



# Physical and Chemical Properties of Biscuits Fortified with Bee Pollen

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**Abstract**— Functional foods, such as bee pollen, contain nutrients and positively affect health. Bee pollen is a natural bee product with high antioxidant potential. Phenolic compounds are found in bee pollen as antioxidants. Other contents of bee pollen are protein, minerals, and ash. Bee pollen contains several essential amino acids required by the human body. Bee pollen has been used to fortify various food products, such as yoghurt, bread, and biscuits. The study aimed to evaluate bee pollen biscuits. The experimental design was a Completely Randomized Design (CRD) with one factor. The factor was substituted for bee pollen (0%, 5%, 10%, 15%, 20%) for wheat flour. The research revealed that the substitution of bee pollen had a significant impact on the total phenolic content, CIE Lab values (Lightness, a\*, b\*), hue angle, and chroma of the biscuits. In contrast, substituted bee pollen did not affect the protein and ash content. The results showed that bee pollen has a positive effect on the quality of the biscuit, indicating its potential as a functional food.

**Keywords**— bee pollen; biscuit; protein; substitute; total phenolic content

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## I. INTRODUCTION

Biscuits are one of the most famous baked sugary goods in the whole world. Biscuits are traditionally made using wheat flour as the primary ingredient. However, in recent years, there has been a growing trend toward incorporating additional natural ingredients that are rich in nutrients. This initiative aims not only to reduce the reliance on wheat flour but also to enhance the nutritional value of the biscuits, thereby promoting them as a healthier food option. For instance, biscuits were manufactured with the supplementation of marine collagen peptide [1], *moringa oleifera* [2-4], and bee pollen [5,6]. Research on bee pollen from Indonesia is limited, yet it is rich in nutrients that are beneficial to health.

Bee pollen is located in the beehive. It is a lumpy powder, available in white, yellow, and brown colours. Bee pollen is a beneficial product that contains protein, carbohydrates, fat, and fiber [7-9]. Bee pollen also had total phenolic and flavonoid compounds. Composition of phenolic compounds in bee pollen, such as gallic acid, rutin, quercetin, kaempferol, and *p*-coumaric acid, [10]. Other bioactive compounds also consisted of phenolic acids, flavonoids, and their glycosides [11]. Chestnut

bee pollen is a rich source of phenolic compounds, including galangin, pinocembrin, hyperoxide, and chrysin, which are known for their antioxidant properties. These bioactive constituents contribute to the protective effects of bee pollen against oxidative stress-induced DNA damage [12]. The bioactive compounds contained in bee pollen make it an antimicrobial and antioxidant [13]. Bee pollen showed antibacterial activity against *Salmonella typhi* NCTC786 BCC712, indicating its potential as a natural antimicrobial agent [14]. According to [15], bee pollen is produced from three types of pollen, including multifloral, forest flowers, and kapok, which exhibit different proximate contents. The composition of bee pollen is affected by several factors, including harvest season and geographical and botanical origins [16-18]. Bee pollen has been extensively utilized as a functional ingredient in biscuit formulation due to its rich nutritional profile. Incorporating bee pollen at substitution levels of up to 10% has been shown to enhance the nutritional quality of biscuits by significantly increasing their ash content, soluble and insoluble dietary fiber, total polyphenolic content, and antioxidant activity as measured by Trolox Equivalent Antioxidant Capacity (TEAC) [6]. Bee pollen has been widely utilized as a functional raw material in the production of

biscuits and cookies due to its rich nutritional and bioactive composition. Substitution of bee pollen in biscuit formulations at levels up to 10% has been reported to enhance ash content, soluble and insoluble dietary fiber, total polyphenolic content, and antioxidant activity, as measured by Trolox Equivalent Antioxidant Capacity (TEAC). In cookie formulations, the addition of bee pollens up to 15% has similarly resulted in increased levels of protein, ash, and total phenolic content.

Furthermore, carotenoid compounds such as lutein and  $\beta$ -carotene have been detected in bee pollen-enriched cookies, contributing to their nutritional and functional properties [5]. These improvements suggest that bee pollen can serve as a valuable additive in developing functional bakery products with potential health benefits [19]. Consequently, this research substitutes wheat flour with bee pollen to raise its nutritional and explores bee pollen from Indonesia value, which can be used for food products. The study aimed to investigate the physical and chemical properties of biscuits that were fortified with bee pollen.

## II. MATERIAL AND METHODS

### A. Material

The main ingredient was multifloral bee pollen from Tumpang, Malang Regency, East Java, Indonesia. Other ingredients included wheat flour (low protein, 10%), margarine, sugar powder, eggs, vanilla flavour, and baking powder. The supporting materials were methanol (Merck), gallic acid (Merck),  $\text{Na}_2\text{CO}_3$  (Merck), and Follin-Ciocalteu reagent (Merck). The equipment used included a UN30 Memmert Oven, a muffle furnace, a Thermo Scientific GENESYS 10S UV Vis spectrophotometer, and a Linshang LS171 Colourimeter.

### B. Experimental design

The experimental design in this research was a Completely Randomized Design (CRD) with one factor. The factor was the proportion of bee pollen to wheat flour at five levels (0:100; 5:95; 10:90; 15:85; and 20:80 (w/w)). Each treatment was replicated three times, resulting in a total of 15 experimental units.

### C. Experimental Procedure

#### *Production of Biscuit Bee Pollen*

Biscuits were produced with a modified method [6]. First; Wheat flour and bee pollen were mixed and blended according to the treatment. Furthermore, 26.7 g of sugar, 0.7 g of baking powder, and 1 g of vanilla flavour were added to the dough. In addition, other ingredients, such as 45 g of margarine and 10 g of eggs, were stirred with a mixer for 10 minutes to obtain a homogeneous dough, and then it was flattened and shaped. The dough was baked at  $150 \pm 10$  °C for 20 minutes. The finished biscuits were analysed for their physical and chemical characteristics.

### D. Parameters

#### *Physical Characteristics*

Analysis of colour (L,  $a^*$ ,  $b^*$ ) used colourimeter [20]. Measurements were carried out in triplicate for each formulation. Furthermore, hue angle and chroma were acquired from formula [21]:

$$\text{Hue} = h^* = \tan^{-1} \left( \frac{a^*}{b^*} \right) \quad \text{eq (1)}$$

$$\text{Chroma} = C^* = \sqrt{(a^{*2} + b^{*2})} \quad \text{eq (2)}$$

#### *Chemical Characteristics*

##### *Protein Content Analysis*

Protein content was analyzed according to method [22].

##### *Ash Content Analysis*

Ash content analysis according to the method [22]. Biscuits were weighed at as much as 1 g and put into a crucible that had been weighed. The sample was then put into the furnace at 550 °C for 5 hours. The sample was then cooled first, and then the sample was weighed as the weight of the ash. Ash content can be calculated using the following formula:

$$\text{Ash content (\%)} = \frac{\text{Ash weight}}{\text{Sample weight}} \times 100 \quad \text{eq (3)}$$

##### *Total Phenolic Content*

Total phenolic content was determined using a modified method from [6]. For the first, 300  $\mu\text{L}$  sample were added 1.5 mL follin ciocalteu, and then mixture was given 1,2 mL  $\text{Na}_2\text{CO}_3$  (7.5%). Lastly, the solvent was incubated for 30 minutes and it was measured by Spectrophotometer UV-Vis with 765 nm.

##### *Data Analysis*

Data were analyzed with using Analysis of Variance (ANOVA) to evaluate significant differences among treatments. Post hoc comparisons were conducted using Duncan's Multiple Range Test (DMRT) at a significance level of  $p < 0.05$ .

## III. RESULT AND DISCUSSION

### F. Characteristics of Bee Pollen

This research used multifloral bee pollen with characterisation in **Table 1**. Bee pollen has a protein content of approximately 10.94% and an ash content of roughly 2.06%. Multifloral bee pollen from the mountain and continental regions (Croatia) has ash content from 1.75% to 2.89% [23]. Bee pollen multifloral from Croatia also contained protein contents (14.73% - 23.29%), which was higher than the result of protein content in this research. Furthermore, bee pollen also showed a brown. Bee pollen's color exhibited lightness,  $a^*$ , and  $b^*$  values at just above 64, 14, and 40, respectively.

Another interesting point is that **Table 1** indicated a hue angle of just under 72 °, which showed a yellow to brown colour. The colour of bee pollen was influenced by botanical origin. For instance, bee pollen from *Salix caprea* was a white green colour. Moreover, bee pollen from *Brassica napus* showed a yellow colour [24]. Lastly, bee pollen has a chroma of exactly 43.31. Chroma value exhibited the intensity of colour.

TABLE 1  
 CHARACTERISTICS OF BEE POLLEN

Protein Content (%)	Ash Content (%)	Lightness	a*	b*	Hue angle (°)	Chroma
10.94±0.87	2.06±0.01	64.44±0.64	14.0±0.40	40.9±1.29	71.0±0.80	43.3±1.21

### G. Physical Characteristics of Biscuit

The physical characteristics of the biscuit were meticulously investigated using a colour test or CIE Lab (L, a\*, b\*). The results, as presented in **Table 2**, revealed a significant impact of bee pollen substitution on the lightness, a value, b value, hue angle, and chroma of biscuit ( $p < 0.05$ ), providing valuable insights for further research and application in the field. Lightness indicated a slight down between 72 at 0% or without bee pollen and 50 with 20% bee pollen (**Table 3**). Moreover, adding bee pollen significantly decreased lightness, from just above 72 to almost 51. Bee pollen is brown. On the contrary, wheat flour is white. Bee pollen and wheat flour were mixed, producing a different colour [25]. Therefore, lightness was reduced [26]. As presented in Table 3, higher levels of bee pollen fortification in biscuits resulted in a progressively darker color. This trend was consistent across all bee pollen-fortified formulations, which exhibited darker coloration compared the control sample prepared solely with wheat flour (100%) [6], [26]. The darker appearance may be attributed to the natural pigments present in bee pollen as well as intensified Maillard reactions during baking. The Maillard reaction is a key process in the browning of baked products, involving interactions between the carbonyl groups of reducing sugars and the amino groups of proteins [27,28]. Since bee pollen contained both proteins and free amino acids [29-31], its incorporation likely enhanced the extent of Maillard browning, thereby contributing to the darker color of the fortified biscuits.

Additionally, a positive value is represented by red, and a negative value is represented by green. **Table 2** illustrates the a\* value of adding different proportions of bee pollen. At first glance, 100% flour was the lowest on a\*. Additionally, 5-20% bee pollen substitution grew significantly at just above 12. The a\* value in this experiment was smaller than that of another experiment, which reached almost 16 [5]. The cause may have different origins, and botanical bee pollen may be used [24,32]. **Table 2** indicates that the b value (positive) corresponds to a yellow colour, and the b value (negative) corresponds to a blue colour. At first sight, the b value fluctuated from just above 28 to roughly 31. The incorporation of bee pollen into the biscuit

formulation led to a darker surface color [6], which can be attributed to the presence of phenolic compounds and the occurrence of Maillard reactions during baking, both of which contribute to increased yellowness and overall colour intensity of the final product [25].

An interesting observation is that the hue angle began at approximately 80° and gradually decreased to around 66° when the bee pollen concentration reached 20% (**Table 2**). This shift in hue angle corresponds to a color transition from yellow to brown, as detailed in **Table 3**. Bee pollen is known to contain a variety of sugars, including fructose, glucose, sucrose, and maltose, each present in varying amounts [23]. The development of the brown coloration is likely due to non-enzymatic browning processes. Specifically, this includes the Maillard reaction, which occurs between reducing sugars and amino acids, as well as sugar caramelization [26], [33,34]. Chroma values began at approximately 28 and showed a gradual increase, reaching up to 32 as indicated in **Table 2**. This steady rise suggests a consistent change in color intensity over the measured period or conditions.

TABLE 2  
 COLOUR OF BISCUIT

Bee pollen:F flour (%)	Lightness	a*	b*	Hue angle (°)	Chroma
0:100	72.37±1.10 <sup>e</sup>	4.69±0.80 <sup>a</sup>	28.18±0.91 <sup>a</sup>	80.57±1.47 <sup>d</sup>	28.57±0.97 <sup>a</sup>
5:95	65.96±2.30 <sup>d</sup>	7.96±1.94 <sup>b</sup>	31.33±1.64 <sup>b</sup>	75.74±3.30 <sup>c</sup>	32.39±1.70 <sup>b</sup>
10:90	60.54±0.85 <sup>c</sup>	10.78±0.35 <sup>c</sup>	30.48±0.70 <sup>b</sup>	70.51±0.71 <sup>b</sup>	32.35±0.67 <sup>b</sup>
15:85	57.12±2.20 <sup>b</sup>	10.39±0.37 <sup>c</sup>	31.48±0.37 <sup>b</sup>	71.74±0.37 <sup>b</sup>	33.15±0.77 <sup>b</sup>
20:80	50.42±1.17 <sup>a</sup>	12.65±0.74 <sup>d</sup>	29.68±1.90 <sup>ab</sup>	66.89±1.98 <sup>a</sup>	32.30±0.70 <sup>b</sup>

Note: different letter on the same column showed that the values were significantly different at  $p < 0.05$  DMRT test.

TABLE 3  
 IMAGE OF THE BISCUIT



### H. Chemical Characteristics of Biscuit

Biscuit was produced from bee pollen and other materials. Bee pollen was used as a substitute for wheat flour in biscuit production. Well, the substitution of bee pollen was not significantly determined by the biscuit's protein contents ( $p > 0.05$ ) (**Table 4**). The protein contents started at just above 5%, then fluctuated from 5.10 % to roughly 6% and remained the same at around 5.10% (20% bee pollen). In this research, the protein content of biscuits was lower than that reported in

previous studies[5,6], [35]. The relatively low protein content observed in the bee pollen biscuits is likely attributed to variations in the protein composition of the bee pollen used. In this study, multifloral bee pollen from East Java, containing less than 11% protein (**Table 1**), was utilized. In contrast, bee pollen from Poland has been reported to contain over 21% protein [6], while that from Turkey contains approximately 17% [5], highlighting geographical differences in nutritional composition. Furthermore, variations in the protein content of biscuits were influenced by the botanical origin of the bee pollen, as demonstrated by [36], which emphasises the significant impact of pollen source on its nutritional composition.

Biscuit bee pollen was also analysed for its ash content. There was no impact addition of bee pollen on the ash content of biscuits ( $p>0.5$ ) (**Table 4**). With an ash content of approximately 2% (**Table 1**), bee pollen, when used as a fortifying ingredient, does not substantially alter the overall composition of the resulting biscuits. Ash contents fluctuated from almost 4% (control) to just under 1.50% (20% bee pollen). This result indicated that the ash content was higher than the result from [5] the previous study. Bee pollen contains ash content, which is influenced by several factors, including geographic and botanical origins. In addition, the structure of bee pollen was made of organic materials, and then its ash content was related to the minerals it carried. Several bee pollen minerals comprise K, Ca, P, S, Na, Mg, and Zn [35], [37].

The addition of bee pollen significantly influenced on total phenolic content of biscuits ( $p<0.05$ ) (**Table 4**). To begin with, the biscuit without the addition of bee pollen had a total phenolic content of approximately 137.85 mg GAE/100 g. Furthermore, wheat flour was replaced with 5-20% bee pollen, resulting in a significant increase from 170.69 mg GAE/100 g to almost 390 mg GAE/100 g. The TPC result (**Table 4**) was lower than that of another researcher regarding cookies from bee pollen (352-403 mg/100g GAE) [5]. On the contrary, biscuit bee pollen was higher than bee pollen biscuit from Hungary [36]. In addition According to **Table 4** the total phenolic content of biscuits supplemented with bee pollen exceeded that of both biscuits and bee pollen-fortified bread reported by [38,39]. The variation in total phenolic content (TPC) observed in bee pollen-fortified biscuits may be explained by differences in the botanical origin of the pollen. Previous studies have highlighted that monofloral bee pollen derived from *Prunus* spp. and *Salix* spp. contains substantially higher levels of phenolic compounds compared to other floral sources [40]. These bioactive compounds are known to play a crucial role in the antioxidant potential of functional foods. Consequently, the incorporation of pollen with a richer phenolic profile contributes to an enhanced TPC in the final baked product. There were phenolic compounds in cookies bee pollen, such as  $\rho$ -coumaric, ferulic acid, and rutin hydrate [5].

TABLE 4  
 CHEMICAL CHARACTERISTICS OF BISCUIT

Bee pollen:Flour (%)	Protein Content (%)	Ash Content (%)	Total Phenolic Content (TPC) (mg GAE/100 g)
0:100	5.10±0.50	1.37±0.06	137.85±13.65 <sup>a</sup>
5:95	5.98±0.66	1.68±0.46	170.69±2.62 <sup>a</sup>
10:90	5.69±0.01	1.49±0.06	200.36±13.68 <sup>a</sup>
15:85	5.25±0.43	1.55±0.09	334.65±18.26 <sup>b</sup>
20:80	5.10±0.25	1.49±0.18	388.25±73.13 <sup>b</sup>

Note: different letter on the same column showed that the values were significantly different at  $p<0.05$  DMRT test.

#### IV. CONCLUSION

In summary, bee pollen fortification significantly influenced both the physical attributes of biscuits (L, a, b\*, hue angle, and chroma) and their chemical properties, particularly the total phenolic content. However, no significant changes were observed in the ash and protein content, indicating that bee pollen primarily enhanced the functional and bioactive qualities of the biscuits without markedly altering their proximate composition.

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#### CONFLICT OF INTEREST

Authors declare no conflict of interest to disclose.

During the preparation of this manuscript, generative AI tools (Grammarly, Quillbot, MS Office AI, and OpenAI) were used to improve the clarity, grammar, and overall readability of the text. The use of these tools was conducted under complete human oversight, and all intellectual and scientific content, data interpretation, and conclusions are entirely the responsibility of the authors. No generative AI tool was used to generate or analyze scientific data, and AI tools are not listed as authors or co-authors.

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