

Article

Comparison of the Benefits of Enriching Wheat Bread with Bee Pollen or Bee Bread in Terms of Soluble Protein and Bioactive Compounds Content and Storage Stability of Texture

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Abstract

The aim of this study was to evaluate the enrichment in the soluble protein and bioactive components of wheat bread by Polish bee pollen or bee bread addition, applying a previously reported recipe. The research phase included a baking trial and laboratory analyses, including water and soluble protein content, total acidity, crumb color of the fresh bread, sensory evaluation and texture profile (TPA) (fresh and after 4 days of storage). The bioactivity of 80% ethanol extracts (1% *w/v*) of used additives and enriched breads was assessed in terms of total phenolic and flavonoid content as well as antioxidant activity. The results confirmed that both additives (at percentages: 1%, 3%, and 5%) enhanced bread's properties in a dose-dependent manner, and the bread with 5% bee bread addition achieved the highest acidity, a 3.7-fold increase in soluble protein content, a 10-fold increase in iron-reducing capacity, and a 6-fold increase in antiradical activity compared to the control. Moreover, in texture tests, the same variant demonstrated the most effective delay in the staling process, maintaining the highest softness after 4 days. However, consumers rated the bread with 5% bee bread variant too intense in flavor. In general, the technological repeatability of the fortification degree of wheat bread with Polish bee products with the recipe used was confirmed; however, the quality of raw additives can deteriorate sensory qualities.

Keywords: wheat bread; bee pollen; bee bread; fortification texture analysis

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1. Introduction

Modern food technology is increasingly focusing on developing products with enhanced health benefits, which stems from growing consumer awareness of the impact of diet on the prevention of lifestyle diseases [1,2]. In this context, bee products, including honey, pollen, bee bread, propolis, and royal jelly, are recognized as extremely valuable natural resources with a broad spectrum of biological activity [3,4]. They exhibit, among others, antioxidant, anti-inflammatory, antimicrobial, anticancer, and immunomodulatory properties [1,4–6]. Their therapeutic potential is connected with the presence of various active substances, including proteins, essential amino acids, lipids, vitamins (B, C, D, and E), trace elements, and phenolic compounds, primarily flavonoids and phenolic acids [1,3,5,6].

Bee pollen is a product collected by worker bees, mixed with nectar and salivary gland secretions, and then transported to the hive in the form of pollen loads [5,7]. Despite its rich chemical composition, the bioavailability of its nutrients for the human body is limited due to the specific structure of the pollen grain's cell wall [4,8]. It consists of two layers: the inner intine (made of cellulose) and the outer exine, whose main component is sporopollenin [7–9]. Exine is characterized by extraordinary resistance to chemical agents and digestive enzymes in the human digestive system, which means that only 10–15% of the health-promoting components of raw pollen can be absorbed [6,8]. A natural solution to this limitation is the beehive production of bee bread, which results from the natural lactic acid fermentation of pollen stored in honeycomb cells. This process involves the activity of *Lactobacillus* bacteria, yeasts, and enzymes secreted by honey bees, leading to biochemical transformations that enhance the nutritional value and bioavailability of pollen constituents [5,9]. During this process, the cell wall of the pollen grain is partially degraded, significantly increasing the bioavailability and digestibility of its components (increasing to 60–80%) [4,8,9]. Bee bread differs from pollen in its higher acidity (the presence of lactic acid), higher content of easily digestible sugars and proteins, and the presence of vitamin K [4,10].

The rich composition and bioactive potential of bee products have stimulated growing interest in their application as functional ingredients in various food products. Their incorporation into dairy products, such as yogurts and cheeses, has been shown to enhance antioxidant capacity and mineral content while also supporting the viability of probiotic microorganisms [11]. In meat products, including sausages and burgers, bee-derived ingredients, particularly propolis and bee pollen, have demonstrated antioxidant and antimicrobial properties, contributing to delayed lipid oxidation and improved shelf life [9,11]. Bee products have also been successfully applied in beverages and fruit juices, where they improve nutritional quality and sensory characteristics [11]. More recently, growing attention has been directed towards their incorporation into cereal-based products, especially bakery goods, which are widely consumed and therefore represent an attractive vehicle for delivering bioactive compounds to consumers. In the bakery sector, however, research has focused mainly on the incorporation of bee pollen, especially in gluten-free bread and cookies [11–14]. The use of pollen was technologically justified by the introduction of proteins into gluten-free bread to improve the texture of the bread. It has been shown that the addition of pollen in amounts of 1–5% significantly increases the content of protein, minerals (K, Ca), and polyphenols, while improving sensory properties and delaying the staling process [12,13]. As a fermented product, bee bread can not only provide a higher dose of bioavailable ingredients but can also uniquely modify the technological properties of dough, as was tested by Ertosun et al. [2,9]. However, the variability in the phytochemical composition of these bee products is rarely considered. Additionally, it is known from the literature that the composition and bioactivity of pollen strongly depend on its botanical, geographical, and climatic origin [1,15].

The aim of this study was to evaluate the enrichment of wheat bread with soluble protein and bioactive compounds introduced via Polish bee bread and bee pollen using a recipe previously reported by foreign authors, and to determine how the quality of additives affects the texture of the obtained functional wheat bread.

2. Materials and Methods

2.1. Raw Materials

The baking process was carried out using “Plony Natury” type 450 cake wheat flour (Jeronimo Martins Polska S.A., Kostrzyn, Poland), fresh baker's yeast “Bakello” (Jeronimo Martins Polska S.A., Kostrzyn, Poland), iodized evaporated salt “o'Sole” (Cenos, Września, Poland) and functional additives: multifloral bee pollen (Mirosław Paźniewski

apiary, Stary Bógpomóz, Poland, harvest year 2024) and bee bread (Miodoland Beekeeping Company, Wola Węgierska, Poland, harvest year 2024). Both additives were selected as they had the best antioxidant properties of several samples at the preliminary stage.

2.2. Bread Preparation

The breads were prepared according to the recipe described by Ertosun et al. [9], while maintaining the exact proportions of the individual ingredients shown in Table 1. For the enriched bread, the standard recipe was modified—an appropriate portion of the flour (1%, 3%, or 5% of the total flour) was replaced with finely ground (particle size max. 1.7 mm) bee pollen or bee bread. Bread without bee products served as a control. Each bread variant was prepared in two independent replicates (2 baking sessions of 2 loaves each).

Table 1. Quantitative content of ingredients in recipes of individual bread samples.

Ingredients	Control	BP1	BP3	BP5	BB1	BB3	BB5
Flour (g)	520.0	514.8	504.4	494.0	514.8	504.4	494.0
Water (mL)	300	300	300	300	300	300	300
Yeast (g)	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Salt (g)	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Bee bread (BB) (g)	0	0	0	0	5.2	15.6	26.0
Bee pollen (BP) (g)	0	5.2	15.6	26.0	0	0	0
Total (g)	826.5	826.5	826.5	826.5	826.5	826.5	826.5

The first step was preparing a yeast starter, which was a form of pre-fermentation (yeast activation). The starter was prepared by mixing 15 g of flour, 15 g of water, and 5 g of fresh yeast. It was then left at an elevated temperature for 15 min, allowing the yeast to begin its intensive metabolic processes. After the activation period, the remaining recipe ingredients were added to the starter. The resulting mixture was kneaded by hand for 5 min, which promoted the proper hydration of the gluten proteins and the formation of the gluten network responsible for the dough's rheological properties. The dough was then covered and left in a warm environment for 35 min, allowing for proper fermentation and achieving the appropriate degree of leavening. The risen dough was divided into two equal portions and placed in prepared baking tins. The baking process was carried out in a kitchen oven at 180 °C for 45 min. After removing from the oven, the loaves were left to cool naturally, which allowed the internal structure to stabilize and the moisture content to even out throughout the product.

2.3. Preparation of Bread Extracts

To determine antioxidant activity and polyphenol content, bread samples (ground mass from the central part of the bread, taken from approximately 1 cm from the crust) were extracted in two types of solvents based on the procedure described by Ivanišová et al. [16]. Extraction was performed using 80% ethanol for 2 h (0.5 g of sample with 20 mL of ethanol solution) with shaking (SK-O330-PRO, Chemland, Stargard, Poland). Simultaneously, aqueous extraction was also performed, using distilled water and the same proportions of sample, solvent, and extraction time. Both extracts were centrifuged at 4000× g (MPW-351R laboratory centrifuge) and decanted. All extractions were performed in two independent replicates.

2.4. Color Analysis

Measurements were taken using a CR 5 colorimeter (Konica Minolta, Tokyo, Japan) in specular reflection exclusion (SCE) mode at an angle of 10°/D 65°, using a 3 mm aperture. Before measurements, the equipment was calibrated for black and white according to the manufacturer's recommendations. Bread crumb samples measuring 30 × 30 × 30 mm were placed in the aperture. Three measurements were taken for each sample at random locations on the cutout bread cube. Based on the results obtained, the whitening index (WI) was calculated for the crumb of the tested breads, according to the formula given by [17].

2.5. Water Content

Water content was determined using an MA 50.R moisture analyzer (Radwag, Radom, Poland) at 120 °C. The test was performed for all types of bread, with two replicates for each sample.

2.6. Titratable Acidity Determination

Total acidity was determined with a modified titration method [18]. A 25 g sample cut from the crumb of a loaf of bread was extracted with 200 mL of distilled water and filtered. A total of 50 cm³ of the solution was titrated with 0.1 M NaOH solution in the presence of 1% phenolphthalein solution until a light pink color was obtained that persisted for 1 min. Acidity was expressed in degrees (1° of acidity corresponds to 0.25 mL of NaOH).

2.7. Soluble Protein Content Determination

Soluble protein was assayed using the Bradford method, based on a modified procedure described by Džugan et al. [19]. To 20 µL of aqueous extract, 200 µL of Bradford reagent (Biorad, Hercules, CA, USA) was added, and after five minutes, absorbance at 595 nm was measured using an EPOCH2 microplate reader (Biotek, Winooski, VT, USA). The obtained results were calculated based on a standard curve prepared for albumin ($y = 0.1264x$; $r^2 = 0.97$).

2.8. Total Phenolic and Flavonoid Content

Total phenolic content (TPC) was determined according to the method of Singleton and Rossi [20], with a modification to allow the results to be read using a microplate reader. Twenty µL of extract was added to each microplate well, followed by 100 µL of Folin–Ciocalteu reagent (Merck, Darmstadt, Germany), diluted tenfold with distilled water. Subsequently, 80 µL of 7.5% sodium carbonate solution was added. The plate was incubated in the dark for one hour. After this time, absorbance at 760 nm was measured using a Biotek EPOCH2 microplate reader and compared to the control. Results are expressed in gallic acid equivalents (mg GAE) based on a standard curve ($y = 0.336x$; $r^2 = 0.9978$).

Total flavonoid content (TFC) was determined using the method of Biju et al. [21]. To 100 µL of ethanol extract, 100 µL of 2% AlCl₃ solution in methanol was added. After 10 min of incubation in the dark, absorbance was measured at 415 nm, and the results were calculated based on a standard curve prepared for quercetin ($y = 0.0655x$; $r^2 = 0.9999$).

2.9. Antioxidant Capacity

The antioxidant activity of the samples was determined using the FRAP (Ferric Reducing Antioxidant Power) method according to the procedure described by Benzie and Strain [22], adapted to measurement using a microplate reader. Each microplate well was

loaded with 20 μ L of 100-fold diluted extracts, followed by the addition of 180 μ L of FRAP reagent. The prepared microplate was incubated for 10 min at 37 °C. Absorbance was measured at 593 nm using an EPOCH2 microplate reader (BioTek, Winooski, VT, USA), relative to a blank control sample. Results were expressed as micromoles of Trolox equivalents (TE) based on the standard curve ($y = 0.1522x$; $r^2 = 0.9997$).

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was used to evaluate the anti-radical activity of the extracts, following the procedure described by Blois [23], modified for microplate use. To 20 μ L of samples (appropriately diluted), 180 μ L of DPPH reagent (0.1 M, diluted to an absorbance of 0.9 ± 0.1) was added. Absorbance was measured at 517 nm using an EPOCH2 microplate reader (BioTek, Winooski, VT, USA) against methanol after 30 min of incubation in the dark. For the control, the 20 μ L sample was replaced with distilled water. The percentage of DPPH radical scavenging was calculated and expressed as micromoles of Trolox equivalents (TE), based on the standard curve ($y = 15.553x$; $r^2 = 0.9970$).

2.10. HPTLC Analysis

High-performance thin-layer chromatography (HPTLC) was used to evaluate the enrichment of bread with bioactive compounds from the polyphenol class derived from pollen and bee bread. Ethanol extracts were applied to an HPTLC plate (Merck, Darmstadt, Germany) in a 5 μ L volume using an automatic applicator (Linomat 5, CAMAG, Muttenz, Switzerland). The chromatogram was developed using a mobile phase composed of ethyl acetate, acetic acid, formic acid, and water (10:1.1:1.1:2.6) in an ADC-2 development chamber (CAMAG). Results were documented after plate derivatization with Natural Product Reagent/Polyethylene glycol 400 (NP/PEG) under 366 nm UV light (TLC Visualizer, CAMAG). The process was controlled using Vision CATS 3.2 software (CAMAG).

2.11. Texture Profile Analysis

Texture Profile Analysis (TPA) was performed using a MultiTest 2.5i texturometer (Mecmesin, Newton House, UK) equipped with a 50 N load cell. The double-compression method using a 30 mm diameter head at a feed rate of 60 mm per minute was used to achieve 50% sample deformation with a compressive force of 0.2 N. The TPA test defined the following parameters: elasticity, hardness, adhesiveness, cohesiveness, and gumminess. Six cubes of $30 \times 30 \times 30$ mm were cut from each bread loaf. Three samples from each loaf were analyzed immediately after baking, and another three were analyzed four days after baking.

2.12. Sensory Evaluation

The sensory evaluation of the bread was performed using a 9-point hedonic scale ranging from 1 ('dislike extremely') to 9 ('like extremely'). Seven sensory attributes were evaluated: color, smell, taste, general appearance, texture, hardness and overall acceptance. Ten trained individuals aged 20 to 25 participated in the study. Samples were coded with random letter codes and presented to the participants in a randomized order to prevent carryover effects. The test was performed at room temperature (21 °C), under neutral artificial lighting.

2.13. Statistical Analysis

Statistical calculations were performed using GraphPad Prism 11 (GraphPad Software, Boston, MA, USA). A one-way analysis of variance (ANOVA) was applied for physicochemical parameters and sensory evaluation results. The significance of differences between mean values for individual samples was verified using the Tukey standard deviation (HSD) test at a significance level of $p < 0.05$. A correlation matrix was constructed to

determine the strength and direction of the relationships between individual qualitative parameters (including bioactive compound content and antioxidant potential). Additionally, a detailed analysis of texture parameters was supplemented with a paired Student's *t*-test, which allowed for the assessment of the significance of texture changes after storage.

3. Results and Discussion

In the first stage of the study, seven variants of bread were baked in replication according to the same recipe, in which flour was replaced with an increasing amount of bee products (Figure 1), which were then assessed in terms of their functional parameters and nutritional value in relation to the control bread.

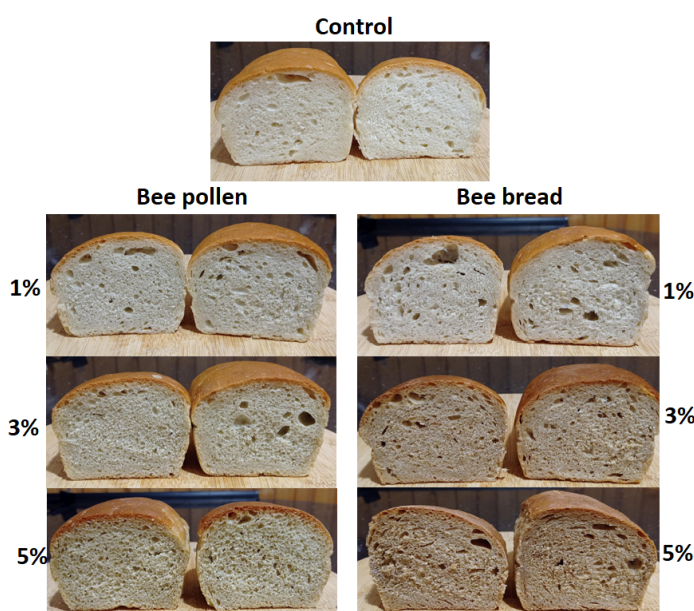


Figure 1. Visual appearance of the prepared breads.

3.1. Physicochemical Evaluation

Bread color was assessed instrumentally using the CIE $L^*a^*b^*$ system, which allows for the precise determination of differences in hue and color saturation between the control bread and the enriched samples. The obtained results are summarized in Table 2.

Table 2. Color characteristics of tested bread crumb.

Sample	L^*	a^*	b^*	Whitening Index (WI) for Bread Crumb
Control	61.95 ± 0.91 ^A	-0.523 ± 0.10 ^D	11.43 ± 0.09 ^C	60.24 ± 0.89 ^A
BP1	58.39 ± 0.63 ^A	-0.920 ± 0.09 ^D	13.35 ± 0.58 ^C	57.24 ± 0.78 ^{AB}
BP3	51.92 ± 1.06 ^{BC}	-0.805 ± 0.15 ^D	15.27 ± 0.51 ^B	49.55 ± 1.17 ^{CD}
BP5	54.96 ± 0.10 ^{ABC}	-0.495 ± 0.00 ^D	17.25 ± 0.01 ^B	51.77 ± 0.10 ^{BD}
BB1	57.63 ± 1.93 ^{AC}	0.522 ± 0.01 ^C	13.81 ± 0.39 ^C	55.43 ± 1.72 ^{ABC}
BB3	50.02 ± 3.18 ^C	3.15 ± 0.10 ^B	19.74 ± 0.21 ^A	46.17 ± 3.03 ^D
BB5	49.85 ± 2.15 ^C	3.98 ± 0.07 ^A	20.85 ± 0.03 ^A	45.54 ± 2.00 ^D

^{A,B,C,D}—means within the column marked with different superscript letters differ significantly ($p < 0.05$); BP1, BP3, BP5—bread with 1, 3 and 5% bee pollen; BB1, BB3, BB5—bread with 1, 3 and 5% bee bread.

The lightest color was found in the control sample, for which the L^* parameter had the highest value (61.95). The addition of additives to the recipe resulted in a significant

darkening of the crumb (a decrease in the L^* value) in all samples tested. This phenomenon is confirmed in the literature. As indicated by Conte et al. [12] and Ertosun et al. [2], the addition of bee products to bread dough causes a linear decrease in the brightness of the crumb. This is due to the naturally darker color of pollen and bee bread compared to white wheat flour. In the conducted study, the lowest L^* parameter values (darkest crumb, 49.85) were recorded for bread with 5% bee bread content (BB5). The stronger darkening of the samples with bee bread results from the fact that it contains more reducing sugars, which promotes more intense browning during baking [2]. Analysis of the a^* parameter revealed a clear change in color tone. The control sample and the samples with pollen showed negative values (greenish hue), while the addition of bee bread caused a sharp shift towards red (positive values, up to +3.98 for BB5). An increase in the proportion of red color (a^* parameter) in enriched bread was also observed by Ertosun et al. [2]. Similarly, a shift in the a^* parameter towards positive values was also observed when using bee bread. This can be explained by the presence of natural pigments in bee products (mainly carotenoids and flavonoids), which undergo color changes when exposed to temperature. Furthermore, as reported by Kieliszek et al. [8], the high content of amino acids and sugars in bee bread accelerates Maillard reactions, leading to the formation of brownish-red melanoidins. An upward trend was observed for the b^* parameter (yellowness), indicating an increase in the proportion of yellow color from the added additives. They contain numerous yellow plant pigments and derivatives, flavonoids, and carotenoids. However, bee pollen is known to exhibit significant color variation depending on the botanical sources used by bees. Therefore, the color parameters of multifloral pollen are typically highly variable [24], varying from light yellow to almost black. Thus, the final color of the bread can be influenced by the color of the bee pollen used. Moreover, the whitening index calculated for the bread crumb confirms a decrease in crumb brightness with the addition of bee products. The initial value of this parameter (60.24) is typical for wheat bread [17]. The addition of bee bread, in particular, caused a significant reduction in this parameter compared to the control sample.

Water content was determined to assess the effect of bee product addition on the moisture content of the finished bread crumb. This parameter is important not only for the technological efficiency of the process, but primarily for the sensory characteristics and physicochemical stability of the product during storage. The obtained results, illustrating the differences in water content between the control and enriched samples, are presented in Figure 2.

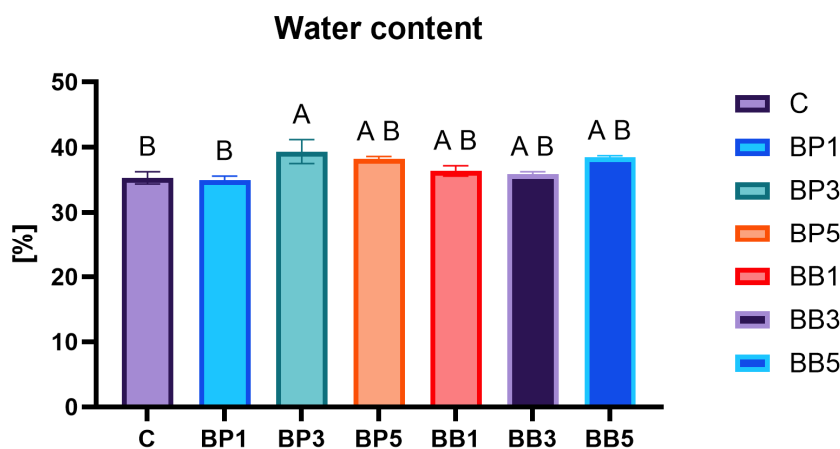


Figure 2. Water content in bread samples. A, B—means marked with different letters differ significantly ($p < 0.05$).

The analysis showed that the addition of bee pollen and bee bread to the recipe changed the water content of the finished product, and this effect was closely correlated with the amount of the additive used (Figure 2). In the control sample, the crumb moisture was 35.3%. A significant increase in water content ($p < 0.05$) was recorded in the sample enriched with 3% bee pollen, where this value increased to 39.35%. Similar results were described by Ertosun et al. [2] for bread enriched with the bee products (from 34.3% in control bread up to 37.7% in bread with 5% of bee bread). They indicated that the introduction of pollen or bee bread to dough modifies its processing properties, increasing its ability to retain water, which is crucial for extending the freshness of the final product. The higher moisture content of enriched samples results from the chemical nature of the additives. Bee products, like pollen and bee bread, are characterized by a high content of reducing sugars (fructose and glucose) and proteins [1,14]. These substances have strong hygroscopic and hydrophilic properties—they compete for water with starch and gluten, leading to stronger water binding in the crumb structure and reduced moisture loss during baking [25].

Titrateable acidity analysis showed that the addition of bee products significantly affected the acidity of the finished bread, with this increase closely correlated with the level of the additive used (Figure 3). In the control sample, the acidity was the lowest at 3.1°. The acidity parameter is often omitted when evaluating enriched bread, but it can significantly affect the stability and taste of the product.

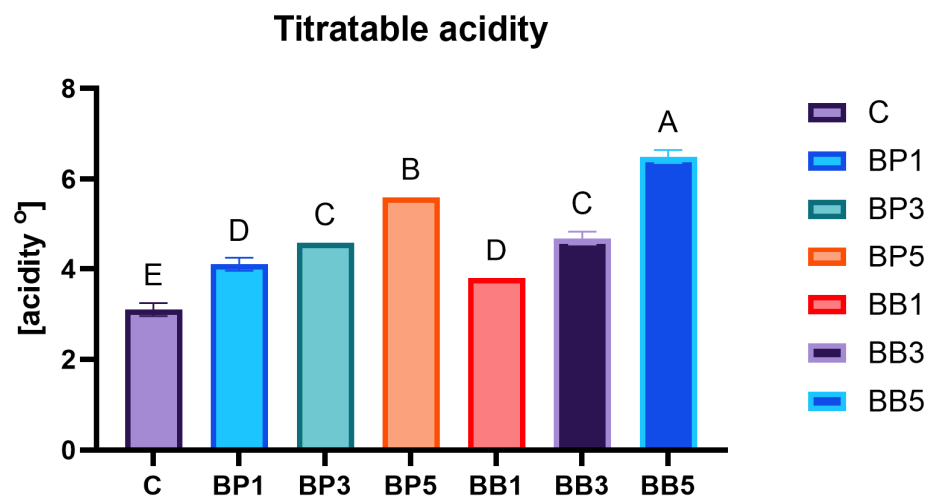


Figure 3. Titrateable acidity of bread samples. A, B, C, D, E—means marked with different letters differ significantly ($p < 0.05$).

In the case of both bee pollen (BP) and bee bread (BB), a linear increase in acidity was observed with increasing the percentage of the additive in the recipe. The observed increase in acidity in all enriched samples results from the natural chemical composition of bee products, which contain a number of organic acids (including gluconic, citric, and malic acids) and phenolic acids [3]. However, the significantly higher acidity of bread with bee bread (especially at 5%) is explained by its chemical composition, resulting from lactic acid fermentation during the bee bread beehive formation. The action of *Lactobacillus* bacteria and bee enzymes leads to the production of significant amounts of lactic acid, which naturally lowers the pH and increases the titrateable acidity of the final product compared to fresh pollen [8]. Our previous research indicates that the lactic acid content in Polish bee bread is almost twice as high as in pollen (on average 4.24% vs. 2.49%, respectively) [7]. From a technological perspective, increased acidity in bread (especially bread with

pollen) is a desirable phenomenon. Lowering the pH of the environment helps inhibit the growth of undesirable microflora, including spore-forming bacteria (*Bacillus* sp.) responsible for bread spoilage, which, according to Ertosun et al. [2], extends the microbiological stability of the product. Moreover, the presence of lactic acid in the product shapes its aroma profile; the characteristic aroma of fermented products may be an undesirable feature of the bread aroma.

3.2. Soluble Protein Content

Traditional wheat bread is a moderate source of these nutritional components, so the introduction of pollen or bee bread into the recipe was intended to enrich the final product with protein. As used bee products are rich source of protein, the first effect of bread supplementation was focused on the changes in soluble protein content depending on the concentration and type of additive (Figure 4).

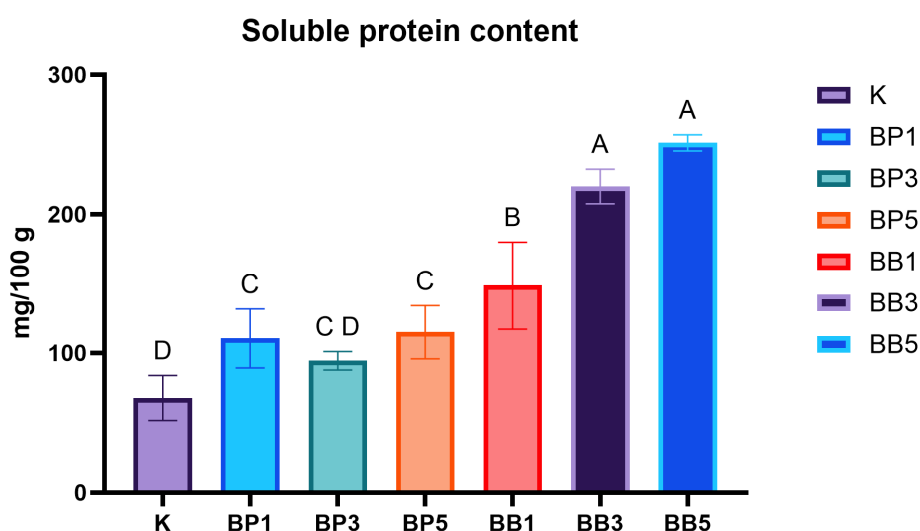


Figure 4. Soluble protein content in bread samples. A, B, C, D—means marked with different letters differ significantly ($p < 0.05$).

The Bradford method allows for the determination of soluble protein fractions. Quantitative analysis revealed a varied effect of the tested additives on the soluble protein content of the bread. In the control sample, the soluble protein content was the lowest, at 0.067 g/100 g of fresh weight. In the case of bee bread addition, a clear linear relationship was observed: as the proportion of raw material in the recipe increased, the content of determined proteins increased significantly. In this case, the soluble protein content increased from more than two-fold to approximately 3.7-fold at the highest bee bread addition. This confirms that bee bread is a more favorable source of protein due to its better bioavailability. Pollen grains are surrounded by a very resistant cell wall (exine), which hinders the extraction of intracellular proteins [26]. In the case of bee bread, the previously mentioned natural fermentation and enzymatic action lead to the degradation of the cell walls, which releases peptides and significantly increases their solubility [8,27], which significantly affects the extraction efficiency. A similar trend in total protein content was observed by Ertosun et al. [2], who noted enrichment led to an increase from 10.5% total protein in the control bread to 12.7 and 12.8%, respectively, in breads with the highest addition of bee pollen and bee bread. In this case, the lack of a significant difference corresponded to the protein content in the pollen and bee bread itself, which also did not differ significantly. Conte et al. [13] observed an increase in total protein content measured

by the Kjeldahl method in gluten-free bread from 3.9 to 4.44 g/100 g with 5% addition of bee pollen. In turn, Yan et al. [28], who added pollen in much larger amounts, achieved enrichment from 14.68 to 17.39 g/100 g with 25% pollen addition. It is important to note that the results we present concern the soluble protein fraction, which constitutes approximately 10% of the total protein in bread. The results obtained are promising and demonstrated higher enrichment in the case of bee bread, which resulted from the better accessibility of protein in this product.

3.3. Antioxidant Properties and Polyphenol Content

The degree of enrichment of bread with bioactive substances was assessed based on the following indicators: total phenolic content, flavonoid content, and antioxidant capacity using the FRAP and DPPH methods. The results are presented in the graphs (Figure 5).

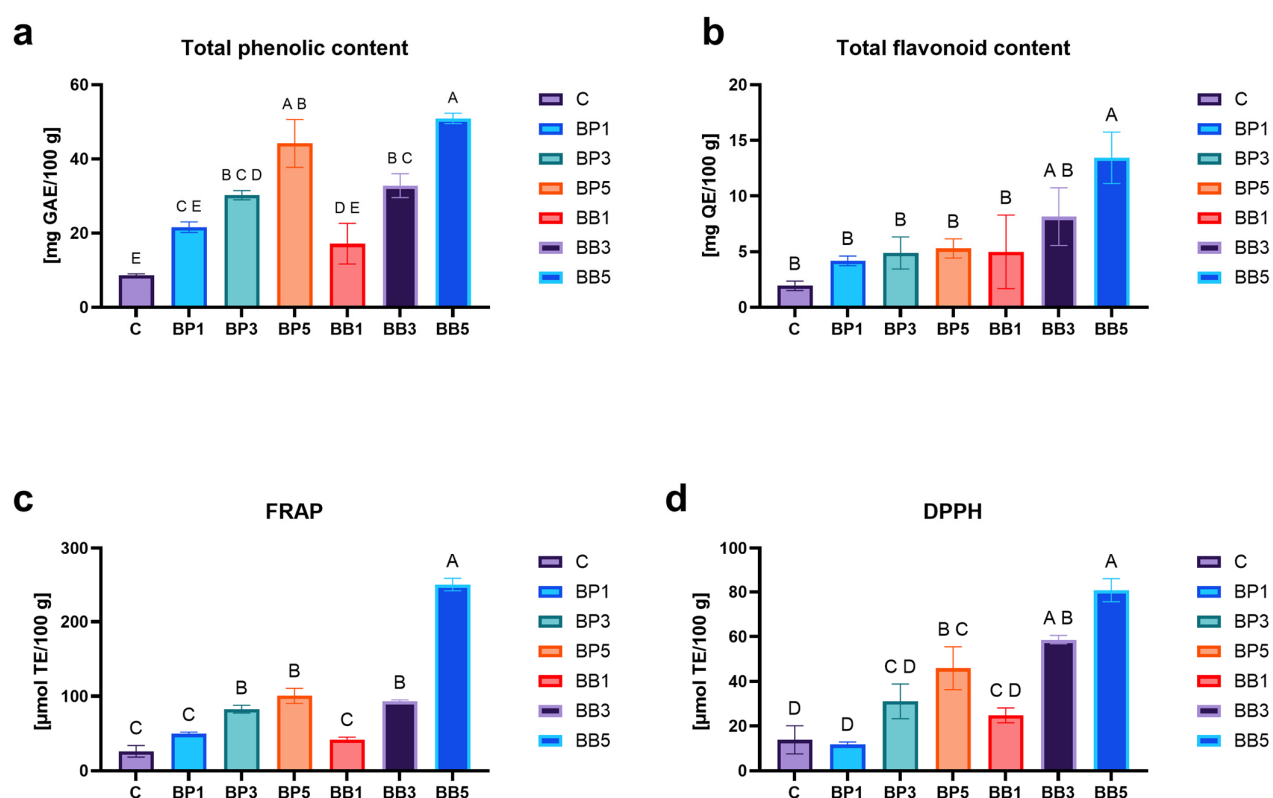


Figure 5. Total phenolic content (a) and total flavonoid content (b), as well as antioxidant capacity ((c)—FRAP method; (d)—DPPH method) of tested breads. A, B, C, D, E—means marked with different letters differ significantly ($p < 0.05$).

Analysis of the total polyphenol content showed that the addition of bee products to the bread recipe resulted in a statistically significant increase in the content of bioactive compounds in the bread. The control sample had the lowest level of phenolic compounds, at 8.62 mg GAE/100 g. The use of additives resulted in a linear increase in the TPC value, which was closely correlated with the percentage of raw material (Figure 5a). Bread enrichment at the level of 1% (BP 1%, BB 1%) resulted in an approximately two-fold increase in phenol content, while the addition of 3% (BP 3%, BB 3%) resulted in an increase to 30.2–32.8 mg GAE/100 g. The highest result was recorded for samples with 5% bee bread (BB 5%), reaching 50.9 mg GAE/100 g, which is more than a five-fold increase compared to the

control sample. The observed rapid increase in phenolic compound content results directly from the rich phytochemical profile of the raw materials. According to Kieliszek et al. [8] and Camacho-Bernal et al. [11], bee pollen and bee bread are concentrated sources of flavonoids including rutin, quercetin, and kaempferol and phenolic acids, which exhibit strong antioxidant properties. Both bee pollen and bee bread are characterized by the variability of their phytochemical profile, which not only depends on the botanical origin but also shows seasonal variation [29,30]. These results are consistent with the research by Ertosun et al. [9], who demonstrated that despite losses caused by high baking temperatures, the final product with added pollen or bee bread is characterized by a much higher antioxidant potential than traditional bread. They obtained a similar degree of enrichment: a 4- and 5-fold increase in total phenol content in the case of 5% addition of pollen and bee bread, respectively. Similarly, a linear increase in TPC with increasing pollen content in gluten-free bread was also observed by Conte et al. [13], who recorded an increase from 28

7 mg/100 g to 442.1 mg/100 g for a 5% pollen addition. Importantly, they determined the fraction of soluble polyphenols, for which the content was almost threefold increased, and the fraction of bioaccessible polyphenols also increased significantly [13].

Determination of total flavonoid content confirmed that enriching bread with bee products significantly increases the content of this group of bioactive compounds in the finished product. The control sample had the lowest flavonoid level, at 1.9 mg QE/100 g. A linear increase in flavonoid content was observed with increasing percentages of bee pollen and bee bread (Figure 5b). For bee pollen, a maximum addition of 5% allowed for a value of 5.3 mg QE/100 g. Significantly higher effectiveness was noted for bee bread. The sample with 3% bee bread (BB 3%) had 8.1 mg QE/100 g, exceeding all samples with bee pollen. The highest value, 13.4 mg QE/100 g, was obtained with 5% bee bread, representing an almost seven-fold increase compared to the control bread. The increase in flavonoid content in the enriched samples was strongly ($p < 0.05$) correlated with the previously discussed TPC results ($r = 0.80$) and results from the chemical composition of the additives: richness in rutin, kaempferol, isorhamnetin [8,11]. The studies by Ertosun et al. [9] confirm that these compounds exhibit high thermal stability, which allows them to survive the baking process and increase the biological activity of the finished product in proportion to the amount of the added bee product. In the cited work, with the highest addition of both bee products, an approximately 5-fold increase in flavonoid content was also obtained compared to the control.

Determination of antioxidant potential using the FRAP (Ferric Reducing Antioxidant Power) method allowed us to assess the ability of the tested extracts to reduce iron(III) to iron(II) ions. The obtained results showed that enriching bread with bee products led to an increase in its reducing power, which is correlated with the previously discussed phenolic compound content (Figure 5c). The control sample had the lowest activity, at 26.1 $\mu\text{mol TE}/100\text{ g}$. This level is typical for wheat bread and results from the presence of endogenous antioxidants and Maillard reaction products [31]. The introduction of bee pollen (BP) resulted in a linear increase in FRAP values. The maximum result for pollen (BP 5%) reached 100.5 $\mu\text{mol TE}/100\text{ g}$, representing a four-fold increase compared to the control. The most pronounced effect was observed for bread with bee bread. While at lower concentrations (1% and 3%) the bee bread results were similar to those for pollen, at a 5% addition, an increase in activity occurred. The BB 5% sample reached 249.9 $\mu\text{mol TE}/100\text{ g}$, which is 10-fold higher than the control. In the work of Ertosun et al. [9], the reducing power (Fe^{3+} to Fe^{2+}) of extracts of enriched breads was also compared. A 3-fold increase in iron-reducing properties was obtained in both products with a 5% enriching additive.

Assessment using the synthetic DPPH radical method showed that enriching bread with bee products significantly increased its antioxidant potential, depending on the type and concentration of the additive (Figure 5d). The control sample showed a low activity of 13.8 $\mu\text{mol TE}/100\text{ g}$. This activity is due to the presence of natural flour antioxidants and Maillard reaction products formed during the baking process, which also exhibit some antioxidant properties. The analysis showed that 1% additions of pollen (BP 1%) and bee bread (BB 1%) did not significantly change the activity compared to the control. A significant increase occurred at higher concentrations. In the case of bee pollen, the highest result was obtained for the 5% sample (45.9 $\mu\text{mol TE}/100\text{ g}$). However, extracts from bread enriched with bee bread demonstrated by far the highest antioxidant activity. The sample with 3% bee bread (BB 3%) achieved a result of 58.7 $\mu\text{mol TE}/100\text{ g}$, exceeding the samples with pollen, while the 5% BB sample achieved a value of 80.8 $\mu\text{mol TE}/100\text{ g}$. This represents an almost six-fold increase in antiradical activity compared to the control bread. In the compared publication by Ertosun et al. [9], the DPPH radical scavenging capacity was expressed as an EC50 parameter. Hence, the trend is reversed—the greater the addition of pollen or bee bread to bread, the lower the value of this parameter. However, the antiradical properties were also more than doubled with the addition of 5% bee products. Despite the use of different methods of expressing the tested properties, the trends are maintained, with a noticeably higher degree of antioxidant enrichment in our case, which may be due to the properties of the additives themselves.

To verify the effect of the type of additive on improving the functional value of bread, additional characterization of pollen and bee bread extracts was conducted. Table 3 presents the results of antioxidant potential (DPPH, FRAP) and polyphenol and flavonoid content determinations of the raw additive used. Based on the results, the theoretical concentrations in bread were calculated and compared to real measured values.

Table 3. Antioxidant properties and polyphenol and flavonoid content of bee pollen and bee bread.

	Bee Pollen	Bread with 5% BP		Bee Bread	Bread with 5% BB	
		Calculated	Measured **		Calculated	Measured **
TPC mg GAE/100 g	936.19 $\pm$ 26.36	55.43	44.19	902.89 $\pm$ 40.93	53.77	50.93
TFC mg QE/100 g	97.71 $\pm$ 8.52	6.82	5.29	192.57 $\pm$ 18.02 *	11.56	13.44
FRAP $\mu\text{mol TE}/100\text{ g}$	2859.40 $\pm$ 279.36	169.03	100.53	2910.21 $\pm$ 120.96	171.57	249.89
DPPH $\mu\text{mol TE}/100\text{ g}$	1954.44 $\pm$ 76.53	111.54	45.89	1958.61 $\pm$ 263.86	111.75	80.83

*—significant difference ($p < 0.05$) between bee pollen and bee bread; **—values are extracted from Figure 5.

Comparing the raw material analysis results (Table 3) with the parameters of the finished baked goods demonstrates a clear and direct relationship between the bioactive compounds content of the additive used and the nutritional value of the final product. Bee pollen and bee bread were characterized by extremely high antioxidant potential, reaching FRAP values of approximately 2800–2900 $\mu\text{mol TE}/100\text{ g}$ and DPPH values of approximately 1950 $\mu\text{mol TE}/100\text{ g}$. The observed linear increase in TPC, TFC, FRAP, and DPPH parameters in the bread with an increase in the additive content (from 1% to 5%) is a direct reflection of the introduction of a concentrated source of bioactive substances into the dough matrix. In the case of DPPH and FRAP parameters, the starting raw materials were characterized by similar activity (differences of 1–2%), which also translated into the results of the finished product, where the differences between the BP and BB series were smaller than in the case of flavonoids, although with a slight predominance of bee bread, which may result from the better thermal stability of its components or the presence of other groups of antioxidants that are not flavonoids. These results are consistent with

those previously described for pollen and bee bread from Poland [7]. Multi-seasonal studies have shown that the content of bioactive compounds is also subject to seasonal fluctuations; the TPC value for Polish bee pollen in three consecutive years ranged from 873.33 to 1469.15 mg GAE/100 g [29]. In the study on bread enrichment, bee pollen used contained fewer total phenols than bee bread (approx. 700 vs. 1250 mg/100 g) [9], while in our case, both products did not differ significantly in this aspect. The opposite trend was observed in the case of total flavonoid content—with a predominance of bee pollen and higher values than those obtained by us (approx. 200–250 mg QE/100 g) [9].

Table 3 also presents a comparison of the theoretical antioxidant enrichment of bread (calculated for a 5% addition of bee pollen and bee bread, assuming the total amount of compounds present in the added amount of the additive was added to the baked bread) and the experimental results. The results clearly show that bee bread is a better source of antioxidant compounds. For the TPC parameter, significantly better agreement was achieved, and flavonoid enrichment was also completely effective. For the FRAP and DPPH parameters, obtained with methods based on completely different mechanisms with different sensitivity against hydrophilic and lipophilic antioxidant fraction, the interactions with the matrix during analysis should be considered. However, significantly higher effectiveness was observed for bee bread enrichment.

Analysis of ethanol extracts using HPTLC confirmed the enrichment of bread with polyphenols derived from bee pollen and bee bread. Figure 6 presents the paths corresponding to the extracts from the control bread, the bee raw materials used (A-bee pollen, B-bee bread), and the enriched breads.

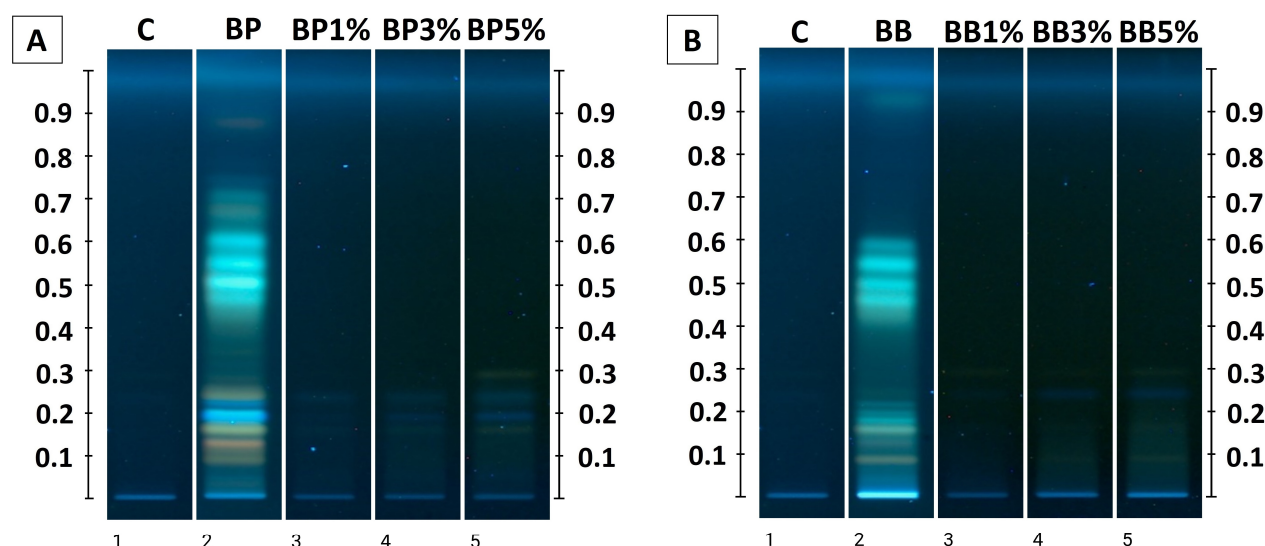


Figure 6. Image of HPTLC plates after NP reagent/PEG 400 derivatization under 366 nm UV light. (A)—breads with added bee pollen; (B)—breads with added bee bread.

HPTLC is useful for quickly confirming the introduction of new bioactive components during food fortification with various additives. In this case, additional bands can also be observed, originating from polyphenols naturally present in pollen and bee bread. The image of the lane corresponding to the control bread extract does not contain any bands, indicating that polyphenolic compounds were not present in concentrations that could be detected by this technique. In contrast, the chromatograms of pollen and bee bread extracts (BP and BB, respectively) show numerous blue, yellow-green, and yellow-orange bands. The images for both bee products were similar, although in the case of pollen the profile was richer. Two main series of bands were visible: yellow-green, orange

and blue in the Rf 0.1–0.3 range and mainly blue in the Rf 0.4–0.6 range. Three less pronounced bands above Rf 0.6 were specific to bee pollen. Based on previous analyses, blue and yellow-green bands correspond to compounds from the phenolic acids and flavonoids groups. Similar images for bee pollen, containing a series of blue bands in the Rf range between 0.6 and 0.8, were obtained by Mosić et al. [32] and Sen et al. [33]. The substances manifesting as a series of blue-stained bands have not yet been precisely identified. However, considering the results obtained by Sen et al. [33], who used the N¹,N⁵,N¹⁰-tricafeoylspermidine standard, it can be speculated that these are compounds of a similar nature. These types of phenolamides have also been identified in pollen, for example, from *Castanea* sp. [30] and *Brassica napus* [34]. Moreover, compounds from this class are proposed as chemotaxonomic markers for determining pollen origin [35]. Compounds appearing above Rf approx. 0.3 did not appear in the extracts of enriched breads, which suggests their thermolability. However, there are other possible explanations for the poorer profiles of fortified bread extracts, e.g., the detection limit of individual substances and limitations resulting from the extraction system used. Compounds with lower Rf values, stained blue and greenish-yellow, were visible in the enriched bread extracts. These may be tentatively labeled flavonoids, e.g., derivatives of quercetin, kaempferol, and isorhamnetin, identified using LC-MS/MS by Mosić et al. [32]. The band pattern for bee bread is slightly different, probably resulting from changes during the fermentation of pollen in the comb; moreover, the raw materials used came from different sources, which could undoubtedly have an impact on their phytochemical profile. There are no data in the literature regarding HPTLC analyses of bee bread extracts that could serve as a comparison. The obtained results confirm the selective enrichment of bread only with certain polyphenols that demonstrate stability during baking. In the tested extracts of enriched breads, an increasing tendency can be observed in the number and intensity of bands in the chromatograms with an increase in the amount of additive.

3.4. Texture Profile Analysis

The TPA test analyzed the texture of baked breads when fresh and after four days of storage at room temperature. The results for individual texture parameters are summarized in Table 4.

Table 4. Texture profile analysis (TPA) results at day 1 and after 4 days storage of wheat bread enriched with bee pollen (BP) and bee bread (BB).

Sample	Hardness [N]		Adhesiveness [N/mm]		Cohesiveness [-]		Stringiness [mm]		Gumminess [N]	
	Day 1	Day 4	Day 1	Day 4	Day 1	Day 4	Day 1	Day 4	Day 1	Day 4
Control	14.28 ± 0.54 ^{AB}	35.73 ± 2.26 ^{*CD}	0.34 ± 0.08 ^C	0.10 ± 0.01 ^{*D}	0.48 ± 0.05 ^A	0.25 ± 0.01 ^{*B}	8.47 ± 0.80 ^{AB}	8.68 ± 0.09 ^A	6.95 ± 0.31 ^{AB}	8.45 ± 0.44 ^{*D}
BP1	13.18 ± 0.47 ^{BC}	34.80 ± 3.08 ^{*CD}	0.35 ± 0.01 ^C	0.11 ± 0.02 ^{*D}	0.50 ± 0.08 ^A	0.28 ± 0.01 ^{*AB}	7.99 ± 0.41 ^B	8.55 ± 0.21 ^A	5.98 ± 0.64 ^{CD}	9.23 ± 0.69 ^{*CD}
BP3	10.88 ± 1.27 ^D	42.70 ± 1.27 ^{*AB}	0.48 ± 0.01 ^{AB}	0.38 ± 0.06 ^C	0.45 ± 0.06 ^A	0.26 ± 0.03 ^{*B}	8.41 ± 0.16 ^{AB}	8.43 ± 0.62 ^A	5.10 ± 0.25 ^D	10.03 ± 0.29 ^{*BD}
BP5	13.98 ± 0.92 ^{AC}	46.63 ± 4.38 ^{*A}	0.54 ± 0.02 ^A	0.50 ± 0.03 ^B	0.45 ± 0.06 ^A	0.25 ± 0.03 ^{*B}	8.11 ± 0.19 ^B	8.32 ± 0.50 ^A	6.25 ± 0.42 ^{BC}	11.65 ± 2.41 ^{*ABC}
BB1	14.10 ± 0.53 ^{AC}	45.10 ± 2.83 ^{*AB}	0.35 ± 0.02 ^C	0.44 ± 0.07 ^{*BC}	0.55 ± 0.06 ^A	0.32 ± 0.02 ^{*A}	7.74 ± 0.33 ^B	8.62 ± 0.19 ^A	7.35 ± 0.19 ^A	14.70 ± 1.76 ^{*A}
BB3	15.18 ± 0.56 ^A	40.03 ± 2.32 ^{*BC}	0.40 ± 0.03 ^{BC}	0.64 ± 0.03 ^{*A}	0.50 ± 0.00 ^A	0.31 ± 0.01 ^{*A}	8.07 ± 0.50 ^B	8.80 ± 0.29 ^A	7.23 ± 0.15 ^A	12.50 ± 1.37 ^{*AB}
BB5	12.15 ± 1.25 ^{CD}	32.00 ± 1.83 ^{*D}	0.42 ± 0.05 ^{BC}	0.67 ± 0.05 ^{*A}	0.48 ± 0.05 ^A	0.31 ± 0.02 ^{*A}	9.29 ± 0.40 ^A	8.50 ± 0.44 ^A	6.05 ± 0.66 ^{BD}	10.03 ± 1.21 ^{*BD}

A,B,C,D—means marked with different letters (in each column) differ significantly ($p < 0.05$); *—means marked with and asterisk differ significantly between day 1 and day 4; BP1, BP3, BP5—bread with 1, 3 and 5% of bee pollen; BB1, BB3, BB5—bread with 1, 3 and 5% of bee bread.

The addition of bee products significantly affected the texture of the bread, which was particularly noticeable in terms of hardness, gumminess, and adhesiveness. However, the trends of the changes varied. For hardness and gumminess, the addition of pollen caused an increase in the parameters related to the additive content, while for bee

bread, these parameters were higher than for the control bread, but decreased with increasing additive content. For bread with bee pollen, the hardness increased more than for the control bread, while for bread with bee bread it increased less, i.e., the bread with bee bread staled less intensively. A clear upward trend with the addition of the additive was observed for adhesiveness, for both pollen and bee bread. No significant changes or only slight changes were noted in the cohesiveness and stringiness of bread. As Conte et al. [12] pointed out, introducing bee products rich in fiber and non-gluten proteins into the recipe may weaken the gluten network. This structure becomes less flexible and more susceptible to starch retrogradation, which results in faster hardening of the crumb. This phenomenon was also confirmed in the research of Ertosun et al. [2], indicating that the addition of bee products significantly modifies the processing properties of the dough, which may lead to an increase in hardness if the water is not properly balanced. In most cases, an increase in the tested parameters was observed after 4 days of bread storage, except for cohesiveness. Only in breads with pollen did a significant increase in adhesiveness occur after 4 days of storage compared to fresh bread. The gumminess of the bread with bee pollen was lower than that of the control, but increased more during storage. An increase in this parameter was observed with the addition of bee bread. Similar changes in texture parameters after 3 days of bread storage were observed by Różyło et al. [36], who examined the effect of water addition on these changes. A higher water content in the recipe promoted better retention of softness and a lower increase in gumminess. This confirms that the water-binding capacity of the bee product additives is also an important factor. Bahadorloo et al. [37] investigated the effect of adding bee pollen (BP) in amounts of 0%, 3%, and 5% and hydroxypropyl methylcellulose (HPMC) in amounts of 3% on the textural and microbiological properties of gluten-free dough based on rice flour. The addition of both bee pollen and HPMC significantly affected the dough's structure, with a significant increase in hardness and a decrease in springiness. Bee pollen alone contributed to increased adhesiveness, gumminess, cohesiveness, and chewiness. Various additives to bread can have a positive impact on its textural properties and stability. The partial substitution of wheat flour with *Cordyceps sinensis* powder led to an increase in bread crumb hardness 24 h after baking, which was associated with decreased crumb porosity and a weakened gluten network. However, the enriched breads demonstrated good textural stability, as texture profile analysis showed no statistically significant changes in hardness or elasticity after 48 h of storage [38].

3.5. Sensory Properties Evaluation

The assessment of laboratory analysis was complemented by a sensory evaluation, which allows for a subjective assessment of the product's attractiveness. The study considered the following qualitative characteristics: appearance, color, hardness, texture, taste, and smell. The results were collected in Figure 7.

Among the parameters assessed, the greatest impact of additives was observed on bread color. The sensory panel rated breads with additives, especially with bee bread (3 and 5%), more highly, considering the darker color to be more attractive. The visible change in crumb color results from two main mechanisms. The first is the presence of natural pigments, such as carotenoids and flavonoids, which are abundant in bee products [8]. The second factor is the intensification of the Maillard reaction, caused by the high content of reducing sugars and amino acids, which leads to browning of the baked product. As noted by Conte et al. [12], crumb darkening correlated with the addition of pollen is usually positively perceived by consumers. The darkening of the bread crumb observed organoleptically was in line with the previously mentioned instrumental measurements and the decrease in the calculated whitening index. The taste and aroma of the breads were rated higher than those of the control bread. The improvement in flavor parameters

in the enriched samples is confirmed by Ertosun et al. [2]. These authors demonstrated that bee products contribute a unique bouquet of volatile aromatic compounds (esters, aldehydes), masking the bland taste of wheat flour. They noted the highest overall acceptance for breads with 3% pollen and 5% bee bread, although in some respects, baked goods with a lower share of additives were rated higher (e.g., color). However, it should be noted that bee bread, due to the lactic acid fermentation process [26], is characterized by a more pronounced, slightly sour taste compared to sweetish pollen. This may explain the lower overall ratings for the bread with the highest bee bread content—the intensity of this addition may have exceeded the acceptance threshold for some respondents accustomed to traditional bread. Bread texture was rated highest for the control bread; the addition of bee pollen worsened the organoleptic perception of texture according to panelists. This is consistent with the instrumental texture assessment, which showed a general deterioration in values such as gumminess, which is one of the most noticeable aspects when chewing bread. The sensory panel rated hardness higher for the bread with bee pollen, which is confirmed by the decrease in this parameter in the TPA. In the case of bee pollen, the bread with a 3% addition scored higher than the control, which also showed a lower instrumental hardness.

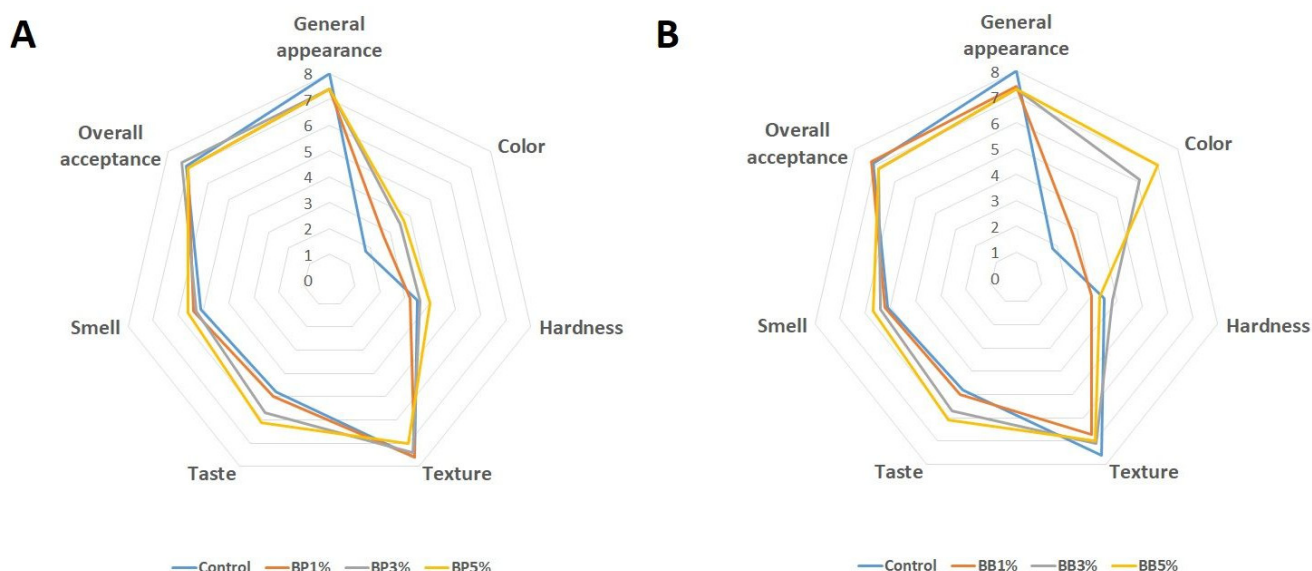


Figure 7. Summary of the sensory evaluation results for the tested bread variants. (A)—bread with bee pollen; (B)—bread with bee bread.

In order to comprehensively assess the strength and direction of the relationship between the examined bread quality parameters, a statistical analysis was performed using the Pearson correlation coefficient. The results of the analysis were presented graphically in the form of a correlation matrix, where the color intensity of the fields corresponds to the value of the coefficient, illustrating the degree of covariation of individual variables (Figure 8).

Analysis of the correlation matrix confirmed a very strong, positive relationship between the share of bee bread additives and parameters describing antioxidant properties. In the case of bee pollen addition, the correlation was weak. Variables such as total polyphenol (TPC) and flavonoid (TFC) content, as well as antioxidant activity measured by FRAP and DPPH methods, were strongly correlated ($r > 0.80$), confirming that the inclusion of bee bread in the recipe effectively increases the health-promoting potential of bread, making it a valuable source of bioactive compounds. A strong positive correlation

($r = 0.65$) was also observed between the amount of bee bread used and the total acidity of the bread. This is an expected result given the natural acidity of bee bread, which directly reduces the pH of the finished product. In terms of color parameters, a strong negative correlation ($r = -0.69$) was observed between the bee bread content and crumb lightness (L^*). This indicates that as the bee bread concentration increased, the crumb became darker, which is typical when bread is fortified with intensely colored ingredients rich in carotenoids and phenolic compounds. At the same time, a positive correlation was observed between the additive content and the b^* parameter ($r = 0.82$), indicating an increase in the yellow color characteristic of the pigments contained in bee pollen. However, it should be remembered that the color is closely related to the botanical origin of the pollen. Among the textural parameters (at day 1), the high positive correlation between adhesiveness and bee pollen content ($r = 0.89$) is noteworthy. This may suggest that the presence of sugars and proteins from bee pollen, as well as changes in the environmental pH, affect the water-absorbing properties of the crumb, increasing its viscosity. Analysis of correlation parameters confirmed the superiority of bee bread over pollen as a bread-enriching additive. This bee product had a significantly stronger effect on the bread's properties. However, since the additives used were of different geographical origin and were initially selected for their highest antioxidant activity, it is not possible to generalize on this basis about the superiority of bee bread over bee pollen in the fortification of wheat bread.

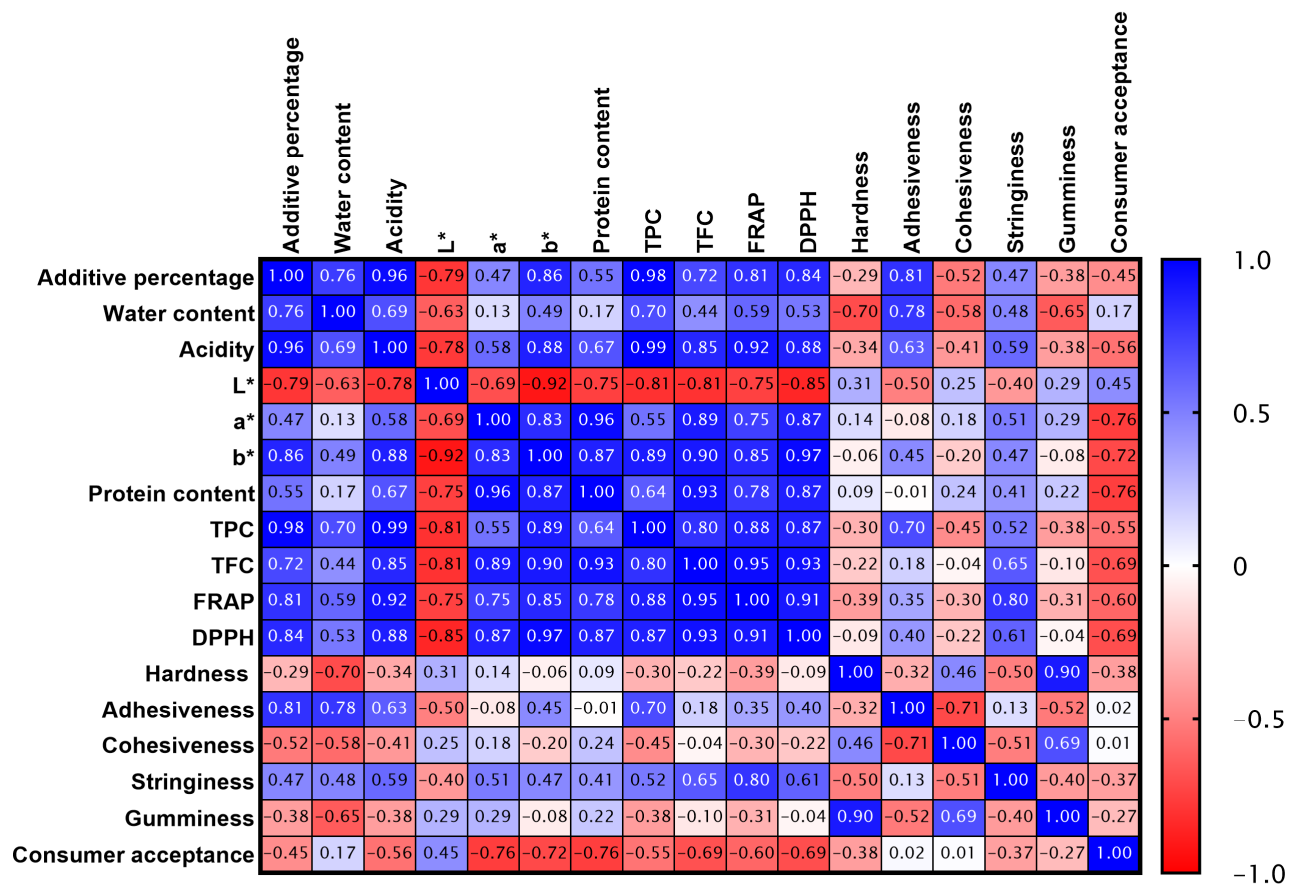


Figure 8. Correlation matrix (Pearson r coefficients) of the obtained results. The color intensity of the table cells corresponds to the value of the coefficient in a range of -1 (red) to 1 (blue).

4. Conclusions

The obtained results clearly confirmed that both used Polish bee products can successfully enrich wheat bread in soluble-protein and polyphenolic compounds; however, the final effect was lower than theoretically predicted. The degree of enrichment was significantly correlated with the amount of additive incorporated to bread and was comparable to the results obtained by other authors with the same recipe, which confirmed its technological repeatability. Higher enrichment of bioactive components was achieved using bee bread, although the variants with the highest addition were not appreciated by the sensory panel. Moreover, the addition of bee bread maintained the texture stability of bread crumb, and the water loss and hardening for this bread was comparable to the control bread, while in the case of bread with bee pollen, a significant increase in hardness was observed at additions above 3%. To conclude, the variable quality of bee products used as additives may affect the degree of enrichment of the bread and, more importantly, can negatively impact the sensory characteristics and consumer acceptability of the fortified bread.

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Institutional Review Board Statement: The sensory tests conducted are not part of a medical treatment experiment involving humans and are not subject to evaluation by the Bioethics Committee <https://www.ur.edu.pl/pl/universytet/nauka/komisja-bioetyczna-ur/informacje-na-temat-badan-podlegajacych-ocenie> (accessed on 10 June 2026). The research did not involve human experimentation in the same way as clinical or psychological research.

Informed Consent Statement: Informed consent for participation in sensory evaluation was obtained from all subjects involved in the study.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

TPA	Texture Profile Analysis
CIE	Comission Internationale de l'Eclairage
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
FRAP	Ferric-Reducing–Antioxidant Power
BB	Bee bread
BP	Bee pollen
SCE	Specular Component Excluded
TPC	Total Phenolic Content
TFC	Total Flavonoids Content
GAE	Gallic Acid Equivalents
QE	Quercetin Equivalents
TE	Trolox Equivalents
HPTLC	High-Performance Thin-Layer Chromatography

NP	Natural Product reagent
ANOVA	Analysis of Variation
HSD	Honestly Significant Difference
R _t	Retardation factor
HPMC	Hydroxypropyl methylcellulose
WI	Whitening index

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