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The Influence of Bee Bread on Antioxidant Properties, Sensory and Quality Characteristics of Multifloral Honey

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Abstract: The aim of this study was to investigate the influence of bee bread addition on the phenolic content, antioxidant properties, sensory and quality characteristics of the multifloral honey. On the base of results obtained, it was stated that an enrichment of honey with bee bread led to an significant increase in total phenolic content (from 30.75 to 158.96 mg GAE/100 g), total flavonoids content (from 2.77 to 21.15 mg QE/100 g), and phenolic acids content (from 11.02 to 35.47 mg CAE/100 g). Gallic acid was the predominating phenolic acid, while quercetin was the main determined flavonoid. A significant elevation of the phenolic content resulted in an increase in antioxidant capacity of the honey. However, an addition of bee bread to the honey led to the unfavorable changes of its sensory characteristics. The decrease in clarity, and uniformity of color and brightness was detected. In the case of consistency, the decrease in smoothness and meltability was found along with an increase in the feeling of sandiness. The assessment of taste showed a significant increase in acid taste, sharpness, bitterness and durability of the aftertaste, with a decrease in sweetness. The addition of bee bread to the honey caused a significant increase in water-insoluble substances content, free acidity, specific conductivity and proline level. At the same time, a decrease in the content of glucose and fructose was observed.

Keywords: honey; bee bread; phenolics; antioxidant properties; sensory properties; quality

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1. Introduction

Honey is a product that is easily metabolized by humans. It provides many nutrients and energy, and has an advantageous influence on the gastrointestinal tract functions [1]. As a carrier of bioactive substances, honey is an important dietary component supporting the treatment of many diseases [2,3]. This is associated, among others, with presence of bee enzymes and phenolic compounds in a honey [2,4]. The phenolic content mainly depends on the geographical and botanical origin of a honey [5–7]. Phenolic compounds, especially flavonoids and phenolic acids supplied to the human body with a honey, play an important role in, e.g., contributing to a deactivation of free radicals. They also have antimicrobial, anti-inflammatory, and anticancer properties [3,4,8,9]. The other bee products, including propolis, pollen, bee bread, or even drone brood homogenate, are also a rich source of bioactive substances possessing antioxidant properties [10–12]. Honey and other bee products may represent an alternative to food additives, and may serve as a source of the biologically active ingredients of functional foods [13,14]. Bee products, including propolis and bee pollen, have been utilized to enrich a honey with substances that are antioxidant in character; however, their too-high content may not be acceptable from a sensory point of view [15,16]. Bee pollen has also been used as an ingredient enriching cakes, fermented milk drinks, or cheeses, while propolis has been added to meat products [13].

Bee bread is a preserved fermentation product being a mixture of flower nectar, pollen, and bee secretion with a wide range of bacteria and yeasts required for fermentation. It is stored in honeycomb cells [17,18]. This product has strong antimicrobial, antioxidant,

antiradical, anti-inflammatory, and anti-cancer activities [17–19]. Already in ancient times, bee bread was used for nutritional and therapeutic purposes in the many cultures. Today, bee bread is considered as a valuable dietary supplement or as a functional food ingredient, and interest in its use has been significantly increasing recently [17,19]. In case of anaerobic conditions, the pollen, honey, and bee secretion mixture undergoes fermentation, during which hydrogen peroxide, organic acids, and antibacterial peptides are formed [18]. In addition, the content of basic nutrients changes significantly during this process [20]. Due to its low pH, bee bread is an excellent environment for development of lactic acid bacteria, including those from *Lactobacillus* and *Bifidobacterium* genera, participating in the fermentation process. Apart from bacteria, yeasts, especially those from the *Saccharomyces* genus, and *Aspergillus* and *Penicillium* molds also play a crucial role in the formation of this bee product [18]. Bee bread has a higher nutritional value and better digestibility when compared to bee pollen, as well as is characterized by a richer chemical composition [17,19]. It contains more proteins, vitamins, minerals, amino acids, and lipids than bee pollen [17,20,21]. The process of transforming pollen into bee bread involves dissolving of pollen sheaths, so it is easier to digest than bee pollen itself. The presence of bee digestive enzymes and honey in a bee bread results in an increase in its nutritional value, while its higher content of carbohydrates and lactic acid prevents development of molds and spoilage bacteria [17,18]. Bee bread contains numerous phenolic compounds, including apigenin, chrysin, kaempferol, and *p*-coumaric acid, and traces of both caffeic and ferulic acids as well as naringenin and quercetin [20–24]. Bee bread is also a valuable source of minerals, especially potassium [25]. The content of these compounds, especially phenolic compounds having antioxidant properties in particular, contributes to the high health-promoting value of bee bread [17,21–24]. With its unique chemical composition, including content of bioactive compounds as well as health-promoting properties, bee bread can be an excellent dietary supplement and a functional food ingredient. However, due to its sensory characteristics, especially its specific aroma and taste, its sensory acceptability may be limited. For this reason, the most natural way of implementing bee bread into a diet is adding it to a honey. In our previous studies, we analyzed the influence of propolis or bee pollen addition to a honey on its antioxidant properties [15,16]. Therefore, the purpose of this study was to determine the effect of honey enrichment with bee bread on its phenolic content and antioxidant activity, as well as its sensory and quality characteristic. These comprehensive research results will indicate the possibility of using bee bread as a bioactive ingredient introduced into the human diet, also taking into account the sensory characteristics and quality parameters of the honey enriched in this way as a functional food.

2. Materials and Methods

2.1. Materials

Multifloral honey purchased from District Beekeeping Cooperative "Pszczelarz", Krakow, Poland and micronized bee bread supplied by the Biopharmaceutical Laboratory "Arria", Krakow, Poland, were used as the investigated materials.

On the basis of the preliminary sensory assessment, the proposed maximum amount of bee bread used as a component of honey was no higher than 25%. For this reason, the honey samples were supplemented with bee bread in the levels of 5, 10, 15, 20, and 25% per mass of honey. The tested samples were prepared in an amount of 500 g by adding the micronized bee bread in the appropriate amount and mixing thoroughly. The samples prepared in this way were stored at room temperature in glass containers until analysis.

2.2. Analytical Methods

2.2.1. Total Phenolic, Flavonoids, Phenolic Acids, Anthocyanins, and Carotenoids Content

The antioxidant properties of multifloral honey and the honey samples supplemented with bee bread were determined using ethanolic/water (50:50, *v*/*v*) extracts with a concentration of 0.2 g/mL. The spectrophotometric analyses were performed using a UV/Vis V-630 spectrophotometer (Jasco, Tokyo, Japan).

The total phenolic content (TPC) was evaluated by a Folin–Ciocalteu method described by Singelton and Rossi [26]. The obtained results were calculated as gallic acid equivalents in mg GAE/100 g of the sample.

The total flavonoid content (TFC) was estimated in reaction with $AlCl_3$ using the method reported by Ardestani and Yazdanparast [27]. The obtained results were calculated as quercetin equivalents in mg QE/100 g of the sample.

The total phenolic acids content (TPAC) was analyzed in reaction with Arnov's reagent using the protocol described by Nalewajko-Sieliwoniuk et al. [28]. The obtained results were calculated as caffeic acid equivalents in mg CAE/100 g of the sample.

The total anthocyanins content (TAC) was determined using the method described by Rababah et al. [29], and the obtained results were expressed as cyanidin-3-glucoside equivalents in mg CGE/100 g of the sample.

The total carotenoids content (TCC) was analyzed using a method described by Boussaid et al. [2] and the obtained results were expressed as β-carotene equivalents in mg β-CE/100 g of the sample.

2.2.2. Determination of Phenolic Compounds Profile

The contents of particular phenolic compounds were determined after extraction of the samples using ethyl acetate. The chromatographic analysis of phenolic compounds present in the investigated samples was performed with the use of HPLC (LC-Net II/ADC, Jasco, Japan) equipped with a DAD detector and Purospher RP-18 column (Merck, Germany) using gradient elution (acetic acid water solution (2.5%, v/v)/acetonitrile) at the flow rate of 1 mL/min and at the temperature of 30 °C [15,16].

2.2.3. Antioxidant Capacity

Determination of total antioxidant capacity (TAC) was performed according to the protocol described by Prieto et al. [30] and the obtained results were calculated as ascorbic acid equivalents (AAE) in μM/100 g.

Determination of antiradical activity in the reaction with $DPPH^{\bullet}$ was analyzed according to the procedure described by Blois et al. [31] and the obtained results were calculated as trolox equivalents (TE) in μM/100 g.

Determination of antiradical activity in the reaction with $ABTS^{\bullet+}$ was conducted according to the method described by Baltrušaitytė et al. [22] and the obtained results were calculated as trolox equivalents (TE) in μM/100 g.

The ferric reducing ability power (FRAP) was determined using the method described by Benzie et al. [32] and the obtained results were calculated as μM Fe(II)/100 g.

The cupric reducing ability (CUPRAC) was performed in reaction with neocuproine (Sigma-Aldrich, Germany) according to the protocol described by Apak et al. [33] and the obtained results were expressed as trolox equivalents (TE) in μM/100 g.

2.2.4. Sensory Characteristic

The sensory characteristic of samples was performed with the use of assessments made by the 14-person sensory panel with confirmed sensory sensitivity. The sensory characteristic was determined by a quantitative description method in accordance with the PN-EN ISO 13299:2016 standard [34]. The parameters assessed during the assessment were color, smell, texture, and taste. The perception intensity was rated on a scale from imperceptible (0 points) to very strongly perceptible (5 points). In order to assess the samples' acceptance, the hedonic scale was used. Color, consistency, smell, and taste were estimated with a scale from "I dislike very much" (0 points) to "I like very much" (7 points) according to the standard PN-ISO 4121:2003 [35].

2.2.5. Analysis of Quality Parameters

The quality parameters of samples were determined in an agreement with the regulations of the Ministry of Agriculture and Rural Affairs in Poland [36]. The gravimetric method was

used in order to determine the content of water-insoluble matter. Free acidity was determined using a titration method. Specific conductivity was determined using a conductometer. The saccharides (i.e., glucose, fructose, and sucrose) and 5-hydroxymethyfurfural (HMF) content was determined by HPLC (LaChrom D-7000, Merck-Hitachi, Tokyo, Japan). The content of proline was determined by the spectrophotometric method.

All the analyzes were performed in triplicate, and all details of the individual analytical procedures are available in our previous papers [15,16].

2.3. Statistical Analyses

The results were calculated as means of the three independent repetitions ± SD. The statistical differences between the mean values were evaluated by a one-way Anova and a Fisher LSD test (significance of 0.05). The Pearson linear correlations between the studied variables were calculated, and their significance was assessed at the level of 0.05. The Statistica 11.0 software (StatSoft Inc., Tulsa, OK, USA) was performed for calculations.

3. Results and Discussion

3.1. Total Phenolic Content

The obtained results for the TPC in honey and the samples enriched with bee bread are presented in Table 1. The TPC in honey was 30.75 mg GAE/100 g. The increasing bee bread content in the samples resulted in a significant increase in TPC, from 64.29 mg GAE/100 g for 5% enrichment to 158.96 mg GAE/100 g for the maximum bee bread level in the multifloral honey. Thus, the increase in phenolic content was above 500%. Socha et al. [37] determined the total phenolic content in the commercial honeys supplemented with bee bread at a level of 10% and 20%. The mean level of those compounds was 73.51 mg GAE/100 g and 138.15 mg GAE/100 g for the honey with 10 and 20% addition of bee bread, respectively. In the other studies focused on the commercial honeys with bee bread addition, the mean TPC was estimated at a level of 179.96 mg GAE/100 g [10]. Majewska et al. [38] demonstrated that phenolic content in the product called as "bee bread in honey" containing over 50% of bee bread was as high as 626 mg GAE/100 g.

Table 1. Total phenolics (TPC), flavonoids (TFC), phenolic acids (TPAC), anthocyanins (TAC), and carotenoids content (TCC) in the honey and samples supplemented with bee bread.

Components	Bee Bread Content (%)					
	0	5	10	15	20	25
TPC (mg GAE/100 g)	$30.75^{a} \pm 0.25$	$64.29^{b} \pm 0.46$	$85.77^{c} \pm 0.91$	$106.61^{d} \pm 0.20$	$138.92^{e} \pm 0.26$	$158.96^{f} \pm 0.21$
TFC (mg QE/100 g)	$2.77^{a} \pm 0.29$	$7.86^{b} \pm 0.42$	$11.85^{c} \pm 0.56$	$13.45^{d} \pm 0.11$	$17.08^{e} \pm 0.46$	$21.15^{f} \pm 0.12$
TPAC (mg CAE/100 g)	$11.02^{a} \pm 0.68$	$16.45^{b} \pm 1.15$	$22.56^{c} \pm 0.33$	$25.08^{d} \pm 0.31$	$29.14^{e} \pm 0.84$	$35.47^{f} \pm 0.93$
TAC (mg CGE/100 g)	$2.01^{a} \pm 0.05$	$4.74^{b} \pm 0.20$	$6.70^{c} \pm 0.08$	$9.34^{d} \pm 0.23$	$12.29^{e} \pm 0.24$	$15.66^{f} \pm 0.72$
TCC (mg β-CE/100 g)	$0.14^{a} \pm 0.00$	$0.55^{b} \pm 0.01$	$0.80^{c} \pm 0.00$	$1.33^{d} \pm 0.00$	$1.52^{e} \pm 0.01$	$2.37^{f} \pm 0.01$

The mean values in lines denoted with different superscripts differ significantly ($p < 0.05$).

The available literature data indicate that the high number of polyphenolic compounds in bee bread, to a large extent, translates into an increase in their levels in the honeys supplemented with that bee product. In the studies on total phenolic content in bee bread originating from Romania and India, Urcan et al. [39] reported that this content ranged from 567 to 1283 mg GAE/100 g, while the samples of bee bread originating from Colombia were characterized by TPC of 250 to 1370 mg GAE/100 g [40]. Mayda et al. [41] determined the TPC in bee bread in a range from 826 to 4342 mg GAE/100 g, and Ivanišová et al. [42] found it in a range from 1236 to 2544 mg GAE/100 g. Sawicki et al. [8] demonstrated that the TPC in bee bread was lower than that in bee pollen and amounted to 823 mg GAE/100 g, on average. Furthermore, Urcan et al. [39] reported that the phenolic compounds profile in the bee bread was very similar to that of the corresponding bee pollen, despite biochemical processes occurring during its fermentation and maturing. They also demonstrated that

the plant species influenced the total phenolic content and profile, while factors such as soil type and climate have a limited influence on a presence of those compounds.

Flavonoids are also thought to be another important group of bioactive substances found in honey. They are transferred to a honey with such bee constituents as pollen and propolis, hence their limited content was determined in honey. The TFC in multifloral honey was assessed to be at a level of 2.77 mg QE/100 g (Table 1). The obtained result was slightly lower than the values found in the literature for Polish multifloral honeys [10,37]. The honeys supplemented with bee bread were characterized by a significant increase in their TFC, from 7.87 mg QE/100 g to 21.15 mg QE/100 g for 5% and the highest (25%) bee bread addition, respectively (Table 1). Thus, the increase in flavonoids content was about 763%. The results of the previous studies indicated that the TPC in the samples of commercial honeys supplemented with bee bread ranged from 13.56 to 38.92 mg QE/100 g [10] and from 26.72 to 48.31 mg QE/100 g [37]. A significant increase in the total flavonoids content in the enriched honeys results from their high content in bee bread itself. According to Mayda et al. [41], the flavonoids content in bee bread ranges from 181 to 444 mg QE/100 g. Sawicki et al. [8] determined the TFC in bee bread as ranging from 181 to 374 mg QE/100 g, and demonstrated that it was lower than that in the bee pollen. In this study, a significant linear Pearson correlation (r = 0.9942) was found between the TPC and TFC. In the analyzed multifloral honey, the TPAC content was found to be at a level of 11.02 mg CAE/100 g and within the range found by Pieszko et al. [43], who determined the TPAC in honey to be within the range from 6.3 to 18.11 mg CAE/100 g. The bee bread added to the honey at a level from 5% to 25% also increased the TPAC, similarly as in the case when honey was supplemented with bee pollen [16]. With the highest level of bee bread (25%), the TPAC increased by over three times when compared to the honey that was not enriched, and amounted to 35.47 mg CAE/100 g (Table 1). A significant Pearson linear correlation (r = 0.9920) was also found between the TPC and TPAC.

In the studied multifloral honey, anthocyanins content was determined at a level of 2.01 mg/100 g (Table 1) and was within a range given in the literature. According to Alqarni et al. [44], the multifloral honeys contained from 1.07 to 1.38 mg of anthocyanins per 100 g, while in the multifloral honeys originating from the various regions of Jordan, the anthocyanins content was determined in a range from 0.91 to 4.10 mg/100 g [29]. The bee bread addition resulted in a significant increase in anthocyanins content. The TAC in honey with 5% bee bread addition amounted to 4.74 mg/100 g, while in the case of maximum bee bread content, it rose to the value of 15.66 mg/100 g. Therefore, it can be supposed that bee bread is also a rich source of those compounds. A significant Pearson linear correlation (r = 0.9942) was also observed between the TPC and TAC.

The results for carotenoids content in the studied honeys are listed in Table 1. The amount of this group of dyes in the multifloral honey was much lower in comparison to that of anthocyanins, and amounted to 0.14 mg/100 g. The determined carotenoids content was at a lower limit of the range found by Boussaid et al. [2]. The carotenoids content in the honeys supplemented with bee bread increased similarly as in the case of bee pollen addition [16], reaching the value of 2.37 mg β-CE/100 g at a 25% level of addition of bee bread (Table 1). A significant Pearson linear correlation (r = 0.9973) was also calculated between the TPC and TCC.

3.2. Phenolic Acids and Flavonoids Content

Phenolic acids and flavonoids are the main components of a honey which determinate its antioxidant properties. Their content is associated with the honey type and its geographical origin [5,7,9,11]. The results obtained on a basis of chromatographic analysis in the form of chromatograms for the honey sample without the addition of bee bread and that with the highest 25% addition of bee bread are shown in Figures 1 and 2. Table 2 lists the results for the phenolic acids identified and determined in the analyzed samples. Gallic acid turned out to be the phenolic acid found in the highest amount. Its content in the honey was 0.217 mg/100 g. The rising bee bread addition resulted in the increase in content of gallic acid in a range from

0.262 to 1.104 mg/100 g. That increase was lower than in the case of the samples enriched with bee pollen, analyzed during the previous study [16], and this implies that bee bread contains a lower amount of that compound. Socha et al. [37] determined gallic acid at a higher level in the commercial honeys supplemented with bee bread. The presence of gallic acid in bee bread at a level of 3.26 mg/100 g was confirmed by Sawicki et al. [8].

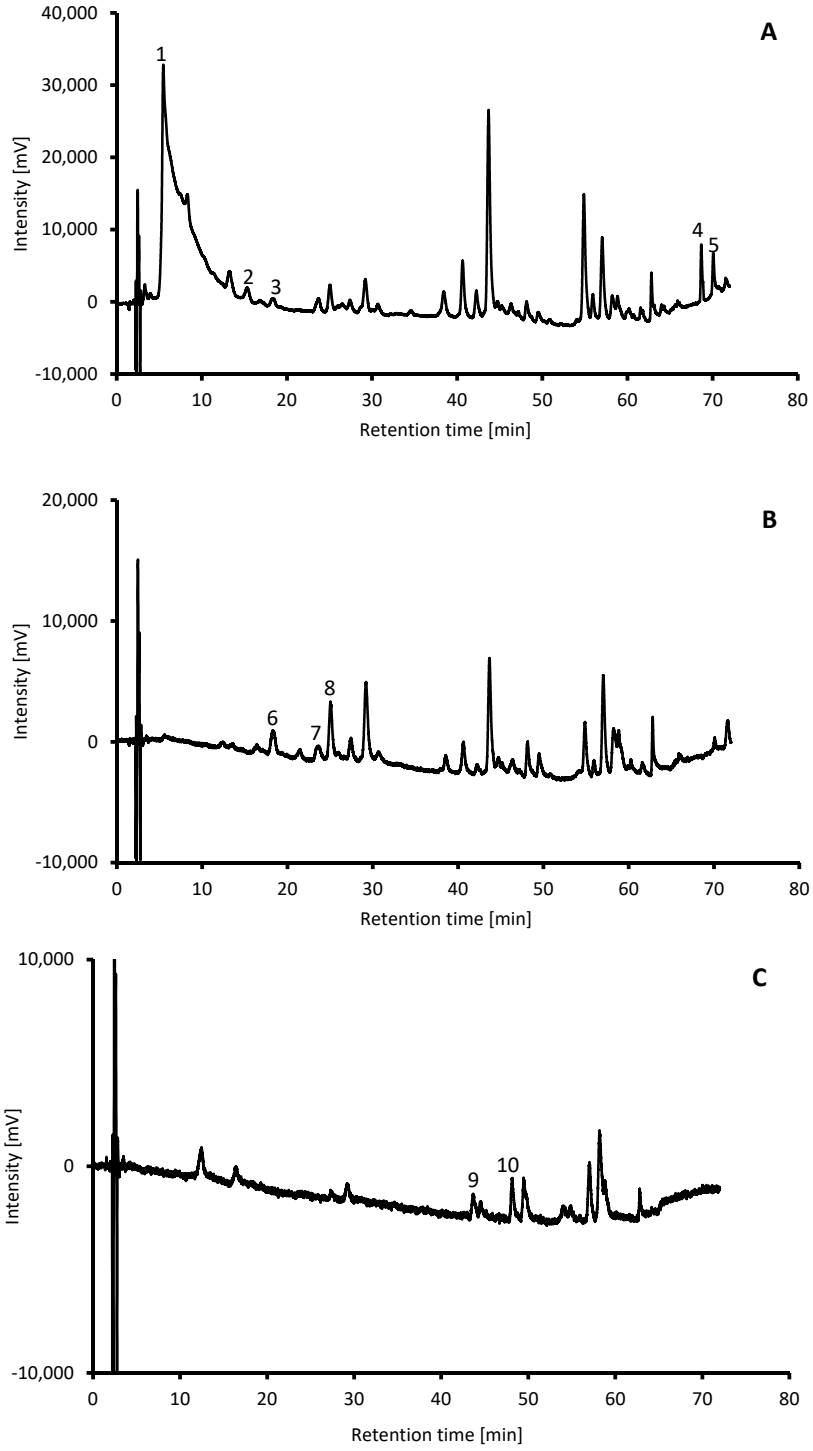

Figure 1. The exemplary chromatograms for the sample of honey without the addition of bee bread with detection at 280 nm (**A**); 320 nm (**B**); and 360 nm (**C**). Designations: 1—gallic acid; 2—protocatechuic acid; 3—*p*-hydroxybenzoic acid; 4—chrysin; 5—galangin; 6—caffeic acid; 7—*p*-coumaric acid; 8—ferulic acid; 9—quercetin; 10—kaempferol.

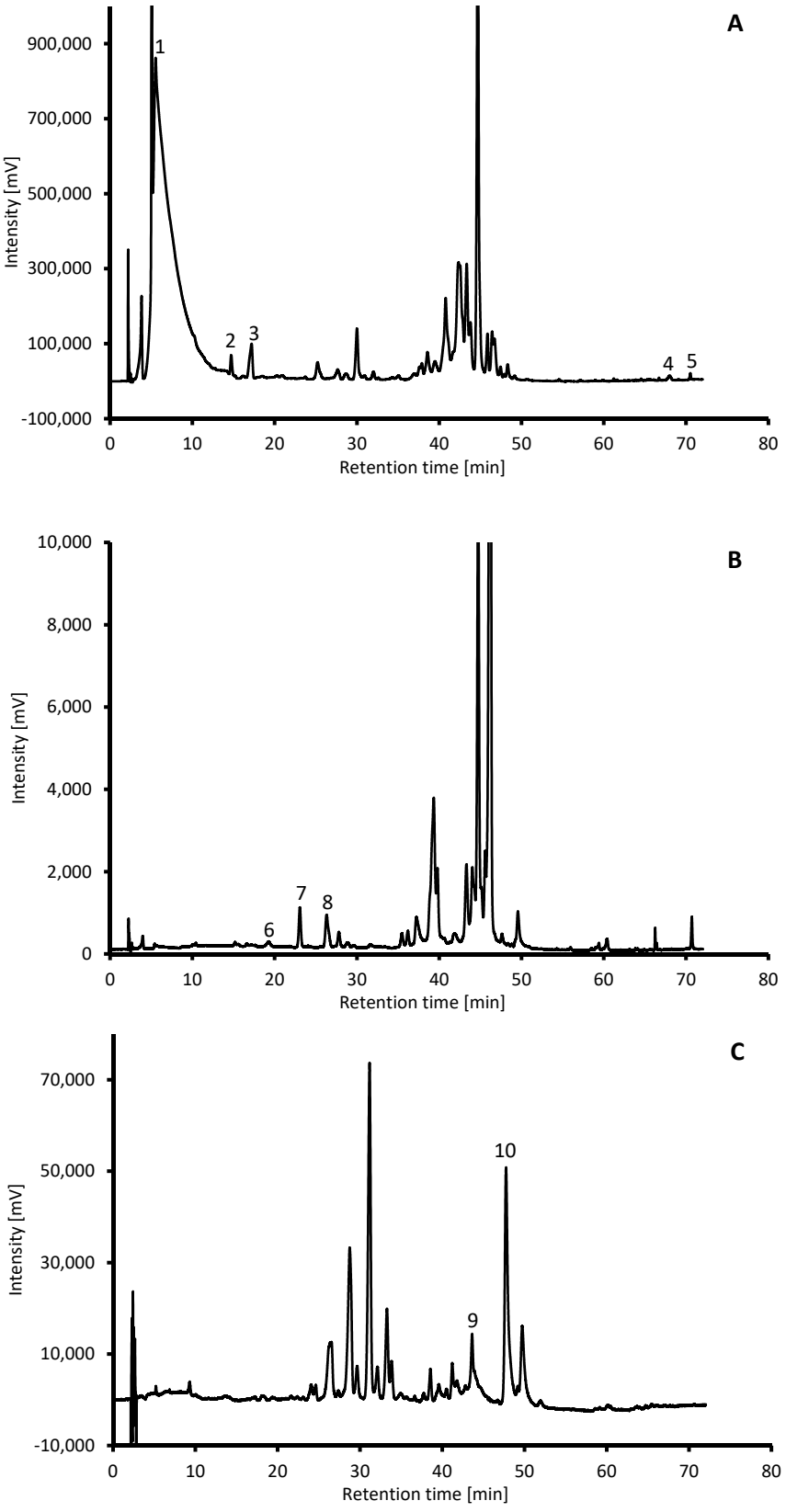

Figure 2. The exemplary chromatograms for the sample of honey with 25% of bee bread with detection at 280 nm (**A**); 320 nm (**B**); and 360 nm (**C**). Designations: 1—gallic acid; 2—protocatechuic acid; 3—*p*-hydroxybenzoic acid; 4—chrysin; 5—galangin; 6—caffeic acid; 7—*p*-coumaric acid; 8—ferulic acid; 9—quercetin; 10—kaempferol.

Table 2. Phenolic acids and flavonoids content (mg/100 g) in the honey and samples supplemented with bee bread.

Phenolic Acid/Flavonoid	Bee Bread Content (%)					
	0	5	10	15	20	25
Ferulic	0.095 ^{a} ± 0.005	0.091 ^{a} ± 0.005	0.176 ^{b} ± 0.006	0.192 ^{c} ± 0.008	0.210 ^{d} ± 0.005	0.229 ^{e} ± 0.005
Gallic	0.217 ^{a} ± 0.004	0.262 ^{b} ± 0.019	0.301 ^{c} ± 0.014	0.396 ^{d} ± 0.010	0.471 ^{e} ± 0.010	1.104 ^{f} ± 0.034
p-Hydroxybenzoic	0.040 ^{a} ± 0.003	0.059 ^{b} ± 0.001	0.089 ^{c} ± 0.002	0.102 ^{d} ± 0.001	0.122 ^{e} ± 0.002	0.140 ^{f} ± 0.006
Caffeic	0.026 ^{a} ± 0.000	0.061 ^{b} ± 0.004	0.069 ^{b} ± 0.002	0.079 ^{c} ± 0.002	0.093 ^{d} ± 0.004	0.159 ^{e} ± 0.014
p-Coumaric	0.136 ^{a} ± 0.006	0.155 ^{b} ± 0.004	0.203 ^{c} ± 0.004	0.313 ^{d} ± 0.006	0.404 ^{e} ± 0.006	0.454 ^{f} ± 0.003
Protocatechuic	0.070 ^{a} ± 0.003	0.068 ^{a} ± 0.001	0.080 ^{b} ± 0.004	0.102 ^{c} ± 0.002	0.127 ^{d} ± 0.001	0.154 ^{e} ± 0.002
Chrisin	0.014 ^{a} ± 0.001	0.023 ^{b} ± 0.001	0.029 ^{c} ± 0.001	0.041 ^{d} ± 0.001	0.054 ^{e} ± 0.000	0.067 ^{f} ± 0.001
Galangin	0.023 ^{a} ± 0.001	0.032 ^{b} ± 0.002	0.043 ^{c} ± 0.002	0.051 ^{d} ± 0.001	0.070 ^{e} ± 0.001	0.107 ^{f} ± 0.002
Kaempferol	0.049 ^{a} ± 0.004	0.113 ^{b} ± 0.003	0.198 ^{c} ± 0.006	0.280 ^{d} ± 0.015	0.330 ^{e} ± 0.008	0.483 ^{f} ± 0.003
Quercetin	0.040 ^{a} ± 0.001	0.293 ^{b} ± 0.011	0.327 ^{c} ± 0.010	0.452 ^{d} ± 0.011	0.522 ^{e} ± 0.002	0.868 ^{f} ± 0.041

The mean values in lines denoted with different superscripts differ significantly ($p < 0.05$).

In the analyzed honey, a large amount of *p*-coumaric acid was also found. The increasing bee bread level also resulted in the rising content of that phenolic acid in honey, reaching 0.454 mg/100 g in the case of the maximum addition of that product (Table 2). Socha et al. [37] analyzed the commercial honeys supplemented with bee bread and found that *p*-coumaric acid content was within a wider range (0.11–1.68 mg/100 g). The presence of *p*-coumaric acid in bee bread was earlier confirmed by Isidorov et al. [20] and Baltrušaitytė et al. [22]. Ferulic acid came next in order in the terms of its content. Its level in the honey was 0.095 mg/100 g (Table 2), and an increasing bee bread addition in the honey influenced the growth in the discussed phenolic acid to the amount of 0.229 mg/100 g for maximum enrichment. The presence of that acid in bee bread was earlier confirmed by Isidorov et al. [20]. Protocatechuic acid was the next determined phenolic acid in terms of its content in the honey. With respect to the analyzed honey sample, it was found to be at an amount of 0.070 mg/100 g (Table 2). The rising bee bread addition in the honey resulted in the increase in that acid content to a level of 0.154 mg/100 g. That increase was lower than in the case of honey enriched with bee pollen [16], and this suggests that bee bread contains a lower amount of that compound. The presence of protocatechuic acid in bee bread was earlier reported by Sawicki et al. [8].

p-Hydroxybenzoic acid content in the honey was found to be at a level of 0.040 mg/100 g (Table 2). When honey was enriched with bee bread, its levels rose to 0.140 mg/100 g for its maximum content (Table 2). The rising bee bread addition in the multifloral honey also influenced caffeic acid content in that honey. Its amount was 0.061 mg/100 g for 5% enrichment and rose to 0.159 mg/100 g for the maximum bee bread addition. The identified increase in the content of caffeic acid was 612% when compared to the honey without the addition of bee bread. Socha et al. [37] determined caffeic acid content within a range from 0.09 to 0.77 mg/100 g in the commercial honeys supplemented with bee bread at a level of 10% or 20%. Isidorov et al. [20] found only the traces of caffeic acid in Polish and Lithuanian bee bread. Flavonoids are a known group of antioxidants present in honey and other bee products. Their content and type significantly determine the honey antioxidant activity. The bee bread addition is responsible for an increase in quercetin content in honey. Quercetin is a flavonoid with the highest share in the entire group of these compounds determined in the analyzed samples. The content of this flavonoid in honey with 25% addition of bee bread increased above 20 times, to the level of 0.868 mg/100 g (Table 2). This value is within the range (0.19–1.1 mg/100 g) reported by Socha et al. [37] for the honeys with 10% or 20% addition of bee bread. Sawicki et al. [8] did not find quercetin in bee bread, while Bakour et al. [17] and Sobral et al. [24] confirmed the presence of quercetin in bee bread, in the form of glycosides. The presence of quercetin in that bee product was also confirmed by Kalifa et al. [18], while Isidorov et al. [20] found traces of that flavonoid in Polish and Lithuanian bee bread. Čeksterytė et al. [45] determined quercetin levels

in bee bread in the range from 27.41 mg/100 g to 49.58 mg/100 g. The high quercetin level in bee bread directly influences its rising content in honey. Kaempferol was another flavonoid present and determined in the analyzed samples. Its content in honey amounted to 0.049 mg QE/100 g. The presence of that flavonoid in the honeys originating from Poland was earlier confirmed by Socha et al. [7,37] and Halagarda et al. [9]. The bee bread addition at a level of 5% resulted in the increase in kaempferol level to 0.113 mg/100 g; whereas, its maximum level resulted in a ten-fold increase in that flavonoid (Table 2). An increase in kaempferol content in the honey supplemented with bee bread was lower than that determined for honey with bee pollen addition [16]. Socha et al. [37] found a lower kaempferol content in the commercial honeys with 10% addition of bee bread, while the content of that compound was much higher in honeys with 20% addition of bee bread. Čeksterytė et al. [45] and Isidorov et al. [20] reported that content of kaempferol in bee bread ranged from 3.99 to 49.68 mg QE/100 g. Baltrušaitytė et al. [22] found that kaempferol is the main flavonoid found in bee bread. Bakour et al. [17] and Sobral et al. [24] confirmed the presence of kaempferol in bee bread, in the form of glycosides. The high kaempferol level in bee bread led to an increase in its content in the studied honeys enriched with that bee product. Galangin is yet another identified and determined flavonoid, whose content was noted to increase when honey was enriched with bee bread. The content of this flavonoid in honey amounted to 0.023 mg QE/100 g, and its presence in the samples of honeys originating from Poland was earlier confirmed by Socha et al. [7,37] and Halagarda et al. [9]. The maximum addition of bee bread (25%) resulted in an elevation of that flavonoid level to 0.107 mg/100 g (Table 3) and that increase was lower than when honey was enriched with bee pollen [16]. Socha et al. [37] determined a higher galangin content in the samples of commercial honeys with 10% or 20% addition of bee bread. Chrysin is a flavonoid, the contents of which were determined to be at the lowest level (Table 3). The presence of that flavonoid in honeys originating from Poland was previously confirmed by Socha et al. [7,37] and Halagarda et al. [9]. Chrysin content in honey with bee bread addition rose from 0.023 mg/100 g to 0.067 mg/100 g for the highest, 25% addition of that ingredient (Table 3). Socha et al. [37] determined a higher content of chrysin in commercial honeys with bee bread addition at a level of 10% or 20%. Isidorov et al. [20] found traces of chrysin in Polish and Lithuanian bee bread. In addition, Baltrušaitytė et al. [22] confirmed the presence of that flavonoid in this bee product.

Table 3. Total antioxidant activity (TAA), antiradical activity ($ABTS^{\bullet+}$, $DPPH^{\bullet}$), and reducing power (FRAP, CUPRAC) of the honey and samples supplemented with bee bread.

Activity	Bee Bread Content (%)					
	0	5	10	15	20	25
TAA (mM AAE/100 g)	$9.24^{a} \pm 0.12$	$10.40^{b} \pm 0.26$	$11.17^{c} \pm 0.20$	$11.74^{d} \pm 0.07$	$12.31^{e} \pm 0.15$	$12.85^{f} \pm 0.31$
$ABTS^{\bullet+}$(mM TE/100 g)	$1.78^{a} \pm 0.02$	$5.22^{b} \pm 0.07$	$8.50^{c} \pm 0.39$	$10.49^{d} \pm 0.19$	$11.82^{e} \pm 0.26$	$13.51^{f} \pm 0.18$
$DPPH^{\bullet}$ (mMTE/100 g)	$0.26^{a} \pm 0.00$	$0.90^{b} \pm 0.00$	$1.44^{c} \pm 0.01$	$1.73^{d} \pm 0.00$	$2.15^{e} \pm 0.01$	$2.47^{f} \pm 0.01$
FRAP (μM Fe(II)/100 g)	$233.9^{a} \pm 0.7$	$486.1^{b} \pm 0.4$	$658.5^{c} \pm 0.8$	$856.2^{d} \pm 0.8$	$1018.4^{e} \pm 0.5$	$1214.9^{f} \pm 0.8$
CUPRAC (μMTE/100 g)	$77.8^{a} \pm 1.4$	$276.2^{b} \pm 1.6$	$298.3^{c} \pm 2.7$	$357.7^{d} \pm 2.5$	$395.6^{e} \pm 2.50$	$429.0^{f} \pm 2.9$

The mean values in lines denoted with different superscripts differ significantly ($p < 0.05$).

3.3. Antioxidant Activity

The presence of polyphenolic compounds in bee products contributes to their antioxidant properties. The obtained results of total antioxidant activity (TAA) determined for the honey and samples supplemented with bee bread are collected in Table 3. The total antioxidant activity of the multifloral honey was at a level of 9.24 mM AAE/100 g (Table 3). The total antioxidant activity of the multifloral honeys analyzed by Meda et al. (2005) was determined within the range of 10.20 to 37.87 mM AAE/100 g. The increasing bee bread addition contributed to the elevation in the honey's antioxidant activity at a level of 10.40 to 12.85 mM AAE/100 g, and this resulted from the replacement of the part of honey

with the bee bread that exhibited a higher antioxidant capacity, when compared to that for the honey. The same trend was previously observed by Habryka et al. [16] when they enriched honeys with bee pollen. It was also found that much lower additions of propolis (below 1%) added to honey resulted in a similar increase in its total antioxidant activity [15]. Furthermore, the significant linear Pearson correlations were found between the TAA and the TPC (r = 0.9700), TFC (r = 0.9810), and TPAC (r = 0.9760).

The basic tests evaluating antioxidant properties are the spectrophotometric methods based on the reactions of reduction in the stable synthetic free radicals or metal ions. The results of analysis of antiradical activity against ABTS and DPPH radicals, and the reductive capacity of metal ions are presented in Table 3. Antiradical activity of the multifloral honey in the reaction with $ABTS^{\bullet+}$ was determined at a level of 1.78 mM TE/100 g. The antiradical activity of the studied samples increased in a range from 5.22 to 13.5 mM TE/100 g along with an increased addition of bee bread to the honey. In addition, Juszczak et al. [10] observed rising antiradical activity of the commercial samples supplemented with bee bread. Furthermore, significant linear correlations were observed between the antioxidant activity against $ABTS^{\bullet+}$ and the TPC (r = 0.9820), TFC (r = 0.9860), and TPAC (r = 0.9840). The antiradical activity of analyzed honey and the samples supplemented with bee bread was also studied in the reaction with $DPPH^{\bullet}$ (Table 3). Similarly, as for the ABTS method, the addition of bee bread in honey led to an increase in the antiradical activity, to the level of 2.47 mM TE/100 g for its highest share. This confirms earlier observations for the commercial honey samples supplemented with bee bread [10,37]. The significant linear Pearson correlations were found between the antioxidant activity against $DPPH^{\bullet}$ and the TPC (r = 0.9930), TFC (r = 0.9950), and TPAC (r = 0.9920).

The reductive capacity of multifloral honey against iron ions was determined at a level of 233.9 µM Fe(II)/100 g (Table 3). That result is within the range reported for the multifloral honeys originated from Poland [10]. The supplementation of honey with bee bread resulted in an increase in the reductive capacity of the studied samples. A 5% level of bee bread led to an increase in the reductive capacity to the level of 486.1 µM Fe(II)/100 g; whereas, for the maximum addition of bee bread (25%), the reductive capacity reached 1214.9 µM Fe(II)/100 g (Table 4). Juszczak et al. [10] also found an increase in the reductive capacity in a range from 424 to 976 µM Fe(II)/100 g of the commercial honeys supplemented with bee bread. In addition, Socha et al. [37] observed an increase in the reductive capacity of the commercial honeys supplemented with bee bread, when compared to the natural honey. The earlier studies indicated that the presence of bee pollen as an ingredient in a honey resulted in a slight increase in the honey reductive capacity [16]. Furthermore, significant linear Pearson correlations were found between the reduction activity against iron ions and the TPC (r = 0.9970), TFC (r = 0.9950), and TPAC (r = 0.9940).

Table 4. The quality parameters of the studied honey and honey supplemented with bee bread.

Parameter	Bee Bread Content (%)					
	0	5	10	15	20	25
Insoluble matter (g/100 g)	$0.06^{a} \pm 0.01$	$1.54^{b} \pm 0.01$	$2.93^{c} \pm 0.01$	$4.79^{d} \pm 0.01$	$6.20^{e} \pm 0.02$	$7.75^{f} \pm 0.02$
Free acidity (mval/kg)	$22.9^{a} \pm 0.2$	$57.1^{b} \pm 0.5$	$85.9^{c} \pm 1.4$	$119.0^{d} \pm 2.0$	$149.3^{e} \pm 1.6$	$187.1^{f} \pm 0.9$
Specific conductivity (mS/cm^3)	$0.50^{a} \pm 0.00$	$0.69^{b} \pm 0.00$	$0.85^{c} \pm 0.01$	$1.02^{d} \pm 0.00$	$1.18^{e} \pm 0.01$	$1.33^{f} \pm 0.01$
Glucose + fructose (g/100 g)	$65.2^{e} \pm 1.4$	$63.6^{e} \pm 0.6$	$60.5^{d} \pm 0.3$	$58.5^{c} \pm 0.5$	$55.4^{b} \pm 0.7$	$53.3^{a} \pm 0.6$
Sucrose (g/100 g)	$1.83^{b} \pm 0.06$	$1.66^{ab} \pm 0.07$	$1.79^{b} \pm 0.06$	$1.45^{a} \pm 0.11$	$1.45^{a} \pm 0.14$	$1.57^{a} \pm 0.08$
Proline content (mg/100 g)	$32.3^{a} \pm 0.4$	$76.5^{b} \pm 1.3$	$122.0^{c} \pm 1.2$	$170.5^{d} \pm 1.1$	$195.6^{e} \pm 0.6$	$286.7^{f} \pm 5.8$

The mean values in lines denoted with different superscripts differ significantly ($p < 0.05$).

The reductive activity of the multifloral honey determined using the CUPRAC method was 77.81 µM TE/100 g (Table 3). Correspondingly, as in the case of the FRAP method, the supplementation of honey with bee bread led to a significant rise in reductive capacity of honey. Indeed, 5% enrichment of honey with bee bread resulted in an increase in its

reductive capacity to the level of 276.2 µM TE/100 g, while for the maximum supplementation, it reached the value of 429.0 µM TE/100 g (Table 3). A similar range of the reductive capacity of the honeys supplemented with bee pollen against copper ions was previously observed by Habryka et al. [16]. Also in this case, significant linear Pearson correlations were found between the reductive activity against copper ions and the TPC (r = 0.9410), TFC (r = 0.9480), and TPAC (r = 0.9320).

3.4. Sensory Characteristics

Figure 3a demonstrates the average results of color assessment of multifloral honey samples with increasing addition of bee bread. The investigated honey was estimated as very bright, clear, and highly uniform. The increasing bee bread addition led to a decrease in the brightness of the honey (Figure 3a). The addition of this ingredient at the highest level of 25% reduced honey brightness to the average value of 1.07. The addition of bee bread also resulted in a reduction in the score for uniformity and clarity of the honey (Figure 3a). The reduced brightness, clarity, and uniformity of the honey samples enriched in bee bread resulted in a clearly visible increase in the assessment of cloudiness.

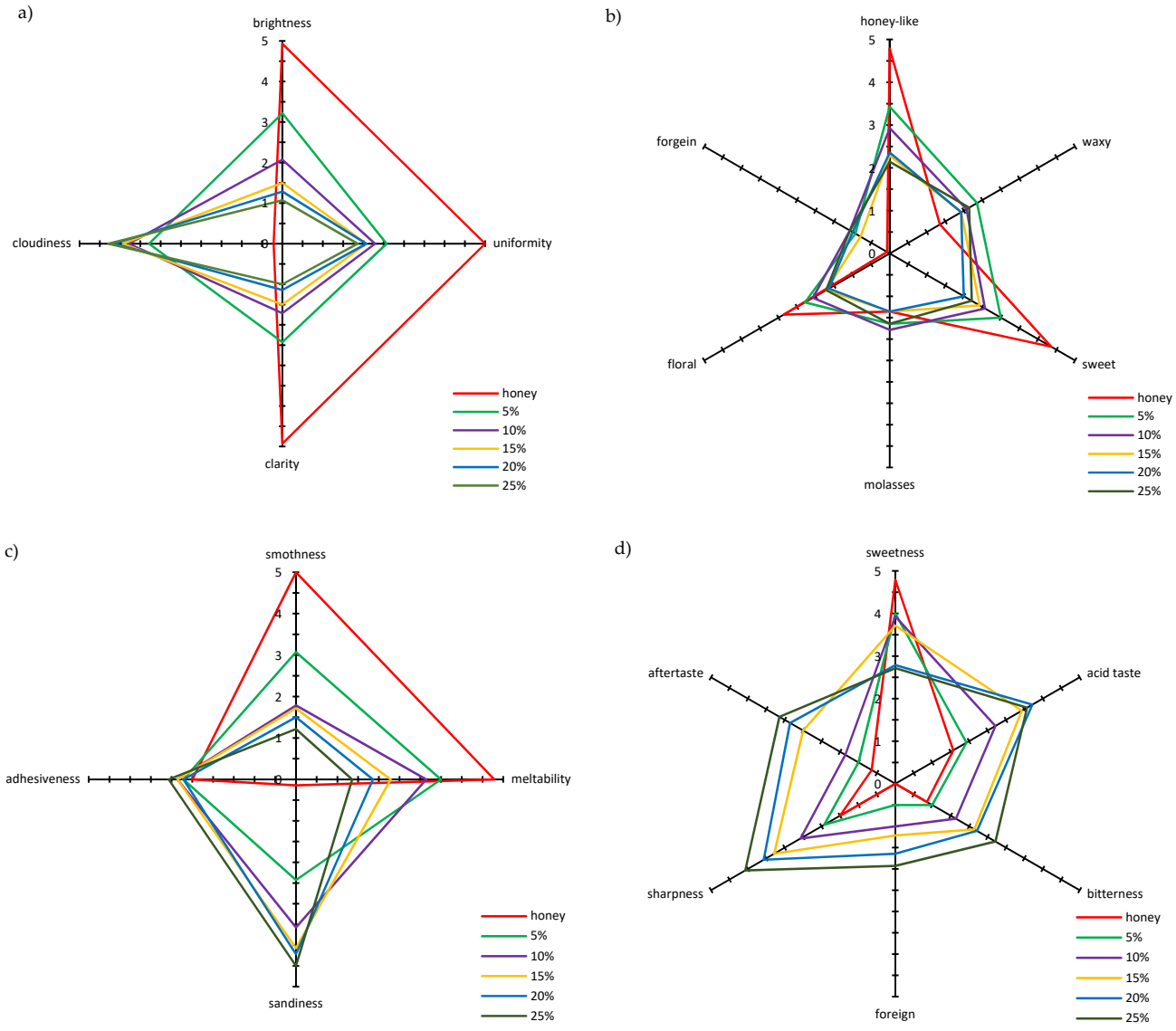

Figure 3. The results of sensory profiling analysis for (**a**) color, (**b**) smell, (**c**) consistency, and (**d**) taste of the multifloral honey and honeys enriched with bee bread.

The smell of multifloral honey without bee bread addition was described as strongly pronounced, sweet, and honey-like, while the floral aroma was described as moderately noticeable (Figure 3b). In contrast, aromas reminiscent of molasses and wax were rated as hardly perceptible, and foreign smell was rated as undetectable. The increasing addition of bee bread to honey significantly reduced the intensity of sweet and honey-like aromas. It was also stated that the addition of bee bread to honey only slightly influenced the intensity of the molasses and wax aromas. Furthermore, there was no significant influence of bee bread supplementation on the intensity of foreign odor, which indicates that this ingredient does not introduce a foreign odor while it clearly changes the natural aroma of honey.

When the texture was assessed, the intensity of smoothness, meltability, sandiness, and adhesiveness was considered (Figure 3c). The texture of studied honey had strongly noticeable meltability and smoothness, and moderate adhesiveness, while its sandiness was described as imperceptible. The increasing supplementation with bee bread significantly influenced the intensity of the texture descriptors (Figure 3c). Sandiness increased to very strongly perceptible for the honeys with bee bread addition at the highest level. An increase in adhesiveness was also observed, with a significant drop in meltability and smoothness, which was described by the evaluators as moderate.

In the taste assessment, the multifloral honey was evaluated as very sweet with other features, i.e., sourness, bitterness, sharpness, and aftertaste being slightly perceptible or perceptible at a threshold level. Intensity of the foreign taste was evaluated as unnoticeable (Figure 3d). The increasing bee bread addition significantly affected the taste descriptors. There was a noticeable drop in sweetness, down to moderate intensity with the maximum bee bread concentration. There was a moderately noticeable rise in the intensity of bitter, sour, and sharp taste notes for the samples with the highest bee bread addition. The presence of bee bread also affected the intensity of aftertaste and perception of foreign taste, from barely perceptible up to moderate.

The overall acceptability of the chosen sensory parameters (i.e., color, smell, texture, and taste) of the honeys supplemented with bee bread, as estimated according to the hedonic scale, is presented in Figure 4. The increasing addition of bee bread to honey decreased the acceptability of color, resulting from a marked rise in cloudiness and also a decrease in clarity and transparency (Figure 3a). The significant linear Pearson correlation was found between the color acceptability evaluated using the hedonic scale and the sensory profiling results. Color acceptability was significantly correlated with honey brightness ($r = 0.93$), clarity ($r = 0.88$), uniformity ($r = 0.82$), and cloudiness ($r = -0.83$). Correspondingly, as in the case of color, the supplementation of honey with bee bread caused a drop in acceptability of smell, following from a decrease in the intensity of honey-like, sweet, and floral smells, and an increase in the intensity of waxy smell (Figure 3b). A general acceptability of the smell was significantly correlated with honey-like ($r = 0.97$), sweet ($r = 0.93$), and floral ($r = 0.98$) smells. The bee bread addition to honey decreased the acceptability of its texture, and this should be associated with an increase in the perception of sandiness, and thus a reduction in the desirable parameters of meltability and smoothness. The values of the linear Pearson correlation coefficients indicate an interdependence between acceptability and smoothness ($r = 0.93$) and meltability ($r = 0.99$) and sandiness ($r = -0.94$). The increasing addition of bee bread also decreased the acceptability of taste, related to a reduced perception of sweetness as well as an increased perception of sour, bitter, sharp, and foreign tastes, and of aftertaste. The taste acceptability was positively correlated with perceptible sweetness ($r = 0.94$), while negatively with perception of sour ($r = -0.98$), bitter ($r = -0.99$), foreign ($r = -0.98$), and sharp tastes ($r = -0.99$), and aftertaste ($r = -0.99$).

Figure 4. The acceptability results evaluation of the studied honey and samples supplemented with bee bread.

3.5. Quality Parameters

Table 4 lists values for the selected parameters characterizing the commercial quality of honeys. The content of water-insoluble substances is one of the parameters of honey quality. These substances include pollen, bee bread, and propolis, fragments of bees, and cells of algae and fungi. The water-insoluble substances in studied honey were at a level of 0.06 g of per 100 g, so this met the relevant requirements [46,47]. The supplementation of honey with bee bread significantly contributed to an increase in water-insoluble substances. After the maximum addition of bee bread of 25%, their content was 7.75 g per 100 g. Juszczak et al. [48] reported that addition of bee products to multifloral honey may result in even a four-fold increase in the insoluble solid substances content.

Honey acidity results from the presence of organic acids, mainly produced from glucose with the participation of relevant bee enzymes. Too-high free acidity may be a sign of growth of microorganisms and fermentation of honey. Free acidity was 22.9 mval/kg for the multifloral honey, and this confirms the earlier literature data for multifloral honeys originating from Poland [48] and meets the relevant legal requirements [46,47]. The bee bread addition to honey led to a significant increase in free acidity. At a 5% addition, free acidity increased to 57.1 mval/kg, and with the highest addition of 25%, it was as high as 187.1 mval/kg (Table 4). Bee bread contains both organic acids, including gluconic and lactic acids that are produced during fermentation, as well as large quantities of amino acids, e.g., proline (Table 4), which increases the acidity of honey with bee bread addition. As Dranca et al. [49] reported, free acidity of bee bread can be at a level of 543 mval/kg.

The conductivity of honey solution depends mainly on the mineral and organic acids content. The quality requirements impose the maximum value of conductivity for nectar honeys at a level of 0.8 mS/cm [46,47]. The specific conductivity of studied honey was measured at 0.50 mS/cm (Table 4), which met the legal requirements. The bee bread addition led to a proportional increase in specific conductivity, which was 1.33 mS/cm for the maximum concentration of bee bread (25%). It indicates that minerals and organic acids are introduced along with bee bread, which also increases free acidity. As Dranca et al. [49] report, the ash content of bee bread is 3.4 g/100 g.

The sum of fructose and glucose in nectar honey cannot be lower than 60 g/100 g of honey [46,47]. In the honey under study, the sum of glucose and fructose content was 65.2 g/100 g; therefore, it conforms to legal requirements [46,47]. The bee bread addition contributed to a significant drop in the sum of glucose and fructose (Table 4). In the case of the highest bee bread concentration, the sum of glucose and fructose dropped to

53.3 g/100 g. Bee bead contains fewer simple sugars than honey itself. As Dranca et al. [49] reported, bee bread contained 19.7 g of fructose, and only 8.8 g of glucose per 100 g. For this reason, when part of the honey is replaced by bee bread, the level of these sugars decreases.

Sucrose content, being another quality requirement for honey, may not exceed 5 g/100 g [46,47]. The studied multifloral honey met this requirement (Table 4). As bee bread does not contain a significant amounts of sucrose [49], adding it to honey does not matter here.

Another factor which influences the quality of honey is the presence of 5-hydroxymethylfurfural (5-HMF), the elevated content of which indicates that the honey was improperly stored, overheated to facilitate its filtration, or adulterated with inverted sugar. In case of multifloral honey, the content of HMF was 10.76 mg/kg, and the addition of bee bread did not significantly influence the results. That fact confirms previous data on the commercial honey samples enriched with bee products [47].

The investigated multifloral honey contained 32.3 mg of L-proline per 100 g. Along with the increasing addition of bee bread, a proportional rise in L-proline content was observed, up to 286.7 mg/100 g for 25% addition of this ingredient. Such a significant increase in the content of this amino acid results from a high content of protein substances in bee bread, which Dranca et al. [49] measured at 18.6 g/100 g.

4. Conclusions

Introduction of bee bread into a honey resulted in a significant increase in the total content of phenolic compounds, flavonoids, and phenolic acids. At the same time, with an increase in the total phenolic content by about 517%, the total content of flavonoids increased by about 763%, and phenolic acids by about 322% in relation to the honey without the addition of bee bread. An increase in the individual phenolic acids and flavonoids was diversified and dependent on the amount of bee bread added. In the samples of the honeys enriched with bee bread, gallic acid, whose content increased by about 509% compared to the unenriched honey, was a dominating phenolic acid, while quercetin, whose content increased by about 2170% when compared to unenriched honey, led among flavonoids. An increase in polyphenols content resulted in the rise in antioxidant, antiradical, and reductive capacities. The addition of bee bread to a honey is an appropriate method for introducing this valuable ingredient into the diet, and honey enriched with bee bread can be treated as a functional food with a targeted health-promoting effect. However, the significant changes in sensory characteristics caused by the introduction of bee bread to honey must also be taken into account.

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