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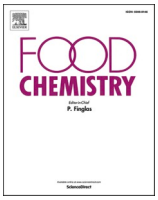

Quality and functional properties of bread containing the addition of probiotically fermented *Cicer arietinum*

Katarzyna Skrzypczak [a], Anna Wirkijowska [b,*], Krzysztof Przygoński [c], Konrad Terpiłowski [d], Agata Blicharz – Kania [e]

[a] *Department of Plant Food Technology and Gastronomy, Sub-department of Fruits, Vegetables and Mushrooms Technology, Faculty of Food Science and Biotechnology University of Life Sciences in Lublin, Skromna 8, 20-704 Lublin, Poland*
[b] *Department of Plant Food Technology and Gastronomy, Department of Engineering and Technology of Grains, Faculty of Food Science and Biotechnology University of Life Sciences in Lublin, Skromna 8, 20-704 Lublin, Poland*
[c] *Prof. Waclaw Dabrowski Institute of Agricultural and Food Biotechnology – State Research Institute, Food Concentrates and Starch Products Department, Starołęcka 40, 61-361 Poznań, Poland*
[d] *Department of Interfacial Phenomena, Maria Curie Skłodowska University, M. Curie Skłodowska Sq. 3, 20-031 Lublin, Poland*
[e] *Department of Biological Bases of Food and Feed Technologies, University of Life Sciences in Lublin, Głęboka 28, 20-612 Lublin, Poland*

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ABSTRACT

This study aimed to determine the impact of supplementation with probiotically fermented chickpea (*Cicer arietinum* L) seeds on the quality parameters and functional characteristics of wheat bread. The addition of chickpea seeds caused significant changes in the chemical composition of the control wheat bread. The legume-supplemented products exhibited higher values of a* and b* color parameters and higher hardness after 24 h of storage than the control. The application of fermented or unfermented chickpeas contributed to an increase in total polyphenol and flavonoid contents, iron chelating capacity, and antioxidant properties of the final product. The variant containing unfermented seeds had the highest riboflavin content (29.53 ± 1.11 μg/100 g d.w.), Trolox equivalent antioxidant capacity (227.02 ± 7.29 μmol·L^{-1} TX/100 g d.w.), and free radical scavenging activity (71.37 ± 1.30 % DPPH inhibition). The results of this preliminary research have practical importance in the production of innovative bakery products with potential properties of functional food.

1. Introduction

The global concerns about environmental and geopolitical changes and the greater awareness of the health effects of plant food consumption are the main factors moderating dietary trends in modern society, hence the growing importance of plant-based proteins and foodstuffs as sustainable alternatives to conventional animal-origin products. According to the latest data (Plant-based food market value worldwide, 2030 – Statista), the global plant-based food market is expected to reach 77.8 billion U.S. dollars in 2025, and an over twofold increase by 2030 is predicted. Additionally, the volume of bread and bakery products on the worldwide market is growing dynamically. The global market for bread and bakery products in 2021 amounted to almost 111.06 million tons and is predicted to achieve 135 million tons by 2025 (Global bread and bakery products market size 2017–2025- Statista). These data not only display the scale of the market's needs and urgent challenges of the food industry but also indicate the potential for development of innovative bakery products that suit consumer demands.

Nowadays some scientific efforts are focused on developing an effective method of incorporation of probiotics into cereal-based baked foods or supplementation of dough with probiotically fermented material before baking to improve the quality of bread. One of the approaches is to apply probiotics during the sourdough bread fermentation process (Akamine et al., 2023) or probiotic spore-forming bacteria (such as *Bacillus coagulans* GBI-30, 6086) exhibiting resistance to very high temperatures prevailing during wheat bread processing (Almada-Érix et al., 2022). As shown by current research, other approaches include microencapsulation of probiotic, formation of edible probiotic-containing films, and addition of probiotics after baking (Mani-López et al., 2023).

Another solution for improving the quality and functional properties of bakery products is to use pulse-derived flours for fortification of

* Corresponding author.
E-mail address: anna.wirkijowska@up.lublin.pl (A. Wirkijowska).

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sourdough (Shrivastava and Chakraborty, 2018; Bojňanskáet al., 2021; Olakanmi et al., 2022). Many current studies analyze the effect of incorporation of fermented raw materials, such as flour or wheat germs, into sourdough (Zhao et al., 2020). The incorporation of legumes (mainly soybeans) or pulse-derived additives is becoming increasingly popular in the breadmaking industry as well. Nevertheless, there are noticeable concerns and reluctance to consume soy products (e.g. due to food intolerance or allergy, concerns about genetically modified seeds) in many societies. Therefore, more extensive use of *Cicer arietinum* L. (chickpea, garbanzo beans), characterized by high protein content and exhibiting beneficial health effects (Gupta et al., 2017), may be an appropriate solution for food production. To the best of our knowledge, bakery products containing whole chickpeas (especially bread supplemented with probiotically fermented *Cicer arietinum* L.) are not popular on the food market, especially in most European countries. The use of such an innovative additive in breadmaking may expand the range of new bakery products with improved quality and desired nutritional and health-promoting properties. However, the technological suitability of chickpeas in breadmaking and the features of bread fortified with this plant-based additive have not yet been fully described so far. Also, the possibility of control fermentation pretreatment of chickpeas to improve the potential functional properties of bakery products still has to be explored.

Current research mainly focuses on fortification of bakery products with chickpeas (and other legumes) in the form of flour. The application of fermented whole chickpea seeds may be a novel technological approach contributing to development of an innovative functional and low-cost ingredient suitable for breadmaking. Therefore, the research was undertaken to verify the hypothesis that the control fermentation with probiotic bacterial strain can be used as a pretreatment of *Cicer arietinum* L seeds to improve the nutritional value, functional properties, and quality of wheat bread. The objective of this investigation was to examine the effect of the addition of chickpea seeds fermented by different probiotic bacterial strains on the physicochemical properties and quality of wheat bread. The study also aimed at preliminary assessment of specific functional attributes and the technological applicability of fermented chickpeas in innovative bakery products rich in bioactive compounds.

2. Material and methods

2.1. Chemicals, reagents and materials

The following chemicals and reagents (of analytical grade and analytically pure) were purchased from POCH S.A. (Gliwice, Poland): sodium nitrite, sodium hydroxide, sodium phosphate dibasic heptahydrate, sodium phosphate monobasic monohydrate, sodium chloride, sodium carbonate, aluminum chloride, iron(II) chloride tetrahydrate, and Folin-Ciocalteu's reagent. HPLC-grade methanol and methanol, riboflavin (European Pharmacopoeia Reference Standard), 1,1-diphenyl-2-picrylhydrazyl, potassium peroxodisulfate, 2,2′-Azinobis-3-Ethylbenzthiazolin-6-Sulfonic acid, and ferrozine solution were obtained from Merck KGaA (Darmstadt, Germany). Gallic acid, quercetin, and Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman- 2-carboxylic acid) standards were purchased from Sigma–Aldrich (St. Louis, MO, USA). De Man, Rogosa, and Sharpe (MRS) broth was obtained from Biocorp (Warsaw, Poland).

All solutions were prepared using purified Milli-Q water (resistivity not less than 18 MΩ/cm) generated by a Milli-Q Plus Water Purification System (Millipore, Bedford, MA, USA).

For bread preparation, wheat flour was purchased from Niedźwiady Mill (Poland) (ash 0.74 % d.w., falling number 248 s, wet gluten content 25 %; the chemical composition is presented in Table 1). Organic (PL-EKO-07) chickpea seeds (*Cicer arietinum* L) were purchased from a local retail organic food store (the chemical composition is presented in Table 1).

2.2. Preparation of inoculums for probiotic fermentation

Two probiotic strains: *Lactobacillus plantarum* 299v (Sanprobi IBS, Sanum Poland) and *Bifidobacterium animalis* subsp. *lactis* BB12 (Chr. Hansen, Poland) were used as starter cultures. Two inoculum variants (each containing only one probiotic strain) were prepared according to a method described previously (Skrzypczak et al., 2021) with modifications. In brief, sterile mediums (MRS broth) were inoculated (2 % v/v) with one of the overnight bacterial cultures and incubated at 37 °C for 18 h. Thereafter, bacterial biomass was harvested by centrifugation (8000 x g/4 °C/15 min). The pellets were washed three times with a sterile NaCl solution (0.85 %) and resuspended (in saline) to half of their initial volumes. Then, two final inoculum variants (in sterile saline solutions) with an equal density level of $OD_{600} = 0.8$ were prepared.

2.3. Preparation of legume-derived raw material for fermentation

The preparation of chickpeas for probiotic fermentation was based on the description of solid-state fermentation presented by Xiao et al. (2015) with some modifications. Briefly, after rinsing in a sieve under running lukewarm tap water and thorough shaking to remove residual

Table 1
Chemical composition of raw materials and bread variants.

Analyzed variant*	Protein[% d.w]	Fat[% d.w.]	CHO[% d.w.]	TDF[% d.w.]	SDF[% d.w.]	IDF[% d.w.]	Ash[% d.w.]	Energy [kcal/100 g]
WF	13.16 ± 0.07^{a}	0.49 ± 0.00^{a}	80.27 ± 0.09^{e}	5.36 ± 0.04^{a}	2.93 ± 0.22^{a}	2.43 ± 0.18^{a}	0.71 ± 0.02^{a}	388.9 ± 0.0^{c}
NFCP	21.08 ± 0.43^{b}	5.83 ± 0.15^{c}	40.64 ± 0.71^{c}	29.39 ± 0.08^{d}	6.00 ± 0.24^{c}	23.38 ± 0.16^{d}	3.07 ± 0.06^{b}	358.1 ± 0.3^{a}
299 V/FCP24	24.28 ± 0.13^{c}	5.39 ± 0.14^{b}	38.98 ± 0.02^{b}	28.10 ± 0.23 ^{cd}	5.16 ± 0.08^{bc}	22.94 ± 0.31^{d}	3.25 ± 0.06^{b}	357.6 ± 1.4^{a}
299 V/FCP48	26.85 ± 0.30^{d}	5.26 ± 0.03^{b}	37.04 ± 0.29^{a}	27.75 ± 0.00^{c}	4.95 ± 0.17^{b}	22.80 ± 0.17^{d}	3.10 ± 0.05^{b}	358.4 ± 0.3^{a}
BB12/FCP24	23.66 ± 0.39^{c}	5.37 ± 0.08^{b}	40.93 ± 0.31^{c}	26.92 ± 0.77^{c}	5.68 ± 0.40^{bc}	21.24 ± 0.37^{c}	3.11 ± 0.01^{b}	360.6 ± 2.0^{ab}
BB12/FCP48	24.16 ± 0.35^{c}	5.19 ± 0.02^{b}	42.78 ± 0.10^{d}	24.65 ± 0.30^{b}	5.43 ± 0.03^{bc}	19.22 ± 0.28^{b}	3.21 ± 0.07^{b}	363.8 ± 0.2^{b}
299 V/24	15.30 ± 0.09^{b}	1.42 ± 0.20^{c}	66.83 ± 0.73^{a}	14.00 ± 0.73^{b}	4.36 ± 0.50^{a}	9.64 ± 0.22^{c}	2.46 ± 0.15^{a}	183.6 ± 1.7^{a}
299 V/48	15.36 ± 0.09^{b}	1.47 ± 0.02^{c}	66.93 ± 0.77^{a}	13.76 ± 0.84^{b}	4.20 ± 0.70^{a}	9.56 ± 0.14^{c}	2.50 ± 0.09^{a}	182.4 ± 2.5^{a}
BB12/24	15.26 ± 0.07^{b}	1.51 ± 0.03^{c}	67.07 ± 0.47^{a}	14.13 ± 0.60^{b}	4.72 ± 0.47^{a}	9.41 ± 0.12^{c}	2.45 ± 0.02^{a}	183.6 ± 0.0^{a}
BB12/48	15.22 ± 0.02^{b}	1.12 ± 0.01^{b}	67.52 ± 0.01^{a}	13.23 ± 0.02^{b}	4.66 ± 0.01^{a}	8.57 ± 0.01^{b}	2.56 ± 0.02^{a}	181.4 ± 0.8^{a}
NF	15.19 ± 0.06^{b}	1.67 ± 0.03^{d}	66.25 ± 0.12^{a}	14.54 ± 0.15^{b}	4.81 ± 0.32^{a}	9.73 ± 0.18^{c}	2.43 ± 0.15^{a}	182.7 ± 0.4^{a}
CON	13.38 ± 0.12^{a}	0.34 ± 0.02^{a}	77.03 ± 0.26^{b}	6.77 ± 0.06^{a}	3.64 ± 0.14^{a}	3.14 ± 0.08^{a}	2.48 ± 0.03^{a}	216.4 ± 0.7^{b}

Explanatory notes: *WF- wheat flour; NFCP- non-fermented chickpeas; 299 V/FCP24(48)– chickpeas fermented with *Lactobacillus plantarum* 299v for 24 h (or for 48 h); BB12/FCP24(48) - chickpeas fermented with *Bifidobacterium animalis* subsp. *lactis* BB12 for 24 (or for 48 h); CON– control wheat bread; NF- bread with unfermented chickpea addition; 299 V/24(48)– bread with the addition of chickpeas fermented with *Lactobacillus plantarum* 299v per 24 or 48 h; BB12/24(48) – bread with the addition of chickpeas fermented with *Bifidobacterium animalis* subsp. *lactis* BB12 for 24 h (or 48 h); CHO– available carbohydrate; TDF- Total dietary fiber; SDF- Soluble dietary fiber; IDF- Insoluble dietary fiber; the energy value for the raw materials is shown in 100 g of d.w. and for bread in 100 g of fresh bread. Values are expressed as the mean (n = 3) ± standard deviation. The mean values in the same column with different letters are significantly different ($p < 0.05$).

water, the seeds (225 g) were transferred into clean conical glass flasks and covered with 900 g of distilled water. Subsequently, the samples were sealed, sterilized (121 °C/15 min), cooled to ambient temperature, inoculated (2 % v/w) with one of the probiotic inoculums, and incubated at 37 °C for 24 h and 48 h. The following sample designations were introduced: 299 V/FCP24 and 299 V/FCP48 (chickpea fermented by *L. plantarum* 299v for 24 h and 48 h, respectively); BB12/FCP24 and BB12/FCP48 (material fermented by *B. animalis* subsp. *lactis* BB12 for 24 h and 48 h, respectively). Uninoculated chickpea samples were used as unfermented variants (NFCP).

The pH value was determined in the fermented material after 0 h, 24 h, and 48 h of incubation (Supplementary material Table 1) using the Hanna Instruments HI 221 pH meter (Hanna Instruments, Poland). The measurements were performed in sterile conditions.

2.4. Breadmaking process

The blend used for the production of the control variant (CON) of dough consisted of wheat flour (600 g), compressed yeast *Saccharomyces cerevisiae* (18 g), and NaCl (9 g). In the legume-supplemented bread variants, wheat flour was replaced by fermented chickpea (37 %; the amount was optimized in pilot studies).

The volume of added water and the mixing time (WA 55.0 %; DDT 3.9 min; stability time 13.7 min; MTI 33 FU; elasticity 35 FU) were determined using a Farinograph-E (Brabender, model 8110142, Duisburg, Germany) according to the AACC method 54–21 (AACC, 2010).

All the dough variants were prepared using the single-stage method with punching as described by Wirkijowska et al. (2020). The dough ingredients were mixed in a BEAR Varimixer Teddy 5 L (Verimixer A/S, Copenhagen, Denmark) at a low speed for 3 min and then at a high speed for 7 min to achieve full gluten network development. The dough was then fermented in a proofing chamber (Tefi Klima pro 100, Debag, Germany) at 30 °C and 85 ± 2 % RH for 90 min. An intermediate punching step with mixing (at a low speed for 30 s) was performed after 60 min of dough fermentation. After this step, the dough was manually divided into equal portions (290 ± 5 g), molded, and placed in baking pans sized 18 × 7.5 × 7.0 cm. The dough was allowed to rest for 30 min in the proofing chamber at 30 °C and 85 ± 2 % RH. Subsequently, the fermented dough samples were baked in a bakery oven Helios pro 100 (Debag, Germany) at 230 °C for 30 min.

The pH values of raw dough (Supplementary material Table 1) were determined immediately before placing the samples in the oven using a Hanna Instruments HI 221 pH meter (Hanna Instruments, Poland).

Three loaves of each formula were baked in two separate bakes, yielding a total of six loaves of each bread variant. After baking, the loaves were left to cool at room temperature for 1 h, placed separately in polyethylene bags, and stored in room conditions (20 °C, 50 % RH) before quality evaluation.

2.5. Chemical composition and bioactive properties of bread variants

The contents of moisture, protein, fat, ash, and dietary fiber were determined in the final products and the main raw materials with standard methods (AACC, 2000; AOAC, 2016). The protein content was determined using a KjeltecTM8400 device (Foss Analytical AB, 8400, Höganäs, Sweden) with the ASN 3100 application (conversion factor 5.7). The fat content was analyzed with the continuous ether extraction method using a SoxtecTM8000 device (Foss Analytical AB, 8000, Höganäs, Sweden) with the AN 310 application.

The dietary fiber content (IDF - water-insoluble fraction of dietary fiber and SDF- water-soluble fraction) was assayed with the enzymatic method using an enzyme kit and procedures from Megazyme (K-TDFR-200A, Bray, Ireland). The total dietary fiber (TDF) was calculated as the sum of fractions IDF and SDF (AACC, 2000; AOAC, 2016). The contents of all chemical components were determined in three repetitions.

The energy value was determined in kilocalories (kcal) per 100 g of wet bread using Atwater factors (4 kcal/g - protein and carbohydrate, 9 kcal/g - fat, and 2 kcal/g - total dietary fiber.

After 24 h of baking, the riboflavin content was determined in the bread variants according to EN 14152 (Foodstuffs - Determination of vitamin B2 by high-performance liquid chromatography, 2014). High-performance liquid chromatography (HPLC) was used with a DIONEX chromatograph combined with a P680 pump (DIONEX, CA, USA), an RF-2000 fluorescence detector (DIONEX, CA, USA), an ASI-100 autosampler (DIONEX, CA, USA), and a TC-100 thermostat (DIONEX, CA, USA). A Supelco Discovery HS C18 column (100 x 4.6 mm, 5 μm) was used for chromatographic separation. 20 μL of each sample was dispensed onto the column. The mobile phase consisted of methanol and ultrapure water (6:4, v/v) with isocratic elution at a flow rate of 1 mL/min. The detection was performed at EX excitation: 450 nm and EM emission: 520 nm. The analysis was performed in six repetitions (n = 6). The method was validated in accordance with the guidelines of the International Conference on Harmonization (ICH) and European Pharmacopoeia, taking into account the specific characteristics of the vitamins analyzed.

For further biochemical analysis, extracts of all the bread variants were prepared as in Shori et al. (2021) with some modifications. Briefly, the samples (approx. 2 cm thick slices) were dried to constant weight at ambient temperature. Next, they were ground and sieved (through a sieve with Ø =2 mm). The extracts were prepared using 80 % aqueous methanol (in a ratio of 1:10). After mixing for 1 h (500 rpm / 25 °C) in Multi-Speed Vortex MSV- 3500 BioSan (BioSan, Lithuania) and shaking (200 rpm /37 °C/ 60 min) in Incu-Shaker Mini (Benchmark Scientific, USA), the samples were centrifuged for 20 min at 16.000 × g (MPV-350R, Warsaw, Poland). Clear supernatants were subjected to the analysis of total phenolic content (TPC), total flavonoid content (TFC), free radical scavenging activity (FRSA), and Trolox equivalent antioxidant capacity (TEAC). In the ferrous ion-chelating activity (FICA) assay, water was used as a solvent instead of 80 % aqueous methanol.

2.5.1. Determination of total polyphenol content (TPC)

The analysis was performed as in Dranca et al. (2020) with some modifications. In short, 1.9 mL of distilled water and 100 μL of Folin-Ciocalteu's reagent were added to 100 μL of each bread extract. The samples were thoroughly mixed and, after 2 min of incubation at ambient temperature, 800 μL of 5 % Na_2CO_3 was added. After mixing, the samples were transferred into a water bath and incubated for 20 min at 45 °C. After cooling to ambient temperature, they were incubated in darkness for 30 min (at ambient temperature). Absorbance was measured at λ = 765 nm (Spectrophotometer UV–Vis Helios Gamma Thermo, USA). The TPC was calculated from the standard curve prepared for gallic acid. The results were expressed as mg of gallic acid equivalents per 100 g of dry weight [mg GAE/100 g d.w.]. The analysis was performed in six repetitions (n = 6).

2.5.2. Determination of total flavonoid content (TFC)

The analysis was conducted as in Boeriu et al. (2020) with some modifications. The following reagents were added to 400 μL of the tested extracts: 40 μL of 5 % $NaNO_2$, 40 μL of 10 % $AlCl_3$, 400 μL of 4 % NaOH, and 120 μL of an 80 % aqueous solution of methanol. The mixtures were thoroughly mixed (Multi-Speed Vortex MSV- 3500 BioSan, Latvia) and incubated in darkness at room temperature for 15 min. Absorbance was measured at λ = 430 nm (Spectrophotometer UV–Vis Helios Gamma Thermo, USA). Quercetin was used to prepare a standard curve. The results were expressed as mg of quercetin equivalents per 100 g of dry weight [mg QE/100 g d.w.]. The analysis was performed in six repetitions (n = 6).

2.5.3. Determination of free radical scavenging activity (FRSA)

The analysis was performed as in Meral and Köse (2019) with slight modifications. In brief, 3 mL of a freshly prepared solution (4 mg/L) of $DPPH^{\bullet}$ in ethanol was added to 1 mL of each sample extract. The

mixtures were vortexed (Multi-Speed Vortex MSV- 3500 BioSan, Latvia) and incubated in darkness for 30 min at room temperature. Absorbance was measured at λ = 517 nm (Spectrophotometer UV–Vis Helios Gamma Thermo, USA) against the control sample (containing ethanol instead of the extract). FRSA, expressed as % of the $DPPH^{\cdot}$ inhibition, was calculated using Equation (1):

$$DPPH^{\cdot}\ Inhibition[\%] = \left[100x\left(A_{control} - A_{sample}\right)\right]/A_{control}, \quad (1)$$

where $A_{control}$ is the absorbance of the control sample, and A_{sample} is the absorbance of the extract-containing sample. The analysis was performed in six repetitions (n = 6).

2.5.4. Determination of Trolox equivalent antioxidant capacity (TEAC)

The analysis was performed as in Meral and Sait Dogan (2013) with slight modifications. The reagent (2,2′-Azinobis-3-Ethylbenzthiazolin-6-Sulfonic Acid) was prepared by incubating a 7 mM ABTS solution with a 2.45 mM solution of $K_2S_2O_8$ for 16 h in darkness at room temperature. Then, the $ABTS^{+}$ solution was diluted with potassium phosphate-saline buffer (pH 7.4) to obtain 0.7 ± 0.001 absorbance (at λ = 734 nm). Afterward, 50 μL of the extract was mixed with 3 mL of the $ABTS^{+}$ solution, mixed vigorously (Multi-Speed Vortex MSV- 3500 BioSan, Latvia), and incubated in darkness at room temperature for 15 min. Absorbance was measured at 734 nm (Spectrophotometer UV–Vis Helios Gamma Thermo, USA) against a blank sample.

The results were calculated based on a Trolox calibration curve and expressed as $\mu mol \cdot L^{-1}$ of Trolox equivalent per 100 g of dry weight [$\mu mol \cdot L^{-1}$ TX /100 g d.w.]. The analysis was performed in six repetitions (n = 6).

2.5.5. Ferrous ion-chelating activity (FICA) assay

The Fe^{2+} chelating activity was analyzed as in Shori et al. (2021) with modifications. Each water bread extract (1 mL) was diluted with 2.7 mL of distilled water. Then, 0.1 mL of a 2 mmol/L $FeCl_2$ solution was added to each sample and, after 3 min of incubation at room temperature, the reaction was stopped by the addition of 0.2 mL of a 5 mmol/L ferrozine solution. After thorough mixing, the samples were incubated for 15 min in darkness at ambient temperature. The absorbance was measured at λ = 562 nm (Spectrophotometer UV–Vis Helios Gamma Thermo, USA) against a blank sample (containing deionized water instead of the bread extract). The activity was calculated using the following equation:

$$FICA[\%] = [(A_0 - A_t) \times 100]/A_0 \quad (2)$$

where A_0 is the absorbance of the blank, and A_t is the absorbance of the tested sample (containing the analyzed bread extract variant). The analysis was performed in six repetitions (n = 6).

2.6. Evaluation of bread quality characteristics

The quality of bread (yield, total baking loss (TBL), specific volume, and crumb moisture) was analyzed within 4 h after cooling. The bread yield and total baking loss were determined as described previously (Wirkijowska et al., 2020). The specific volume (cm^3/g) of the bread was calculated by dividing its volume by weight, and the crumb moisture was analyzed according to the standard AACC 2000 method (Method 44–15.02).

After 24 h of baking, 1 cm thick slices of the bread variants were analyzed in a 3Color K9000Neo spectrophotometer (3Color, Narama, Poland) with a standard light source (D65), a standard visual field (10°), and a 12.3-mm diameter hole. Their color characteristics were determined (using the CIE L*a*b* system) by analyzing the following parameters: L* - brightness, a* - transition from green (-a) to red (+a), and b* - changes in the range from blue (-b) to yellow (b). Also, the surface of all the baked products (in 0.3 cm thick slices) was observed using a metallurgical optical microscope MA200 (Nikon, Japan).

2.7. Texture profile analysis (TPA) of bread variants

The textural properties of the final products were determined after 24 h and 72 h of baking. The breads were stored in plastic bags at room temperature. The samples were prepared and analyzed as in Wirkijowska et al. (2020). Using the testXpert II software, the following properties were calculated: hardness [N] (maximum force during the first compression), elasticity [-] (strain quotient during the second and first compression cycle), cohesiveness [-] (quotient of the surface area under the force–time curve in the second and first compression cycle), and chewiness [N] (product of hardness, elasticity, and cohesiveness) (n = 8).

2.8. Statistical analysis

The statistical analysis was performed in the STATISTICA 13.1 program (StatSoft, Inc., USA) applying the Tukey HSD test in the analysis of variance (ANOVA). The significance of the differences between the mean values of the tested parameters was verified by the Tukey test with a significance level of $p < 0.05$.

3. Results and discussion

3.1. Chemical composition assay and analysis of bioactive properties

The trend in fortification of bakery products has emerged in response to the current demand for food with increased dietary fiber and protein content (Waters et al., 2012). In the present study, the introduction of each variant of leguminous raw materials contributed to significantly higher contents of protein, fat, TDF (and its fractions), and ash but lower concentrations of CHO compared to wheat flour (Table 1). Moreover, the fermented additives were characterized by significantly lower fat levels and higher protein contents than those in NFCP. Apart from 299 V/FCP48, the fermented additives did not cause significant differences in the SDF level, compared with NFCP. In terms of the TDF values, variants 299 V/FCP48, BB12/FCP24, and BB12/FCP48 exhibited statistically significant differences from NFCP (Table 1). In the case of IDF, only BB12/FCP24 and BB12/FCP48 differed from NFCP, i.e. the fermented chickpea variants had lower contents of the fraction.

The legume-derived additive modified the chemical composition of the bread. In comparison to CON, the content of protein, fat, TDF, and IDF increased in all the chickpea-supplemented products (Table 1), which may be associated with the hydrolysis of complex plant proteins into amino acids and short-chain peptides by extracellular proteases, resulting in an overall increase in the nitrogen content (Enujiugha et al., 2003).

The legume-based additive did not influence significantly the SDF and ash concentrations in the bread. However, the chickpea-fortified products did not differ from each other (but differed from CON) in their protein, CHO, and TDF contents as well as the energy value (Table 1). Interestingly, the final products containing seeds fermented for 48 h (regardless of the strain used) showed lower values of TDF, IDF, and SDF than analogous products containing chickpeas fermented for 24 h. These results are comparable to the findings reported by Lambo et al. (2005), who analyzed changes in the content of fat and dietary fiber in barley and oat fiber concentrate induced by the activity of *Lactobacillus* strains. The lower TDF and IDF levels in 299 V/48 and BB12/48 (compared to 299 V/24 and BB12/24, respectively, and the NF) were probably caused by the hydrolysis of dietary fiber polysaccharides in chickpeas induced by microbial enzymes, which was more effective after the longer time of fermentation. This assumption is consistent with the results of the chemical composition of raw material, i.e. the TDF and IDF values in 299 V/FCP24, 299 V/FCP48, BB12/FCP24, and BB12/FCP48 (Table 1).

All the chickpea-supplemented breads exhibited higher values of TPC than CON (Table 2). BB12/48 had the highest concentration of

Table 2
Comparison of the bioactive properties of bread variants.

Product variant*	TPC [mg GAE /100 g d.w.]	TFC [mg QE/ 100 g d. w.]	FRSA [DPPH• inhibition %]	TEAC [μmol·L^{-1} TX/ 100 g d. w.]	FICA activity [%]	Vitamin B_2 [μg/100 g d.w.]
299 V/ 24	29.88 ± 1.53^{a}	114.38 ± 3.54^{d}	63.25 ± 0.64^{b}	193.47 ± 3.68^{b}	17.77 ± 1.32^{c}	63.14 ± 1.05^{b}
299 V/ 48	35.46 ± 1.97^{b}	83.78 ± 1.80^{bc}	69.91 ± 2.15^{c}	209.86 ± 1.72^{c}	13.22 ± 0.87^{b}	74.70 ± 1.32^{c}
BB12/ 24	37.62 ± 3.25^{b}	78.86 ± 2.34^{ab}	61.96 ± 0.84^{b}	190.27 ± 5.89^{b}	21.20 ± 0.63^{d}	71.42 ± 1.78^{c}
BB12/ 48	46.24 ± 6.45^{c}	86.96 ± 2.45^{c}	71.00 ± 0.62^{c}	194.68 ± 2.28^{b}	26.47 ± 0.73^{f}	62.88 ± 1.37^{b}
NF	36.57 ± 2.51^{b}	80.15 ± 4.48^{b}	71.37 ± 1.30^{c}	227.02 ± 7.29^{d}	23.42 ± 0.32^{e}	79.79 ± 2.56^{d}
CON	29.04 ± 1.89^{a}	73.69 ± 2.69^{a}	53.68 ± 1.80^{a}	181.94 ± 1.31^{a}	5.33 ± 1.42^{a}	51.53 ± 1.93^{a}

Explanatory notes: *CON– control wheat bread; NF- bread with unfermented chickpea addition; 299 V/24(48)– bread with the addition of chickpeas fermented with *Lactobacillus plantarum* 299v for 24 or (48 h); BB12/24(48) – bread with the addition of chickpeas fermented with *Bifidobacterium animalis* subsp. *lactis* BB12 for 24 (or 48 h); TPC- total polyphenol content; TFC- total flavonoid content; FRSA- free radical scavenging activity TEAC- Trolox equivalent antioxidant capacity; FICA- ferrous-ion chelating activity. The values are expressed as the mean (n = 6) ± standard deviation. The mean values in the same column with different letters are significantly different ($p < 0.05$).

polyphenols (46.24 ± 6.45 mg GAE /100 g d.w.). Furthermore, the products containing seeds fermented for 48 h (regardless of the bacterial strain used) exhibited higher TPC values than the variants with chickpeas fermented for 24 h. This indicates a pronounced influence of the metabolic activity of bacteria on changes in TPC during the fermentation of chickpeas. The results correspond to those reported by Magro et al. (2019), who indicated an increase in the content of phenolic compounds in lentils during the fermentation process. Similarly, Bautista-Expósito et al. (2018) additionally reported changes in the peptide and polyphenol composition in lentils fermented by *L. plantarum*. Microbial enzymes may also enhance the release of conjugated phenols, improve bioavailability, and increase the content of biologically active compounds from fermented raw material (Magro et al., 2019). Moreover, Bautista-Expósito et al. (2018) reported the ability of different strains of *L. plantarum* to synthesize various enzymes (β-glucosidases, carbohydrolases, and various esterases) involved in the release of bound phenols into plant cell matrices. This supports our assumption that the increased TPC in the chickpea-supplemented bread may be attributed to the enzymatic specificity and activity of the bacterial strains involved in the fermentation process.

The results of the present study suggest that *Bifidobacterium animalis* subsp. *lactis* BB12 had a greater impact on changes in the polyphenol content during chickpea fermentation (in comparison to *L. plantarum* 299v), which was reflected in the higher TPC values in variants BB12/24 and BB12/48 (Table 2). However, an additional effect of the yeast activity on changes in the polyphenol content during the fermentation of dough containing fermented seeds cannot be excluded either. Moreover, it has been suggested that the generation of ethanol (occurring in the breadmaking process during fermentation) may also facilitate the extraction of phenolic components, contributing to the higher antioxidant activity of the final product (Jayaram et al., 2014).

All the chickpea-supplemented products had higher flavonoid content than the control bread, but variant BB12/24 did not differ statistically significantly from CON (Table 2). The highest TFC (114.38 ± 3.54 mg QE/100 g d.w.) was noted in variant 299 V/24. The increase in the TFC value may have been caused by the enhancement of flavonoid release from plant raw material at the decreasing level of pH during the fermentation process (Haile and Kang, 2019).

The present results correspond with the findings reported by Gomaa et al. (2021), who analyzed the effect of probiotic fermentation of legumes (konjac and carob pods) on TFC. They found significantly higher concentrations of flavonoids in fermented legume-derived materials than in unfermented ones. The results of their study also suggest that the flavonoid content in fermented plant-based material not only is modified by the duration of fermentation but also depends on the starter culture. Our findings are consistent with those presented by Bouhlal et al. (2019), who noted that the addition of lentil flour to wheat flour significantly increased TPC and TFC and enhanced the DPPH scavenging capacity of bread (which also corresponds to our observations). Moreover, legume-derived additives were shown to be a powerful ingredient enhancing the antioxidative potential of wheat flour and bakery products. This was also supported by our TEAC and FRSA analyses (Table 2). All the chickpea-fortified baked products exhibited stronger antioxidant properties than CON. The highest values of TEAC (227.02 ± 7.29 μmol·L^{-1} Trolox/100 g d.w.) were noted in NF. Noteworthy, variants 299 V/48 and BB12/48 had higher values of this parameter than the analogous products supplemented with chickpeas fermented for 24 h. The FRSA analysis revealed a similar trend (Table 2). The highest values of antioxidative activity were noted in NF (71.37 ± 1.30 DPPH• inhibition %), followed by variants BB12/48 and 299 V/48 (71.00 ± 0.62 DPPH• inhibition % and 69.91 ± 2.15 DPPH• inhibition %, respectively). However, the differences in this parameter between these three variants of bread were not statistically significant ($p > 0.05$). As indicated by these results, the addition of even unfermented chickpeas enhances the antioxidant properties of wheat bread.

The concentration of phenolic compounds and the free radical scavenging properties of bakery products may also be affected by the concentration of glutathione. The component is present in the yeast structure and can be released from cells (during dough fermentation), leading to increased antioxidant activity (Wei et al., 2003). Also, reducing sugars generated during the fermentation and Maillard reaction products (MRPs) formed during the baking process increase the concentration of Folin-Ciocalteau's reagent reducers. This may lead to overestimation of the real content of polyphenols.

Troadec et al. (2023) indicated that fermentation conditions strongly moderated the content of reducing sugars. In the production of yeast bread, they noticed that fermentation enhanced the formation of aromatic components from yeast metabolism in the dough but inhibited the generation of these compounds from the Maillard reaction in the crust. In the case of sourdough bread, fermentation promoted the Maillard reaction, and metabolites of lactic acid bacteria had a positive effect on the sensory characteristics of both the crust and the crumb. Therefore, it is important to undertake additional research to elucidate unequivocally the effect of introducing chickpea seeds fermented by probiotic bacterial strains on the formation of MRPs in wheat bread.

Furthermore, MRPs (especially melanoidins) exhibit antioxidant properties associated with the presence of amino acids, such as glycine and lysine, characterized by high free-radical scavenging activity (Kitts, 2021). They may have influenced the results obtained in the antioxidative capacity assay.

Variant BB12/48 not only had the highest TPC value but also exhibited the highest value (26.47 ± 0.73 %) of ferrous ion-chelating activity (Table 2). This activity was higher in all the chickpea-supplemented products than in the CON variant. The present results correspond to the findings reported by Li et al. (2020), who observed that soybean fermentation significantly increased this parameter. Moreover, the authors indicated that whole soybean flour fermentation with *Lactobacillus casei* increased the riboflavin content almost three times, compared to the control. Riboflavin plays a key role in the proper functioning of the nervous, immune, and visual systems (preventing

cataracts). Although this bioactive component can be synthesized in the human gastrointestinal tract by intestinal microflora, the amounts produced are insufficient to meet the body's total requirements (Suwannasom et al., 2020). The deficiency of this component causes many dysfunctions of body systems that may lead to various disorders and diseases. Hence, one of the preventive and therapeutic strategies for riboflavin deficiency is supplementation and fortification of foods with this vitamin. Furthermore, it has been demonstrated that foodstuffs with elevated levels of B-group vitamins may eliminate the need for chemical fortification with vitamin B_2 (LeBlanc et al., 2017). The riboflavin content in chickpeas (raw material) may reach 173.33 μg/100 g (Kumari, 2023), but food processing causes substantial losses of this component (e.g. cooking leads to a 48.5 % lower amount of this vitamin).

Among all the tested bread variants, products with the addition of unfermented chickpea seeds had the highest concentrations of riboflavin (79.79 ± 2.56 μg/100 g d.w.) (Table 2). All the products containing fermented seeds were characterized by higher levels of vitamin B_2 than CON. Interestingly, products containing chickpea seeds fermented for 48 h by *L. plantarum* 299v exhibited a higher level of vitamin B_2 than variant 299 V/48. In the bread supplemented with chickpeas fermented by *Bifidobacterium animalis* subsp. *lactis* BB12, variant BB12/24 had a higher riboflavin level than BB12/48. These differences may be associated with the different metabolic activities (enzyme specificity and activity), growth rates, and nutritional requirements of the chickpea-fermenting strains. The vitamin concentration in fermented plant-based food depends not only on the conditions of the process but also on the metabolic activity of microorganisms involved in the fermentation process (Adebo et al., 2022). Therefore, the lower riboflavin content in BB12/48 may have been an effect of vitamin utilization by *B. animalis* subsp. *lactis* BB12 during the seed fermentation process (Ejigui et al., 2005). The present results are also comparable with the findings presented by Thompson et al. (2020), who found a significant increase in the riboflavin content (from 42.83 ± 1.2 μg/100 g fresh weight to 91.6 ± 0.6 μg/100 g fresh weight) in a mixture of cauliflower and white bean after fermentation by *L. plantarum* Lp900. In addition, the authors observed differences in the vitamin content depending on the strain used. The product obtained with the use of *L. plantarum* 299v exhibited the lowest level of vitamin B_2 (75.64 ± 0.82 μg/100 g fresh weight). This confirms our assumption that the different contents of vitamin B_2 observed in the variants of bread containing fermented chickpeas may be the result of the application of the different probiotic strains. The results suggest that using chickpeas as an additive to bread is an efficient and relatively cheap method for increasing not only the protein content but also the riboflavin concentration.

These findings (Tables 1 and 2) indicate that probiotically fermented chickpeas may be a high-potential approach to improve not only nutritional but also functional values of bakery products.

3.2. Evaluation of bread quality characteristics

The yield, specific volume, and crumb moisture are important determinants of bread quality and the economic viability of production. Moreover, the bread yield and total baking loss (TBL) are primarily important for bread producers, as they do not directly relate to the quality of the product itself but to the profitability of production; hence, these parameters are often disregarded in scientific publications.

The present results (Table 3) indicate that the addition of chickpeas had a positive effect on all the analyzed bread parameters except its volume. The yield of the CON bread was comparable to that reported in earlier studies (Wirkijowska et al., 2020) and significantly lower compared to all the chickpea-fortified variants (Table 3). The highest values of this parameter were exhibited by the BB12/FCP48 products. All the bread variants with the addition of fermented chickpeas were characterized by lower values of total baking loss in comparison to CON and NF.

The specific volume declined significantly decrease in all the chickpea-enriched products (compared to CON), with the lowest value (36 % decrease) recorded in BB12/48 (Table 3). However, there were no significant differences between the specific volume values of all the bread variants with the addition of chickpea seeds. Mohammed et al. (2014) reported a similar decrease in the bread loaf volume (by approx. 30 %) after addition of 30 % of chickpea flour.

The study showed a significantly higher moisture level in the fresh crumb of the chickpea-enriched baked products, compared to CON (Table 1). Moreover, the bread variants containing the fermented additives did not differ in the measured parameter. This may be explained by the fact that chickpeas (as well as chickpea flour) exhibit higher water absorption capacity than wheat flour suppressing the amount of generated water vapor, which results in a reduced loaf volume and increased crumb hardness (Mahommed et al., 2014). The low baking loss, higher bread yield, and moisture content in fresh crumbs indicate the economic attractiveness of the investigated components. Also, it has been indicated that the addition of chickpea flour (fermented or not) has a significant effect on the color of the bread crumb and crust, compared to wheat bread (Xiao et al., 2016). The chickpea-fortified baked products were characterized by significantly lower color brightness (Table 2, Fig. 1a), which was probably associated with the partial replacement of wheat flour with the chickpeas. This may have contributed to an increase in the protein content in the dough (compared to CON) and intensified the formation of MRPs during the baking process, resulting in a darker color of the final products (Dall'Asta et al., 2013).

Among all the baked products, slightly lower values of the L^* index were recorded in NF. In addition, the variants supplemented with chickpeas fermented for 48 h (regardless of the bacterial strain used) were characterized by lower color brightness than analogous products containing chickpeas fermented for 24 h. Nevertheless, the differences in the L^* values between all the chickpea-enriched bread variants were not statistically significant.

The a^* and b^* values in the chickpea-supplemented products were

Table 3
Physical properties of bread variants.

Product variants*	Bread yield[%]	TBL[%]	Specific volume of bread [cm^3/g]	Crumb moisture[%]	Color parameter		
					L^*	a^*	b^*
299 V/24	157.1 ± 0.9^{c}	13.6 ± 0.5^{a}	2.3 ± 0.1^{a}	50.7 ± 0.2^{bc}	71.41 ± 1.48^{a}	4.05 ± 0.36^{b}	18.94 ± 0.43^{bc}
299 V/48	152.9 ± 1.0^{b}	14.8 ± 0.6^{b}	2.3 ± 0.1^{a}	50.0 ± 0.6^{bc}	70.67 ± 1.05^{a}	4.14 ± 0.36^{b}	18.83 ± 0.39^{b}
BB12/24	157.3 ± 0.9^{c}	13.6 ± 0.5^{a}	2.4 ± 0.2^{a}	50.8 ± 0.3^{bc}	71.87 ± 0.52^{a}	4.10 ± 0.39^{b}	19.80 ± 0.33^{cd}
BB12/48	159.6 ± 0.8^{d}	13.0 ± 0.4^{a}	2.2 ± 0.0^{a}	49.7 ± 0.6^{b}	71.51 ± 1.20^{a}	4.15 ± 0.23^{b}	19.50 ± 0.33^{bcd}
NF	158.7 ± 1.1^{cd}	15.0 ± 0.6^{b}	2.4 ± 0.1^{a}	50.9 ± 0.3^{c}	70.92 ± 0.94^{a}	4.08 ± 0.19^{b}	20.14 ± 0.32^{d}
CON	136.7 ± 0.8^{a}	15.2 ± 0.5^{b}	3.5 ± 0.2^{b}	42.7 ± 0.2^{a}	76.93 ± 2.36^{b}	2.17 ± 0.45^{a}	15.11 ± 1.24^{a}

Explanatory notes: *CON– control wheat bread; NF- bread with unfermented chickpea addition; 299 V/24(48)– bread with the addition of chickpeas fermented with *Lactobacillus plantarum* 299v for 24 h (or 48 h); BB12/24(48) – bread with the addition of chickpeas fermented with *Bifidobacterium animalis* subsp. *lactis* BB12 for 24 h (or 48 h); TBL-total baking loss. The values are expressed as the mean (n = 6) ± standard deviation. The mean values in the same column with different letters are significantly different ($p < 0.05$).

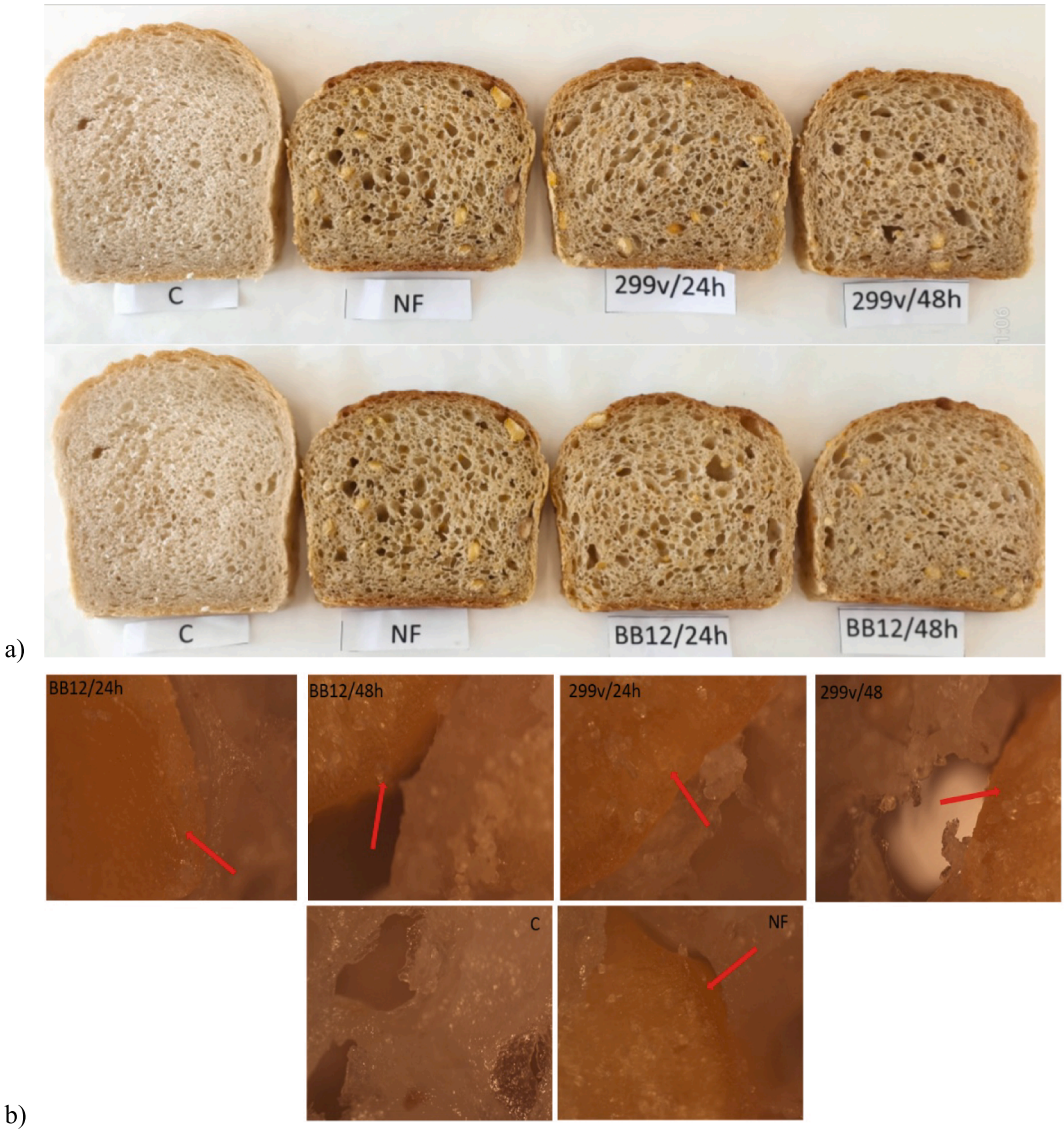

Fig. 1. Representative images of the macrostructure (a) and microstructure (x 40 magnification) of crumbs of the bread variants.

significantly higher than in CON (Table 3). Similar observations were presented by Gadallah and Aljebreen (2023) in their analysis of the color of pan bread fortified with fermented chickpea flour. Interestingly, slightly higher values of a^* were recorded in products containing chickpeas fermented for 48 h (Table 3) than those fermented for 24 h or the NF variant. However, there were no significant differences ($p > 0.05$) in the* values between all the chickpea-enriched variants (Table 3).

The chickpea-containing products were characterized by higher saturation of the crumb with the yellow color (b^*) than CON. This may be associated with the high content of carotenoids in *C. arietinum*, including lutein (7.70 μg/g), zeaxanthin (5.76 μg/g), and β-carotene (0.40 μg/g), contributing to the natural yellow-orange coloration of seeds (Ashokkumar et al., 2014). Moreover, riboflavin, which is naturally present in legume raw material and can be synthesized by some of the bacterial strains, may have increased the share of the yellow-orange component in the color of the chickpea-fortified baked products (Prodanov et al., 2004; LeBlanc et al., 2017; Suwannasom et al., 2020). This is consistent with the content of vitamin B_2 determined in the present study (Table 2).

The lower b^* values recorded for variants 299 V/48 and BB12/48 (in comparison to NF) may be related to the metabolic activity of bacteria during the chickpea fermentation, which may lead to modifications of the carotenoid composition. As shown previously, the degradation of α-carotene and β-carotene in carrot juices fermented for 24 h with *Bifidobacterium lactis* Bb-12 and *Bifidobacterium bifidum* (strain B7.1 and B32) ranged from 15 to 45 %, depending on the starter culture (Kun et al., 2008).

3.3. Texture profile analysis (TPA) of bread variants

After 24 h of storage, all the chickpea-enriched bread variants exhibited higher values of hardness than CON (Table 4). The value of this parameter increased significantly ($p < 0.05$) in all the baked products after 72 h of storage. The bread variants containing chickpeas fermented by *L. plantarum* 299v (especially variant 299 V/48) had the highest value of this parameter after both periods of storage. The results are comparable with the findings presented by Shrivastava and Chakraborty (2018) showing an increase in the hardness of wheat bread induced by incorporation of fermented chickpea flour. Interestingly, NF exhibited the slightest change in the analyzed property (with an increase of only over 2 N).

The springiness of the tested products after 24 h of storage reached from 0.930 ± 0.03 (299 V/48) to 0.988 ± 0.02 (CON), and the chickpea-

Table 4
Comparison of the texture profiles of bread variants.

	Hardness[N]		Springiness		Chewiness[N]		Cohesiveness	
Storage timeProduct variant*	24 h	72 h	24 h	72 h	24 h	72 h	24 h	72 h
299 V/24	5.72 ± 1.28^{bcA}	14.39 ± 2.00^{cB}	0.974 ± 0.05^{abB}	0.840 ± 0.04^{aA}	3.53 ± 0.47^{bA}	4.55 ± 0.92^{cdB}	0.646 ± 0.06^{aB}	0.374 ± 0.05^{aA}
299 V/48	7.05 ± 1.80^{cA}	14.96 ± 1.87^{cB}	0.930 ± 0.03^{aB}	0.828 ± 0.05^{aA}	4.04 ± 0.92^{cA}	4.73 ± 0.70^{dA}	0.623 ± 0.07^{aB}	0.383 ± 0.04^{aA}
BB12/24	5.39 ± 0.97^{bcA}	11.70 ± 1.26^{bB}	0.946 ± 0.02^{abB}	0.866 ± 0.04^{aA}	3.01 ± 0.44^{bA}	3.96 ± 0.65^{cdB}	0.593 ± 0.03^{aB}	0.389 ± 0.02^{aA}
BB12/48	5.02 ± 0.79^{abA}	10.91 ± 1.81^{bB}	0.940 ± 0.04^{abB}	0.881 ± 0.05^{aA}	2.79 ± 0.33^{abA}	3.70 ± 0.48^{bcB}	0.595 ± 0.06^{aB}	0.388 ± 0.04^{aA}
NF	5.11 ± 1.32^{bA}	7.44 ± 1.47^{aB}	0.964 ± 0.04^{abB}	0.824 ± 0.07^{aA}	2.84 ± 0.52^{abA}	2.54 ± 0.52^{aA}	0.586 ± 0.04^{aB}	0.419 ± 0.07^{aA}
CON	3.29 ± 0.34^{aA}	8.14 ± 0.86^{aB}	0.988 ± 0.02^{bB}	0.864 ± 0.04^{aA}	2.08 ± 0.18^{aA}	2.90 ± 0.37^{abB}	0.641 ± 0.05^{aB}	0.411 ± 0.01^{aA}

Explanatory notes: * CON– control wheat bread; NF- bread with unfermented chickpea addition; 299 V/24(48) – bread with the addition of chickpeas fermented with *Lactobacillus plantarum* 299v for 24 (or 48 h); BB12/24(48) – bread with the addition of chickpeas fermented with *Bifidobacterium animalis* subsp. *lactis* BB12 per 24 h (or 48 h). The values are expressed as the mean (n = 8) ± standard deviation. The mean values in the same column (the tested texture parameter) among all variants of bread at one of the analyzed storage time points (after 24 or 72 h) followed by different lowercase letters (a-d) are significantly different ($p < 0.05$), whereas uppercase letters (A-B) indicate the statistically significant differences ($p < 0.05$) between the values of the selected texture feature recorded after 24 h and 72 h for the same bread variant.

containing bread variants did not differ from each other. Moreover, only 299 V/48 differed significantly from CON exhibiting a lower value of springiness. These values are comparable with the results reported by Da Costa et al. (2020) in their analysis of sandwich bread containing 30 % of whole chickpea flour. After 72 h of storage, the springiness values in all the final products declined. The greatest decrease was noted in the NF variant (15 %), while BB12/48 exhibited the smallest change (6 %) in the analyzed parameter. No statistically significant differences were recorded between all the tested products stored for 3 days. This corresponds to the results reported by Serventi et al. (2018), who demonstrated no significant impact of chickpea addition on bread springiness. Furthermore, the trend of changes observed by the authors was similar to that noted in our study.

The lowest chewiness (2.08 ± 0.18) was recorded in CON after 24 h of storage, whereas NF was characterized by the lowest value of this parameter after 72 h (Table 3). The results indicate that, during consumption, these variants required the least force to crush the bread to a uniform state before swallowing in comparison to the other tested products. In contrast, the highest values of this feature were exhibited by 299 V/48 in both periods of storage. Also, the longer storage time (3 days) contributed to an increase in the value of this parameter. The exceptions were 299 V/48 and NF demonstrating no significant changes in chewiness after both storage times.

The results of the statistical analysis indicated a significant decrease in the cohesiveness of all the bread variants after 72 h of storage (Table 3). The most notable changes in the values of this parameter were recorded in products with chickpeas fermented by *L. plantarum* 299v (39 % in 299 V/24 and 42 % in 299 V/48). However, in each of the analyzed storage periods, the bread variants were not significantly different from each other. This is consistent with the results reported by Serventi et al. (2018), who did not observe significant changes in the cohesiveness of bread with chickpea additives.

The chickpeas incorporated in all the final products were firmly embedded into the structure of the bread mass (Fig. 1) and did not fall out during cutting. The seeds were surrounded and stabilized by the bread crumb, which was also visible during the observation of the microstructure of the bread variants (Fig. 1 b).

4. Conclusions

The study provides significant insight into the impact of probiotically fermented chickpeas on the quality and functional properties of bread. The variant containing chickpeas fermented by *Bifidobacterium animalis* subsp. lactis for 48 h had the highest values of TPC (46.24 ± 6.45 mg GAE /100 g d.w.), TFC (86.96 ± 2.45 mg QE/100 g d.w.), FRSA (71.00 ± 0.62 $DPPH^{\bullet}$ inhibition %), and FICA activity (26.47 ± 0.73 %), while 299 V/48 exhibited the highest TEAC (209.86 ± 1.72 $\mu mol \cdot L^{-1}$ TX/100 g d.w). Generally, the findings indicated that the applied additives (fermented and not fermented) increased the protein content and the riboflavin concentration and enhanced the free radical scavenging activity, iron ion chelating ability, and antioxidant capacity of the baked products. However, the use of the unfermented *Cicer arietinum* additive yielded the highest values of antioxidant activity of the wheat bread. In turn, the inclusion of the fermented chickpeas reduced the negative changes in the springiness and chewiness of the final products after 72 h of storage. The variants containing fermented chickpeas did not differ significantly in the a* and L* color parameters from NF, but exhibited significantly lower values of b*. The statistical analysis indicated that the incorporation of fermented seeds had a significant impact on the enhancement of the characteristics of wheat bread (CON). However, further in-depth investigations are necessary to conclusively validate the hypothesis that incorporation of probiotically fermented chickpeas in bread dough can improve bakery products. Nevertheless, the promising results of this preliminary research provide knowledge that may contribute to the development of innovative bread products with higher nutritional value and reinforced functional properties. However, analyses based on chromatographic techniques (HPLC/LC-MS) have to be done to determine individual bioactive compounds. Comprehensive analyses of organoleptic properties and consumer preferences for this novel fortified bread are also required.

CRediT authorship contribution statement

Katarzyna Skrzypczak: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Anna Wirkijowska:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Krzysztof Przygoński:** Writing – review & editing, Methodology, Investigation. **Konrad Terpiłowski:** Writing – review & editing, Investigation. **Agata Blicharz – Kania:** Writing – review & editing, Methodolog, y, Investigation.

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.

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2024.139117.

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