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# Quality and chemical safety of wheat bread enriched with untreated, milled, and *Lactiplantibacillus plantarum* fermented red lentils (*Lens culinaris* L.)

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A R T I C L E I N F O

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A B S T R A C T

This study investigated the effects of untreated, milled, and fermented with *Lactiplantibacillus plantarum* No. 122 red lentils (*Lens culinaris* L.) on the quality and safety parameters of wheat bread (WB). The quantity (5, 10, 15, 20 and 25 %) and type of lentils added significantly influenced WB specific volume. Bread with 10 % of fermented non-milled (FNM) and 15 % of fermented milled (FM) lentils exhibited lower porosity (average, 52.4 %), while the highest total titratable acidity (1.12°N) was observed in bread enriched with 25 % of (FM) lentils. Enrichment with red lentils increased acrylamide levels in most breads to 14–44 μg/kg. A moderate correlation (−0.415–0.449) was found between acrylamide levels and certain VOCs of WB. Breads containing 10 and 15 % (FM) and (FNM) lentils showed overall acceptability scores (average, 4.1) similar to control bread. In conclusion, adding 5 % of non-fermented/fermented lentils to WB allowed for high acceptability without increasing acrylamide concentrations.

## 1. Introduction

Recent data indicate a rise in global lentil production (FAO, 2021), driven by consumers' preference for sustainable plant-based proteins, such as grain legumes and pulses (Dhull et al., 2023). Lentils are particularly valued for their high protein content (20.6–31.4 g/100 g) (Jarpa-Parra et al., 2014), complex carbohydrates, essential minerals, vitamins, antioxidants and phytoestrogens (Dhull et al., 2023; Joshi et al., 2017; Siva et al., 2017). Lentil proteins possess remarkable functional properties as emulsifying, fat-absorption, and foaming

*Abbreviations:* −b*, blueness; BI, browning index; CFU, colony forming units; MRS, de Man, Rogosa, Sharpe; DM, dry matter; GABA, gamma-aminobutyric acid; GC–MS, gas chromatography–mass spectrometry; GC–ECD, gas chromatograph with an electron capture detector; -a*, greenness; LAB, lactic acid bacteria; L*, lightness; nd, not determined; a*, redness; RT, retention time; TPA, Texture Profile Analysis; ΔE*, total colour change; TTA, °N, total titratable acidity; SPME, solid-phase microextraction; SE, standard error; VOC, volatile compound; b*, yellowness.

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capacities (Khazaei et al., 2019). However, despite these benefits, lentils also contain antinutritional factors, which can be mitigated through various pre-processing techniques, including fermentation (Bartkiene et al., 2018; Campos-Vega et al., 2010; Patterson et al., 2017). The presence of antinutritional factors such as saponins, phytic acid, tannins, and trypsin inhibitors is undesirable because they can diminish the bioavailability and digestibility of proteins, minerals, and starches (Dewan et al., 2024; Mazı et al., 2023).

Lentils find broad application, ranging from everyday home cooking to the industrial manufacture of bread, crackers, extruded snacks, and energy bars (Kaale et al., 2023). Incorporating lentil flour or sourdough into bread formulations offers substantial nutritional benefits, such as increased protein and fibre content (Cacak-Pietrzak et al., 2024; Neagu Dragomir et al., 2025; Rizzello et al., 2014). Although lentil flour is increasingly utilized in bakery formulations, optimizing its substitution level for wheat flour remains essential, as lentil incorporation markedly influences dough rheology, textural properties, and the sensory quality of the final product (Previtali et al., 2014; Ropciuc et al., 2025). Furthermore, the effects of particle size on flour functionality, dough characteristics and bread quality are also significant (Pang et al., 2021) but understudied in lentil-based bread formulations. It was reported that particle size can significantly affect water absorption and rheological properties of dough (Dayakar Rao et al., 2016; Kadan et al., 2008). Mockus et al. (2023) reported the effects of lentil particle size on various sourdough parameters (Mockus et al., 2023). Nevertheless, investigations into how such lentil-based sourdoughs affect the technological and sensory quality of bread are still limited. Therefore, the optimization of lentil flour substitution levels is critical to balance the nutritional advantages with the potential adverse effects on dough rheology and bread quality.

Fermentation, one of the key methods for processing legumes, greatly alters the composition and technological behavior of lentils and shapes the sensory qualities of lentil-based products, ultimately influencing consumer acceptance (Asensio-Grau et al., 2020; Badia-Olmos et al., 2024; Padhy et al., 2025). Fermentation can induce desirable changes in lentil, such as improved protein digestibility through proteolysis and generation of free amino acids and peptides (Alrosan et al., 2021). However, applying fermentation processes, such as sourdough fermentation, in bakery production may alter the acrylamide concentration in the finished products, as a harmful acrylamide is generated during thermal processing through the Maillard reaction between reducing sugars and asparagine at temperatures exceeding 120 °C (Codină et al., 2021; Lopez-Moreno et al., 2024; Zhou et al., 2022). Physiological implications of acrylamide formation were reported (Komoike et al., 2020; Liu et al., 2020; Matoso et al., 2019) as well as distinct concentrations of this compound in wheat breads (Bartkiene et al., 2023; Crawford et al., 2019; Galluzzo et al., 2021; Juodeikiene et al., 2018; Klupsaite et al., 2022, 2023; Mollakhalili-Meybodi et al., 2021; Zhou et al., 2022). In 2024, the wheat bread segment dominated the global bread market, as reported in industry analyses, reflecting both the functional properties of wheat for baking, such as gluten network development and volume retention, and its prevalent use in bread-making practices (*Market Data Forecast* ., 2025). Moreover, previous study revealed that consumers preferred refined wheat bread over whole wheat bread or breads with certain alternative grains as chia seeds, Tartary buckwheat, and quinoa (Bakke & Vickers, 2007; Kušar et al., 2023). Considering the aforementioned points, it is essential to improve wheat bread formulations to minimize acrylamide formation, enhance nutritional quality, and preserve consumer acceptability. Returning to the importance of fermentation in bakery, the genus *Lactobacillus* conclusively demonstrated to be effective in reducing acrylamide formation in bakery products (Codină et al., 2021). According to Nasiri Esfahani et al. (2017) the incorporation of *Lactobacillus*-based sourdough in combination with yeast fermentation led to a decrease in acrylamide levels in whole wheat bread, with the magnitude of reduction being specific to the *Lactobacillus* strain employed (Nasiri Esfahani et al., 2017). Thus, variations in the *Lactobacillus* species used for fermentation are likely to influence the extent of acrylamide formation in wheat breads containing legume ingredients, reflecting species-specific metabolic activities. Moreover, it is important to mention that the incorporation of sourdough in bread production has been shown to enhance the sensory and nutritional qualities of the final product, including improved flavour, texture, nutritional composition, and extended shelf life (D'Amico et al., 2023; Wang & Wang, 2024). In addition, sourdough fermentation contributes to an increased concentration of bioactive compounds and a reduction in microbial spoilage of bread (Wang & Wang, 2024). Furthermore, several studies have reported that consumption of specific sourdough breads may positively influence postprandial glycaemic response, promote satiety, and improve gastrointestinal tolerance (Ribet et al., 2023).

While the use of fermented legumes in bakery products has been previously investigated, limited attention has been given to how lentil particle size and the choice of lactic acid bacteria strain influence both technological and safety aspects (i.e. acrylamide formation) of wheat bread. Unlike previous studies on legume fermentation, this research employs *Lactiplantibacillus plantarum* No. 122 strain, which was previously isolated from spontaneously fermented rye sourdough and characterized for good antimicrobial and acidification activity. Application of the probiotic *L. plantarum* gives additional value, because this strain is linked to various health benefits (Aljohani et al., 2025). Therefore, the present study introduces a novel approach by applying L. *plantarum* No. 122 for the fermentation of milled and unmilled red lentils and assessing their impact on the quality, chemical safety (acrylamide content), and acceptability of wheat bread. Furthermore, data on the acrylamide content of bakery products fermented with L. *plantarum* remain limited, highlighting the need to explore their potential as a promising approach for reducing acrylamide formation.

We hypothesize that quality and chemical safety of wheat bread can be related to the pre-treatment (milling or fermentation) and quantity of the red lentils used. Therefore, the aim of this study was to evaluate the effect of untreated, milled, and *Lactiplantibacillus plantarum* No. 122–fermented red lentils (*Lens culinaris* L.) on wheat bread quality and safety parameters. Research objectives were to investigate the impact of red lentil form (untreated vs. milled vs. fermented with L. *plantarum* No. 122) and varying substitution levels (5, 10, 15, 20, 25 %) on overall acceptability, specific volume, crumb porosity, moisture content, baking mass loss, texture hardness, crumb and crust colour, volatile compound (VOC) profile and acrylamide concentration of wheat bread were investigated; to explore correlations between acrylamide concentration and VOCs in wheat bread enriched with red lentils; to identify the optimal red lentil type and substitution level that balances quality, safety, and overall acceptability in wheat bread. The study is expected to demonstrate that optimized technology for incorporating red lentils into a wheat bread recipe can significantly improve the quality and safety of wheat bread, providing additional scientific support for the inclusion of fermented legumes in cereal products.

## 2. Materials and methods

### *2.1. Characteristics of lentils, lactic acid bacteria used for lentils fermentation, and fermentation conditions*

Red lentils (nutritional composition per 100 g: total carbohydrates 63.1 g, protein 23.9 g, fat 2.2 g) were supplied by Ltd. 'Galinta ir partneriai' (Kaunas, Lithuania). To evaluate an influence of the lentils' particle size on sourdough characteristics and quality and safety parameters of wheat bread, the samples were ground using a Laboratory Mill 120 (Perten Instruments AB, Stockholm, Sweden) to achieve a particles size of 1–2 mm.

The *Lactiplantibacillus plantarum* No. 122 strain, obtained from Lithuanian University of Health Sciences (Kaunas, Lithuania), was cultured in De Man, Rogosa and Sharpe (MRS) broth (Biolife, Milano, Italy) at 30 °C under anaerobic conditions for 24 h. Fresh LAB culture (3 mL; 8.6 $\log_{10}$ CFU/mL) was inoculated into 100 g of lentils (1:1 *w*/w lentil-to-water ratio) and fermented for 24 h at 30 °C under anaerobic conditions in a chamber incubator (Memmert GmbH Co. KG, Schwabach, Germany). The initial LAB count averaged 5.02 $\log_{10}$ CFU/g, increasing to 8.04 and 8.01 $\log_{10}$ CFU/g in fermented non-milled and milled lentils, respectively. Non-fermented samples (mixed with water) were analysed and (or) used as non-fermented controls in bread preparation. The pH, colour coordinates, viable LAB count, free amino acid profile, gamma-aminobutyric acid (GABA) concentration and volatile compound (VOC) profile of the prepared lentil samples are given in **Supplementary File 1** (Tables S1, **S2**, and **S3**) and described by Mockus et al. (2023).

### 2.2. Wheat bread preparation and analysis methods

#### 2.2.1. Wheat bread preparation

The bread formula consisted of 1.0 kg of wheat flour, 1.5 % (*w*/w) salt, 3 % (w/w) instant yeast and 1000 mL water, used as the control bread. Control samples were prepared without the addition of non-fermented or fermented red lentils. Experimental bread groups were prepared by incorporating 5, 10, 15, 20 and 25 % of either non-fermented non-milled, non-fermented milled, fermented non-milled and fermented milled red lentils into the base recipe (lentils/water the ratio was 1:1, w/w). The term “non-milled” refers to hulled whole red lentils seeds. In total, 20 groups of dough and bread were prepared and analysed:

- Control bread (i.e. without lentil additions) (C);
- 5 groups with 5, 10, 15, 20 and 25 % of non-fermented (NF) and non-milled (NM) lentils (NF-NM);
- 5 groups with 5, 10, 15, 20 and 25 % of fermented (F) NM lentils (F-NM);
- 5 groups with 5, 10, 15, 20 and 25 % of NF milled (M) lentils (NF-M); and
- 5 groups with 5, 10, 15, 20 and 25 % of fermented F milled (M) lentils (F-M).

The dough was mixed using a dough mixer (KitchenAid Artisan, Ohio, USA) at low speed for 3 min, followed by 7 min at high-speed. After mixing, the dough was rested at 22 ± 2 °C for 15 min for relaxation. Afterwards, the dough was divided into 375 g, shaped in loaves and proofed at 32 ± 2 °C with 80 % relative humidity (RH) for 60 min. The bread was baked in a deck oven (EKA, Borgoricco PD, Milano, Italy) at 220 °C for 25 min. After cooling for 12 h at 22 ± 2 °C, the bread samples were analysed for: overall acceptability; total titratable acidity (TTA); specific volume; shape coefficient; crumb porosity and texture hardness; moisture content; mass loss after baking; acrylamide concentration; crust and crumb colour characteristics; and VOC profile.

The experimental design is summarized in Fig. 1.

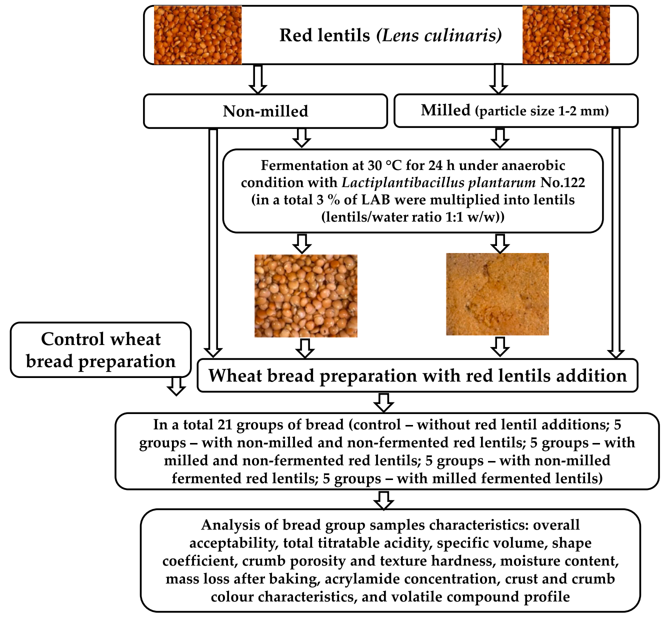



**Fig. 1.** The experimental design.

#### 2.2.2. Bread analysis methods

*2.2.2.1. Overall acceptability.* The overall acceptability of the breads was assessed by 10 trained judges following the ISO method (*ISO 11136*, 2014). A 5-pont Likert scale was used, ranging from 5 (extremely like) to 0 (extremely dislike). Each bread group sample was identified by a randomly generated three-digit code and presented individually on a plate and one at a time. Participant received approximately one-quarter of a slice of each of the bread sample. To clean their palates between samples, participants were provided with water.

*2.2.2.2. Quality parameters.* The quality parameters under scrutiny were:

- *Total titratable Acidity*. TTA was determined for a 10 g bread sample homogenized with 90 mL of distilled water. The results were expressed as the mL of 0.1 mol/L NaOH (°N) required to titrate the mixture to a pH of 8.2.
- *Bread volume*: Bread volume was measured by the American Association of Cereal Chemists (AACC) method (AACC, 2025). The bread loaf was placed in a container and millet were used to measure the volume displaced. The specific volume was calculated by dividing the loaf's weight by its volume.
- *Bread porosity*: porosity was determined using a 7–8 cm thick slice taken from the centre of a loaf of bread (LST 1442:1996). The crumb was shaped into 3 cylinders, each with a volume of 27 $cm^3$, using a special Zhuravliov device. These cylinders were weighed and porosity was calculated using formula (**Eq. 1**):

$$Porosity\ (\%) = 100 \times \frac{V - \frac{m}{q}}{V} \quad (1)$$

where $V$ is the total volume of the 3 crumb cylinders ($cm^3$), $m$ is the total weight of the 3 crumb cylinders (g); and $q$ is the crumb density (1.31 $cm^3$/g).
- *Crumb texture hardness*. Bread crumb firmness was measured using Texture Profile Analysis (TPA) with a Stevens-LFRA Texture Analyzer (Poland). Bread slices (2 cm thick) were compressed to 10 % of their original height at a crosshead speed of 10 mm/s. The peak compression force was recorded as crumb firmness. Results were averaged from 3 replicates across 3 separate baking sets.
- *Moisture content*. Moisture content was determined following the International Association for Cereal Science and Technology (ICC) Standard Method 110/1 (ICC-Standard No. 110/1, 2025).
- *Mass loss after baking*. Mass loss was calculated as a percentage by comparing the weight of the dough before baking to the weight of the baked loaf.

*2.2.2.3. Colour coordinates, total colour change (ΔE) and browning index (BI) of crumb and crust.* Crust and crumb colour parameters were evaluated using a CIE L*a*b* system with a CromaMeter CR-400 (Konica Minolta, Tokyo, Japan) (Carocho et al., 2020). The total colour change (ΔE*) between the control bread and bread samples with lentils was calculated using the equation described by Wrolstad and Smith (2017) (**Eq. 2**).

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (2)$$

where $\Delta L^* = L^*_{sample} - L^*_{control\ sample}$; $\Delta a^* = a^*_{sample} - a^*_{control\ sample}$; $\Delta b^* = b^*_{sample} - b^*_{control\ sample}$

The browning index (BI) was calculated according to the method of Ramírez-Jiménez et al. (2000) (**Eq. 3**).

$$BI = 100 - L^* \quad (3)$$

*2.2.2.4. Acrylamide determination.* Acrylamide concentration was determined following the method of Zhang et al. (2006), with modifications as outlined in Bartkiene et al. (Bartkiene et al., 2023). 2 g of homogenized bread sample were weighed into a 50 mL centrifuge tube and diluted with 20 mL of distilled/deionized water. The tube was briefly vortexed (ZX3 Advanced VELP, Italy) for 10 min and centrifuged (Hermle Z 306, Germany) at 4000 rpm for 10 min.

10 mL of the aqueous supernatant solution, in 15 mL centrifuge tubes, were clarified with 100 μL of Carrez I {85 mM $K_4[Fe(CN)_6] \times 3H_2O$} and 100 μL of Carrez II (250 mM $ZnSO_4 \times 7H_2O$) solutions, followed by centrifugation at 4000 rpm for 10 min. A standard acrylamide solution (30.4 μg/L) was prepared by dissolving 15.2 mg of acrylamide analytical (99.8 % purity) in 1000 mL deionized water in a volumetric flask.

3 mL of the supernatant (or standard solution) was derivatized, in a glass sample tube, with 1.5 g of potassium bromide (KBr), 1 mL of potassium bromate solution (0.1 M, $KBrO_3$) and 0.3 mL of sulfuric acid solution [50 % (*v*/v), $H_2SO_4$]. The mixture was shaken and refrigerated (~4 °C) for 2 h. The derivative was neutralized by adding 250 μL of sodium thiosulfate solution (1 M, $Na_2S_2O_3x5H_2O$) until the orange colour disappeared. Approximately 1.5 g of sodium chloride (NaCl) was added to the derivative mixture, which was then extracted two times with 5 mL ethyl acetate ($CH_3COOC_2H_5$).

The collected ethyl acetate was concentrated with a rotary evaporator (Christ CT 02–50, Germany) at 40 °C under reduced pressure. The residue was dissolved in 0.5 mL of ethyl acetate or 3 mL for standards. The solutions were treated with 100 mg of anhydrous sodium sulphate ($Na_2SO_4$). 20 μL of triethylamine [$(C_2H_5)_3N$] (20 μL of triethylamine in 0.5 mL of the concentrated derivatization solution) was added to the solution in a 15 mL centrifuge tube, mixed and centrifuged at 4000 rpm for 10 min. The supernatant was analysed with a gas chromatograph with an electron capture detector (GC–ECD). System (Shimadzu GC-17 A, Japan) was equipped with a thermostated Rxi-5Sil MS column [Restek, Germany; 30 m × 0.25 mm-φ inner diameter (ID) × 0.25 μm-φ stationary phase film thickness], and an integrator to measure peak areas. Operating conditions included an injection volume of 1 μL, an initial column temperature of 70 °C (1 min hold), a gradient of 3 °C/min to 140 °C (0.5 min hold) and 15 °C/min to 280 °C (4 min hold). The mobile phase was nitrogen (flowrate: 18 cm/s), with a split of 3.0, and with injector and detector (detector current: 2 nA) temperatures set at 250 and 260 °C, respectively.

*2.2.2.5. Volatile compound (VOC) profile analysis.* VOC analysis of bread samples was performed using gas chromatography–mass spectrometry (GC–MS). A solid-phase microextraction (SPME) device with a Stable-flex™ fibre coated with a 50 μm PDMS-DVB-Carboxen™ layer (Supelco, Bellefonte, Pennsylvania, USA) was used. For headspace extraction, 2 g of bread sample and 10 mL of 1 M phosphate buffer (pH = 3) with 25 % (*w/v*) NaCl were placed in a 20 mL extraction vial, mixed and sealed with a polytetrafluoroethylene septum. The mixture was equilibrated at 60 °C for 30 min before exposing the fibre to the headspace of the vial for 10 min.

Desorption was performed in splitless injection mode for 2 min and the prepared samples were analysed using a GCMS-QP2010 (Shimadzu, Kioto, Japan) gas chromatograph and mass spectrometer. The injector temperature was set at 250 °C, the ion source at 220 °C and the interface at 260 °C. Helium was used as the carrier gas at a flowrate of 0.65 mL/min. VOC separation was performed using a low polarity Stabilwax™-DA column (Restek, Bellefonte, PA, USA; 30 m × 0.25 mm-φ ID × 0.25 μm-φ film thickness). The temperature program started at 40 °C (3 min hold), increased by 5 °C/min to 250 °C, and held for 5 min. VOC was based on mass spectrum libraries (NIST11, NIST11S, FFNSC2).

### 2.3. Statistical analysis

For data interpretation, the results were expressed as the mean values ± standard errors (SE). Physical and chemical characteristics of bread samples were based on $n = 3 \pm$ SE, while overall acceptability was evaluated with $n = 10 \pm$ SE. To assess the effects of fermentation, milling and varying quantities of lentils on bread quality parameters, data were analysed using multivariate ANOVA followed by Tukey-HSD post-hoc tests, performed in R Statistical Software (v4.1.2; R Core Team, 2021, Vienna, Austria). Additionally, Pearson correlation coefficients were calculated between various parameters. Results were considered statistically significant at $p \leq 0.05$.

## 3. Results and discussion

### 3.1. Overall acceptability and other quality characteristics of wheat bread enriched with red lentils

The overall acceptability scores for the bread samples are presented in Fig. 2. In general, increasing the lentil content in the bread formula reduced the overall acceptability scores. The lowest overall acceptability was observed for breads prepared with 20 and 25 % of both non-fermented and fermented lentils. However, the addition of 5 % lentils, whether non-fermented or fermented, had no significant impact on sensory acceptability of the bread. Notably, fermentation enabled the incorporation of higher quantities of lentils without negatively affecting bread overall acceptability. Breads enriched with 10 and 15 % fermented milled or non-milled lentils achieved overall acceptability scores comparable to the control samples. Similar to our results, Previtali et al. . (2014) found that raising the lentil flour level to 20 % and 25 % exerted a detrimental impact on the wholemeal durum wheat bread's sensory quality. As observed in our study, prior research likewise indicates that the acceptability of legume-enriched breads, including those containing lentils, can be enhanced through fermentation or germination. It was reported that wheat bread supplemented with germinated lentils had higher sensory scores, while the addition of wheat-legume sourdough improved certain sensory properties of wheat bread and provided a good acceptability (Atudorei et al., 2022; Rizzello et al., 2014). Perri et al. (2021) demonstrated that an integrated biotechnological approach, combining sprouting with sourdough fermentation, allows for the incorporation of up to 30 % lentil sourdough without compromising overall acceptability, as it effectively mitigates beany off-flavors and improves the aroma profile.

Wheat bread quality parameters are depicted in Fig. 3 (**a** – **f**). When comparing the specific volumes of breads prepared with non-fermented non-milled lentils, it was found that supplementation with 5, 10, 15 and 20 % of these lentils increased the specific volume by an average of 19.7 % (Fig. 3a). The highest specific volume was observed in breads containing 5 % fermented non-milled lentils. In most cases, fermented non-milled lentil supplementation increased specific volume compared to control breads, except for those prepared with 10 % fermented non-milled lentils. Breads enriched with 20 and 25 % of non-fermented milled lentils exhibited reduced specific volumes compared to the control, while those with 5, 10 and 15 % non-fermented milled lentils had similar specific volumes to the control. Conversely, breads prepared with 25 % fermented milled lentils showed, on average, a specific volume 15.5 % higher than the control. Factors such as lentil quantity (QL), non-fermented or fermented (NoF/F), non-milled or milled (NoM/M)),

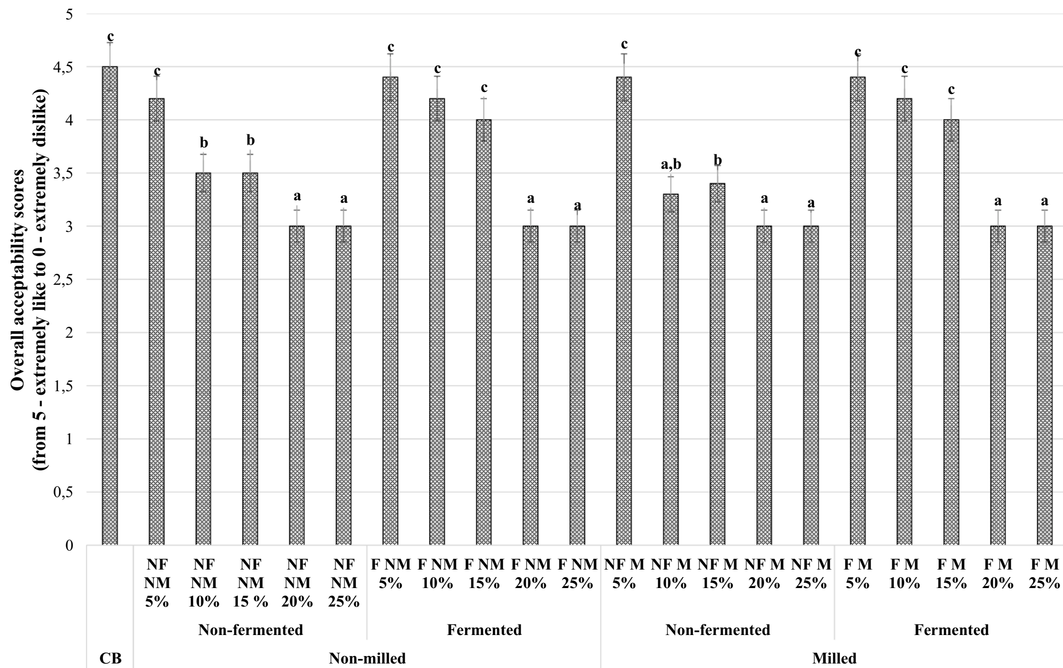



**Fig. 2.** Overall acceptability of bread samples (NF – non-fermented; F – fermented with *Lactiplantibacillus plantarum* No.122 strain; NM – non-milled; M – milled; 5 %, 10 %,15 %, 20 %, 25 % –quantities of non-fermented non-milled, non-fermented milled, fermented non-milled and fermented milled red lentils in bread formula. Data expressed as mean values (n = 10) ± standard error (SE); a–c Mean values within the columns with different letters are significantly different ($p \leq 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and their interactions (except NoF/F × NoM/M, ($p = 0.168$)) significantly affected bread specific volume ($p \leq 0.001$) (Table 1). Bread samples with 10 % fermented non-milled lentils and 15 % fermented milled lentils displayed lower porosity compared to the control (Fig. 3b). Despite this, the analysed factors and their interactions did not significantly affect bread porosity (Table 1). The highest moisture content was found in breads containing 20 % non-fermented milled lentils (Fig. 3c). A moderate negative correlation was established between moisture content and mass loss after baking ($r = -0.419$, $p < 0.001$). Although factors and interactions had no significant effect on moisture content, factors such as NoF/F, NoM/M, and their interactions (QL × NoF/F or QL × NoF/F × NoM/M) significantly influenced mass loss after baking (Table 1). Lentil supplementation generally increased mass loss after baking (Fig. 3d), with a strong positive correlation observed between mass loss after baking and specific volume ($r = 0.691$, $p < 0.001$). In most cases, red lentil supplementation increased bread texture hardness, except in breads prepared with 5 % non-fermented non-milled, 5 % fermented non-milled, or 5 % fermented milled lentils (Fig. 3e). Increasing the content of fermented lentils in the bread formula also increased TTA, with the highest TTA observed in breads enriched with 25 % fermented milled lentils (Fig. 3f). Most analysed factors (except NoF/F) and their interactions significantly influenced bread TTA (Table 1). Legume flours are well documented to impair baked product texture, as their incorporation typically weakens dough structure and baking properties, reduces bread volume and crumb elasticity, and enhance loaf hardness (Rizzello et al., 2014). These effects are mostly caused by the fibre and non-gluten proteins found in lentils, as well as the fact that the proteins in pulses might compete with those in cereals for water absorption (Perri et al., 2021). Consistent with our findings, Cacak-Pietrzak et al. (2024)reported that increasing the proportion of lentil flour used to fortify wheat flour led to a significant, linear rise in the crumb hardness of wheat sourdough bread. Our results are also comparable to those in previous studies on various legume addition to bread. When lupin flour or lupin sourdough was added to wheat bread, their reduced dough pH, while lupin flour alone increased crumb hardness, an effect not observed when sourdough was included (Nigro et al., 2025). It was reported that addition of wheat-legume flour sourdough to wheat bread resulted in diminished pH in dough, increased hardness, similar moisture and specific volume compared to wheat bread (Rizzello et al., 2014). In our study, breads made with non-milled lentils showed greater volume than those made with milled lentils, likely because the finer particles in the milled fraction interfered more with gluten network formation (Ropciuc et al, 2025). Our findings also showed that fermentation improved bread volume when non-milled lentils were used. It was reported flour with broader and coarser particle size distribution increased specific volume of bread when used with sourdough fermentation (Cardinali et al., 2024). This could be attributed to the sourdough ability to enhance gas retention capacity in the dough due to slight acidification, production of exopolysaccharides, synergy with yeast and endogenous flour enzyme activation (Cardinali et al., 2024; Ertop & İbrahim Tuğkan, 2018; Gobbetti et al., 2005; Hu et al., 2022; Woo et al., 2023). Our finding also showed that fermentation efficiency depends on substrate particle size, because finer particles improve nutrient availability for LAB and a lower pH can be obtained. Most breads with fermented milled lentils had a higher TTA than breads with fermented non-milled lentils. However, this could also lead to lower specific volume, because excessive acidification during sourdough fermentation can lead to the hydrolysis and depolymerization of gluten proteins (Thiele et al., 2004), weakening the gluten network and reducing specific volume in the final bread. The present study further demonstrated that increasing the proportion of lentils generally reduced the specific volume of bread. Similarly, it was reported that at higher levels of wheat flour substitution, dough rheological properties tend to deteriorate (Marchini et al., 2021).

### *3.2. Wheat bread crust and crumb chromaticity parameters, total colour change (ΔE) and browning index (BI)*

The colour characteristics of bread crust and crumb are presented in Table 2. For crust lightness (L*), the highest values were observed in control bread samples and breads containing 10 % non-fermented non-milled red lentils, as well as 20 and 25 % non-fermented milled red

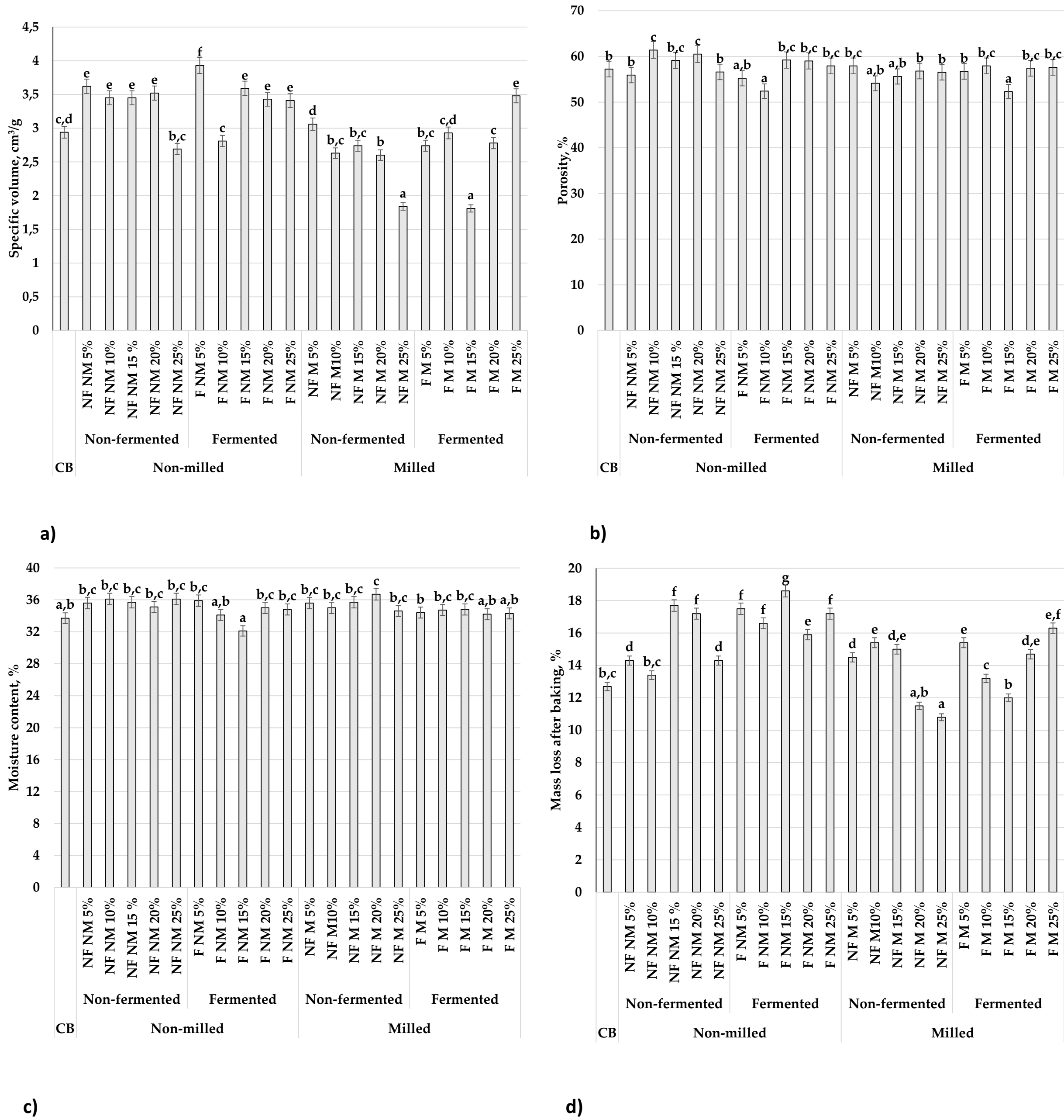
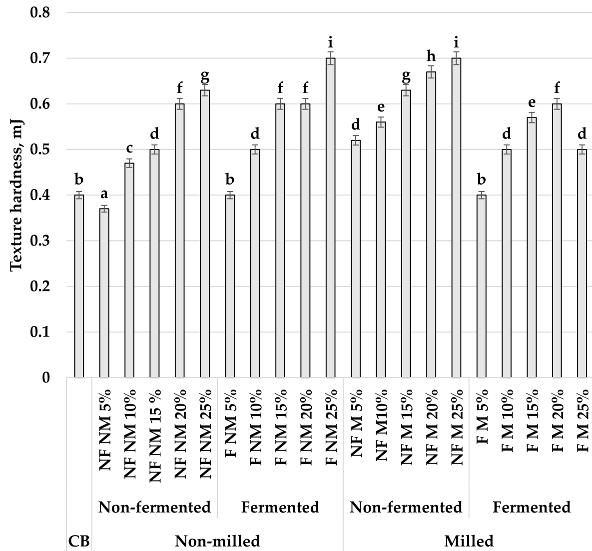
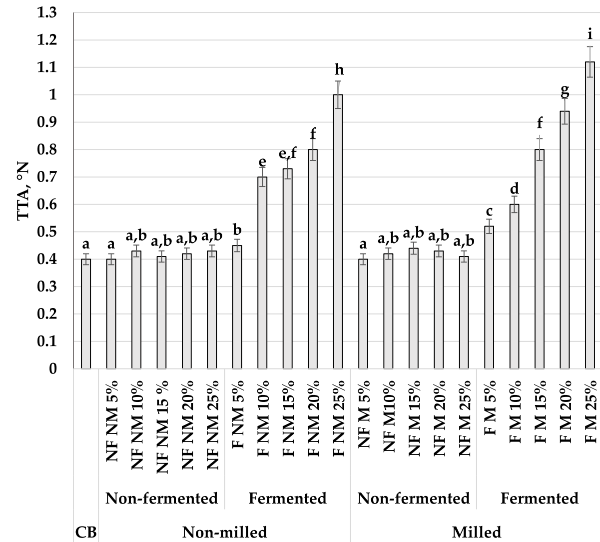



**Fig. 3.** Bread quality parameters: a – specific volume, b – porosity, c – moisture content, d – mass loss after baking, e – texture hardness, f – total titratable acidity (TTA – total titratable acidity; °N – Neiman degree; NF – non-fermented; F – fermented with *Lactiplantibacillus plantarum* No.122 strain; NM – non-milled; M – milled; 5 %, 10 %,15 %, 20 %, 25 % – quantities of non-fermented non-milled, non-fermented milled, fermented non-milled, and fermented milled red lentils in bread formula. Data expressed as mean values (n = 3) ± standard error (SE); a–i Mean values within the columns with different letters are significantly different ($p \leq 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

lentils. NoF/F factor significantly affected crust L* and a* coordinates (Table 4). However, no significant correlations were found between crust L* and other bread parameters. For crust redness (a*), the highest values were observed in breads with 5 % non-fermented non-milled lentils and 15 % fermented milled lentils. The highest yellowness (b*) was found in breads with 20 and 25 % non-fermented milled lentils and 15 % fermented milled lentils. The QL factor and the interaction QL × NoF/F × NoM/M significantly influenced crust b* values (Table 4). For crumb lightness (L*), the highest values were observed in breads with 5 % non-fermented non-milled lentils. However, the analysed factors did not significantly affect crumb L* values (Table 4). Control breads, along with samples containing 5 and 10 % non-fermented non-milled lentils, 5 and 10 % fermented non-milled lentils, 5, 10, and 20 % non-fermented milled lentils, and 5 and 20 % fermented milled lentils, exhibited more pronounced greenness (−a*) compared to redness (+a*). Most analysed factors and their interactions (except QL and NoF/F × NoM/M) significantly influenced crumb a* values (Table 4). The addition of lentils reduced crumb yellowness (b*) in samples with 5–25 % non-fermented non-milled lentils, 15 % non-fermented milled lentils, and 15 and 25 % fermented milled lentils. Most analysed factors, except QL and QL × NoF/F, significantly affected crumb b* values (Table 4). The total colour change (ΔE*) and browning index (BI) for bread crust and crumb are summarized in Table 3. ΔE* values ranged from 1.61 to 16.1 for crust and 1.24 to 6.79 for crumb, with greater differences observed in the




e)




f)

**Fig. 3.** (*continued*).

**Table 1**
Significance of the analysed factors and their interactions on the analysed bread quality parameters.

| Factors and their interactions | Bread parameters | | | | | |
|---|---|---|---|---|---|---|
| | Specific volume | Porosity | Moisture content | Mass loss after baking | Texture hardness | Total titratable acidity |
| | Significance (p) value | | | | | |
| Quantity of lentils (QL) | <0.001 | 0.340 | 0.375 | 0.392 | 0.035 | 0.003 |
| Non-fermented or fermented (NoF/F) | <0.001 | 0.351 | 0.089 | 0.006 | 0.417 | 0.028 |
| Non-milled or milled (NoM/M) | <0.001 | 0.300 | 0.834 | <0.001 | 0.506 | 0.006 |
| QL × NoF/F | <0.001 | 0.361 | 0.428 | 0.014 | 0.139 | 0.003 |
| QL × NoM/M | <0.001 | 0.370 | 0.248 | 0.052 | 0.018 | 0.001 |
| NF/F × NoM/M | 0.168 | 0.210 | 0.706 | 0.341 | 0.051 | 0.005 |
| QL × NoF/F × NoM/M | <0.001 | 0.466 | 0.659 | 0.006 | 0.296 | 0.001 |

Factors or their interaction is significant, when $p \leq 0.05$

crust. Among the crust samples, breads with 10 % non-fermented non-milled lentils exhibited the closest ΔE* values to the control crust, while crust samples with 10 and 15 % non-fermented milled lentils showed the largest differences. For crumb ΔE*, breads with 10 % non-fermented milled lentils or 5 and 20 % fermented milled lentils had the closest values to the control crumb, whereas breads with 5 % non-fermented non-milled lentils showed the largest differences. BI values ranged from 48.16 to 61.38 for crust and 19.51 to 26.48 for crumb. Crust BI values were higher at all substitution levels for breads with fermented lentils compared to the control. The highest increase in crust BI (22.3 %) was observed in breads with 10 and 15 % non-fermented milled lentils. For crumb BI, the lowest value was found in breads with 5 % non-fermented non-milled lentils, while the highest value was observed in breads with 25 % fermented non-milled lentils.

The colour of bread is influenced by several factors, including the Maillard reaction, crust caramelization, and the natural pigments provided by the ingredients in the recipe (Martins et al., 2017). Similar trends in crumb colour changes of wheat bread were reported by Portman et al. (2018) in wheat breads with lentil flour. Kotsiou et al. (Kotsiou et al., 2023) also observed significant changes in crust and crumb colour when lentil flour was included in wheat bread formulations, noting that crumb colour differences were primarily attributed to lentil flour addition rather than products of the Maillard reaction or caramelization. Previous study of Hajas et al. (2022) showed that addition of red lentils reduced lightness of gluten-free cookies and significantly redness. Moreover, variations in reducing sugar levels and lysine concentration in fermented and non-fermented lentil play a relevant role in browning reactions and further affect bread crust colour (Turfani et al., 2017). Contrary to our results, Cacak-Pietrzak et al. (2024) found higher crumb ΔE* values for lentil-wheat sourdough bread. These differences may occur due to differences in used lentil colour, bread formula, and breadmaking. In the present study, the elevated BI values observed in all breads containing fermented lentils, which also exhibited the highest total titratable acidity (TTA), indicate that the microbial fermentation enhanced the availability of Maillard reaction precursors (reducing sugars and/or amino acids), thereby counteracting the inhibitory effect of the reduced pH (Qi et al. 2025). In general, crust and crumb colour are one of the main sensory attributes strongly influencing consumer acceptability (Cabello-Olmo et al., 2023; Zarzycki et al., 2024). Our study observed a moderate negative correlation between bread overall acceptability and crumb redness (a*) ($r = -0.314$, $p = 0.012$), further supporting that inherent lentil pigments act as a 'sensory barrier', challenging consumer expectations for a traditional wheat bread appearance. Therefore, bread manufacturers should give particular attention to consumer expectations concerning colour in order to preserve the appeal and market competitiveness of wheat–legume products.

### 3.3. Acrylamide concentration

The acrylamide concentration (μg/kg) in bread samples is presented in Fig. 4. When comparing all the bread samples together, acrylamide

**Table 2**
Bread crust and crumb colour characteristics.

| Bread Samples | Crust | | | Crumb | | |
|---|---|---|---|---|---|---|
| | L* | a* | b* | L* | a* | b* |
| | NBS | | | | | |
| CB | 49.4 ± 0.33$^{e,f}$ | 10.2 ± 0.12$^{a}$ | 23.7 ± 0.21$^{d}$ | 76.1 ± 0.38$^{b}$ | −1.15 ± 0.014$^{a}$ | 20.9 ± 0.07$^{b}$ |
| NF NM 5 % | 48.2 ± 0.29$^{e}$ | 13.2 ± 0.11$^{c}$ | 23.5 ± 0.12$^{d}$ | 80.5 ± 0.94$^{d}$ | −0.190 ± 0.018$^{f}$ | 15.8 ± 0.14$^{a}$ |
| NF NM 10 % | 49.5 ± 0.48$^{e,f}$ | 11.7 ± 0.08$^{b}$ | 24.2 ± 0.33$^{d}$ | 75.0 ± 0.77$^{a,b}$ | −0.010 ± 0.002$^{h}$ | 16.9 ± 0.11$^{a}$ |
| NF NM 15 % | 46.4 ± 0.41$^{d}$ | 10.7 ± 0.09$^{a}$ | 20.1 ± 0.26$^{c}$ | 74.7 ± 0.05$^{a,b}$ | 0.320 ± 0.021$^{k}$ | 16.6 ± 0.15$^{a}$ |
| NF NM 20 % | 47.5 ± 0.48$^{d}$ | 10.7 ± 0.07$^{a}$ | 22.1 ± 0.21$^{c,d}$ | 75.4 ± 0.49$^{b}$ | 0.470 ± 0.033$^{l}$ | 15.9 ± 0.09$^{a}$ |
| NF NM 25 % | 46.9 ± 0.88$^{d}$ | 10.2 ± 0.04$^{a}$ | 21.6 ± 0.19$^{c}$ | 78.2 ± 0.76$^{c}$ | 0.680 ± 0.014$^{m}$ | 17.1 ± 0.12$^{a}$ |
| F NM 5 % | 41.4 ± 0.74$^{b}$ | 12.3 ± 0.05$^{b}$ | 20.1 ± 0.20$^{c}$ | 76.7 ± 0.38$^{b,c}$ | −0.510 ± 0.020$^{c}$ | 17.6 ± 0.10$^{a}$ |
| F NM 10 % | 47.1 ± 0.16$^{d}$ | 11.0 ± 0.02$^{a}$ | 22.7 ± 0.21$^{d}$ | 76.8 ± 0.73$^{b,c}$ | −0.060 ± 0.005$^{h}$ | 18.2 ± 0.11$^{a,b}$ |
| F NM 15 % | 38.8 ± 0.53$^{a,b}$ | 10.6 ± 0.03$^{a}$ | 14.8 ± 0.13$^{b}$ | 73.8 ± 0.62$^{a}$ | 0.040 ± 0.003$^{h,j}$ | 17.9 ± 0.13a$^{,b}$ |
| F NM 20 % | 40.2 ± 0.59$^{b}$ | 12.1 ± 0.04$^{b}$ | 18.7 ± 0.16$^{c,d}$ | 74.3 ± 0.51$^{a}$ | 0.060 ± 0.005$^{h,j}$ | 18.4 ± 0.14$^{a,b}$ |
| F NM 25 % | 39.4 ± 0.44$^{a,b}$ | 12.1 ± 0.06$^{b}$ | 18.0 ± 0.14$^{c,d}$ | 73.5 ± 0.49$^{a}$ | 0.070 ± 0.003$^{j}$ | 19.8 ± 0.12$^{b}$ |
| NF M 5 % | 47.3 ± 0.63$^{d}$ | 12.6 ± 0.05$^{b}$ | 21.0 ± 0.19$^{d}$ | 75.3 ± 0.99$^{a,b}$ | −0.290 ± 0.019$^{d}$ | 18.7 ± 0.10$^{a,b}$ |
| NF M 10 % | 38.6 ± 0.85$^{a,b}$ | 11.1 ± 0.07$^{a}$ | 14.6 ± 0.13$^{b}$ | 75.7 ± 0.40$^{a,b}$ | −0.230 ± 0.021$^{e}$ | 19.9 ± 0.11$^{b}$ |
| NF M 15 % | 37.6 ± 0.33$^{a}$ | 10.7 ± 0.04$^{a}$ | 12.7 ± 0.11$^{a}$ | 75.7 ± 0.61$^{a,b}$ | 0.010 ± 0.001$^{h}$ | 17.3 ± 0.15$^{a}$ |
| NF M 20 % | 50.0 ± 0.48$^{e,f}$ | 12.1 ± 0.08$^{b}$ | 24.2 ± 0.22$^{e}$ | 76.1 ± 0.48$^{a,b}$ | −0.150 ± 0.011$^{g}$ | 18.9 ± 0.14$^{a,b}$ |
| NF M 25 % | 51.8 ± 0.31$^{f}$ | 11.3 ± 0.04$^{a}$ | 24.5 ± 0.23$^{e}$ | 78.3 ± 0.65$^{b,c}$ | 1.42 ± 0.012$^{n}$ | 18.8 ± 0.11$^{a.b}$ |
| F M 5 % | 43.1 ± 0.28$^{c,d}$ | 12.0 ± 0.03$^{b}$ | 20.7 ± 0.16$^{d}$ | 77.0 ± 0.42$^{b,c}$ | −0.970 ± 0.024$^{b}$ | 20.1 ± 0.13$^{b}$ |
| F M 10 % | 40.3 ± 0.39$^{b}$ | 12.6 ± 0.05$^{b}$ | 18.6 ± 0.14$^{c,d}$ | 76.9 ± 0.46$^{b,c}$ | 0.510 ± 0.014$^{l}$ | 18.7 ± 0.12$^{a,b}$ |
| F M 15 % | 46.3 ± 0.26$^{d}$ | 13.4 ± 0.08$^{c}$ | 24.3 ± 0.23$^{e}$ | 77.5 ± 0.74$^{b,c}$ | 0.510 ± 0.023$^{l}$ | 17.7 ± 0.14$^{a}$ |
| F M 20 % | 45.3 ± 0.35$^{d}$ | 12.6 ± 0.06$^{b}$ | 21.5 ± 0.20$^{d}$ | 76.3 ± 0.73$^{b}$ | −0.130 ± 0.010$^{g}$ | 19.9 ± 0.17$^{b}$ |
| F M 25 % | 40.4 ± 0.27$^{b}$ | 11.9 ± 0.09$^{b}$ | 17.2 ± 0.15$^{c}$ | 75.1 ± 0.65$^{a,b}$ | 0.270 ± 0.021$^{k}$ | 17.9 ± 0.10$^{a}$ |

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, CB – control breads, NM – non-milled; M – milled; 5 %, 10 %,15 %, 20 %, 25 % – quantities of non-fermented non-milled, non-fermented milled, fermented non-milled, and fermented milled red lentils in bread formula. Data expressed as mean values (n = 3) ± standard error (SE); a–n Mean values within the columns with different letters are significantly different ($p \leq 0.05$).

concentrations significantly varied between them ($p \leq 0.05$). Most lentil-enriched bread samples (except breads with 5 % non-fermented non-milled, 5 % fermented non-milled, 5 % non-fermented milled, 5 % fermented milled, 25 % non-fermented milled and 20 % fermented milled red lentils) exhibited higher acrylamide levels compared to the control bread (Fig. 4). A more than 30 % increase in acrylamide concentration was found in breads with 15 % non-fermented non-milled lentils, 10 % fermented non-milled lentils, 15 and 20 % non-fermented milled lentils, and 15 and 25 % fermented milled lentils (on average by 38.9, 35.3, 35.3, 47.6, 47.6 and 54.2 %, respectively) compared to the control bread. However, none of the tested factors or their interactions had a statistically significant effect on acrylamide levels, and no correlations were observed between acrylamide content and other bread parameters.

When comparing acrylamide concentrations across groups with the same percentage of lentil supplementation, an increase in acrylamide levels was noted as the proportion of lentils in the formulation increased: 13.5 μg/kg in groups with 5 % of red lentils; 16.0 μg/kg in groups with 10 % of red lentils; 16.0 μg/kg in groups with 15 % of red lentils; 18.0 μg/kg in groups with 20 % of red lentils; and 24.8 μg/kg in groups with 25 % of red lentils. The similar trend was also observed by Portman et al. (2021), who found that higher proportions of lentil (*L. culinaris*. Medik.) in the wheat-lentil composite flour led to a considerable rise in acrylamide content in bread, cookies, and extrudates made from this flour. According to Sá and House (2024), pulses are less likely than cereals to

**Table 3**
The total colour change and browning index of bread crust and crumb.

| Bread Samples | Crust | Crumb | Crust | Crumb |
|---|---|---|---|---|
| | ΔE* | ΔE* | BI | BI |
| CB | nd | nd | 50.6 ± 0.1$^{ab}$ | 23.9 ± 0.4$^{bcd}$ |
| NF NM 5 % | 3.4 ± 0.4$^{abc}$ | 7.1 ± 1.9$^{f}$ | 51.8 ± 0.3$^{abc}$ | 19.5 ± 2.9$^{a}$ |
| NF NM 10 % | 1.7 ± 0.7$^{ab}$ | 4.4 ± 0.6$^{cde}$ | 50.5 ± 0.7$^{ab}$ | 25 ± 0.8$^{bcd}$ |
| NF NM 15 % | 4.6 ± 2$^{abcd}$ | 4.9 ± 0.2$^{cdef}$ | 53.6 ± 1.4$^{abcde}$ | 25.3 ± 1.1$^{bcd}$ |
| NF NM 20 % | 2.7 ± 2$^{abc}$ | 5.4 ± 0.4$^{ef}$ | 52.5 ± 1.5$^{abc}$ | 24.6 ± 0.5$^{bcd}$ |
| NF NM 25 % | 3.3 ± 2.3$^{abc}$ | 5 ± 0.6$^{def}$ | 53.1 ± 1.9$^{abcd}$ | 21.8 ± 1.8$^{ab}$ |
| F NM 5 % | 9.1 ± 1$^{def}$ | 3.5 ± 0.4$^{bcde}$ | 58.6 ± 0.7$^{defg}$ | 23.3 ± 0.4$^{abcd}$ |
| F NM 10 % | 2.7 ± 0.3$^{abc}$ | 3.1 ± 0.3$^{bcde}$ | 52.9 ± 0.2$^{abcd}$ | 23.2 ± 0.7$^{abcd}$ |
| F NM 15 % | 13.8 ± 1.8$^{fg}$ | 4.1 ± 0.8$^{bcde}$ | 61.2 ± 1.5$^{f}$ | 26.2 ± 1.1$^{cd}$ |
| F NM 20 % | 10.7 ± 0.8$^{ef}$ | 3.4 ± 0.6$^{bcde}$ | 59.8 ± 0.6$^{fg}$ | 25.7 ± 0.5$^{bcd}$ |
| F NM 25 % | 11.7 ± 0.1$^{efg}$ | 3.3 ± 1.9$^{bcde}$ | 60.6 ± 0.4$^{fg}$ | 26.5 ± 1.8$^{d}$ |
| NF M 5 % | 5 ± 0.4$^{abcd}$ | 2.7 ± 1.2$^{bcd}$ | 52.7 ± 1.6$^{abcd}$ | 24.7 ± 1$^{bcd}$ |
| NF M 10 % | 14.1 ± 0.3$^{fg}$ | 1.6 ± 0.7$^{ab}$ | 61.4 ± 0.8$^{f}$ | 24.3 ± 0.4$^{bcd}$ |
| NF M 15 % | 16.1 ± 2$^{g}$ | 4.6 ± 0.9$^{cdef}$ | 62.4 ± 1.3$^{f}$ | 24.3 ± 2.6$^{bcd}$ |
| NF M 20 % | 2.2 ± 0$^{abc}$ | 2.4 ± 0.6$^{abc}$ | 50 ± 0.5$^{ab}$ | 23.9 ± 0.5$^{bcd}$ |
| NF M 25 % | 5.1 ± 5$^{abcd}$ | 4.1 ± 1$^{bcde}$ | 48.2 ± 7$^{a}$ | 21.7 ± 0.7$^{ab}$ |
| F M 5 % | 7.3 ± 1.5$^{cde}$ | 1.6 ± 0.9$^{ab}$ | 56.9 ± 1.8$^{cdefg}$ | 23 ± 1.4$^{abcd}$ |
| F M 10 % | 10.7 ± 1$^{ef}$ | 2.9 ± 0.7$^{bcde}$ | 59.7 ± 0.8$^{fg}$ | 23.1 ± 0.4$^{abcd}$ |
| F M 15 % | 4.8 ± 0.5$^{abcd}$ | 4 ± 0.2$^{bcde}$ | 53.7 ± 1.7$^{abcde}$ | 22.5 ± 0.8$^{abc}$ |
| F M 20 % | 5.4 ± 2.1$^{bcd}$ | 2.2 ± 0.5$^{abc}$ | 54.8 ± 2.2$^{bcdef}$ | 23.7 ± 1.8$^{bcd}$ |
| F M 25 % | 11.2 ± 1$^{efg}$ | 3.5 ± 0.4$^{bcde}$ | 59.6 ± 1$^{efg}$ | 24.9 ± 1.2$^{bcd}$ |

ΔE* – the total colour change, BI – browing index, NF – non-fermented; F – fermented with *Lactiplantibacillus plantarum* No.122 strain, CB – control breads, NM – non-milled; M – milled; 5 %, 10 %,15 %, 20 %, 25 % – quantities of non-fermented non-milled, non-fermented milled, fermented non-milled, and fermented milled red lentils in bread formula, nd – not determined. Data expressed as mean values (n = 3) ± standard error (SE); a–g Mean values within the columns with different letters are significantly different ($p \leq 0.05$).

**Table 4**
Significance of the analysed factors and their interactions on bread crust and crumb colour coordinates.

| Factors and their interactions | Bread crust and crumb colour parameters | | | | | |
|---|---|---|---|---|---|---|
| | Crust | | | Crumb | | |
| | L* | a* | b* | L* | a* | b* |
| | Significance (p) value | | | | | |
| Quantity of lentils (QL) | 0.900 | 0.143 | 0.013 | 0.527 | <0.001 | 0.291 |
| Non-fermented or fermented (NoF/F) | 0.005 | 0.048 | 0.851 | 0.888 | 0.001 | 0.001 |
| Non-milled or milled (NoM/M) | 0.210 | 0.590 | 0.459 | 0.891 | 0.824 | 0.009 |
| QL × NoF/F | 0.071 | 0.326 | 0.256 | 0.537 | <0.001 | 0.273 |
| QL × NoM/M | 0.095 | 0.171 | 0.686 | 0.538 | 0.001 | 0.086 |
| NF/F × NoM/M | 0.223 | 0.123 | 0.401 | 0.856 | 0.197 | 0.039 |
| QL × NoF/F × NoM/M | 0.149 | 0.407 | 0.049 | 0.089 | 0.022 | 0.036 |

Factors or their interaction is significant, when $p \leq 0.05$

**Table 5**
Significance of the analysed factors and their interactions on bread volatile compounds profile.

| Dependent Variable | Factors and their interactions | | | | | | |
|---|---|---|---|---|---|---|---|
| | Quantity of lentils (QL) | Non-fermented or fermented (NoF/F) | Non-milled or milled (NoM/M) | QL × NoF/F | QL × NoM/M | NF/F × NoM/M | QL × NoF/F × NoM/M |
| Ethanol | <0.001 | <0.001 | 0.015 | <0.001 | 0.075 | 0.049 | <0.001 |
| 3-methyl-1-butanol | <0.001 | <0.001 | 0.030 | <0.001 | <0.001 | <0.001 | <0.001 |
| 2-methyl-pyrazine | <0.001 | 0.058 | 0.169 | <0.001 | <0.001 | <0.001 | <0.001 |
| 2-Ethylpyrazine | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| 1-Hexanol | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.029 |
| (*Z*)- 3-Hexen-1-ol | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| 2-ethyl-6-methylpyrazine | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Nonanal | <0.001 | <0.001 | 0.008 | <0.001 | 0.058 | <0.001 | <0.001 |
| 2-ethyl-3-methylpyrazine | <0.001 | 0.908 | 0.344 | <0.001 | <0.001 | <0.001 | <0.001 |
| 1-octen-3-ol | <0.001 | <0.001 | 0.008 | <0.001 | 0.018 | <0.001 | <0.001 |
| Heptan-1-ol | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Acetic acid | <0.001 | <0.001 | 0.281 | <0.001 | 0.160 | <0.001 | 0.003 |
| 3-Furaldehyde | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Benzaldehyde | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.027 | <0.001 |
| 2-methylpropanoic acid | <0.001 | 0.009 | <0.001 | <0.001 | 0.006 | <0.001 | 0.004 |
| 3-Furanmethanol | <0.001 | <0.001 | <0.001 | <0.001 | 0.050 | 0.469 | <0.001 |
| 2-methylhexanoic acid | <0.001 | 0.014 | <0.001 | <0.001 | 0.421 | <0.001 | 0.002 |
| (Z)-3-Nonen-1-ol | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Hexanoic acid | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.002 |
| 2-butyltetrahydrofuran | <0.001 | <0.001 | <0.001 | <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 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Phenethyl alcohol | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Heptanoic acid | <0.001 | 0.035 | <0.001 | <0.001 | <0.001 | 0.009 | <0.001 |
| γ-Amylbutyrolactone | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.054 | <0.001 |
| Octanoic acid | 0.009 | <0.001 | 0.015 | 0.012 | 0.042 | 0.165 | <0.001 |
| Nonanoic acid | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.947 | <0.001 |
| Decanoic acid | <0.001 | <0.001 | 0.009 | 0.052 | <0.001 | <0.001 | <0.001 |

Factors or their interaction is significant, when $p \leq 0.05$.

**Table 6**
Significant correlations between acrylamide concentration and volatile compounds.

| Volatile compounds | r | p |
|---|---|---|
| 2-methyl-pyrazine | 0.263* | 0.037 |
| 2-ethyl-6-methylpyrazine | 0.407** | <0.001 |
| 2-ethyl-3-methylpyrazine | 0.449** | <0.001 |
| Heptan-1-ol | −0.305* | 0.015 |
| Acetic acid | 0.426** | <0.001 |
| 2-methylpropanoic acid | −0.415** | <0.001 |
| 3-Hydroxy-2,4,4-trimethylpentyl 2-methylpropanoate | −0.271* | 0.031 |
| Octanoic acid | −0.258* | 0.041 |
| Nonanoic acid | −0.303* | 0.016 |

r – Pearson correlation between acrylamide concentration and volatile compound; p – significance of the correlation; * – correlation is significant at the 0.05 level (2-tailed); ** – correlation is significant at the 0.01 level (2-tailed).

generate acrylamide due to the presence of amino acids and natural antioxidants that prevent the production of this compound. However, pulse–wheat composite flours can elevate acrylamide levels when they introduce precursors (initial amount of asparagine, dietary fibre) that enhance its formation or when processing conditions unintentionally promote it. It was reported that finer wheat flour particle sizes lead to increased acrylamide formation in chapatti and poori flatbreads (Hitlamani & Ashok Inamdar, 2025). In our study, this tendency was not observed with the non-milled and milled red lentils. In general, the formation of acrylamide in bread is influenced by multiple factors as the presence of reducing sugars, free asparagine, baking temperature, water activity, fermentation conditions, sourdough microorganisms, and the antioxidant activity of ingredients (Cheng et al., 2015; Mollakhalili-Meybodi et al., 2021). Strategies for reducing acrylamide include adjusting pH, lowering baking temperatures and durations, selecting raw materials with fewer precursors, and incorporating additives such as acids, amino acids, hydrogen carbonates, proteins or antioxidants (Constantinou & Koutsidis, 2016; Li et al., 2014). Sourdough incorporating a blend of *Lactobacillus* strains alongside yeast was effective in reducing acrylamide formation in whole-wheat bread (Nasiri Esfahani et al., 2017). It was stated that fermentation with lactic acid bacteria (LAB) ability to reduce acrylamide levels is related primarily to pH reduction rather than the consumption of precursor nutrients like asparagine and reducing sugars (Sarion et al., 2021). In our study, breads with fermented red lentils showed higher total titratable acidity (TTA) than those with non-fermented lentils, but acrylamide levels were comparable in these both groups or even elevated in the fermented lentil breads. Although non-milled fermented red lentils contained the lowest levels of free asparagine (Mockus et al., 2023), breads incorporating these lentils did not correspondingly show the lowest acrylamide content. Probably, the variations in other factors as reducing sugars, water activity, and actions of fermenting microorganisms as well as combination of them lead to the indistinct trend in acrylamide formation among breads in the present study. Therefore, the findings of our study underscore the necessity of optimizing wheat bread formulations for each newly incorporated ingredient, given the absence of a consistent pattern in acrylamide content between tested breads. Based on the results, wheat bread can be enriched with red lentils, but it is recommended to use the following lentil additions for optimal quality and safety: 5 % non-milled non-fermented; 5 % fermented non-milled; 5 and 25 % non-fermented milled; and 5 and 20 % fermented milled red lentils.

### 3.4. Bread volatile compound profile

The volatile compound (VOC) profile, expressed as a percentage of the total identified VOC content, is presented in Fig. 5 and in **Supplementary File 2** (**Tables S2–S7**). Alcohols were the dominant VOC class with a high abundance across nearly all bread samples. Among the

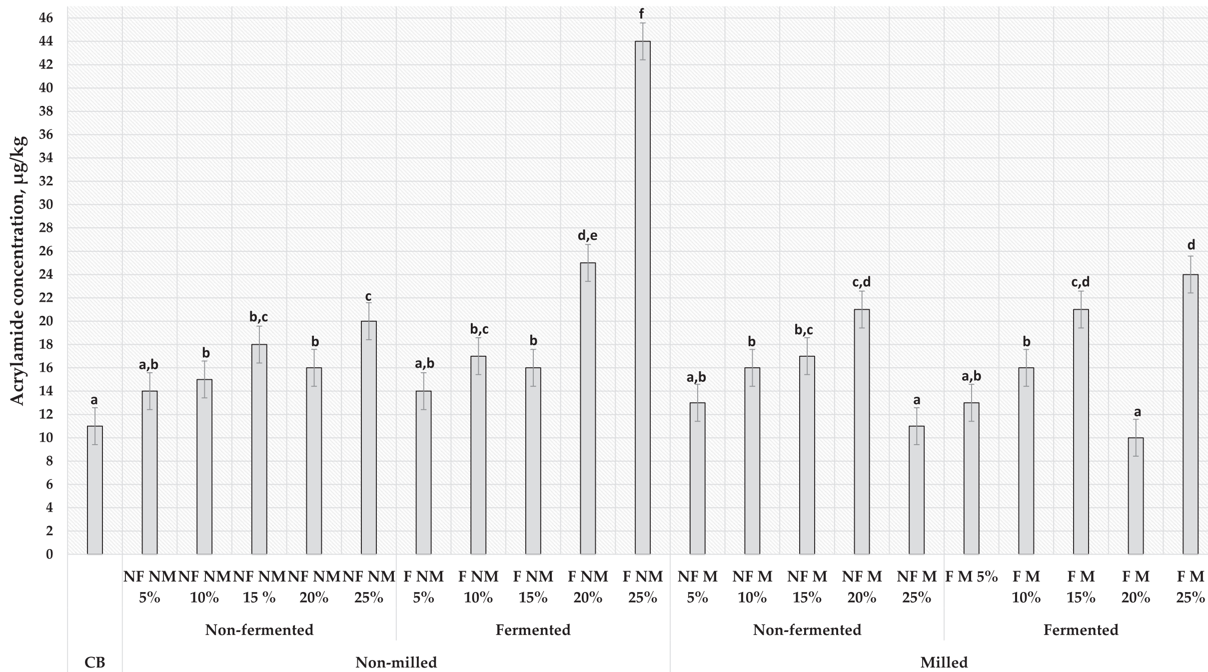



**Fig. 4.** Acrylamide concentration (µg/kg) in bread samples (NF – non-fermented; F – fermented with. *Lactiplantibacillus plantarum* No.122 strain; NM – non-milled; M – milled; 5 %, 10 %,15 %, 20 %, 25 % – quantities of non-fermented non-milled, non-fermented milled, fermented non-milled and fermented milled red lentils in bread formula; Data expressed as mean values ($n$ = 3) ± standard error (SE); a–f Mean values within the columns with different letters are significantly different (p ≤ 0.05). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

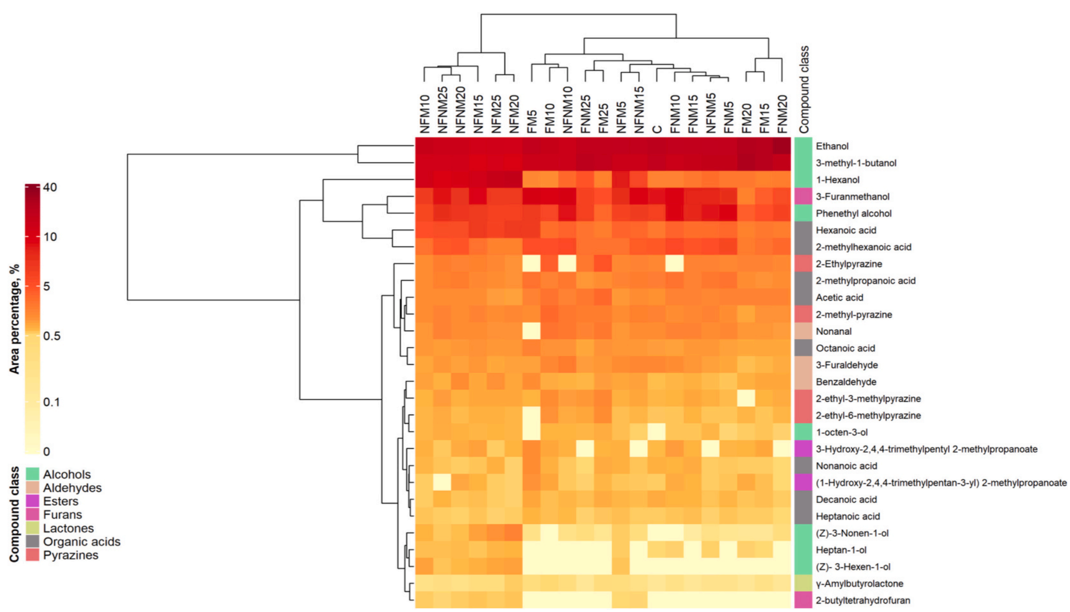



**Fig. 5.** Volatile compound (VOC) profile (% from the total volatile compounds content) in bread samples (NF – non-fermented; F – fermented with *Lactiplantibacillus plantarum* No.122 strain; NM – non-milled; M – milled; 5, 10,15, 20, 25 – quantities of non-fermented non-milled, non-fermented milled, fermented non-milled and fermented milled red lentils in bread formula. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

alcohols identified, ethanol and 3-methyl-1-butanol were the primary VOCs in all bread samples (Fig. 5). Ethanol, a product of fermentation, contributes to strong, ethereal, alcohol, and medicinal odour for bread and is usually common for wheat sourdough bread (Pétel et al., 2017). 3-Methyl-1-butanol, which is derived from leucine/isoleucine via the Ehrlich pathway during fermentation with LAB or yeast, provides malt, sweet, oil, alcohol, whiskey, fruity, banana, and almond like odour to bread (De Luca et al., 2021; Pétel et al., 2017). Also, this VOC is positively correlated with the aroma of wheat bread (Quílez et al., 2006). In our study, fermented milled red lentils had the highest free leucine/isoleucine content, and that could probably correlate with the highest 3-methyl-1-butanol abundance in breads with 15 and 20 % of this lentil addition. Moreover, this compound at low concentration (≤ 10 ppm) is also reported as one of the “beany flavor markers” in lentils, but the odour of it transforms to sweet at higher concentrations (Vurro et al., 2024). The third most abundant alcohol was 1-hexanol, with percentage abundance ranging from 2.1 to 22.1 % of the total VOCs in breads prepared with 10 % fermented milled lentils and 20 % non-fermented milled lentils, respectively. This compound was previously reported in red lentil (Paucean et al., 2018). 1-Hexanol imparts ethereal, oily, alcoholic, woody, green, floral, fruity, sweet, and aroma characteristics and is generated mainly via fermentation processes and/or the oxidation of lipids (De Luca et al., 2021). Phenethyl alcohol percentage abundance ranged from 5.7 to 9.5 % of the total VOCs in breads with 10 % non-fermented milled lentils and 10 % fermented non-milled lentils, respectively. Phenethyl alcohol, with a rose-like aroma, is a metabolite of yeast fermentation and positively impacts the flavour and scent of baked goods (Dueñas-Sánchez et al., 2014). Other alcohols accounted for less than 2 % of the total VOC content.

When analysing other VOC groups of tested samples, 3 aldehydes, 2 esters, 2 furans, and 1 lactone were identified and these compounds (except for 3-furan-methanol) constituted less than 4 % of the total VOC profile. Furans and lactones contribute to sweet–toasty, caramel, creamy, and buttery notes (Pétel et al., 2017; Reineccius, 2005). The percentage abundance of 3-furan-methanol ranged from 2.9 to 12.0 % in breads prepared with 20 and 5 % fermented milled lentils, respectively. 3-furan-methanol, which is generated through Maillard reactions and caramelization (Ozolina et al., 2011), was described as having burnt, warm, mild oily odour notes (Poinot et al., 2008). 8 organic acids were also identified in the VOC profile. Hexanoic acid was the most prevalent and accounted for approximately 7 % of the total VOC content in breads with 15 and 20 % non-fermented milled lentils. Hexanoic acid, derived from LAB fermentation or lipid breakdown, are associated with ethereal and fatty odours, respectively (Pétel et al., 2017). Additionally, 4 pyrazines were identified, all contributing less than 4 % of the total VOC content. It was reported that, the two most abundant pyrazines in wheat bread, 2-methylpyrazine and 2-ethyl-3-methylpyrazine, significantly influence bread flavour (Zhao et al., 2021). 2-methylpyrazine elicit nutty, toasted, cocoa-chocolate, green, odour and as other pyrazines are mainly produced during Maillard reaction in sourdough breads (Pétel et al., 2017).

In our study, most analysed factors and their interactions significantly affected the VOC content in the bread profile (Table 5). Furthermore, significant correlations were observed between acrylamide concentration and certain VOCs (Table 6). However, a more comprehensive interpretation of these correlations requires deeper insight into the formation mechanisms of these compounds in the tested bread types. In this study, weak positive correlations were observed between bread overall acceptability and specific organic acids, including 2-methylpropanoic acid ($r = 0.26$, $p = 0.04$), 2-methylhexanoic acid (r = 0.26, p = 0.04), heptanoic acid ($r = 0.27$, $p = 0.03$), octanoic acid ($r = 0.25$, $p = 0.05$), nonanoic acid (r = 0.25, p = 0.05) and decanoic acid ($p = 0.27$, p = 0.04). These positive correlations suggest that higher concentrations of these compounds are associated with increased bread acceptability. This finding is essential as it indicates that the secondary metabolites produced during fermentation (such as these organic acids and higher alcohols) successfully mask or mitigate the typical undesirable 'beany' or 'grassy' off-flavors often associated with raw pulse ingredients (Pétel et al., 2017). Thus, the fermentation of red lentils enhances consumer acceptability by producing a complex, 'sourdough-like' aroma profile that overcomes the innate sensory limitations of the raw materia. It is known that a wide spectrum of VOCs contributes to bread aroma (De Luca et al., 2021; Pétel et al., 2017; Pico et al., 2015). Within the bread crumb, VOCs are primarily generated through enzymatic reactions during dough kneading and the fermentation of sugars by yeast and lactic acid bacteria (LAB) (Pico et al., 2015). Lipid oxidation also contributes to the formation of aldehydes, ketones and alcohols, while some aldehydes are produced via the Ehrlich pathway during yeast metabolism (Birch et al., 2013). In the crust, VOCs are mainly formed through thermal reactions. In the present study, bread with fermented red lentils appeared to have richer VOC profiles due to higher levels of alcohols, acids and slightly elevated furan and pyrazine formation. This could indicate more complex aroma development due to microbial metabolism. It was reported that fermentation process significantly affects the VOC profile by producing alcohols, aldehydes, acids, esters and ketones (Bianchi et al., 2008; Birch et al., 2013; Cho & Peterson, 2010; Hansen & Schieberle, 2005). Secondary fermentation processes, such as the glycolysis of pyruvic acid, and pathways like the Ehrlich pathway contribute to the formation of higher molecular weight alcohols (Birch et al., 2013; Czerny & Schieberle, 2002).

## 4. Conclusions

The pre-treatment (milling or fermentation) and quantity of incorporated red lentils affected certain quality parameters of wheat bread. Fermentation enhanced wheat bread acidity and enabled greater red lentil substitution without compromising acceptability, yet simultaneously elevated acrylamide concentrations in the majority of formulations. Increasing the red lentil content in the bread formula corresponded with a rise in acrylamide. Interpreting the moderate yet significant correlations between acrylamide concentrations and certain volatile compounds demands a more thorough examination of the complex formation mechanisms operating in the respective bread systems. Based on this study findings, it is recommended to supplement wheat bread with 5 % non-fermented non-milled, fermented non-milled, non-fermented milled or fermented milled red lentils. These formulations allow to produce wheat-lentil bread with high overall acceptability without significantly increasing acrylamide concentration. However, this study has several limitations. Only red lentils and a single L. *plantarum* strain (No. 122) were evaluated, which may restrict generalizability to other lentil varieties or LAB strains. The focus on wheat bread alone leaves the effects on other bread types unclear. Additionally, the laboratory-scale setting and the limited consumer panel may not fully reflect industrial production outcomes or broader population preferences.

## CRediT authorship contribution statement

**Vytaute Starkute:** Writing – original draft, Visualization, Validation, Methodology, Data curation. **Elena Bartkiene:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Project administration, Funding acquisition, Data curation, Conceptualization. **Ernestas Mockus:** Visualization, Validation, Methodology, Investigation, Data curation. **Emilis Radvila:** Investigation, Formal analysis. **Daiva Matuzeviciute:** Investigation, Formal analysis. **Kamile Balynaite:** Investigation, Formal analysis. **Arvydas Bredikis:** Investigation, Formal analysis. **Gabriele Ilgunaite:** Investigation, Formal analysis. **Akvile Juskaite:** Investigation, Formal analysis. **Vaneck Cho:** Investigation, Formal analysis. **João Miguel Rocha:** Writing – review & editing, Investigation. **Darius Cernauskas:** Validation, Methodology, Investigation, Formal analysis. **Romas Ruibys:** Investigation. **Erika Mozuriene:** Validation, Formal analysis. **Meleksen Akin:** Writing – review & editing. **Tanya Curtis:** Writing – review & editing. **Dovile Klupsaite:** Writing – review & editing, Writing – original draft, Validation, Methodology.

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## 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.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.103362.

## Data availability

Data will be made available on request.

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