001 and r = 0.750, p ≤ 0.001,

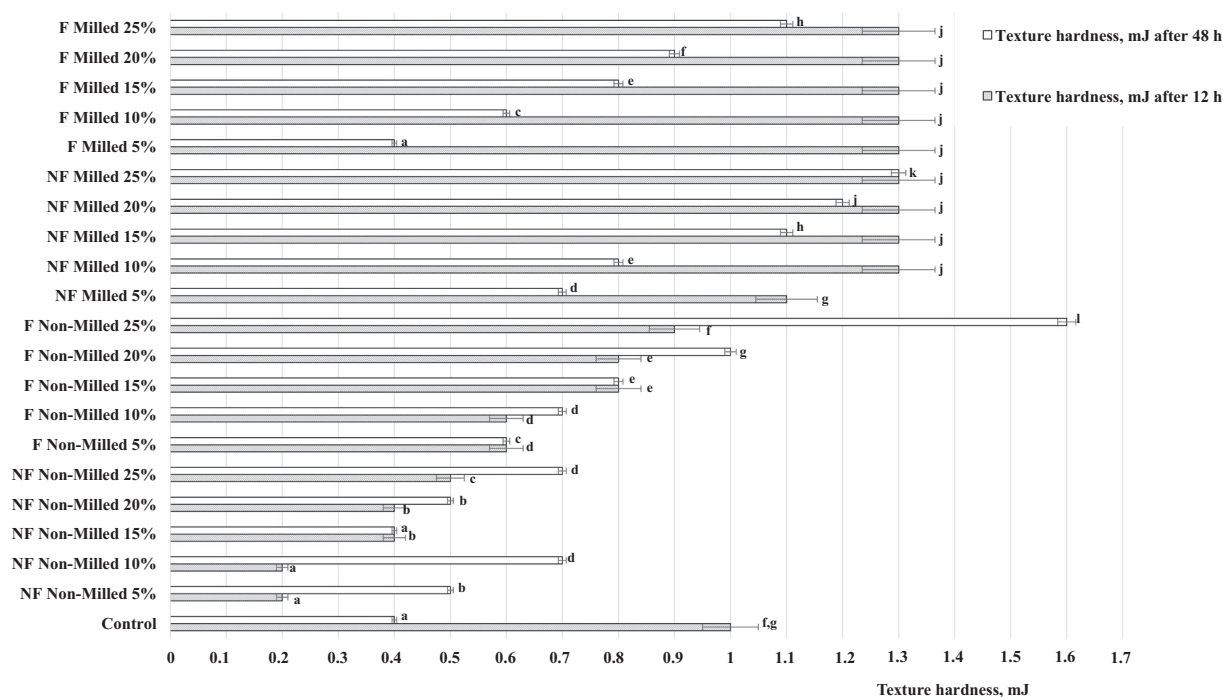


Fig. 2. Bread texture hardness after 12 and 24 hours of storage (NF – non-fermented; F – fermented with *Lactiplantibacillus plantarum* No.122 strain; 5%, 10%, 15%, 20%, 25% – quantities of non-fermented non-milled, non-fermented milled, fermented non-milled, and fermented milled green lentils in bread formula. Data expressed as average ($n = 3$) \pm SD; ^{a–j} – Mean values within the columns with different letters are significantly different between bread texture hardness after 24 h and between texture hardness after 48 h ($p \leq 0.05$).

respectively). The majority of the factors that were examined and their interactions had a significant impact on the main bread parameters (except ‘fermentation’ * ‘milling’, and ‘quantity of lentils’ * ‘fermentation’ * ‘milling’ interactions on bread specific volume; ‘milling’ on bread shape coefficient; ‘quantity of lentils’ * ‘fermentation’ interaction on bread moisture content; ‘milling’ and ‘fermentation’ * ‘milling’ interaction on bread mass loss after baking) (Table 2).

Increased TTA mainly affects shelf life and sensory quality of bread. TTA affects dough mixing behaviour, as well as the gluten, starch, and arabinoxylans that give dough its structure (Arendt et al., 2007). TTA is important for the activation of cereal or bacterial enzymes. TTA induce proteolysis and due to that free amino acids participate in flavor development, improve elasticity and specific volume of bread (Su et al., 2019). However, an overabundance of acids may significantly reduce the strength of the gluten network and hence hinder the ability to retain gas (Blanco et al., 2011). Increased TTA slowed the bread’s short-term retrogradation by softening the crumb of both fresh and stored bread (Su et al., 2019). Decreased pH in dough with wheat–legume (lentil included) sourdough (Rizzello et al., 2014), and increased TTA in breads containing lupin sourdough (Nigro et al., 2025) were also reported in other studies. Turfani et al. found that 24% wheat flour substitution by green lentils flour led to reduced bread volume, while 6 and 12% had no effect (Turfani et al., 2017). In our study, lower specific volume of bread with non-fermented milled lentils was also found, but at lower (5–15% addition). Because lentil flour has a lower fat content and higher fiber and non-gluten protein content, it disrupts the development of gluten networks, which significantly reduces bread volume and increases density and crumb hardness. (Kotsiou et al., 2021). However, by replacing 15% of the wheat flour with a legume sourdough consisting of such legumes as chickpea, lentil, and bean flours, a bread with a considerably larger specific volume may be produced than the control bread produced with the same proportion of unfermented legume flours (Gobbetti et al., 2020).

Small, uniform pore structures create softer, elastic crumb, while large pores with thick walls form crumb with increased firmness (Oates,

2001). Bread with greater crumb firmness could have a reduced specific volume (Rathnayake et al., 2018). Therefore, with larger pores, baked goods may not retain their shape and their shape retention coefficient and specific volume may be lower. Moreover, the complexity of other factors, including fermentation, weakening of the gluten structure, and the size (whole or flour), quantity, and distribution of green lentil particles, may also influence the specific volume.

In comparison bread hardness after 12 h, bread samples with non-fermented/fermented non-milled green lentils had softer texture than control breads (Fig. 2). In opposite, the texture of bread samples with non-fermented/fermented milled lentils was harder than control bread samples. All factors that were examined and their interactions had a significant impact on bread texture hardness after 12 h of storage ($p \leq 0.001$). In general, after 48 hours of storage, the higher hardness was observed in most breads with lentils, except for bread samples with 15% of non-fermented non-milled and with 5% fermented milled lentils. The latter bread samples shared similar hardness as control. Strong and moderate, respectively, positive correlations were found between bread texture hardness after 12 and 48 h of storage with bread TTA ($r = 0.642$, $p \leq 0.001$ and $r = 0.519$, $p \leq 0.001$, respectively). Also, strong positive correlation was found between bread texture hardness after 48 h of storage and moisture content, ($r = 0.629$, $p \leq 0.001$).

Since hardness and consumers’ perceptions of bread freshness are closely tied, hardness is an essential quality attribute of bread (Onyango et al., 2010; Wang et al., 2024). Specifically, hardness significantly impacts the sensory experience of bread, influencing both chewability and overall palatability, and directly relates to consumer acceptance and product quality (Gao & Zhou, 2021; Mudgil et al., 2016).

Lentil flour was found to increase the hardness of wheat bread, and the more flour added, the higher the firmness (Cacak-Pietrzak et al., 2024; Previtali et al., 2014). This occurs due to the greater content of protein, which may disrupt the gluten network, fibers and resistant starch, low content of insoluble carbohydrates, as well as the fact that lentil flour causes the crumb walls around the air cells to thicken (Mohammed et al., 2012). A slight impact on bread volume and texture

Table 3
Bread crust colour characteristics

Samples	Crust		
	L*	a*	b*
Control	41.4±1.84 ^a	12.1±0.79 ^{g,h,i,j}	18.9±0.51 ^{c,d,e,f,g,h}
NF Non-Milled 5%	42.1±0.71 ^{a,b}	11.8±0.53 ^{f,g,h,i,j,k}	17.7±0.56 ^{b,c,d}
NF Non-Milled 10%	42.3±0.30 ^{a,b,c}	10.4±0.43 ^{a,b,c,d}	13.1±0.66 ^a
NF Non-Milled 15%	43.4±1.12 ^{a,b,c,d}	12.4±0.31 ^{i,j,k}	20.3±0.56 ^{h,i}
NF Non-Milled 20%	45.8±0.46 ^{e,f}	12.7±0.25 ^{j,k,l}	23.4±0.12 ⁱ
NF Non-Milled 25%	50.0±0.78 ^g	11.4±0.19 ^{e,f,g,h,i}	25.0±0.63 ^k
F Non-Milled 5%	45.8±1.11 ^{e,f}	10.7±0.37 ^{a,b,c,d,e}	20.1±0.82 ^{h,i}
F Non-Milled 10%	42.1±0.82 ^{a,b}	11.3±0.31 ^{d,e,f,g,h}	19.2±0.22 ^{d,e,f,g,h}
F Non-Milled 15%	42.3±0.29 ^{a,b,c}	9.90±0.19 ^a	17.0±0.40 ^b
F Non-Milled 20%	46.6±0.45 ^f	13.0±0.05 ^{k,l}	18.6±0.44 ^{c,d,e,f,g}
F Non-Milled 25%	44.0±0.29 ^{b,c,d,e}	11.1±0.11 ^{c,d,e,f,g}	17.5±0.23 ^{b,c}
NF Milled 5%	44.7±0.38 ^{d,e,f}	13.3±0.22 ^l	18.3±0.15 ^{b,c,d,e,f}
NF Milled 10%	44.3±0.70 ^{c,d,e}	12.2±0.18 ^{h,i,j,k}	21.0±0.97 ⁱ
NF Milled 15%	42.1±0.14 ^{a,b}	10.7±0.06 ^{a,b,c,d,e}	17.9±0.26 ^{b,c,d}
NF Milled 20%	42.1±0.17 ^{a,b}	11.3±0.27 ^{d,e,f,g,h}	18.1±0.24 ^{b,c,d,e}
NF Milled 25%	44.3±0.32 ^{c,d,e}	10.2±0.19 ^{a,b,c}	18.9±0.66 ^{c,d,e,f,g,h}
F Milled 5%	43.3±0.34 ^{a,b,c,d}	11.2±0.18 ^{d,e,f,g}	19.7±0.39 ^{f,g,h,i}
F Milled 10%	42.9±0.30 ^{a,b,c,d}	10.1±0.15 ^{a,b}	18.6±0.69 ^{c,d,e,f,g}
F Milled 15%	44.7±0.67 ^{d,e,f}	10.9±0.35 ^{b,c,d,e,f}	18.3±0.06 ^{b,c,d,e,f,g}
F Milled 20%	48.9±0.41 ^g	11.8±0.18 ^{f,g,h,i,j}	19.4±0.17 ^{e,f,g,h}
F Milled 25%	50.0±0.33 ^g	12.0±0.07 ^{g,h,i,j}	19.8±0.14 ^{g,h,i}

L* – lightness; a* – redness or –a* greenness; b* – yellowness or –b* blueness; NBS – National Bureau of Standards units; NF – non-fermented; F – fermented with *Lactiplantibacillus plantarum* No.122 strain; 5%, 10%, 15%, 20%, 25% – quantities of non-fermented non-milled, non-fermented milled, fermented non-milled, and fermented milled green lentils in bread formula. Data expressed as average (n = 3); ^{a-j} – Mean values within the lines with different letters are significantly different (p ≤ 0.05).

Table 4
Impact of analysed factors and how they interact with bread crust colour coordinates

Factors	Dependent Variable		
	L*	a*	b*
	p values		
,quantity of lentils'	<0.001	<0.001	<0.001
,fermentation'	<0.001	<0.001	<0.001
,milling'	0.074	0.239	0.165
,quantity of lentils' * ,fermentation'	<0.001	<0.001	<0.001
,quantity of lentils' * ,milling'	0.013	<0.001	<0.001
,fermentation' * ,milling'	<0.001	0.197	<0.001
,quantity of lentils' * ,fermentation' * ,milling'	<0.001	<0.001	<0.001

L* lightness; a* redness or –a* greenness; b* yellowness or –b* blueness; Factor or factors interaction is significant, when p ≤ 0.05.

is anticipated when using non-ground seeds because not all of their constituents will interact with the gluten, while existing interactions will be weaker (De Lamo & Gómez, 2018). The variations in bread firmness over time are mostly explained by the diffusion of the moisture, the relation of starch and gluten, and the retrogradation of starch (Ju et al., 2020). Legume flour has greater water-holding capacity and that could lead to softer texture initially, but the starch retrogradation value could grow later at high replacement levels (Moreno-Araiza et al., 2023). Moreover, legumes include starch, which may hasten the bread's staling process (Arendt et al., 2007). Fermentation with lactic acid bacteria (sourdough usage) affects protein fraction of flour and rheological properties of bread dough but improves the activity of endogenous and exogenous enzymes, including proteolysis of the gluten subunit, and that could lower the level of starch retrogradation (Arendt et al., 2007).

3.3. Crust colour coordinates of wheat bread enriched with green lentils

Bread crust colour coordinates are shown in Table 3. Compared to control bread, higher crust L* coordinates were found for samples with

non-fermented non-milled (20% and 25%), fermented non-milled (5%, 20%, and 25%), non-fermented milled (5%, 10%, and 25%), and fermented milled (15%, 20%, and 25%), while the lightness of the rest samples was similar. Compared to control bread samples with 15 and 20% of non-fermented non-milled as well as samples with 20% of fermented non-milled, and samples with 5% of non-fermented milled lentils showed higher crust a* coordinates; and samples with 15% of non-fermented non-milled; with 20% of non-fermented non-milled; with 25% of non-fermented non-milled; with 5% of fermented non-milled; with 10% of fermented non-milled; with 10% of non-fermented milled; with 5% of fermented milled; with 20% of fermented milled; and with 25% of fermented milled showed higher crust b* coordinates. Most of the factors that were examined and their interactions had a significant impact on bread colour coordinates, except milling (was not significant on all colour coordinates) and, fermentation' * ,milling' interaction was not significant on bread crust a* coordinate (Table 4).

Flavonoids, chlorophyll, and carotenoids in bread ingredients can influence the final bread color. Flavonoid pigments come in orange-red, purple-blue, and white-cream hues (Giusti et al., 2023). Temperature and pH can have a substantial impact on flavonoid stability and may elicit a shift in hue or a reduction in color intensity while baking (Khoo et al., 2017). Degradation of anthocyanins, which are a group of flavonoids, during baking may be the cause of the yellowish appearance of the crumb (Sui et al., 2015). Carotenoids provide yellow-orange-red hue, while chlorophyll gives the green hue (Mortensen, 2006). Chlorophyll stability is influenced by pH (unstable in acidic pH (3.5–5)), oxygen, light, other food ingredients, and temperature (Magalhães et al., 2024). High temperatures in baking process leads to chlorophyll degradation and produced magnesium chlorophyll gives brown color (Ning et al., 2017; Paciulli et al., 2017). Deterioration of carotenoids is also available at higher temperature during food thermal processing, which causes changes in color intensity (Ordóñez-Santos et al., 2021). The colour of the legume flour and the increased frequency of the Maillard reaction due to higher level of lysine in lentil are linked to colour changes of produced breads (Turfani et al., 2017). Depending on the storage circumstances and length of time, the green colour of lentils gradually shifts from yellow to dark brown (Miralí et al., 2016). Decrease in a* of bread could be related with the presence of flavonoids and chlorophyll a and b, in green lentils, while higher yellowness and lightness could be imparted due to the carotenoids content (Mishra et al., 2022). The presence of natural light-colored pigments like carotenoids imparts lighter color to the bread and this can counteract the browning effects typically associated with the Maillard reaction. Moreover, the presence of phenolic compounds in green lentils may have an inhibiting effect as α-dicarbonyl trapping agents on Maillard reaction and increase L* value (Amarowicz et al., 2010; Lund & Ray, 2017). In general, increased level of reducing sugars, proteolysis as well as acidification and release or conversion of phenolic compound during fermentation contributes to colour formation of bread during baking (Lin et al., 2024). Changes in L*, a*, and b* colour coordinates of bread with lentil flour were also observed in other studies and mainly depended on the quantity and type (milling fraction, sprouted) of lentil added. Studies showed that when 10% lyophilized and roasted lentil seeds flour were substituted for wheat flour in wheat bread recipes, the resulting bread crust had higher a* values and lower brightness and b* values (Kotsiou et al., 2023). The same color coordinates were significantly lower in wheat breads made with 20 g/100g green lentil flour (Gallo et al., 2022).

3.4. Acrylamide and saccharides concentration in bread

The acrylamide concentration in bread samples is shown in Fig. 3. In all cases, the addition of milled lentils (both non-fermented and fermented ones) resulted in a higher increase in acrylamide concentration in the bread samples, compared to those prepared with non-milled lentils. Also, in comparison bread samples prepared with non-milled

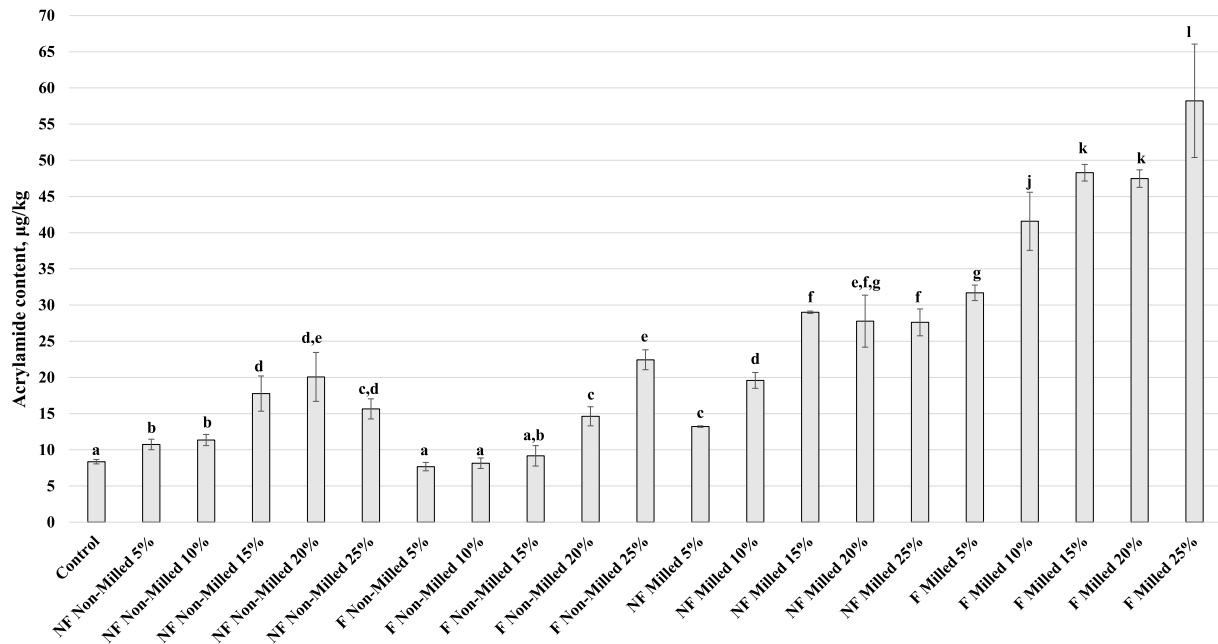


Fig. 3. Acrylamide concentration (µg/kg) in bread samples (NF – non-fermented; F – fermented with *Lactiplantibacillus plantarum* No.122 strain; 5%, 10%, 15%, 20%, 25% – quantities of non-fermented non-milled, non-fermented milled, fermented non-milled, and fermented milled green lentils in bread formula. Data expressed as mean values (n = 2) ± SD; ^{a-l} – Mean values within the columns with different letters are significantly different (p ≤ 0.05).

Table 5

Sugars concentration (g/100g) in bread samples

Samples	Sugars concentration, g/100g		
	Fructose	Sucrose	Maltose
Control	0.28±0.03 ^a	0.34±0.04 ^a	1.22±0.03 ^{d,e,f,g}
NF Non-Milled 5%	0.29±0.04 ^a	0.35±0.05 ^a	1.26±0.01 ^{e,f,g,h}
NF Non-Milled 10%	0.30±0.04 ^a	0.36±0.05 ^a	1.27±0.0 ^{f,g,h,i}
NF Non-Milled 15%	0.33±0.07 ^a	0.40±0.07 ^a	1.23±0.05 ^{d,e,f,g}
NF Non-Milled 20%	0.30±0.04 ^a	0.42±0.03 ^a	1.23±0.04 ^{d,e,f,g}
NF Non-Milled 25%	0.30±0.05 ^a	0.42±0.05 ^a	1.20±0.02 ^{d,e,f}
F Non-Milled 5%	0.29±0.07 ^a	0.40±0.09 ^a	1.32±0.06 ^{f,g,h,i}
F Non-Milled 10%	<0.20	0.39±0.06 ^a	1.10±0.10 ^{b,c,d,e}
F Non-Milled 15%	0.29±0.06 ^a	0.35±0.07 ^a	1.17±0.08 ^{c,d,e,f}
F Non-Milled 20%	<0.20	<0.20	1.00±0.06 ^{a,b}
F Non-Milled 25%	0.32±0.06 ^a	0.38±0.07 ^a	1.00±0.08 ^{a,b}
NF Milled 5%	0.33±0.06 ^a	<0.20	1.41±0.03 ^{h,i}
NF Milled 10%	0.34±0.06 ^a	<0.20	1.43±0.01 ⁱ
NF Milled 15%	0.29±0.07 ^a	0.38±0.08 ^a	1.31±0.03 ^{f,g,h,i}
NF Milled 20%	0.30±0.06 ^a	0.40±0.07 ^a	1.26±0.11 ^{e,f,g,h}
NF Milled 25%	<0.20	0.42±0.06 ^a	1.32±0.02 ^{f,g,h,i}
F Milled 5%	0.34±0.05 ^a	<0.20	1.38±0.08 ^{g,h,i}
F Milled 10%	0.33±0.04 ^a	<0.20	1.21±0.04 ^{d,e,f}
F Milled 15%	0.29±0.04 ^a	<0.20	1.08±0.01 ^{b,c,d}
F Milled 20%	0.29±0.04 ^a	<0.20	1.02±0.01 ^{a,b,c}
F Milled 25%	0.29±0.03 ^a	<0.20	0.87±0.02 ^a

NF – non-fermented; F – fermented with *Lactiplantibacillus plantarum* No.122 strain; 5%, 10%, 15%, 20%, 25% – quantities of non-fermented non-milled, non-fermented milled, fermented non-milled, and fermented milled green lentils in bread formula. Data expressed as average (n = 2); ^{a-i} – Mean values within the lines with different letters are significantly different (p ≤ 0.05).

non-fermented lentils, bread prepared with 15%, 20%, and 25% lentils showed, on average, a 2.06 times higher acrylamide concentration compared to the control samples. However, when non-milled fermented lentils were used, bread prepared with 15% of non-milled fermented lentils showed a similar acrylamide concentration as the control samples. The highest acrylamide increase showed sample groups prepared with fermented and milled lentils. Almost all of the analysed bread samples (except bread with 25% fermented milled lentils) had acrylamide concentrations lower than the referenced EU limit (Regulation

No. 2017/2158, 304 OJ L, 2017) of 50 µg/kg for wheat-based bread. This indicates that the acrylamide levels in the samples were below the recommended threshold, in line with the EU guidelines to reduce acrylamide formation in food. The results suggest that the bread production processes used were effective in keeping acrylamide concentrations below the recommended limit. Acrylamide concentration in bread showed strong positive correlation with bread TTA (r=0.731, p≤0.001) and most of analysed factors and their interactions were significant on acrylamide content in bread (p≤0.001), except ,quantity of lentils‘ * ,fermentation‘ * ,milling‘ interaction. Also, moderate positive correlation was found between acrylamide and moisture contents in bread (r=0.513, p≤0.001). As well as acrylamide concentration showed positive moderate correlation with bread crust L* coordinate (r=0.430, p≤0.001).

The observed increase in acrylamide levels in milled samples compared to non-milled ones can be explained by the structural changes caused by milling (Hölzle et al., 2025). Milling breaks down cell walls, increasing the exposure of starch and proteins, which in turn enhances the release of free amino acids, including asparagine, and accessible reducing sugars (S. Wang et al., 2017). It was found that milling increases the amount of reducing sugar in milled wheat flour (Motttram et al., 2002). The content of sugars and free amino acids in cereal flours is known to rise as the degree of extraction during milling increases (Mesías & Morales, 2015).

It can be speculated that enzymatic activity of lentils-based sourdough could cause release of free amino acids in dough, which also led to a higher acrylamide formation in breads with fermented lentils (Kaur & Halford, 2023; Zhou et al., 2022). The presence of free amino acids results from the synergistic proteolytic activity of strain-specific intracellular peptidases from LAB in sourdough and endogenous proteases found in legumes (Graça et al., 2021). The proteolytic activity of *Lactiplantibacillus plantarum* as well as the presence of proteases in lentils were reported (Paventi et al., 2024; Riaz et al., 2024). However, our previous work showed that fermented and milled lentils have a lower asparagine concentration, in comparison with non-fermented lentils (Supplementary file 1. Table S2). It was reported that acrylamide and free asparagine do not necessarily have a direct link, because other amino acids besides asparagine have been shown to either raise or

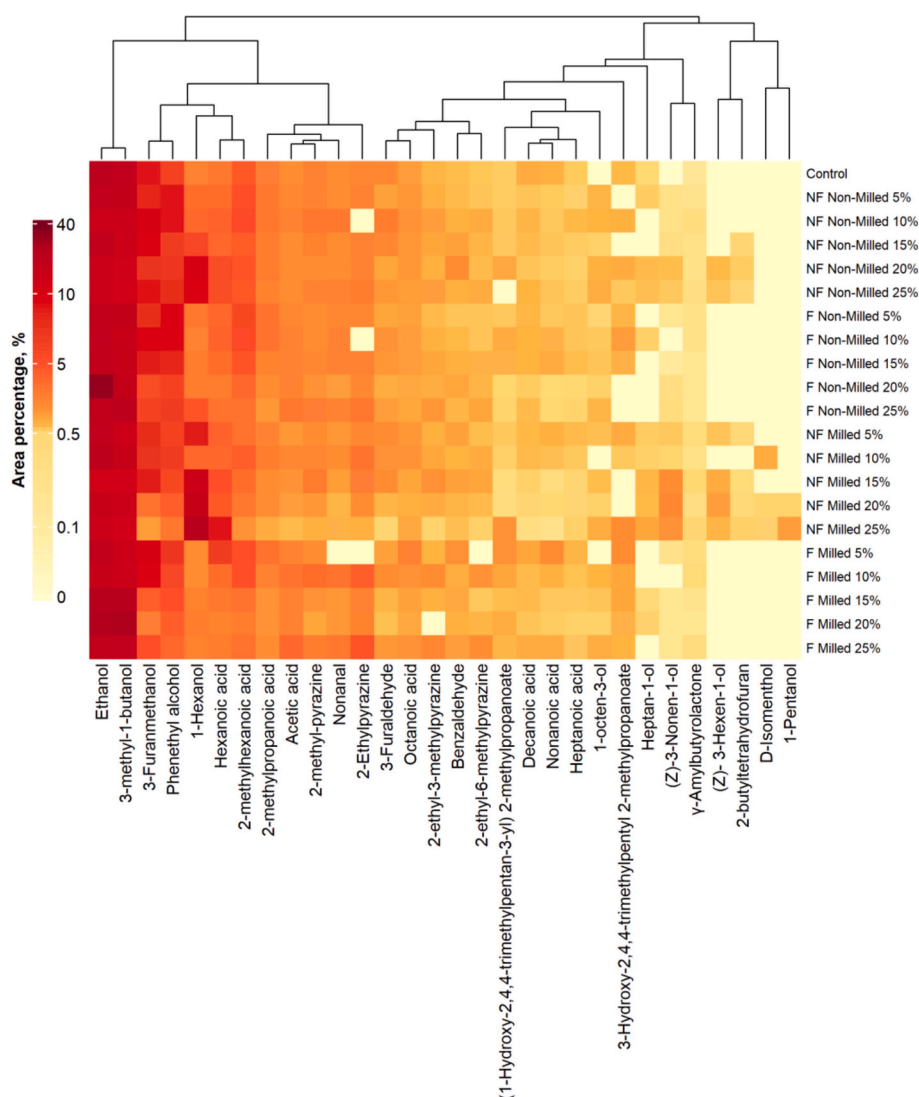


Fig. 4. Volatile compound (VC) profile (% from the total VC content) in bread samples (NF – non-fermented; F – fermented with *Lactiplantibacillus plantarum* No.122 strain; 5%, 10%, 15%, 20%, 25% – quantities of non-fermented non-milled, non-fermented milled, fermented non-milled, and fermented milled green lentils in bread formula.

decrease the amount of acrylamide (Çelik & Gökmen, 2020). Higher concentration of alanine was found to be neutral, whereas glutamine raised the concentration of acrylamide. Greater concentration of cysteine, glycine, or lysine were shown to decrease acrylamide content (Streekstra & Livingston, 2020). In other study, when glycine and proline were added, crust acrylamide was reduced in white pan bread (Shen et al., 2019). However, findings of such studies are diverse and mainly depends on the analysed food matrix or model system. Rather than being caused by a single amino acid or sugar, the observable influence is the result of the intricacy of underlying mechanisms (Claeys et al., 2005).

In general, higher acrylamide level in tested breads could be related with the mechanical and biological treatment of these legumes. The combination of milling and fermentation may create favorable conditions for acrylamide formation (Lemos et al., 2023).

When LAB are used, the pH is lowered and TTA increased by the creation of organic acid, which usually slows down the Maillard reaction. But LAB can also increase the activity of enzymes that drive the Maillard reaction and break down carbohydrates and proteins, making reducing sugars and free amino acids more accessible. This process is typical in bread production. This complex effect means that while acidity may reduce browning, the increased sugar or free amino acid content could counterbalance this and still promote Maillard reactions,

enhancing acrylamide formation. Studies have shown that higher TTA levels can lead to increased Maillard reactions, enhancing acrylamide formation (El-Sayed et al., 2023).

The positive correlation between moisture and acrylamide can be explained by the fact that moisture level affects the thermal conditions during baking, influencing acrylamide production (Mesias et al., 2024). Higher moisture levels can affect the temperature and heat distribution during cooking, even though acrylamide normally occurs in low moisture level. The cooking temperature may be reduced in products with higher moisture content, which could prolong the cooking period and produce circumstances in which acrylamide formation persists. Moreover, increased moisture may influence the caramelization and Maillard reaction, both of which contribute to acrylamide production. As a result, the correlation may be explained by the interaction of the moisture content with other cooking parameters as well.

Mustafa et al. (Mustafa et al., 2005) reported that darker crusts generally correlate with higher acrylamide due to extended baking times. Although our study showed that lighter crust was associated with higher acrylamide levels, this relationship was not strong. This could be explained by the complexity of factors in bread that contribute to the Maillard reaction, but more profound research in model systems is needed to analyze certain factors' influence.

Table 6

Correlations between volatile compounds and acrylamide content in bread samples

Volatile compound	Correlation with acrylamide content	
	r	p
Ethanol	0.127	0.321
3-methyl-1-butanol	0.302*	0.016
1-Hexanol	0.043	0.738
(Z)-3-Hexen-1-ol	0.016	0.903
1-octen-3-ol	0.213	0.094
Heptan-1-ol	-0.018	0.888
(Z)-3-Nonen-1-ol	0.087	0.496
1-Pentanol	0.088	0.491
Phenethyl alcohol	-0.681**	<0.001
D-Isomenthol	0.010	0.936
Nonanal	-0.038	0.768
3-Furaldehyde	-0.334**	0.008
Benzaldehyde	0.327**	0.009
3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	0.156	0.222
(1-Hydroxy-2,4,4-trimethylpentan-3-yl) 2-methylpropanoate	0.287*	0.023
3-Furanmethanol	-0.402**	0.001
2-butyltetrahydrofuran	-0.077	0.546
γ-Amylbutyrolactone	0.285*	0.024
Acetic acid	0.386**	0.002
2-methylpropanoic acid	-0.475**	<0.001
2-methylhexanoic acid	-0.444**	<0.001
Hexanoic acid	-0.050	0.696
Heptanoic acid	-0.022	0.863
Octanoic acid	0.013	0.922
Nonanoic acid	0.109	0.394
Decanoic acid	-0.029	0.824
2-methyl-pyrazine	0.018	0.887
2-ethylpyrazine	0.439**	<0.001
2-ethyl-6-methylpyrazine	0.356**	0.004
2-ethyl-3-methylpyrazine	0.190	0.136

r – Pearson correlation; p – significance; * – Correlation is significant at the 0.05 level (2-tailed); ** – Correlation is significant at the 0.01 level (2-tailed). Significant correlations marked in bold.

Fructose, sucrose, and maltose concentrations in bread samples are shown in Table 5. All bread samples showed similar fructose concentration (on average, 0.29 g/100 g). However, in bread samples, prepared with 20% of fermented non-milled, 5% of non-fermented milled, and 10% of non-fermented milled, as well as in all bread groups prepared with fermented milled lentils, saccharose content was lower than that <0.20 g/100 g. In other bread groups sucrose concentration was, on average, 0.39 g/100 g. The lowest maltose concentration showed breads, prepared with 25% of fermented milled lentils (0.87 g/100 g). Bread samples, prepared with 10% of non-fermented non-milled, 5% of fermented non-milled, non-fermented milled, and fermented milled, 10% of non-fermented milled, 15% of non-fermented milled, and 25% of non-fermented milled showed the highest maltose content (on average, 1.35 g/100 g). Sucrose and maltose concentrations in bread showed negative moderate and weak, respectively, correlations with acrylamide concentration ($r=-0.465$, $p<0.001$ and $r=-0.385$, $p=0.002$, respectively).

According to literature research, sugars, particularly reducing sugars like glucose and fructose, are essential for the Maillard reaction, which leads to acrylamide production (Henao Toro et al., 2022). A reduction in sugar concentration is generally expected to decrease acrylamide formation, as noted in several studies (Liyanaage et al., 2021; Orsák et al., 2022). It was reported that fructose was more reactive than glucose and sucrose, because its addition to soft biscuits resulted in elevated acrylamide level (Hamlet et al., 2007). In a case of whole wheat flour matrix, fructose was also more reactive than glucose, but the reaction with sucrose produced acrylamide levels that were comparable to those seen for fructose and glucose (Taeymans et al., 2004). Thermal processing has the ability to hydrolyze sucrose and maltose and this probably explains

the observed correlation in our study (Mesías & Morales, 2015). Diverse findings on acrylamide content have been reported in relation to the concentration of reducing sugars incorporated into the product formulation. It was reported that increasing the amount of fructose (in wheat bread) or sucrose (in cookies), the amount of acrylamide remained similar or significantly increased, respectively (Mesías & Morales, 2015).

However, factors such as baking temperature, time, and the presence of amino acids can also significantly influence acrylamide levels (Schouten et al., 2022). The temperature and duration of the thermal treatment during baking have an impact on the amount of acrylamide. Beginning at around 120 °C, acrylamide production accelerates until it reaches 200 °C, after which it degrades (Mesías & Morales, 2015). For example, higher temperatures and longer baking times can exacerbate acrylamide formation, even with lower sugar content (Bachir et al., 2024). However, in our study, the baking conditions were the same for all tested breads.

The amount of free asparagine influenced by cereals or legumes cultivation, fertilization, harvest year, and processing methods such as milling, fermentation, and sprouting (Mesías & Morales, 2015; Streekstra & Livingston, 2020). In the Maillard reaction, amino acids may compete with asparagine to prevent the synthesis of acrylamide, but they can also react with acrylamide through Michael addition once it has formed, encouraging its removal (Stadler, 2006). Moreover, the majority of the asparagine that reacts produces additional Maillard products rather than acrylamide (Streekstra & Livingston, 2020).

While lower sugar levels are generally expected to reduce acrylamide formation, our findings reveal an unexpected result. This suggests that specific interactions among ingredients, moisture content, and baking conditions may play a critical role in influencing acrylamide production. Further research is necessary to explain these dynamics, as understanding these relationships could lead to more effective strategies for minimizing acrylamide in baked products while considering the overall formulation and processing techniques.

3.5. Bread volatile compound profile

A heat map was generated to show the variation in the volatile compound (VC) profile (% of the total VC content) of tested bread samples (Fig. 4). In all bread samples, the main VCs (% of the total VC content) were ethanol, 3-methyl-1-butanol, phenethyl alcohol, and 3-furanmethanol. No correlation between VC and overall acceptability of bread samples was observed, except for (Z)-3-nonen-1-ol, which showed only a weak positive correlation with overall acceptability ($r=0.271$, $p=0.031$). Correlations between acrylamide content in bread samples and separate VC were found (Table 6). Strong negative correlation between acrylamide and phenethyl alcohol ($r=-0.681$, $p<0.001$) was observed. Results also revealed significant negative and positive correlations between acrylamide content and 3-furanmethanol ($r=-0.402$, $p=0.001$), 2-methylpropanoic acid ($r=-0.475$, $p<0.001$), 2-methylhexanoic acid ($r=-0.444$, $p<0.001$), and 2-ethylpyrazine ($r=0.439$, $p<0.001$). Most of the analysed factors and their interactions were significant on most of the VC formation in bread (Table 7). Acrylamide and volatile compounds are both produced in the Maillard reaction, which takes place during bread baking. Maillard reaction is highly associated with the sensory qualities of bread, because reaction products contribute to the bread aroma development, i.e. volatile compound profile (Conceição et al., 2024). Therefore, the correlation between these was calculated to see if there is a potential link in their formation. This could be useful for maintaining the sensory quality and chemical safety of bread with green lentils.

3-Methyl-1-butanol, hexanal, 1-hexanol corresponds to beany notes (Xu et al., 2019). (Z)-3-nonen-1-ol odor is described as fresh, waxy, green, melon, rind tropical, and mushroom. Greater yeast content was shown to favor the creation of 2-methyl-1-propanol and 3-methyl-1-butanol, which is a common VC in wheat bread aroma, whilst lower

Table 7

Impact of analysed factors and how they interact with bread volatile compounds

Volatile compounds	Factors						
	,quantity of lentils'	,fermentation'	,milling'	,quantity of lentils' * ,fermentation'	,quantity of lentils' * ,milling'	,fermentation' * ,milling'	,quantity of lentils' * ,fermentation' * ,milling'
	p values						
Ethanol	<0.001	<0.001	0.983	<0.001	0.409	0.359	<0.001
3-Methyl-1-butanol	0.021	<0.001	0.813	<0.001	<0.001	0.010	<0.001
1-Hexanol	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
(Z)- 3-Hexen-1-ol	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
1-Octen-3-ol	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Heptan-1-ol	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
(Z)-3-Nonen-1-ol	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
1-Pentanol	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Phenethyl alcohol	<0.001	0.851	<0.001	0.045	0.042	0.851	<0.001
D-Isomenthol	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Nonanal	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001
3-Furaldehyde	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Benzaldehyde	<0.001	0.642	0.002	<0.001	<0.001	<0.001	<0.001
3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
(1-Hydroxy-2,4,4-trimethylpentan-3-yl) 2-methylpropanoate	0.005	<0.001	<0.001	<0.001	<0.001	0.001	<0.001
3-Furanmethanol	<0.001	0.047	<0.001	<0.001	<0.001	<0.001	<0.001
2-Butyltetrahydrofuran	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
γ -Amylbutyrolactone	<0.001	<0.001	<0.001	<0.001	<0.001	0.849	<0.001
Acetic acid	<0.001	<0.001	0.080	<0.001	0.359	<0.001	0.026
2-Methylpropanoic acid	<0.001	0.344	<0.001	<0.001	0.109	<0.001	<0.001
2-Methylhexanoic acid	<0.001	0.015	<0.001	<0.001	0.474	<0.001	0.001
Hexanoic acid	<0.001	<0.001	<0.001	<0.001	<0.001	0.016	<0.001
Heptanoic acid	<0.001	<0.001	0.080	<0.001	<0.001	0.002	0.009
Octanoic acid	<0.001	0.884	0.944	0.409	<0.001	<0.001	0.357
Nonanoic acid	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Decanoic acid	<0.001	<0.001	0.006	<0.001	<0.001	<0.001	<0.001
2-Methyl-pyrazine	<0.001	0.613	0.003	<0.001	<0.001	<0.001	<0.001
2-Ethylpyrazine	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2-Ethyl-6-methylpyrazine	<0.001	0.124	0.104	<0.001	<0.001	0.001	<0.001
2-Ethyl-3-methylpyrazine	<0.001	0.678	0.582	<0.001	<0.001	0.003	<0.001

Factor or factors interaction is significant, when $p \leq 0.05$.

yeast amounts were found to favor the formation of hexanal in the crust of whole grain wheat bread (Salim-ur-Rehman et al., 2006). Mildly warm, honey-like phenylethyl alcohol is produced during fermentation or as a byproduct of the Maillard reaction (Chulibert et al., 2024). 3-Furaldehyde is typical VC of wheat bread VC profile (Mulders, 1973). Lipid oxidation forms benzaldehyde, while thermal processing of food generates furan and furan derivatives (Schöpf et al., 2022). Lactones are produced from non-hydroxy fatty acids by microorganisms (Silva et al., 2021). 2-methylpropanoic and acetic acid are typical VC for triticale bread and triticale bread with sourdough VC profile (Galoburda et al., 2020). The Maillard reaction is strongly associated with the synthesis of such pyrazines in bread crust as 2-ethylpyrazine (*nutty, woody, buttery notes*) and (1-hydroxy-2,4,4-trimethylpentan-3-yl) 2-methylpropanoate (*fruity, sweet notes*) (do Rosário et al., 2024; Liu et al., 2023). Changes in VC profile of breads with differently prepared green lentils could be related with the fact that enzyme activation, improved exposure to oxygen and cell damage during milling enhance the beany aroma of lentil (Bao et al., 2016). Moreover, during sourdough production, microorganisms, with the help of their enzymes, decompose carbohydrates, proteins and lipids and produce such flavour precursors as amino acids, peptides, etc. The aroma profile of produced breads is greatly impacted by the microorganism species, chemical composition of lentils used, and the fermentation conditions (Senanayake et al., 2023). To guarantee that the changes in VC brought about by fermentation lessen the undesirable sensory qualities, optimization is thus constantly required.

4. Conclusions

This study showed that the wheat bread can be enriched with 5, 10, 15, and 20% of green lentils, as well as with 25% of the non-milled fermented and non-milled non-fermented lentils without negative impact on bread overall acceptability. However, addition of lentils increases hardness of bread after 48 hours of storage (except for bread prepared with 15% of non-fermented non-milled and with 5% fermented milled lentils). Also, in all cases, the addition of milled lentils (both non-fermented and fermented ones) resulted in a higher increase in acrylamide concentration in wheat bread, compared to those prepared with non-milled lentils. Bread prepared with 5, 10 and 15% of non-milled fermented lentils had a similar acrylamide concentration as the control bread. Correlations between acrylamide content in bread and certain VC were found. Most of the analysed factors and their interactions were significant on most of the VC formation in bread, similarly as for acrylamide. Finally, optimization of the technology is always needed to ensure the safest bread formula. To ensure the safest bread version with the lowest acrylamide concentration, its enrichment with 5, 10, 15% of fermented non-milled green lentils can be recommended.

Practical implications of study findings for bakers: this study will provide guidance for practical uses of non-treated and treated green lentils in improving wheat bread quality and safety, as well as maintenance the sensory acceptability by including an optimal quantity of these legumes.

Practical implications of study findings for consumers: Green lentils are a good source of nutritional compounds and possess numerous health benefits. Therefore, our study highlights that the addition of

certain quantities of non-treated and treated green lentils to wheat bread can enhance particular qualities of wheat bread without impairing its sensory acceptability, though negative texture changes during storage are still possible.

We acknowledge limitations, including our focus on a single lentil type and same baking conditions; future research with varied varieties lentils and large-scale baking is necessary for wider application

CRediT authorship contribution statement

Vytaute Starkute: Data curation, Investigation, Formal analysis. **Elena Bartkiene:** Resources, Writing – review & editing, Conceptualization, Writing – original draft, Supervision. **Ernestas Mockus:** Visualization, Data curation, Methodology, Investigation, Formal analysis. **João Miguel Rocha:** Writing – review & editing. **Darius Cernauskas:** Investigation, Formal analysis, Data curation, Methodology. **Erika Mozurienne:** Investigation, Formal analysis, Data curation. **Romas Ruibys:** Investigation, Formal analysis. **Gul Ebru Orhun:** Investigation, Formal analysis. **Dovile Klupsaite:** Methodology, Investigation, Writing – review & editing, Formal analysis, Writing – original draft, Data curation.

Funding

No funding was received for conducting this study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work is based upon the work from COST Action CA21149 ACRYRED — Reducing acrylamide exposure of consumers by a cereals supply-chain approach targeting asparagine. COST is a funding agency for research and innovation networks. Also, part of this research is supported by the Baltic-German University Liaison Office by the German Academic Exchange Service (DAAD) with funds from the Foreign Office of the Federal Republic Germany.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2025.102900>.

Data availability

Data will be made available on request.

References

- AACC. (2003). AACC Method 10-05.01. <http://methods.aaccnet.org/summaries/10-05-01.aspx>.
- Aggarwal, A., & Drewnowski, A. (2019). Plant- and animal-protein diets in relation to sociodemographic drivers, quality, and cost: Findings from the Seattle Obesity Study. *The American Journal of Clinical Nutrition*, 110(2), 451–460. <https://doi.org/10.1093/ajcn/nqz064>
- Amarowicz, R., Estrella, I., Hernández, T., Robredo, S., Troszyńska, A., Kosińska, A., & Pegg, R. B. (2010). Free radical-scavenging capacity, antioxidant activity, and phenolic composition of green lentil (*Lens culinaris*). *Food Chemistry*, 121(3), 705–711. <https://doi.org/10.1016/j.foodchem.2010.01.009>
- Arendt, E. K., Ryan, L. A. M., & Bello, F. D. (2007). Impact of sourdough on the texture of bread. *Food Microbiology*, 24(2), Article 2.
- Bachir, N., Akkoun, H., Pujola, M., Sepulcre, F., & Haddarah, A. (2024). Impact of amino acids and sugars after thermal processing on acrylamide formation in synthetic potato models and real potatoes. *Food Science & Nutrition*, 12(2), 1046–1055. <https://doi.org/10.1002/fsn3.3818>
- Bakke, A., & Vickers, Z. (2007). Consumer Liking of Refined and Whole Wheat Breads. *Journal of Food Science*, 72(7), S473–S480. <https://doi.org/10.1111/j.1750-3841.2007.00440.x>
- Bao, Z., Li, Y., Zhang, J., Li, L., Zhang, P., & Huang, F. R. (2016). Effect of particle size of wheat on nutrient digestibility, growth performance, and gut microbiota in growing pigs. *Livestock Science*, 183, 33–39. <https://doi.org/10.1016/j.livsci.2015.11.013>
- Blanco, C. A., Ronda, F., Pérez, B., & Pando, V. (2011). Improving gluten-free bread quality by enrichment with acidic food additives. *Food Chemistry*, 127(3), 1204–1209. <https://doi.org/10.1016/j.foodchem.2011.01.127>
- Cacak-Pietrzak, G., Sujka, K., Książek, J., Bojarszczuk, J., Ziarno, M., Studnicki, M., Krajewska, A., & Dziki, D. (2024). Assessment of Physicochemical Properties and Quality of the Breads Made from Organically Grown Wheat and Legumes. *Foods*, 13(8). <https://doi.org/10.3390/foods13081244>. Article 8.
- Calabrese, M., De Luca, L., Basile, G., Sorrentino, G., Esposito, M., Pizzolongo, F., Verde, G., & Romano, R. (2024). Reducing the acrylamide concentration in homemade bread processed with L-asparaginase. *LWT*, 209, Article 116770. <https://doi.org/10.1016/j.lwt.2024.116770>
- Çelik, E. E., & Gökmen, V. (2020). Formation of Maillard reaction products in bread crust-like model system made of different whole cereal flours. *European Food Research and Technology*, 246(6), 1207–1218. <https://doi.org/10.1007/s00217-020-03481-4>
- Chelladurai, V., & Erkinbaev, C. (2020). Lentils. In A. Manickavasagan, & P. Thirunathan (Eds.), *Pulses: Processing and Product Development* (pp. 129–143). Springer International Publishing. https://doi.org/10.1007/978-3-030-41376-7_8
- Chigwedere, C. M., Wanasundara, J. P. D., & Shand, P. J. (2022). Sensory descriptors for pulses and pulse-derived ingredients: Toward a standardized lexicon and sensory wheel. *Comprehensive Reviews in Food Science and Food Safety*, 21(2), 999–1023. <https://doi.org/10.1111/1541-4337.12893>
- Chulibert, M. E., Roppolo, P., Buzzanca, C., Alfonzo, A., Viola, E., Sciarba, L., ... Settanni, L. (2024). Exploring the Addition of Mango Peel in Functional Semolina Sourdough Bread Production for Sustainable Bio-Reuse. *Antioxidants*, 13(11). <https://doi.org/10.3390/antiox13111278>. Article 11.
- Claeys, W. L., de Vleeschouwer, K., & Hendrickx, M. E. (2005). Effect of Amino Acids on Acrylamide Formation and Elimination Kinetics. *Biotechnology Progress*, 21(5), 1525–1530. <https://doi.org/10.1021/bp050194s>
- Conceição, L. d. S., Almeida, B. S. d., Souza, S. F. d., Martinez, V. O., Matos, M. F. R. d., Andrade, L. L., ... Pinto Matos, L. C. (2024). Critical conditions for the formation of Maillard Reaction Products (MRP) in bread: An integrative review. *Journal of Cereal Science*, 118, Article 103985. <https://doi.org/10.1016/j.jcs.2024.103985>
- De Lamo, B., & Gómez, M. (2018). Bread Enrichment with Oilseeds. A Review. *Foods*, 7(11). <https://doi.org/10.3390/foods7110191>. Article 11.
- Dewan, M. F., Shams, S.-N.-U., & Haque, M. A. (2024). Impact of Processing on the Bioactive Compounds and Antinutritional Factors of Lentil (L.)—A Review. *Legume Science*, 6(3), Article e253. <https://doi.org/10.1002/leg3.253>
- Dhull, S. B., Kinabo, J., & Uebersax, M. A. (2023). Nutrient profile and effect of processing methods on the composition and functional properties of lentils (*Lens culinaris* Medik): A review. *Legume Science*, 5(1), Article e156. <https://doi.org/10.1002/leg3.156>
- El-Sayed, A. A., Abdelhady, M. M., Jaafari, S. A., Alanazi, T. M., & Mohammed, A. S. (2023). Impact of Some Enzymatic Treatments on Acrylamide Content in Biscuits. *Processes*, 11(4). <https://doi.org/10.3390/pr11040141>. Article 4.
- Gallo, V., Romano, A., Miralles, B., Ferranti, P., Masi, P., Santos-Hernández, M., & Recio, I. (2022). Physicochemical properties, structure and digestibility in simulated gastrointestinal environment of bread added with green lentil flour. *LWT*, 154, Article 112713. <https://doi.org/10.1016/j.lwt.2021.112713>
- Galoburda, R., Straumite, E., Sabovics, M., & Kruma, Z. (2020). Dynamics of Volatile Compounds in Triticale Bread with Sourdough: From Flour to Bread. *Foods*, 9(12). <https://doi.org/10.3390/foods9121837>. Article 12.
- Ganesan, K., & Xu, B. (2017). Polyphenol-Rich Lentils and Their Health Promoting Effects. *International Journal of Molecular Sciences*, 18(11), 2390. <https://doi.org/10.3390/ijms18112390>
- Gao, J., & Zhou, W. (2021). Oral processing of bread: Implications of designing healthier bread products. *Trends in Food Science & Technology*, 112, 720–734. <https://doi.org/10.1016/j.tifs.2021.04.030>
- Giusti, M. M., Miyagusuku-Cruzado, G., & Wallace, T. C. (2023). Flavonoids as Natural Pigments. In *Handbook of Natural Colorants* (pp. 371–390). John Wiley & Sons, Ltd.. <https://doi.org/10.1002/9781119811749.ch17>
- Gobbetti, M., Angelis, D., Maria, D. C., Raffaella, P., & Andrea, & Rizzello, C. G. (2020). The sourdough fermentation is the powerful process to exploit the potential of legumes, pseudo-cereals and milling by-products in baking industry. *Critical Reviews in Food Science and Nutrition*, 60(13), 2158–2173. <https://doi.org/10.1080/10408398.2019.1631753>
- Graça, C., Lima, A., Raymundo, A., & Sousa, I. (2021). Sourdough Fermentation as a Tool to Improve the Nutritional and Health-Promoting Properties of Its Derived-Products. *Fermentation*, 7(4). <https://doi.org/10.3390/fermentation7040246>. Article 4.
- Hamlet, C., Saad, P., Liang, L., Jayaratne, S. N., & Skingle, M. (2007). Exploiting processing conditions to reduce acrylamide in cereal-based foods, Report No. C021. <https://www.nal.usda.gov/research-tools/food-safety-research-projects/exploiting-processing-conditions-reduce-acrylamide>.
- Henao Toro, S. J., Gómez-Narváez, F., Contreras-Calderón, J., & Ariseto, A. P. (2022). Acrylamide in sugar products. *Current Opinion in Food Science*, 45, Article 100841. <https://doi.org/10.1016/j.cofs.2022.100841>
- Hölzle, E., Breiting-Utzmann, C., Blumberg, O., Klass, N., Remezov, A., Schödl, S., Sisichka, A., Tränkle, K., Steliopoulos, P., & Oellig, C. (2025). Influence of chia and flaxseeds on acrylamide formation in sweet bakery products. *Food Chemistry*, 463, Article 141344. <https://doi.org/10.1016/j.foodchem.2024.141344>

- ICC Method, M. (1976). 110/1 Determination of the Moisture Content of Cereals and Cereal Products (Practical method). <https://icc.or.at/publications/icc-standards/standards-overview/110-1-standard-method>.
- ISO 11136:2014 Sensory analysis—Methodology—General guidance for conducting hedonic tests with consumers in a controlled area. <https://www.iso.org/cms/render/live/en/sites/isoorg/contents/data/standard/05/01/50125.html>, (2014).
- Jarpa-Parra, M. (2018). Lentil protein: A review of functional properties and food application. An overview of lentil protein functionality. *International Journal of Food Science & Technology*, 53(4), 892–903. <https://doi.org/10.1111/ijfs.13685>
- Ju, Q., Li, Y., Sun, H., Chen, J., Yuan, Y., Hu, Y., Fujita, K., & Luan, G. (2020). Effect of potato flour on quality and staling properties of wheat–potato flour bread. *Food Science & Nutrition*, 8(10), 5474–5482. <https://doi.org/10.1002/fsn3.1829>
- Kaur, N., & Halford, N. G. (2023). Reducing the Risk of Acrylamide and Other Processing Contaminant Formation in Wheat Products. *Foods*, 12(17). <https://doi.org/10.3390/foods12173264>. Article 17.
- Kho, H. E., Azrina, A., Teng, T. S., & Lim, S. M. (2017). Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & Nutrition Research*, 61(1), Article 1361779. <https://doi.org/10.1080/16546628.2017.1361779>
- Klupsaite, D., Starkute, V., Zokaityte, E., Cernauskas, D., Mockus, E., Kentra, E., ... Bartkiene, E. (2023). The Contribution of Scalded and Scalded-Fermented Rye Wholemeal Flour to Quality Parameters and Acrylamide Formation in Semi-Wheat-Rye Bread. *Foods*, 12(5). <https://doi.org/10.3390/foods12050937>. Article 5.
- Kotsiou, K., Palassaros, G., Matsakidou, A., Mouzakis, C.-K., Biliaderis, C. G., & Lazaridou, A. (2023). Roasted-sprouted lentil flour as a novel ingredient for wheat flour substitution in breads: Impact on dough properties and quality attributes. *Food Hydrocolloids*, 145, Article 109164. <https://doi.org/10.1016/j.foodhyd.2023.109164>
- Kotsiou, K., Sacharidis, D.-D., Matsakidou, A., Biliaderis, C. G., & Lazaridou, A. (2021). Impact of Roasted Yellow Split Pea Flour on Dough Rheology and Quality of Fortified Wheat Breads. *Foods*, 10(8). <https://doi.org/10.3390/foods10081832>. Article 8.
- Lemos, A. C., Borba, V. S. de, Scaglioni, P. T., & Badiale-Furlong, E. (2023). Processing parameters in breadmaking and bioaccessibility of acrylamide and 5-hydroxymethylfurfural. *Food Research International*, 174, 113523. doi: <https://doi.org/10.1016/j.foodres.2023.113523>.
- Lemos, A. C., V. S. de B., Cerqueira, M. B. R., Pereira, A. M., Scaglioni, P. T., & Badiale-Furlong, E. (2024). White and wholewheat bread consumption and the risk of exposure to acrylamide and 5-hydroxymethylfurfural. *Food Chemistry*, 460(Pt 3), Article 140662. <https://doi.org/10.1016/j.foodchem.2024.140662>
- Lin, S., Zhang, X., Wang, J., Li, T., & Wang, L. (2024). Effect of lactic acid bacteria fermentation on bioactive components of black rice bran (*Oryza sativa* L.) with different milling fractions. *Food Bioscience*, 58, Article 103684. <https://doi.org/10.1016/j.fbio.2024.103684>
- Liu, F., Chen, Y., Chen, J., Xu, E., Pan, H., Chen, S., Ye, X., & Cheng, H. (2023). Characteristic aroma improvement mechanisms of heat-sterilized bayberry juice regulated by exogenous polyphenols. *Food Chemistry*, 427, Article 136644. <https://doi.org/10.1016/j.foodchem.2023.136644>
- Liyanage, D. W. K., Yevtushenko, D. P., Korschuh, M., Bizimungu, B., & Lu, Z.-X. (2021). Processing strategies to decrease acrylamide formation, reducing sugars and free asparagine content in potato chips from three commercial cultivars. *Food Control*, 119, Article 107452. <https://doi.org/10.1016/j.foodcont.2020.107452>
- LST. (1996). Bread and bread products. In *Porosity (LST 1442:1996)*. Lithuanian: Standards Board (LST).
- Lund, M. N., & Ray, C. A. (2017). Control of Maillard Reactions in Foods: Strategies and Chemical Mechanisms. *Journal of Agricultural and Food Chemistry*, 65(23), 4537–4552. <https://doi.org/10.1021/acs.jafc.7b00882>
- Magalhães, D., Gonçalves, R., Rodrigues, C. V., Rocha, H. R., Pintado, M., & Coelho, M. C. (2024). Natural Pigments Recovery from Food By-Products: Health Benefits towards the Food Industry. *Foods*, 13(14). <https://doi.org/10.3390/foods13142276>. Article 14.
- Mesías, M., Delgado-Andrade, C., & Morales, F. J. (2024). Chapter 7—Acrylamide in bakery products. In V. Gökmen, & B. A. Mogol (Eds.), *Acrylamide in Food* ((Second Edition), pp. 133–160). Academic Press. <https://doi.org/10.1016/B978-0-323-99119-3.00012-6>.
- Mesías, M., & Morales, F. J. (2015). *Acrylamide in Bakery Products*. <https://doi.org/10.1016/B978-0-12-802832-2.00007-3>
- Mirali, M., Purves, R. W., & Vandenberg, A. (2016). Phenolic profiling of green lentil (*Lens culinaris* Medik.) seeds subjected to long-term storage. *European Food Research and Technology*, 242(12), 2161–2170. <https://doi.org/10.1007/s00217-016-2713-1>
- Mishra, G. P., Ankita, A., S. M., S., Tontang, M. T., Choudhary, P., Tripathi, K., ... Dikshit, H. K. (2022). Morphological, Molecular, and Biochemical Characterization of a Unique Lentil (*Lens culinaris* Medik.) Genotype Showing Seed-Coat Color Anomalies Due to Altered Anthocyanin Pathway. *Plants*, 11(14). <https://doi.org/10.3390/plants11141815>. Article 14.
- Mockus, E., Starkute, V., Klupsaite, D., Bartkevicius, V., Borisova, A., Sarunaite, L., ... Bartkiene, E. (2024). Changes in Chemical Composition of Lentils, Including Gamma-Aminobutyric Acid and Volatile Compound Formation during Submerged and Solid-State Fermentation with *Pediococcus acidilactici*. *Foods*, 13(8). <https://doi.org/10.3390/foods13081249>. Article 8.
- Mockus, E., Zokaityte, E., Starkute, V., Klupsaite, D., Ruibys, R., Rocha, J. M., ... Bartkiene, E. (2023). Influence of different lactic acid bacteria strains and milling process on the solid-state fermented green and red lentils (*Lens culinaris* L.) properties including gamma-aminobutyric acid formation. *Frontiers in Nutrition*, 10, Article 1118710. <https://doi.org/10.3389/fnut.2023.1118710>
- Mohammed, I., Ahmed, A. R., & Senge, B. (2012). Dough rheology and bread quality of wheat–chickpea flour blends. *Industrial Crops and Products*, 36(1), 196–202. <https://doi.org/10.1016/j.indcrop.2011.09.006>
- Mollakhaili-Meybodi, N., Khorshidian, N., Nematollahi, A., & Arab, M. (2021). Acrylamide in bread: A review on formation, health risk assessment, and determination by analytical techniques. *Environmental Science and Pollution Research*, 28(13). <https://doi.org/10.1007/s11356-021-12775-3>. Article 13.
- Moreno-Araiza, O., Boukid, F., Suo, X., Wang, S., & Vittadini, E. (2023). Pretreated Green Pea Flour as Wheat Flour Substitutes in Composite Bread Making. *Foods*, 12(12). <https://doi.org/10.3390/foods12122284>. Article 12.
- Mortensen, A. (2006). Carotenoids and other pigments as natural colorants. *Pure and Applied Chemistry*, 78(8), 1477–1491. <https://doi.org/10.1351/pac200678081477>
- Mottram, D. S., Wedzicha, B. L., & Dodson, A. T. (2002). Acrylamide is formed in the Maillard reaction. *Nature*, 419(6906), 448–449. <https://doi.org/10.1038/419448a>
- Mudgil, D., Barak, S., & Khatkar, B. S. (2016). Optimization of bread firmness, specific loaf volume and sensory acceptability of bread with soluble fiber and different water levels. *Journal of Cereal Science*, 70, 186–191. <https://doi.org/10.1016/j.jcs.2016.06.009>
- Mulders, E. J. (1973). The odour of white bread. *Zeitschrift Für Lebensmittel-Untersuchung Und Forschung*, 151(5), 310–317. <https://doi.org/10.1007/BF01883343>
- Mustafa, A., Andersson, R., Rosén, J., Kamal-Eldin, A., & Åman, P. (2005). Factors Influencing Acrylamide Content and Color in Rye Crisp Bread. *Journal of Agricultural and Food Chemistry*, 53(15), 5985–5989. <https://doi.org/10.1021/jf050020q>
- Nigro, G., Gasparre, N., Vurro, F., Pasqualone, A., & Rosell, C. M. (2025). Lupin flour as a wheat substitute in conventional and sourdough breadmaking: Impact on bread physicochemical properties and volatile profile. *European Food Research and Technology*. <https://doi.org/10.1007/s00217-025-04694-1>
- Ning, J., Hou, G. G., Sun, J., Wan, X., & Dubat, A. (2017). Effect of green tea powder on the quality attributes and antioxidant activity of whole-wheat flour pan bread. *LWT - Food Science and Technology*, 79, 342–348. <https://doi.org/10.1016/j.lwt.2017.01.052>
- Oates, C. G. (2001). Bread Microstructure. In *Bread Staling (1st Edition, p. 14)*. CRC Press.
- Oduro-Yeboah, C., Sulaiman, R., Uebersax, M. A., & Dolan, K. D. (2023). A review of lentil (Medik) value chain: Postharvest handling, processing, and processed products. *Legume Science*, 5(2), Article e171. <https://doi.org/10.1002/leg3.171>
- Onyango, C., Mutungi, C., Unbehend, G., & Lindhauer, M. G. (2010). Rheological and baking characteristics of batter and bread prepared from pregelatinized cassava starch and sorghum and modified using microbial transglutaminase. *Journal of Food Engineering*, 97(4), 465–470. <https://doi.org/10.1016/j.jfoodeng.2009.11.002>
- Ordóñez-Santos, L. E., Esparza-Estrada, J., & Vanegas-Mahecha, P. (2021). Ultrasound-assisted extraction of total carotenoids from mandarin epicarp and application as natural colorant in bakery products. *LWT*, 139, Article 110598. <https://doi.org/10.1016/j.lwt.2020.110598>
- Orsák, M., Kotíková, Z., Podhorecká, K., Lachman, J., & Kasal, P. (2022). Acrylamide formation in red-, purple- and yellow-fleshed potatoes by frying and baking. *Journal of Food Composition and Analysis*, 110, Article 104529. <https://doi.org/10.1016/j.jfca.2022.104529>
- Osemwota, E. C., Alashi, A. M., & Aluko, R. E. (2022). Physicochemical and functional properties of albumin, globulin and glutenin fractions of green lentil seed. *International Journal of Food Science and Technology*, 57(7), 3967–3981. <https://doi.org/10.1111/ijfs.15608>
- Paciulli, M., Palermo, M., Chiavaro, E., & Pellegrini, N. (2017). Chlorophylls and Colour Changes in Cooked Vegetables. In *Fruit and Vegetable Phytochemicals* (pp. 703–719). John Wiley & Sons, Ltd. <https://doi.org/10.1002/9781119158042.ch31>
- Padovani, R. M., Lima, D. M., Colugnati, F. A. B., & Rodriguez-Amaya, D. B. (2007). Comparison of proximate, mineral and vitamin composition of common Brazilian and US foods. *Journal of Food Composition and Analysis*, 20(8), 733–738. <https://doi.org/10.1016/j.jfca.2007.03.006>
- Pal, R. S., Bhartiya, A., Yadav, P., Kant, L., Mishra, K. K., Aditya, J. P., & Pattanayak, A. (2017). Effect of dehulling, germination and cooking on nutrients, anti-nutrients, fatty acid composition and antioxidant properties in lentil (*Lens culinaris*). *Journal of Food Science and Technology*, 54(4), 909–920. <https://doi.org/10.1007/s13197-016-2351-4>
- Parker, J. K. (2024). COST Action ACRYRED, CA21149, Supported by COST (European Cooperation in Science and Technology), Working Group WG3, Grant Period (GP1). Deliverable 3.1 – Overview of research activities in Europe to reduce acrylamide in cereal-based foods for the field of food chemistry including sensory aspects, processing, and test development. <https://acryred.eu/wp-content/uploads/2024/07/WG3-GP1-Deliverable.pdf>.
- Paventi, G., Di Martino, C., Crawford, T. W., Jr., & Iorizzo, M. (2024). Enzymatic activities of *Lactiplantibacillus plantarum*: Technological and functional role in food processing and human nutrition. *Food Bioscience*, 61, Article 104944. <https://doi.org/10.1016/j.fbio.2024.104944>
- Previtali, M. A., Mastromatteo, M., De Vita, P., Ficco, D. B. M., Conte, A., & Del Nobile, M. A. (2014). Effect of the lentil flour and hydrocolloids on baking characteristics of wholemeal durum wheat bread. *International Journal of Food Science & Technology*, 49(11), 2382–2390. <https://doi.org/10.1111/ijfs.12559>
- Rathnayake, H. A., Navaratne, S. B., & Navaratne, C. M. (2018). Porous Crumb Structure of Leavened Baked Products. *International Journal of Food Science*, 2018(1), Article 8187318. <https://doi.org/10.1155/2018/8187318>
- Regulation No. 2017/2158, 304 OJ L. <http://data.europa.eu/eli/reg/2017/2158/oj/eng>, (2017).
- Riaz, F., Hameed, A., & Asghar, M. J. (2024). Grain nutritional and antioxidant profiling of diverse lentil (*Lens culinaris* Medikus) genetic resources revealed genotypes with high nutritional value. *Frontiers in Nutrition*, 11. <https://doi.org/10.3389/fnut.2024.1344986>

- Rizzello, C. G., Calasso, M., Campanella, D., De Angelis, M., & Gobbetti, M. (2014). Use of sourdough fermentation and mixture of wheat, chickpea, lentil and bean flours for enhancing the nutritional, texture and sensory characteristics of white bread. *International Journal of Food Microbiology*, 180, 78–87. <https://doi.org/10.1016/j.ijfoodmicro.2014.04.005>
- do Rosário, D. K. A., da Silva Mutz, Y., Vieira, K. M., Schwan, R. F., & Bernardes, P. C. (2024). Effect of self-induced anaerobiosis fermentation (SIAF) in the volatile compounds and sensory quality of coffee. *European Food Research and Technology*, 250(2), 667–675. <https://doi.org/10.1007/s00217-023-04393-9>
- Ryan, E., Galvin, K., O'Connor, T. P., Maguire, A. R., & O'Brien, N. M. (2007). Phytosterol, Squalene, Tocopherol Content and Fatty Acid Profile of Selected Seeds, Grains, and Legumes. *Plant Foods for Human Nutrition*, 62(3), 85–91. <https://doi.org/10.1007/s11130-007-0046-8>
- Saffarionpour, S. (2024). Off-Flavors in Pulses and Grain Legumes and Processing Approaches for Controlling Flavor-Plant Protein Interaction: Application Prospects in Plant-Based Alternative Foods. *Food and Bioprocess Technology*, 17(5), 1141–1182. <https://doi.org/10.1007/s11947-023-03148-4>
- Salim-ur-Rehman, Paterson, A., & Piggott, J. R. (2006). Flavour in sourdough breads: A review. *Trends in Food Science & Technology*, 17(10), 557–566. <https://doi.org/10.1016/j.tifs.2006.03.006>
- Schöpf, A., Oellig, C., & Granvogel, M. (2022). Formation of furanoic compounds in model systems with saccharides, amino acids, and fatty acids, analyzed with headspace-solid phase micro-extraction-gas chromatography–tandem mass spectrometry. *Journal of Food Bioactives*, 20, 61–71. <https://doi.org/10.31665/JFB.2022.18329>
- Schouten, M. A., Tappi, S., Glicerina, V., Rocculi, P., Angeloni, S., Cortese, M., ... Romani, S. (2022). Formation of acrylamide in biscuits during baking under different heat transfer conditions. *LWT*, 153, Article 112541. <https://doi.org/10.1016/j.lwt.2021.112541>
- Senanayake, D., Torley, P. J., Chandrapala, J., & Terefe, N. S. (2023). Microbial Fermentation for Improving the Sensory, Nutritional and Functional Attributes of Legumes. *Fermentation*, 9(7). <https://doi.org/10.3390/fermentation9070635>. Article 7.
- Shapla, U. M., Solayman, M., Alam, N., Khalil, M. I., & Gan, S. H. (2018). 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: Effects on bees and human health. *Chemistry Central Journal*, 12(1), 35. <https://doi.org/10.1186/s13065-018-0408-3>
- Shen, Y., Chen, G., & Li, Y. (2019). Effect of added sugars and amino acids on acrylamide formation in white pan bread. *Cereal Chemistry*, 96(3), 545–553. <https://doi.org/10.1002/cche.10154>
- Silva, R., Coelho, E., Aguiar, T. Q., & Domingues, L. (2021). Microbial Biosynthesis of Lactones: Gaps and Opportunities towards Sustainable Production. *Applied Sciences*, 11(18). <https://doi.org/10.3390/app11188500>. Article 18.
- Soltan, S. S. A. (2013). The protective effect of soybean, sesame, lentils, pumpkin seeds and molasses on iron deficiency anemia in rats. *World Applied Sciences Journal*, 23(6), 795–807.
- Stadler, R. H. (2006). 2—The formation of acrylamide in cereal products and coffee. In K. Skog, & J. Alexander (Eds.), *Acrylamide and Other Hazardous Compounds in Heat-Treated Foods* (pp. 23–40). Woodhead Publishing. <https://doi.org/10.1533/9781845692018.1.23>
- Stadler, R. H., Blank, I., Varga, N., Robert, F., Hau, J., Guy, P. A., ... Riediker, S. (2002). Acrylamide from Maillard reaction products. *Nature*, 419(6906), 449–450. <https://doi.org/10.1038/419449a>
- Streekstra, H., & Livingston, A. (2020). Chapter 10—Acrylamide in bread and baked products. In S. P. Cauvain (Ed.), *Breadmaking* (Third Edition), pp. 289–321. Woodhead Publishing. <https://doi.org/10.1016/B978-0-08-102519-2.00010-4>
- Su, X., Wu, F., Zhang, Y., Yang, N., Chen, F., Jin, Z., & Xu, X. (2019). Effect of organic acids on bread quality improvement. *Food Chemistry*, 278, 267–275. <https://doi.org/10.1016/j.foodchem.2018.11.011>
- Sui, X., Yap, P. Y., & Zhou, W. (2015). Anthocyanins During Baking: Their Degradation Kinetics and Impacts on Color and Antioxidant Capacity of Bread. *Food and Bioprocess Technology*, 8(5), 983–994. <https://doi.org/10.1007/s11947-014-1464-x>
- Taeymans, D., Wood, J., Ashby, P., Blank, I., Studer, A., Stadler, R. H., ... Whitmore, T. (2004). A Review of Acrylamide: An Industry Perspective on Research, Analysis, Formation, and Control. *Critical Reviews in Food Science and Nutrition*, 44(5), 323–347. <https://doi.org/10.1080/10408690490478082>
- Trindler, C., Annika Kopf-Bolanz, K., & Denkel, C. (2022). Aroma of peas, its constituents and reduction strategies – Effects from breeding to processing. *Food Chemistry*, 376, Article 131892. <https://doi.org/10.1016/j.foodchem.2021.131892>
- Turfani, V., Narducci, V., Durazzo, A., Galli, V., & Carcea, M. (2017). Technological, nutritional and functional properties of wheat bread enriched with lentil or carob flours. *LWT*, 78, 361–366. <https://doi.org/10.1016/j.lwt.2016.12.030>
- Vurro, F., De Angelis, D., Squeo, G., Caponio, F., Summo, C., & Pasqualone, A. (2024). Exploring Volatile Profiles and De-Flavoring Strategies for Enhanced Acceptance of Lentil-Based Foods: A Review. *Foods*, 13(16). <https://doi.org/10.3390/foods13162608>. Article 16.
- Wang, H., Liu, W., Zhang, P., & Lian, X. (2024). The Mechanism Underlying the Increase in Bread Hardness in Association with Alterations in Protein and Starch Characteristics During Room-Temperature Storage. *Foods*, 13(23), 3921. <https://doi.org/10.3390/foods13233921>
- Wang, N., Hatcher, D. W., Toews, R., & Gawalko, E. J. (2009). Influence of cooking and dehulling on nutritional composition of several varieties of lentils (*Lens culinaris*). *LWT - Food Science and Technology*, 42(4), 842–848. <https://doi.org/10.1016/j.lwt.2008.10.007>
- Wang, S., Yu, J., Xin, Q., Wang, S., & Copeland, L. (2017). Effects of starch damage and yeast fermentation on acrylamide formation in bread. *Food Control*, 73(Part B), 230–236. <https://doi.org/10.1016/j.foodcont.2016.08.002>
- Xu, M., Jin, Z., Lan, Y., Rao, J., & Chen, B. (2019). HS-SPME-GC-MS/olfactometry combined with chemometrics to assess the impact of germination on flavor attributes of chickpea, lentil, and yellow pea flours. *Food Chemistry*, 280, 83–95. <https://doi.org/10.1016/j.foodchem.2018.12.048>
- Yan, F., Wang, L., Zhao, L., Wang, C., Lu, Q., & Liu, R. (2023). Acrylamide in food: Occurrence, metabolism, molecular toxicity mechanism and detoxification by phytochemicals. *Food and Chemical Toxicology*, 175, Article 113696. <https://doi.org/10.1016/j.fct.2023.113696>
- Zhang, L.-L., Guan, E.-Q., Yang, Y.-L., Zhang, T.-J., Zhang, Y.-L., Liu, Y.-X., Li, M.-M., Zhang, K.-G., & Bian, K. (2022). The globulin aggregation characteristics induced by salt and alkali and its effects on dough processing quality. *Journal of Cereal Science*, 104, Article 103437. <https://doi.org/10.1016/j.jcs.2022.103437>
- Zhang, Y., Dong, Y., Ren, Y., & Zhang, Y. (2006). Rapid determination of acrylamide contaminant in conventional fried foods by gas chromatography with electron capture detector. *Journal of Chromatography A*, 1116(1–2). Article 1–2.
- Zhou, L., Liang, W.-J., Gao, Q., Dong, W.-M., & He, J.-S. (2022). Inhibitory effects of amino acids on the formation of acrylamide in glucose-asparagine simulation system. *Journal of Food Safety and Quality*, 13(8), 2565–2572.