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ORIGINAL ARTICLE

Total phenolic and antioxidant bioaccessibilities of cookies enriched with bee pollen

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Abstract

The purpose of this study was to investigate the use of bee pollen (BP; 5%, 10%, 15%) as a functional ingredient in cookies. Evaluation of physicochemical and sensory properties with total phenolic content (TPC) and antioxidant capacities in extractable, hydrolyzable, and bioaccessible fractions of enriched cookies was studied. With the addition of BP, carbohydrate content of the cookies decreased, while ash, total protein and total fat content, spread ratio (SR), the browning index, and hardness of the cookies increased. TPC of the enriched cookies were determined as 352.70–401.13 mg/100 g, of which 92.27%–93.16% were hydrolyzable phenolic content, and only 8.55%–9.26% of the TPC were bioaccessible. In addition, the cookies produced with the addition of BP were accepted in terms of their sensory properties. In the light of the information obtained, it can be said that BP is an ingredient that can improve the quality criteria while improving the functional properties of the cookies.

Practical applications

There is limited information about functional and physico-chemical properties of pollen-fortified cookies. There are scarcely any studies in the literature on the hydrolyzable and bioaccessible of TPC and antioxidant capacity in pollen (none in products with pollen). In this study, the cookies were fortified with BP and the physicochemical, sensory attributes, extractable, hydrolyzable, and bioaccessible of TPC, antioxidant capacities (two different methods; ABTS and DPPH) of the developed products were evaluated.

1 | INTRODUCTION

Pollen, the male reproductive cell of seeded plants, is the main source of protein in many creatures, especially honey bees (DeGrandi-Hoffman et al., 2012). Honeybees (*Apis Mellifera*) harvest the pollen from plant, stored on the back leg of bees and enrich it with salivary enzymes to obtain small granular grains called "bee pollen." These bee pollens are also named as "apicultural pollen," "corbicular pollen," and "bee collected pollen" (CarlosFuenmayor et al., 2014).

Bee Pollen (BP) is known as one of the oldest nutritional supplements in history and contains almost all nourishing components for a diet and due to these qualities, it has been described as "only perfectly complete food" and "the world's best food product" in recent years (Ares et al., 2018; Kieliszek et al., 2018; Morais et al., 2011).

BP; has a very rich content in various amino acids, carbohydrates, saturated and unsaturated fatty acids, lipids, sterols, vitamins, terpenes, phenolic substances, enzymes, and minerals, especially proteins (Avni et al., 2014; Kieliszek et al., 2018; Kostić et al., 2020). These nutritional profile satisfy the main source of carbohydrate and protein nutrition of the entire colony with a protein content ranging from 10% to 40%, carbohydrates between 13% and 55%, and lipids ranging from 1% to10% (Avni et al., 2014). In many studies, pollen has been reported to contain 15–19 amino acids and all essential amino acids. In pollen, the main amino acids that can be found are: cystine, lysine, tryptophan, histidine, phenylalanine, arginine, methionine, aspartic acid, cool, leucine, proline, isolysin, glutamine, and valine. It also contains vitamins A, B complex, C and E, K, Na, Ca, Mg, S, Si, Cu, I, Fe, Mn, Ni, Cl, P, Ti, B, and Zn minerals (Komosinska-Vassev et al.,

2015; Rzepecka-Stojko et al., 2012). Twelve percent of BP is water and 3%–20% is fiber (Blackmore et al., 2007; Bogdanov, 2006). Pollen, containing ~1.6% phenolic compounds, has especially flavonoids, leukotrienes, catechins, phenolic acid, flavanol, and flavanol glucosides. It is defined as a free radical scavenger of the antioxidant activity of BP and an inhibitor of lipid peroxidation. This activity is associated with the phenolic content of the pollen (Adaskeviciute et al., 2019; Ali & Kunugi, 2020; Aličić et al., 2020; Fadzilah et al., 2017).

Pollen's addition to a food matrix generally improves the nutritional, functional, techno-functional, and sensory properties due to the concentration, of the newly formulated food products. This improvement potential is dependent on the physical, functional, thermal, and textural properties of pollen (Conte et al., 2018; Krystyjan et al., 2015).

BP, which has a rich biochemical and phytochemical content, also has many therapeutic properties such as antimicrobial, antitumoral, antibacterial, immunomodulator, anti-inflammatory, as well as nutritional properties (Cornara et al., 2017; Mărgăoan et al., 2019; Pasupuleti et al., 2017; Touzani et al., 2019) because it is also rich in phytochemicals, including phenolic acids, flavonoids, and carotenoids. Phenolic compounds in the food matrix are determined by classical methods using organic solvents. However, the linked forms remaining in the extraction residue, which make up a significant portion of the total phenolic compounds, are often ignored. Therefore, it is important to examine the total amount of phenolic substances in extractable, hydrolyzable, and bioaccessible fractions (Altiner et al., 2021).

Bioaccessibility of phenolic compounds is influenced by differences in cell wall structure, differences in concentration in plant tissues, structure of plant tissues, and conjugation. Although it has a high nutritional value, it is shown that the availability of nutrients and bioactive compounds of BP is low when the pollen is ingested by humans (Zuluaga et al., 2015). This is owing to the outer layer of BP, known as exine that is very elastic, strong, and firm, and it is made of sporopollenin, which protects the compounds that are within the pollen and ensures chemical and enzymatic resistance to pollen (Atkin et al., 2011; Bogdanov, 2006; Zuluaga et al., 2015). Owing to these situations, the practical application of pollen in food processing is still has shortcomings. Given the fact that cookie is most popular bakery foods for all consumer age groups, addition of BP in cookie production to provide a functional cookie and broaden the applications of pollen to innovate in terms of phenolic rich bioaccessible fractions (Krystyjan et al., 2015).

Previous studies have shown that pollen has been used for many years in both alternative medicine and human nutrition for its nutritional, physiological, functional, and therapeutic and disease prevention functions (Cornara et al., 2017; Pasupuleti et al., 2017; Touzani et al., 2019). However, information on the practical applications of BP in food processing is still extremely deficient. Although extractable fractions of TPC and antioxidant capacity were determined in the studies (Asmae et al., 2021; Atsalakis et al., 2017; Ivanišová et al., 2015), the studies in which the TPC and antioxidant capacity of the hydrolyzable and bioaccessible fractions were determined are very limited (Altiner et al., 2021). In addition, studies on physical, chemical, and phenolic and antioxidant enrichment that can be achieved by addition of pollen to bakery products are scarcely available in the literature (Conte et al., 2018; Krystyjan et al., 2015). The aim of this study was to enrich the cookie, one of the most popular bakery products in the world, with BP and to determine physicochemical features, the TPC, and antioxidant capacities in the extractable, hydrolyzable, bioaccessible fractions of the cookies with BP addition.

2 | MATERIALS AND METHODS

2.1 | Materials

The flour used in the production of cookies, with moisture (%), ash (%), protein (N*5.7) (%), fat (%), wet gluten (%), and sedimentation (mL) contents of 13.0 ± 0.12; 0.65 ± 0.02; 1.2 ± 0.12; 9.8 ± 0.25; 24.0 ± 0.5; 18 ± 1, respectively, were obtained from Toru Flour. Other ingredients to be included in the cookie formulation (fine granulated sucrose, brownulated granulated sucrose, skimmed milk powder, salt, sodium bicarbonate, ammonium bicarbonate, shortening, and high fructose corn syrup) were purchased from the market. BP, which was used in cookie production, collected in Turkey (Bursa, Cumalikizik), every day between 08:00 and 12:00, between April and September 2020 at 15-day intervals for 6 months. These samples were mixed prior to analysis to represent the entire hive. All samples were stored in the freezer at −18°C until analysis.

2.2 | Preparation of cookies

In cookie production, AACCI Method No. 10.54 was applied (AACC, 1995). Dry ingredients except flour and ammonium bicarbonate were mixed thoroughly in a bowl and the prepared dry mixture and shortening were transferred to the bowl of the mixer (Kitchenaid 5KSM125, USA) and mixed for 3 min. In a separate container, the liquid mixture prepared with water, HFCS, and ammonium bicarbonate, and then mixture was added mixer and mixed for 1 min. To this mixture, flour (flour-BP mixture in cookies to which BP was added) was added and the cookie dough was mixed for 30 s. The dough was taken from the bowl of the mixer, divided into 4 equal parts, and placed on the tray, each giving an oblong shape. The dough of 1 cm thickness was rolled out by going forward once and by going backward once with a rolling pin and shaped with a mold (4 cm diameter). It was cooked in an oven at 190 ± 2°C (Venarro DEF—P4K) for 11 min, and necessary measurements were made after reaching room temperature (~30 min). The formulation for the production of BP cookies is shown in Table 1.

2.3 | Proximate composition of cookies and BP

In order to determine the proximate composition of BP used in cookie production, protein, fat, and moisture content analyzes were performed according to AOAC Method Nos. 990.03, 948.22, and 930.15, respectively (AOAC, 2012).

TABLE 1 Recipe for cookie formulation

Components (g)	Control[a]	5%	10%	15%
Flour	**100**	**95**	**90**	**85**
Sucrose (fine granulated)	32	32	32	32
Brownulated granulated sucrose	10	10	10	10
Skimmed milk powder	1.0	1.0	1.0	1.0
Salt	1.25	1.25	1.25	1.25
Sodium bicarbonate	1.0	1.0	1.0	1.0
Ammonium bicarbonate	0.5	0.5	0.5	0.5
All-purpose shortening (fat)	40	40	40	40
High-fructose corn syrup (HFCS)	1.5	1.5	1.5	1.5
Deionized water (mL)	22	22	22	22
BP	–	**5**	**10**	**15**

Note: Ingredients of the recipe, the number of which changes are marked in bold font.
*Cookie ingredients were determined in AACCI Method No: 10.54 (AACC, 1995).

The proximate compositions of cookies were determined according to the methods of the AACC International, American Association of Cereal Chemists (2010): the crude protein content by the Combustion Method (using LECO FP628 instrument) with a nitrogen to protein conversion factor of 5.7 (method number 46-30.01); fat content by the Soxhlet method (Buchi E816; method number 30-25.01), and ash content by carbonization (method number 08.01.01), moisture content by air-oven method (method number 44-15.02). The total carbohydrate content was calculated by subtracting the sum of moisture, protein, fat, and ash percentages from 100%. The content was calculated according to the Atwater system (FAO, 2002).

2.4 | Physical analysis of cookies

Diameter and thickness of cookies were determined using caliper according to AACCI Method No. 10.54. The spread ratio of the cookies was calculated using Equation (1) (AACC, 1995):

$$\text{Spread Ratio} = \frac{\text{Diameter}}{\text{Thickness}} \times 100 \quad (1)$$

The color of the cookies was determined with Hunter colorimeter (PCE-CSM3). L^*, a^*, and b^* values were measured. L^* value to define the colors between white and black (L = 100–0); a value is green (−a) and red (+a). The b value was used to describe the colors between blue (−b) and yellow (+b). The samples were measured five times at approx. 20°C (Elgün, et al., 2002). Total color difference (ΔE) (Karshenas et al., 2018) (Equation 2) and browning index (BI; Farokhian et al., 2016) (Equation 3) values were calculated.

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

$$\text{BI} = \frac{X \times 0.31}{0.172} \times 100, \text{ where } X = \frac{(a + 1.75L)}{(5.645L + a - 0.3012b)} \quad (3)$$

The texture analysis of the cookies was determined by the texture analyzer (TA.XTPlus Texture Analyzer) 24 hr after the cookies were baked. A cylinder probe was used in the texture analyzer to determine the properties of the texture of the cookies (TPA, Texture Profile Analysis). In TPA analysis, a cylinder probe, pretest speed 0.8 mm/s, test speed 1 mm/s, load cell 5 g, and compression ratio 50% was applied on the cookies. Measurements were conducted three times and the results are mean values.

2.5 | Sensory evaluation

Sensory evaluation of cookies was performed by 40 untrained panelists, 20 male and 20 female, between the ages of 18 and 40 who were not smoking and healthy. The evaluation was made on a 9-point hedonic scale and the most liked cookie was given 9 points and the least liked one was given 1 point. The cookies were evaluated under the headings of appearance, texture, taste characteristics, eagerness to buy, and overall sensory acceptability.

2.6 | Extraction of extractable, hydrolyzable, and bioaccessible fractions

Extractable and hydrolyzable fractions were extracted according to Vitali et al. (2009) with slight modifications. The extractable and hydrolyzable fractions were used for TPC and antioxidant capacity. Briefly, 2 g of each sample was taken in 20 ml of HCl (conc)/methanol/water (1:80:10, vol/v vol) and these samples were shaken for 2 hr at room temperature with an orbital shaker (Mipro/MLS3535; 250 rpm at 20°C). The obtained extracts were centrifuged at 3,500 *g* for 10 min (Hettich/Universal 320R). The supernatant was used to determine the extractable fractions of the TPC and antioxidant capacity of the cookies.

After the extractable fraction, 20 ml of methanol/H_2SO_4 concentrate (10:1) was added to the residue and incubated in a water

TABLE 2 Proximate composition of control and enriched cookies

Samples	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)
Control	6.38 ± 0.01	0.54 ± 0.01^{c}	21.19 ± 0.25^{d}	9.60 ± 0.07^{b}	62.29 ± 0.05^{a}
5% BP	6.35 ± 0.21	0.56 ± 0.03^{bc}	22.17 ± 0.24^{c}	9.67 ± 0.32^{b}	61.25 ± 0.15^{b}
10% BP	6.32 ± 0.29	0.62 ± 0.01^{ab}	22.98 ± 0.97^{b}	9.91 ± 0.10^{ab}	60.17 ± 0.07^{c}
15% BP	6.31 ± 0.01	0.64 ± 0.01^{a}	25.32 ± 0.47^{a}	10.36 ± 0.02^{a}	57.37 ± 0.19^{d}

Note: Mean values ± standard deviation. Within columns, values with the different superscripts differ significantly from each other according to LSD test ($p < .05$).

TABLE 3 Physical attributes of control and enriched cookies

				Color				
Samples	Diameter (cm)	Spread Ratio	Hardness (*N*)	*L**	*a**	*b**	ΔE	BI
Control	6.13 ± 0.11^{b}	3.90 ± 0.11^{c}	36.48 ± 0.27^{d}	74.18 ± 0.33^{a}	7.49 ± 0.33^{c}	23.90 ± 0.22^{b}	–	59.06 ± 0.06^{d}
5% BP	6.29 ± 1.01^{b}	4.12 ± 0.11^{b}	41.58 ± 0.01^{c}	65.20 ± 1.65^{b}	10.82 ± 0.61^{b}	47.51 ± 0.29^{a}	25.47 ± 0.19^{c}	61.76 ± 0.16^{c}
10% BP	6.61 ± 0.33^{a}	4.16 ± 0.33^{b}	56.09 ± 0.69^{b}	61.77 ± 1.87^{c}	13.91 ± 1.20^{a}	48.87 ± 0.25^{a}	28.61 ± 0.37^{b}	63.21 ± 0.11^{b}
15% BP	6.81 ± 0.30^{a}	4.93 ± 0.30^{a}	81.84 ± 0.80^{a}	57.35 ± 1.05^{d}	15.23 ± 0.27^{a}	49.74 ± 0.83^{a}	31.79 ± 0.22^{a}	64.30 ± 0.07^{a}

Note: Mean values ± standard deviation. Within columns, values with the different superscripts differ significantly from each other according to LSD test ($p < .05$).

bath at 85°C for 20 hr. It was then centrifuged at 3500 *g* for 10 min at 4°C in a centrifuge (Hettich/Universal 320R). The obtained supernatant was used as the hydrolyzable fraction.

Bioaccessible fractions of cookies were made according to Bouayed et al., 2012, by in vitro enzymatic digestion extraction that mimics the conditions in the gastrointestinal tract.

2.7 | Determination of total phenolic content and antioxidant capacity

The extractable, hydrolyzable, and bioaccessible fractions of TPC of cookies was determined using Folin-Ciocalteu method (Singleton et al., 1999). In the evaluation of the samples, a gallic acid calibration curve was prepared with 10 points in the range of 0.001–0.1 mg/ml and the TPC was determined in 3 repetitions. The results obtained are given as mg gallic acid equivalent/g.dw.

Antioxidant capacity of cookies extractable, hydrolyzable, and bioaccessible extracts was determined by two different methods. First method was radical cation decolorization assay (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); ABTS; Apak et al., 2004) and the second one was free radical scavenging assay (2,2-diphenyl-1-picrylhydrazyl; DPPH; Brand-Williams et al., 1995). A calibration curve was prepared, Trolox (6-hydroxy-2,5,7,8-tetrame thylchromane-2-carboxylic acid) and the results were given as µmole TE/g dw for each method.

TPC, ABTS, and DPPH results were calculated as the sum of extractable and hydrolyzable fraction extracts. It was called total TPC/antioxidant capacity.

Bioaccessibility (%) was calculated by dividing the results obtained from the bioaccessible fraction by the sum of the extractable and hydrolyzable fractions and multiplying by 100.

2.8 | Statistical analysis

The results were expressed as mean ± standard deviation (*SD*). Differences between samples were analyzed using analysis of variance (ANOVA) with SPSS software version 24.0 (IBM) and LSD (Least significant difference) test was used to compare the groups. The differences between the mean values were found to be statistically significant at a 5% level of significance.

3 | RESULT AND DISCUSSION

3.1 | Proximate composition of cookies and bee pollen

BP, which is one of the leading apicultural products, contains nutritionally valuable substances and polyphenolic compounds (Kroyer and Hegedűs, 2001). In previous studies, it has been determined that the protein content of BP varies between 10% and 40% and the fat content between 1% and 10% (CarlosFuenmayor et al., 2014; Feás et al., 2012; Mayda et al., 2020; Sattler et al., 2015; Villanueva et al., 2002). In this study, protein and fat content of BP used in cookie production were determined as 16.85% and 2.83%, respectively, and these values were similar to previous studies. Wheat flour used in produced cookies had 9.8% protein and 1.2% fat contents.

The proximate composition of control and enriched cookies containing BP are shown in Table 2. The moisture content of cookies decreased from 6.35% to 6.31% with the addition of BP compared with the control sample (6.38%). The moisture content obtained was within the acceptable moisture level. In all proximate analysis (except moisture and carbohydrate), the lowest content was found in the control, the highest was found in the 15% BP cookie. As the BP ratio added to the cookies increased, the ash, fat, and protein contents increased,

FIGURE 1 Figures of control and enriched cookies

whereas the carbohydrate content decreased significantly. It is thought that such an increase is due to the substitution of BP, which has a higher protein and fat content than flour, to flour in different proportions in cookie production.

3.2 | Physical analysis of cookies

The physical characteristics (diameter, spread ratio, hardness, and color) of cookies are given in Table 3. SR is one of the most important physical properties in evaluation the quality of cookies; it is related to texture, grain finesse, bite, and overall mouthfeel (Bose & Shams-Ud-Din, 2010; Jothi et al., 2014). BP addition had a high and positive effect on the SR. The highest SR value was measured in 15% BP cookies, whereas the lowest SR value was measured in control. It is seen that these values of the cookies enriched with the addition of BP increased significantly ($p < .05$) with the increase of the BP addition ratio (except 5%). This difference may be due to the change in the amount of protein in the dough or the ability of the dough to hold gas during the baking process (Ramadan et al., 2012). These results are similar to earlier observations on cookies (Adegbanke et al., 2019; Igbabul et al., 2018; Şahan et al., 2013). The diameter values of cookies tend to be similar to the SR of cookies.

The texture of the cookies is an important parameter that contributes to the acceptance of the cookies by the consumers. The effect of replacement of wheat flour with BP on the textural properties of cookies is shown in Table 3. The texture values of the cookies were found between 36.48 and 81.84 N. BP substitution in cookies production caused the cookies to harden and the hardness of the cookies increased significantly ($p \leq .05$) as the added ratio increased. This can be attributed to the reduced gluten content in cookies (wheat flour substituted by BP), which is not sufficient to obtain good viscoelastic dough. The hardness of the cookies is related to the formation of the gluten network formed by the gluten and gliadin found in wheat flour (Rosell, 2009). The gluten matrix is important for the best rheological aspects such as extensibility and flexibility (Lazaridou et al., 2007). Therefore, the reduction of these proteins (due to wheat flour substitution) may have effect on the formation of a viscoelastic dough, which may possibly lead to increase hardness.

Color is one of the most important factors in the selection of food products (Calvo et al., 2001). The color formation that occurs during baking in bakery products is generally known as browning. When the color of the cookies was examined (Table 3 and Figure 1), it was seen that the L^* and BI values of the cookies varied between 57.35–74.18 and 59.06–64.30, respectively. The addition of BP significantly browned the surface of the cookies compared with the control sample, and the increase of addition ratio significantly increased this browning. L^* and BI values, which increase significantly with the addition of BP, are predicted to be the result of the nonenzymatic chemical reaction, the Maillard reaction. As a result of the Maillard reaction between reducing sugar and amino acids, colored compounds known as melanonids are formed and thus the color of the cookies is formed with increasing temperature and baking time (Köksel and Gökmen, 2008; Mundt & Wedzicha, 2007). This formation varies depending on the amount of sugar and protein in the ingredients used for baking. Since the protein content of BP used in cookie production (16.85%) is higher than the substituted wheat flour (9.8%) and the reducing sugar in the dough comes from BP, the amount of melanoidin formed in enriched cookies may be higher than the control samples. As in this study, L^* values decreased when wheat flour was replaced with ingredients with high protein content (Gallagher et al., 2005.; O'Brien et al., 2003; Inkaya et al., 2009; Şahan et al., 2013; Krystyjan et al., 2015; Sahin, 2020).

The highest a^* and b^* values of the cookies were found in the sample with 15% (15.23; 49.74) and the lowest in the control sample (7.49; 23.90). The color of the BP pellets varied from pale yellow to yellow-brown due to the presence of flavonoids and carotenoids (Almeida-Muradian et al., 2005; Conte et al., 2020; Melo & Almeida-Muradian, 2010; Stanley & Linskens, 1974). Similar results were seen with ingredients added cookies with high carotenoid color pigments (Alshehry, 2020; Krystyjan et al., 2015; Sahin, 2020; Saleh & Ali, 2020; Turksoy & Özkaya, 2011).

Furthermore, total color differences, significant enough ($\Delta E > 5$) to clear, large color deviation (Collar et al., 2014), were observed between control and BP-enriched cookies (Table 3).

3.3 | Sensory evaluation of cookies

Sensory evaluation using the 9-point hedonic scale is widely used by food scientists and technologists (Gajera et al., 2010). Table 4 and

Samples	Appearance	Texture	Taste	Eagerness to buy	Overall sensory acceptability
Control	5.88 ± 0.08^{a}	5.81 ± 0.02^{b}	5.75 ± 0.01^{b}	5.50 ± 0.05^{a}	5.56 ± 0.06^{b}
5% BP	6.06 ± 0.16^{a}	6.44 ± 0.05^{a}	5.75 ± 0.05^{b}	5.25 ± 0.02^{a}	5.56 ± 0.02^{b}
10% BP	6.13 ± 0.23^{a}	5.94 ± 0.04^{b}	6.00 ± 0.24^{a}	5.44 ± 0.08^{a}	6.25 ± 0.12^{a}
15% BP	4.38 ± 0.18^{b}	4.75 ± 0.05^{c}	5.06 ± 0.16^{c}	4.13 ± 0.13^{b}	4.63 ± 0.07^{c}

Note: Mean values ± standard deviation. Within columns, values with the different superscripts differ significantly from each other according to LSD test ($p < .05$).

TABLE 4 Sensory evaluation of control and enriched cookies

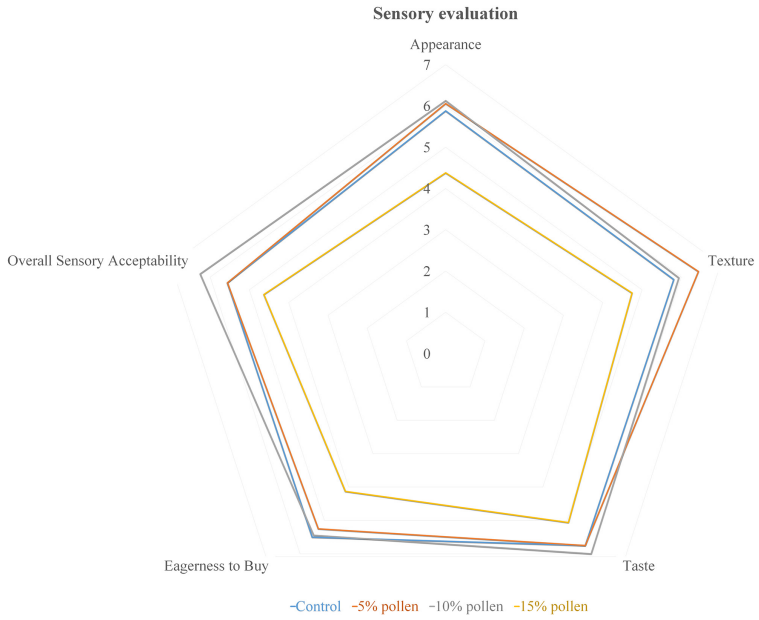

FIGURE 2 Sensory evaluation of control and enriched cookies

Figure 2 show the results of evaluation of appearance, texture, flavor, eagerness to buy, and acceptance of cookies.

Browning is desirable in cookie because it enhances the appearance and flavor of cookies. The appearance of the enriched cookies (except 15%) higher scores than the control sample, but the color of the cookie with an additional ratio of 15% was darker than desired, so it had the lowest score.

The addition of BP to the cookies increased their hardness values (Table 3). In texture evaluation, cookies with 5% additional BP had the highest value, whereas 15% additional BP cookies had lowest value.

When the taste of the samples was evaluated, it was observed that biscuit with 15% addition BP was the least approved cookies, while at 10% ratio was the most approved cookies.

When the panelists were asked whether they would buy the cookies they tasted, it was seen that they were least willing to purchase the cookie with the addition of 15% BP. It was seen that there was no significant difference between the purchase desire of other produced cookies. Therefore, it is clear that evaluators will buy cookies formulated with BP (except 15%).

Overall acceptance refers to how consumers or panelists generally accept the product. In this study, the overall acceptability score of the BP added cookies ranged from 6.25 (10% BP addition) to 4.63 (15% BP addition), control and enriched cookies were liked by evaluators (except 15%). Krystyjan et al. (2015) produced BP-enriched biscuits demonstrating that the replacement of wheat flour with BP (up to 10%) without detrimental effects in overall quality and acceptance. Similar results were found in this study. While the sensory properties were not a problem at the addition ratio of up to 10%, all sensory property scores decreased at the higher ratio (15%). According to the evaluation, the most preferred cookie was 10% BP additional cookie.

3.4 | Total phenolic content and antioxidant capacity of cookies

The phenolic compounds content of honey, pollen, and all other bee products varies depending on the regions where they are obtained. The rich phenolic and nonphenolic contents of BP make it important antioxidant sources (Meda et al., 2005), which decrease free oxygen radicals in the cells and dispose of their adverse impact (Clarkson & Thompson, 2000). In previous studies with different honey pollens, caffeic acid, gallic acid, rutin hydrate, and p-coumaric acid phenolic compounds were determined affluently (Kaškonienė et al., 2015; Solgajová et al., 2014; Guler & Kara, 2020). The chemical

TABLE 5 Total phenolic content and antioxidant capacity of bee pollen

Bee pollen	TPC (mg/g GAE)	Antioxidant capacity (μmole Trolox/g)	
		ABTS	DPPH
Extractable	65.50 ± 0.25	10.18 ± 0.10	15.54 ± 0.27
Hydrolyzable	395.41 ± 10.15	57.22 ± 0.12	62.37 ± 0.68
Total	460.91 ± 10.40	67.40 ± 0.22	77.91 ± 0.95
Bioaccessible	39.18 ± 0.12	12.81 ± 0.17	1.69 ± 0.02
*Bioaccessibility (%)	8.50 ± 0.17	19.00 ± 0.23	2.17 ± 0.11

structures of phenolic compounds significantly affect their antioxidant activities. In addition, the number and position of hydroxyl and methoxyl groups in the benzene ring and the possibility of electron delocalization in double bonds are other important factors affecting antioxidant activity (Xiang & Ning, 2008). Phenolic compounds such as hydroxybenzoic, sinapic, caffeic acid, and p-coumaric acids have been found to have significant antioxidant activity (Nakajima et al., 2007; Zhang et al., 2009). As an example of nonphenolic compounds, BP is rich in carotenoids. Carotenoids are involved in the scavenging of reactive oxygen species, singlet molecular oxygen and peroxyl radicals, and are effective deactivators of electronically excited sensitizing molecules involved in the generation of radicals and singlet oxygen. For these reasons, it is thought to be effective on antioxidant capacity (Stahl & Sies, 2003).

TPC and antioxidant capacity values of BP used in cookie production are given in Table 5. Previous research showed that BP had a high TPC and antioxidant capacity (Asmae et al., 2021; Altiner et al., 2021; Atsalakis et al., 2017; Fadzilah et al., 2017; Özkök & Silici, 2017; Ivanišová et al., 2015; Ulusoy & Kolayli, 2014; Yeşiltaş et al., 2014) that varies due to the ecological diversity and predominance of the plant species that it was collected from. Ulusoy and Kolayli (2014) indicated that the TPC of BP samples collected from Turkey is 44.0–124.1 mg GAE/g. In another study in which the TPC values of pollen samples collected from different regions of Turkey were determined, it was found to be 12.0–36.7 mg GAE/g (Yeşiltaş et al., 2014). Fadzilah et al. (2017), in a study on pollen collected in Malaysia, reported that the TPC of the samples was 33.46–135.93 mg GAE/g. Asmae et al. (2021), BP found TPC values of 8.07–32.38 mg GAE/g. Yeşiltaş et al. (2014) declared that antioxidant capacities of the pollens according to ABTS, CUPRAC, DPPH, and FRAP methods were 15.2–33.6 mg TE/g, 20.7–89.4 mg TE/g, 5.7–15.2 mg TE/g, and 5.2–15.7 mg TE/g, respectively. Özkök and Silici (2017) indicated in a study performed with 20 BPs that the mean and the mean antioxidant capacities were 42.37 mg AAE/g. Altiner et al. (2021) reported that antioxidant capacities of BP samples were 72.19–111.40 μmole TE/g, 96.34–192.58 μmole TE/g, 50.22–74.80 μmole TE/g by ABTS, CUPRAC, and DPPH methods, respectively. It was determined that only extractable forms were taken into account in most of the studies conducted to determine the amount of TPC and antioxidant capacity of BP. However, due to the complex structure of pollen, the hydrolyzable fraction, which is insoluble form, should also be determined and evaluated in order to find these values. Therefore, in this study, TPC and antioxidant capacity were analyzed in the extractable, hydrolyzable, and bioaccessibility fractions and these values were similar to Altiner et al. (2021). Furthermore, the extractable fractions have lower antioxidant capacity and phenolic contents than the hydrolyzable fractions in all of the methods used.

The results of extractable, hydrolyzable, and bioaccessible polyphenol fractions in BP cookies are given in Table 6. The TPC of the BP cookies in the study varied between 352.70 and 401.13 mg/100 g GAE and the hydrolyzable fraction was 92.49%–92.95% and the extractable fraction was 7.05%–7.51% of TPC. The extractable and hydrolyzable polyphenol fractions of the enriched cookies were significantly affected by the addition of BP, this effect being more evident at the increasing percentages of fortification. These findings were consistent with those observed in cereals and cereal-based baked perfectly (Conte et al., 2020; Vitali et al., 2009).

Although in vitro techniques do not show a real biological effect, they represent a good approximation of these extracts' antioxidant effects and chemical mechanisms. In order to evaluate how efficiently the total polyphenols detected in cookies can be digested, adsorbed, and metabolized by the human body, in vitro bioaccessibility analysis was performed on cookies. As with the extractable and hydrolyzable fractions, an increase in the proportion of BP added to the cookies also increased the bioaccessibility of TPC, but only cookies produced with the highest addition ratio (15%; 34.29 mg/g GAE) were significantly higher than the control (30.33 mg/GAE). The bioaccessibility of the BP cookies was found to be very low (Bioaccessibility [%]: 8.55–9.26). This is thought to be caused by the outer layer of BP, known as exine, which is very elastic, strong, and tough and protects the compounds in the pollen and provides chemical and enzymatic resistance to the pollen. Most phenolic compounds in cereal-based matrices are in the insoluble bound forms and these forms can be determined by analyzing the hydrolyzable fractions. Therefore, it is extremely important to determine these fractions in TPC and antioxidant capacity analysis of cereal products. As far as determined, this study is the second study for the fortification of cookies with the use of BP, but the hydrolyzable fractions and bioaccessibility of TPC were not studied in the first study (Krystyjan et al., 2015). Therefore, it is difficult to compare the obtained data with the literature.

According to the results of all assays (ABTS, DPPH) applied to determine the antioxidant capacity, in extractable, hydrolyzable, and bioaccessibility fractions of the BP-enriched cookies were higher

TABLE 6 Total phenolic content and antioxidant capacity of control and enriched cookies

Samples	TPC (mg/100 g GAE)	Antioxidant capacity (μmole Trolox/g)	
		ABTS	DPPH
Extractable			
Control	22.33 ± 0.23^{c}	0.73 ± 0.18^{c}	15.84 ± 0.22^{b}
5% BP	24.84 ± 0.61^{b}	0.99 ± 0.19^{c}	15.98 ± 0.02^{b}
10% BP	26.40 ± 0.42^{b}	3.00 ± 0.05^{b}	16.10 ± 0.15^{b}
15% BP	30.14 ± 0.80^{a}	4.18 ± 0.04^{a}	16.83 ± 0.29^{a}
Hydrolyzable			
Control	308.48 ± 2.34^{b}	25.47 ± 0.48^{c}	108.18 ± 2.94^{b}
5% BP	327.86 ± 8.87^{b}	36.37 ± 0.32^{c}	121.90 ± 0.25^{a}
10% BP	354.07 ± 3.65^{a}	57.83 ± 0.36^{b}	126.07 ± 0.25^{a}
15% BP	370.99 ± 0.39^{a}	83.83 ± 0.80^{a}	130.41 ± 0.59^{a}
Total			
Control	330.81 ± 2.59^{b}	26.20 ± 0.46^{c}	124.02 ± 3.17^{b}
5% BP	352.70 ± 9.50^{b}	37.36 ± 0.34^{c}	137.88 ± 0.27^{a}
10% BP	380.46 ± 9.23^{a}	60.83 ± 0.36^{b}	142.17 ± 0.39^{a}
15% BP	401.13 ± 0.69^{a}	88.01 ± 0.79^{a}	147.24 ± 0.62^{a}
Bioaccessible			
Control	30.33 ± 0.04^{b}	8.18 ± 0.35^{c}	1.72 ± 0.03^{d}
5% BP	32.65 ± 1.00^{ab}	8.70 ± 0.38^{bc}	1.96 ± 0.07^{c}
10% BP	33.28 ± 0.89^{ab}	9.79 ± 0.64^{ab}	2.38 ± 0.02^{b}
15% BP	34.29 ± 0.39^{a}	10.68 ± 0.16^{a}	2.59 ± 0.08^{a}
*Bioaccessibility (%)			
Control	9.17 ± 0.34	31.83 ± 0.70^{a}	1.39 ± 0.06^{b}
5% BP	9.26 ± 0.04	23.43 ± 0.32^{ab}	1.42 ± 0.04^{b}
10% BP	8.76 ± 0.71	16.16 ± 0.20^{bc}	1.68 ± 0.01^{a}
15% BP	8.55 ± 0.08	12.19 ± 0.13^{c}	1.76 ± 0.13^{a}

Note: Mean values ± standard deviation. Within columns, values with the different superscripts differ significantly from each other according to LSD test ($p < .05$).

than the control samples. However, in ABTS analysis, a significant difference was determined in cookies with 10%–15% additions, or only in 15% additions in DPPH analysis. For the same reason as in the TPC analysis, the bioaccessibility of the (exine) BP-cookies was determined to be extremely low.

4 | CONCLUSION

The addition of pollen increased the texture and SR, which are important quality criteria of cookie, compared with the control samples. Except 15% BP sample, a significant increase in the overall sensory acceptability was observed in all the pollen-enriched biscuits. As the addition ratio increased, the browning index and the brittleness increased, the acceptability of the cookies decreased, but the 10% BP sample was the most desired cookie. In addition, this study showed that the addition of BP increased the nutritional value of the cookie, while also increasing the polyphenol contents and antioxidant capacity values. However, pollen has low bioaccessibility due to its outer shell layer. In the future, product enrichment studies are planned to be carried out by destroying the exine layer and to be carried out in vitro and in vivo.

With the results obtained in this study, it can be concluded that BP is a suitable functional component for the production of cookies, and it has been proven that these new products can hold an important place in the growing market of apitherapy products.

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CONFLICT OF INTEREST

The author has declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Ayse Neslihan Dundar: Conceptualization; Data curation; Methodology; Supervision; Writing-original draft; Writing-review & editing.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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