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The Effect of Adding Bee Pollen at Different Concentrations to Marshmallow Candy on Water Content, Water Activity, Texture, Color, Ash Content, Reducing Sugar, Antioxidant Activity, and Organoleptic Properties

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***Abstract**. Marshmallow is one of the confectionery product that made from gelatin and sugar that have the weakness of lacking nutrients but favored by people of all ages. Bee pollen can be the one of natural addition that can enrich the nutrient in marshmallows. The aim of this study was to evaluate bee pollen addition with different concentrations reviewed from physicochemical and organoleptic test of marshmallows. Tresearch method used was a experimental using Complete Randomized Design (CRD) with 4 treatments and 5 replications. Four treatments were applied: P0 (without bee pollen addition), P1 (5%), P2 (10%), and P3 (15%). The study resulted significant differences (P<0.01) in water activity, texture, Lab color, ash content, reducing sugar, antioxidant activity, color, aroma, dan overall organoleptic acceptance, and no significant differences (P>0.05) in moisture content and organoleptic texture. The values resulted increased in moisture content (36.85-37.64%), color a* (0.14-3.38), color b* (25.39-33.27), ash content (0.806-2.404%), reducing sugar (0.372-6.677%), and decreased in antioxidant activity (92.96-503.92 mg/ml) and fluctuated in water activity (0.74-0.86), texture (0.68-1.16 N), and color L* (79.70-91.92). The results of the organoleptic test were still acceptable up to treatment P3. Overall, this study resulted the best results in treatment P3 with a bee pollen concentrations of 15% to increase the nutritional content of marshmallows.*

***Keywords:** Bee pollen, Marhsmallow, Organoleptic, Physicochemical*

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INTRODUCTION

Gelatin is one of the most commonly used food additives and functions as a stabilizer, emulsifier, and foaming agent. Gelatin is extracted through the hydrolysis of collagen from various animal sources, such as cattle, pigs, and fish (Arpi et al., 2018). The use of gelatin in the food sector continues to increase due to its numerous benefits and excellent gelling properties. The use of gelatin in marshmallow production plays a crucial role in enhancing its chewiness (elasticity) (Adi et al., 2022). Marshmallow is one of the most popular confectionery products and has promising business prospects. The marshmallow industry is highly appealing because marshmallows are favored by people of all ages and can be innovated with various colors, flavors, aromas, and shapes. The principle of marshmallow production involves the rapid incorporation of air bubbles to form a stable foam (Gumansalangi et al., 2019).

Manufacturers strive to attract consumer attention by creating new products with unique flavors and numerous health benefits, including those with high nutritional value. Nutrient-rich foods contain essential components such as vitamins, carbohydrates, proteins, fats, fiber, and minerals. One such product that meets these criteria is marshmallows enriched with bee pollen. The addition of bee pollen to marshmallows not only enhances their nutritional value and product quality but also serves as a natural coloring agent due to its carotenoid content. Marshmallows are a popular snack among various consumer groups. However, conventional marshmallows typically contain only sugar and lack significant nutritional value. The incorporation of bee pollen can improve their nutrient content, making them a more functional food option. Consumers today are increasingly inclined toward nutritious food choices, yet marshmallows with bee pollen have not been introduced to the market. Therefore, to meet consumer demand, this product will be developed using bee pollen as an additional ingredient.

Bee pollen is the pollen collected from flowers that adheres to the legs of bees. It is serves as the primary nutritional source for honeybees. Bee pollen contains vitamins A, B, C, D, and E (Firman, 2017). According to Mujahidah (2020), the main components of bee pollen include fiber (6.12%), carbohydrates (64.20%), fat (19.65%), moisture content (4.32%), ash content (2.12%), and protein (11.60%). Bee pollen exhibits a color range from golden yellow to blackish hues, which can influence the color of the resulting marshmallow. Additionally, the incorporation of bee pollen has been found to enhance the texture acceptability of snack bars (Aini et al., 2020). Alcala-Orozco et al. (2024) stated that bee pollen can be utilized to improve the nutritional profile and commercial value of food products. Given its diverse nutritional content, bee pollen is expected to enhance the overall quality of marshmallow candy.

However, limited studies have investigated the physicochemical and sensory impacts of bee pollen incorporation in marshmallow formulations. This research aims to examine the effect of adding bee pollen at different concentrations on the moisture content, water activity, texture, color, ash content, reducing sugar, antioxidant activity, and organoleptic properties of marshmallows. This study hypothesizes that increasing bee pollen concentrations will enhance antioxidant activity and color while maintaining acceptable texture and taste.

LITERATURE REVIEW

Marshmallow is a type of soft candy made from gelatin and sugar, primarily sucrose and various forms of glucose. Marshmallows have a chewy texture and melt in the mouth when eaten, as they are produced by mixing flavorings, water, sugar, and gelatin, which are then whipped at high speed until they expand into a foam-like consistency (Jannah et al., 2023). The texture of marshmallows can vary depending on the formulation, desired density, and the equipment used. The ingredients typically used in marshmallow production include water, sugar, gelling agents, coloring agents, and flavorings (Cahyaningrum et al., 2021). The principle behind marshmallow production involves rapidly forming air bubbles within the mixture and trapping them to create a stable foam, resulting in a light and soft texture (Sebayang et al., 2017). Gelling agents, or gel-forming agents, are food additives used to stabilize and thicken various food products such as candies, jellies, and desserts. Factors that can influence the stability of the gel include temperature, humidity, pH, and the concentration of the additive (Pitaloka et al., 2024).

The process of marshmallow formation involves two main mechanisms, gelatinization and aeration. Gelatin dissolved in hot water functions as a gel-forming agent. When gelatin is mixed with a sugar solution, it forms a network capable of trapping air. The whipping process, which generates air bubbles trapped within the mixture and produces a stable foam, is known as aeration. Aeration is the process of introducing air bubbles into a liquid, transforming it into a foam containing air bubbles (Mayangsari et al., 2017). According to Maharani (2016), gelatin can maintain foam stability by increasing its viscosity, preventing air bubbles from breaking easily, and resulting in marshmallows with a soft and chewy texture.

According to Saputra et al. (2015), gelatin is typically obtained from the conversion of collagen found in the skin and bones of cattle and pigs. Gelatin is a multifunctional macromolecular protein polymer produced through the thermal hydrolysis of collagen tissues present in the skin, bones, and connective tissues of animals and fish. It is opaque, tasteless, brittle (when dry), colorless, and edible (Ahmad et al., 2017). In the production of red dragon fruit (*Hylocereus polyrhizus*) marshmallows, a gelatin concentration of 5% resulted in the lowest moisture content of 18.09% (Zulfajri et al., 2018).

Similarly, bee pollen, a nutrient-rich natural product, has gained attention for its potential in food development due to its high content of vitamins, minerals, and bioactive compounds such as flavonoids and carotenoids. Bee pollen contains significant amounts of phosphorus, potassium, calcium, magnesium, and iron, making it a valuable ingredient for enhancing nutritional value (Anis et al., 2021). Additionally, its flavonoid content (2.5-3.12%) not only provides antioxidant properties but also exhibits antibacterial activity, which can influence a product's final water activity (Aw) and moisture content (Utomo et al., 2017; Sari et al., 2021). The natural pigments in bee pollen, such as carotenoids, contribute to its vibrant colors, ranging from golden yellow to dark hues, and can enhance the visual appeal of food products (Sari et al., 2021). When incorporated into food formulations, bee pollen has been shown to improve texture and sensory acceptance. For instance, adding 10% bee pollen to snack bars enhances their texture quality (Aini et al., 2020), while a 3-4% concentration in bread results in a softer texture (Conte et al., 2018). These properties make bee pollen a promising ingredient for developing functional foods, much like gelatin's role in creating the desirable texture of marshmallows.

RESEARCH METHODS

The research was conducted at the Animal Product Technology Laboratory of the Faculty of Animal Science and the Food Quality Control and Safety Laboratory of the Faculty of Agricultural Technology, Brawijaya University, Malang, East Java, from August to October 2024.

Materials

The research materials used in this study were marshmallows with the addition of bee pollen at different concentrations. The ingredients for marshmallow production included powdered bee pollen obtained from the Kembang Joyo honeybee farm, granulated sugar, bovine bone gelatin, cornstarch, and mineral water. Meanwhile, the materials used for analysis included Nelson reagent A, Nelson reagent B, 1 N NaOH, distilled water (aquadest), PA-grade methanol, and a 500 ppm DPPH solution. The equipment used for marshmallow production included a digital scale, 650 mL thin-walled containers, measuring cups, parchment paper, spatulas, pots, a stove, a knife, a mixer, a wooden spatula, spoons, a thermometer, a teflon pan, and a mixing bowl. The instruments used for analysis included an oven (WTC Binder), petri dishes, desiccator, tongs, a scale (Matsunaga), an Aw meter (Aw Rotronic Hygrometer), a texture analyzer (Crane Scale), a color reader (Konica Minolta), a furnace, porcelain dishes, organoleptic evaluation sheets, a pen, a water bath, a spectrophotometer, an incubator, and a UV-Vis spectrophotometer.

Research Method

The research employed an experimental method with treatments based on different concentrations of bee pollen. A Completely Randomized Design (CRD) was used, consisting of four treatments with five repetitions. The treatments involving the addition of bee pollen at different concentrations were as follows:

P0 = Control treatment with addition of 0% bee pollen

P1 = Addition of 5% bee pollen

P2 = Addition of 10% bee pollen

P3 = Addition of 15% bee pollen

Research Variables

The observed variables in this study included water content, water activity (Aw), texture, color, ash content, reducing sugar, antioxidant, and organoleptic. The marshmallow sample testing procedures were as follows:

1. **Moisture Content (%)**

Moisture content analysis was conducted using the gravimetric method (AOAC, 2005). An empty petri dish was heated in an oven at 105°C for 24 hours, then cooled in a desiccator for 30 minutes before weighing (W0). A 2 gram sample was weighed and placed into the preweighed petri dish (W1). The sample was then dried in an oven at

105°C for 12 hours, cooled in a desiccator for 30 minutes, and reweighed along with the dried sample (W2). The moisture content was calculated using the following formula:

Moisture Content (%) = $\frac{W1-W2}{W1-W0}$ x 100%

2. Water Activity (Aw)

Water activity (Aw) is a crucial parameter of food properties related to shelf life. The water activity analysis was conducted using an Aw meter (Ulfah et al., 2018). A 10 g sample was weighed, cut into small pieces, and placed into the Aw meter container until it covered the surface. The sensor probe was then installed. The Aw meter was turned on, and the container was placed in the designated compartment, then covered with the Aw sensor. The process was monitored until the screen displayed "Reset," at which point the "Enter" button was pressed. The display then changed to "Dwell," and the measurement continued. The first beep indicated that the status changed to "Running," and the second beep signaled the appearance of the "Stop" message, which took approximately 10 minutes. The Aw value was read from the display panel and recorded.

3. Texture (N)

Texture analysis was conducted using a texture analyzer, with the results expressed in Newtons (N) (Wijayanti et al., 2018). A 1 × 1 cm sample was placed directly beneath the probe. The load was released, and the reading was taken once the instrument came to a complete stop. The value displayed on the monitor represented the texture measurement in Newtons (N).

4. Color

Color analysis using the L*a*b* system was conducted with a color reader, which measured three aspects, lightness (L*), redness (a*), and yellowness (b*) (Arifin et al., 2020). A 5 × 5 cm marshmallow sample was placed in a petri dish, which was then covered with a clear plastic sheet. The color reader was positioned on the sample's surface and turned on by pressing the ON button. The L*a*b* values were then recorded.

5. Ash Content

Ash content analysis was conducted using the ashing method with a furnace (AOAC, 2005) as follows:

1) The porcelain dish was dried in an oven at a temperature of 100-105°C for 60 minutes, then cooled in a desiccator for 15–30 minutes.

2) The weight of the porcelain dish was measured and recorded as the dish weight (W0 g).
3) A 2 g sample was weighed and placed into the cooled porcelain dish, and the total weight (dish + sample) was recorded as W1 g.
4) The sample in the porcelain dish was incinerated in a furnace at a temperature of 550-600°C for 4 hours until white ash was obtained.
5) The porcelain crucible containing the ash was cooled in a desiccator for 15–30 minutes, then weighed (W2 g).
6) Ash content is calculated with following formula:

$$\text{Ash Content (\%)} = \frac{\text{W2} - \text{W0}}{\text{W1} - \text{W0}} \times 100\%$$

Description:

W0 = weight of the empty dish (g)

W1 = weight of the dish + sample before ashing (g)

W2 = weight of the dish + ash after ashing (g)

6. Reducing Sugar

The reducing sugar content was analyzed using the spectrophotometric method (%) (Al-Kayyis and Susanti, 2016) as follows:

- The Somogyi-Nelson method (Spectrophotometry) is based on the principle that reducing sugars reduce Cu^{2+} ions to Cu^{+} ions. The Cu^{+} ions then reduce arsenomolybdate compounds, forming a blue-green colored complex.
- The procedure for reducing sugar analysis is as follows:
  1) A 1.0 ml sample extract solution with a concentration of 1.0 mg/ml was added to an alkaline Cu reagent (a mixture of Nelson reagent A and B).
  2) The solution was homogenized and heated in a water bath at 100°C for 20 minutes.
  3) The solution was homogenized again and reheated in a water bath at 100°C for 10 minutes.
  4) Approximately 4 ml of 1N NaOH was added until the solution reached a pH of 7-8.
  5) The solution was allowed to stand for the previously determined optimal time (OT), then 7 ml of distilled water was added.

6) The absorbance of the solution was measured using a visible spectrophotometer at a wavelength of 747.2 nm.

7. Antioxidant (mg/ml)

The antioxidant IC_{50} (mg/ml) was analyzed using the AOAC (2005) method as follows:

1) The sample was prepared by making a stock solution of 100 ppm for each sample by dissolving it in 10 ml of PA methanol.
2) The sample solution was diluted using PA methanol to create concentration variations of 5, 6, 7, 8, and 9 ppm.
3) A 50 ppm DPPH solution was prepared by dissolving DPPH powder in 100 ml of PA methanol.
4) A blank or control solution was prepared, consisting of 2 ml of PA methanol and 1 ml of 50 ppm DPPH solution.
5) Each sample solution (2 ml) was mixed with 2 ml of the 50 ppm DPPH solution.
6) The mixture was incubated for 30 minutes at 27°C until a color change occurred due to DPPH activity.
7) Absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm.
8) The IC_{50} value of the extract was determined by plotting the percentage of inhibition curve against sample concentration and analyzing it using linear regression.

8. Organoleptic

The organoleptic test was conducted by six semi trained panelists using a hedonic scale according to Sari and Dominica (2022), 1 (strongly dislike), 2 (dislike), 3 (moderately like), 4 (like), and 5 (strongly like). The organoleptic testing procedure followed Fadila and Juahrtini (2021) as follows:

1) The test samples were presented randomly.
2) Each sample was labeled with a unique three digit code.
3) Each panelist (either six trained panelists or 30 untrained panelists) was provided with a scoring sheet for sample evaluation.
4) Panelists tasted each prepared sample.
5) Panelists completed the scoring sheet by evaluating color, taste, aroma, texture,

and overall acceptability for each coded sample.

6) The collected data from all panelists will be statistically analyzed.

Data Analysis

The data obtained from the research results were analyzed using analysis of variance (ANOVA) with a Completely Randomized Design (CRD). If significant differences were found between treatments, further analysis was conducted using Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Moisture Content

Table 1. Average Moisture Content Value (%)

Treatments	Average (%)
P0	36.85 ± 1.02
P1	37.03 ± 0.06
P2	37.41 ± 0.47
P3	37.74 ± 0.23

The analysis of variance conducted in this study indicated that the addition of bee pollen at different concentrations did not significantly affect ($P>0.05$) the moisture content of marshmallows. Table 1 shows that the moisture content values exceed the Indonesian National Standard (SNI) 3547.2-2008 for soft jelly candy, which sets a maximum moisture content of 20%. Zainedi et al. (2022) reported that marshmallows with the addition of butterfly pea (*Clitoria ternatea* L.) extract had a relatively high moisture content, ranging from 34.28% to 38.11%. Meanwhile, jelly candies with bee pollen addition had moisture content range from 10.50% to 11.21%, with the highest level found at a 4.5% bee pollen concentration, resulting in 11.21% moisture content (Agustiani, 2017). The high moisture content in this study may be attributed to the significant amount of water used and the presence of high-moisture ingredients, such as gelatin, during the manufacturing process.

The highest average moisture content was found in P3, while the lowest moisture content was found in P0. The addition of bee pollen did not have a significant effect on the moisture content of the marshmallow. However, a higher proportion of bee pollen tended to result in a higher moisture content. This finding aligns with Agustiani (2017), who noted that as the addition of bee pollen increases in jelly candies, the moisture content also increases. Bee pollen has hygroscopic properties, allowing it to absorb moisture from the air or other materials, leading to an increase in moisture content in the final product, particularly when stored in environment with high humidity (Anis et al., 2021).

Several factors can contribute to the high moisture content, including the amount of water and sugar used, as well as the cooking time during production. The moisture content in the product originates both from the water used during processing and from the raw materials. In the marshmallow production process of this study, water and sugar were used in a 1:1 ratio with 10 g of gelatin, which contributed to the higher moisture content. Using a relatively large amount of water can result in a higher moisture content levels in marshmallows (Evandani et al., 2018). Ginting et al. (2015) reported that using water and sugar in a 1:1.6 ratio with 13.65 g of gelatin resulted in a water content of 19.38%. In the present study, the sugar was heated only to boiling point at 65-70°C for 4 minutes. Jannah et al. (2023) stated that sugar is generally dissolved at 112°C for 5 minutes, and that insufficient cooking time prevents optimal evaporation of water, resulting in higher moisture content in the marshmallow.

The purpose of measuring moisture content is to determine the water level in the product, which in turn helps estimate its shelf life. Moisture content also plays a crucial role in determining the quality of food products. One way to preserve texture and contol moisture content is to store marshmallows in a dry, airtight container to prevent the product from being affected by environmental humidity and to minimize excessive water absorption by the sugar. A high moisture creates favorable conditions for bacterial and fungal growth. Such microbial proliferation can lead to change in the product’s appearance, alterations in flavor, and a reduction in shelf life (Nadia et al., 2023).

Water Activity (Aw)

Table 2. Average Aw Value

Treatments	Average
P0	$0.74^{a} \pm 0.005$
P1	$0.86^{c} \pm 0.013$
P2	$0.79^{b} \pm 0.025$
P3	$0.74^{a} \pm 0.005$

Description: The numbers marked with superscript different letters (a, b, c) indicate highly significant different treatments (P<0.01) in the water activity (Aw) of the marshmallows.

The analysis of variance showed that the concentration of bee pollen addition had a highly significant effect ($P<0.01$) on the water activity (Aw) of the marshmallows. Based on Table 2, the results indicated that the addition of 15% bee pollen caused a notable decrease in Aw compared to the control. Meanwhile, the addition of 5% bee pollen produced the highest Aw value among all treatments.

Water activity, also reffered to as free water, must be carefully considered, as food products with high Aw values are generally more susceptible to spoilage due to microbial growth. According to Bintoro et al. (2024), marshmallows with the addition of konjac glucomannan have Aw value ranging from 0.60 to 0.61. Hartel et al. (2018) stated that the Aw value of semi-moist products, such as marshmallows, generally falls between 0.60 and 0.75. The results indicate that P0 and P3 have met the standard. The protein content in bee pollen can influence Aw values. This finding aligns with Mustamin et al. (2023), who stated that hydrophilic groups (which readily absorb water) are protein components capable of binding water. When water content decreases, the distance between protein molecules shortens, forming a denser network, thereby enhancing water-binding capacity.

The Aw value in marshmallows can be influenced by other ingredients, such as the use of sugar and gelatin, as gelatin has the ability to bind water (Fera, 2018). The sugar content in marshmallow production helps control and prevent moisture loss. The sugar cooking process can also affect the Aw value during marshmallow production, as it causes water evaporation (Setiawan, 2020). Additionally, Aw can be influenced by other factors, such as pH and air humidity. The pH value can be used as a determinant of Aw in a product, as a pH of 6-7 allows the enzyme invertase to maximize its hydrolysis reaction of sucrose, which can lower the Aw value (Hartel et al., 2018). The Aw value also affects the shelf life of food products, as higher Aw values make products more susceptible to spoilage.

Texture

Table 3. Average Texture Values

Treatments	Average (N)
P0	$0.68^{a} \pm 0.10$
P1	$0.74^{ab} \pm 0.08$
P2	$1.16^{c} \pm 0.26$
P3	$1.04^{bc} \pm 0.21$

Description: The numbers marked with superscript different letters (a, b, c) indicate highly significantly different treatments (P<0.01) in the texture of the marshmallows.

The analysis of variance in this study showed that the concentration of bee pollen addition had a highly significant effect (P<0.01) on the texture of the marshmallows. Based on Table 3, the results indicated a highly significant difference due to the addition of 5% bee pollen, which led to an increase in texture test values. The addition of 10% bee pollen produced the highest texture value (chewiness) compared to other treatments.

The chewiness of marshmallows with the addition of carrot extract (*Daucus carota* L.) has a texture value ranging from 1.05 N to 2.54 N (Jannah et al., 2023). Based on this study, P2 with the addition of 10% bee pollen met the standard. Bee pollen, used as an additional ingredient in marshmallows, had a highly significant effect. According to Agustiani (2017), the texture of jelly candy with bee pollen addition ranges from 6.33 N to 6.88 N. This finding aligns with Risti (2024), who stated that fat, carbohydrate, protein, and moisture content in food products can influence texture.

Marshmallows without the addition of bee pollen (P0) exhibited the lowest texture value at 0.68 N. This may have been influenced by several factors during the production process, such as gelatin concentration and moisture content. Treatment P3, with the addition of 15% bee pollen, resulted in a lower texture value of 1.04 N compared to P2, which had a texture value of 1.16 N. This may be due to the relatively high 15% bee pollen concentration in P3, leading to suboptimal interactions between bee pollen and the base ingredients. Excessive bee pollen concentration may prevent base ingredients such as gelatin and sugar from effectively absorbing water, as pollen particles could obstruct water-binding agents. Consequently, the marshmallow becomes less dense and softer due to weakened water and gel binding.

Color (L, a*, b*)

Table 4. Average Color Values

Treatments	L	a*	b*
P0	$91.92^{a} \pm 1.02$	$0.14^{a} \pm 0.07$	$25.39^{a} \pm 0.61$
P1	$86.74^{b} \pm 0.71$	$1.32^{b} \pm 0.25$	$29.60^{b} \pm 0.55$
P2	$81.52^{c} \pm 2.03$	$2.74^{c} \pm 0.69$	$31.97^{c} \pm 1.33$
P3	$79.70^{c} \pm 2.12$	$3.38^{c} \pm 0.25$	$33.27^{c} \pm 0.45$

Description: The numbers marked with superscript different letters (a, b, c) indicate significantly different treatments (P<0.01) in the L*a*b* color of the marshmallows.

Lightness (L)

The lightness (L*) value represents the brightness level, ranging from 0 for black to 100 for white. The analysis of variance in this study showed that the concentration of bee pollen addition had a highly significant effect (P<0.01) on the lightness of the marshmallows. Based on the data in Table 4, the results indicate that increasing bee pollen concentration decreases the lightness (L*) of the marshmallows. According to Fitriansyah (2015), the lightness (L*) value of whey marshmallows with a 3.5% gelatin concentration was 76.75. In bread with bee pollen addition has been shown to lower lightness (L*) values, ranging from 56.36 to 66.44 (Conte et al., 2018). Variation in lightness (L*) values in marshmallows are influenced by differences in ingredient composition and production methods.

Bee pollen, when used as an additional ingredient in marshmallow products, has a highly significant effect on their lightness (L*) value. In this study, bee pollen powder had a lightness (L*) value of 28.83. Marshmallows with higher bee pollen concentration exhibit stronger pigmentation effects, making the final product appear darker and resulting in lower lightness (L*) values. This finding is consistent with Cuevas et al. (2024), who stated that bee pollen contains natural pigments, including carotenoids, which contribute to a darker color in the final product. Carotenoids play an important role in imparting color to pollen grains (Ningsih et al., 2023). The pigments present in bee pollen influence the lightness (L*) value, and increasing its concentration produces marshmallow colors that more closely resemble the narural color of bee pollen.

Redness (a*)

The redness (a*) value represents the intensity of red color, ranging from 0 to 100, and green color, ranging from 0 to -80. The analysis of variance in this study showed that the concentration of bee pollen addition had a highly significant effect ($P<0.01$) on the redness (a*) value of marshmallows. Based on the data in Table 4, the results indicate that adding bee pollen increases the redness (a*) value. According to Jannah et al. (2023), the redness (a*) value of carrot extract marshmallows ranges from 16.67 to 43.03. The increase in redness (a*) value in carrot extract marshmallows was due to the high carotenoid content in carrots. The redness (a*) value of marshmallow made with fruit extract concentrate as a corn syrup substitute ranges from -0.84 to 15.11 (Goztok et al., 2022). The pigments in the fruit extract contribute to an increase in the redness (a*) value. Bread with bee pollen addition also shows an increasing redness (a*) value, ranging from 6.71 to 10.51 (Conte et al., 2018).

Based on this study, bee pollen powder exhibited a redness (a*) value of 10.63. The increase in redness (a*) value was presumed to be due to the carotenoid content in bee pollen. Bee pollen contains vitamins, minerals, proteins, carotenoids, carbohydrates, and amino acids (Sadiah & Bayu, 2024). The color of bee pollen is influenced by the presence of pigments, specifically carotenoids. Carotenoids are a group of polyenes, ranging from yellow to red, that are highly diverse and responsible for the coloration of plant-derived products, and they play an important role in human health (El Ghouizi et al., 2023). One of the factors affecting the redness (a*) value was the sugar dissolution process. Consistent heating temperatures led to color changes in the dough (browning). Fitriansyah (2015) stated that caramelization reactions occured when sugar was heated, resulting in the formation of a brown color.

Yellowness (b*)

The yellowness (b*) value represents the intensity of yellow color, ranging from 0 to 70, and blue color, ranging from 0 to -70. The analysis of variance in this study showed that the concentration of bee pollen addition had a highly significant effect ($P<0.01$) on the yellowness (b*) value of marshmallows. Based on the data in Table 7, indicated that the addition of bee pollen increased the yellowness (b*) value. According to Jannah et al. (2023), the yellowness (b*) value of carrot extract marshmallows ranges from 24.37 to 56.47. The high yellowness (b*) value in carrot extract marshmallows was suspected to be due to the beta-carotene content in carrots. Marshmallows with 1.5% cinnamon addition yielded the highest yellowness (b*) value of 18.07, with higher cinnamon concentrations resulting in darker colors in the final marshmallow product (Setiawan, 2020). Bread with bee pollen addition showed an increase in the yellowness (b*) value, ranging from 33.56 to 43.64 (Conte et al., 2018).

Based on this study, bee pollen powder exhibited a yellowness (b*) value of 22.67. Bee pollen contains carotenoids, polyphenols, and flavonoids, which possess natural antioxidant activities (Wardaniati & Taibah, 2019). The increase in yellowness (b*) value was presumed to be due to the carotenoid content in bee pollen. This finding aligns with Sari et al. (2021), who stated that carotenoid compounds are natural pigments found in plants that are responsible for yellow, orange, and red colors. An increase in the yellowness (b*) value indicated that the marshmallow color with bee pollen addition became more yellow. Conversely, a lower yellowness (b*) value indicated a decrease in the yellow color of the marshmallow. One factor that could affect the color of marshmallow was the dissolution process of bee pollen. The dissolution of bee pollen occured simultaneously with the dissolution of gelatin, which helped protect the natural color of bee pollen.

Ash Content

Table 5. Average Ash Content Values

Treatments	Average (%)
P_0	$0.806^{a} \pm 0.328$
P_1	$1.667^{b} \pm 0.027$
P_2	$2.300^{c} \pm 0.217$
P_3	$2.404^{c} \pm 0.323$

Description: The numbers marked with superscript different letters (a, b, c) indicate significantly different treatments (P<0.01) in the ash content of the marshmallows.

The results of the analysis of variance showed that the addition of bee pollen to marshmallows had a highly significant effect ($P<0.01$) on the ash content, as shown in Table 5. High ash content is an indicator of high mineral content (Murdinah, 2015). According to Tendean et al. (2021), marshmallows contain ash content between 0.1% and 0.5%. The ash content in food products refers to the mineral residue remaining after the complete combustion of organic materials. In marshmallows, ash content typically originates from additives that contain minerals, such as gelatin and sugar. Kostic et al. (2022) stated that the ash content in bee pollen was relatively high compared to other food products. Local bee pollen from Indonesia generally has an ash content of about 3.5% to 5.8%, depending on the plant source of the bee pollen. The ash content in bee pollen is an indicator of the amount of minerals present, such as calcium, phosphorus, magnesium, and potassium, which are beneficial to human health (El Ghouizi et al., 2023).

The ash content in marshmallows with the addition of bee pollen in Table 5. indicating that these values were still within the safe limits set by the Indonesian National Standard (SNI). According to BSN (2008), in SNI 3547.2-2008, the maximum ash content for soft candies is 3%. Based on the research findings of this research, the best treatment was P3, with 15% bee pollen addition, resulting in a total ash content of 2.404%. This was because higher ash content in the marshmallow corresponded to higher mineral content obtained from bee pollen addition. This is in accordance with Saras (2023), who stated that bee pollen contains 2.5-5.0% minerals, which had the potential to increase the ash content in the food product which it was added. Another study by Wandita and Rosida (2023) on the ash content in marshmallows with the addition of chayote extract and butterfly pea flower extract revealed that the best treatment was a proportion of chayote extract and butterfly pea flower extract (70:30) with 12% gelatin addition. This formulaiton resulted in a product with an ash content of 1.789%.

Reducing Sugar

Table 6. Average Reducing Sugar Values

Treatments	Averages
P_0	$0,372^a \pm 0,072$
P_1	$2,240^b \pm 0,227$
P_2	$5,052^c \pm 0,903$
P_3	$6,677^d \pm 0,721$

Description: The numbers marked with superscript different letters (a, b, c) indicate significantly different treatments (P<0.01) in the reducing sugar of the marshmallows.

The analysis of variance results indicated that the addition of bee pollen to marshmallows has a highly significant effect (P<0.01) on the reducing sugar content. The increase in reducing sugar content in marshmallows with the addition of bee pollen was influenced by the higher percentage of bee pollen in each treatment, which contributes to the rise in reducing sugar levels. Another factor affecting this increase is the presence of fructose in bee pollen and the heating process, which induced sucrose inversion, leading to an increase in reducing sugar content in marshmallows. According to Komosinska-Vassev et al. (2015), bee pollen contains 15-50% sugars, consisting of fructose and glucose.

Murtiningsih et al. (2018) stated that high-temperature heating of sucrose led to the breakdown of sucrose into glucose and fructose, a process known as inversion. This inversion resulted in an increase in reducing sugars. According to Silaen and Ginting (2019), reducing sugars include all monosaccharides (glucose, fructose, and galactose) and disaccharides (lactose, maltose, and sucrose), while polysaccharides (such as starch) are not classified as reducing sugars. Reducing sugars were closely related to enzyme activity; the higher the enzyme activity, the greater the amount of reducing sugars produced.

The reducing sugar content in marshmallows with the addition of bee pollen ranges from 0.372% to 6.677%, indicating that these values remain within the safe limits set by the Indonesian National Standard (SNI). For soft candies such as marshmallows, jelly, or milk-based candies, BSN (2008) in SNI 3547.1:2008 specifies that the reducing sugar content should not exceed 10-15% of the product's weight. Based on these findings, the best treatment was P3 with 15% bee pollen addition, resulting in a total reducing sugar content of 6.677%. This was because higher reducing sugar levels in marshmallows corresponded to increased enzyme activity, which was attributed to the addition of bee pollen. This finding is consistent with the study by Sarofa et al. (2019) on the characteristics of marshmallows made from banana peel, which reported reducing sugar levels as ranging from 5% to 5.17%.

Antioxidant

Table 7. Average Antioxidant Values

Treatments	Average
P0	$503.927^{a} \pm 37.099$
P1	$242.282^{b} \pm 19.937$
P2	$152.442^{c} \pm 8.966$
P3	$92.962^{d} \pm 2.929$

Description: The numbers marked with superscript different letters (a, b, c) indicate significantly different treatments (P<0.01) in the antioxidant of the marshmallows.

IC_{50}, or Inhibitory Concentration 50%, is a parameter used to measure the effectiveness of an antioxidant compound in inhibiting oxidation. Specifically, IC_{50} is defined as the concentration of a compound required to inhibit 50% of free radical activity in a test system. The lower the IC_{50} value, the stronger the antioxidant activity of the compound (Putri, 2020). Based on the analysis of variance for antioxidant activity testing using the IC_{50} method, the addition of bee pollen to marshmallows showed a highly significant effect ($P<0.01$). The analysis results indicate that the mean IC_{50} values increased from P0 to P3, ranging from 503.927 mg/ml to 92.962 mg/ml. However, the highest value in treatment 3 (P3) still fell within the category of inactive antioxidants. According to Susiloningrum and Sari (2021), antioxidant strength levels are classified as very strong (IC_{50} <50 ppm), strong (IC_{50} 51-100 ppm), moderate (IC_{50} 101-150 ppm), weak (IC_{50} >151 ppm), and inactive (IC_{50} >500 ppm).

The IC_{50} antioxidant values, which indicate the inactive antioxidant content in marshmallows with increasing bee pollen additions in each treatment, may be influenced by several factors, one of which is the effect of temperature and processing methods. Marshmallow production involves intensive heating and whipping processes. Antioxidant compounds in bee pollen, such as vitamins and polyphenols, may degrade due to high temperatures or mechanical whipping, thereby reducing their antioxidant activity. This is consistent with the findings of Juszczak et al. (2021), who stated that natural antioxidants in bee pollen, such as flavonoids and polyphenols, are highly sensitive to high temperatures. As the temperature increases, these compounds degrade, leading to a decrease in antioxidant activity.

Another factor influencing the inactivity of antioxidant values is the interaction between bee pollen components and marshmallow ingredients. Bee pollen contains high levels of antioxidants, but these compounds may not interact effectively with other ingredients such as sugar and gelatin in marshmallows. High sugar content can reduce antioxidant effectiveness because antioxidant compounds may bind to sugar, rendering them biologically inactive. Meanwhile, proteins in gelatin can interact with polyphenol compounds from bee pollen, forming protein-polyphenol complexes that lower antioxidant bioavailability. This aligns with Juszczak et al. (2021), who stated that adding bee pollen to food products can increase phenolic content and antioxidant activity. However, different food ingredients can result in varying interactions, and different processing methods, such as heating, can affect the stability of antioxidant compounds in bee pollen.

The study on antioxidant activity in marshmallows with the addition of beetroot reported a value of 83.42% (Gumansalangi and Djarkasi, 2019). Meanwhile, another study on the addition of bee pollen in snack bars showed that the antioxidant activity of bee pollen snack bars was 10.77 mg ascorbic acid per gram, meaning that each 1 g of product had antioxidant activity equivalent to 10.77 mg of ascorbic acid. The antioxidant content in bee pollen snack bars is still relatively low (Aini et al., 2020). According to the research by Marjan et al. (2016), a food product can be claimed as a functional food if it has a free radical inhibition factor of 50%.

The study by Sari et al. (2020) on the antioxidant activity of corn silk marshmallows showed the highest result in treatment P1 with a gelatin concentration of 2.50%, achieving 8.43% inhibition, while the lowest antioxidant activity was found in treatment P5 with a gelatin concentration of 7.50%, showing 5.71% inhibition. The decrease in antioxidant activity corresponded to the increase in gelatin concentration, as higher gelatin concentrations trapped more oxygen during the whipping process, accelerating oxidation and reducing antioxidant potential. This is supported by Zulfajri (2018), who stated that vitamin C functions as an antioxidant by donating electrons, causing it to be oxidized in the antioxidant process and forming dehydroascorbic acid. Consequently, the more gelatin added, the more oxygen is trapped during mixing, leading to increased oxidation of vitamin C. This results in a decrease in vitamin C content as an antioxidant in red dragon fruit marshmallows. Based on the research findings on bee pollen marshmallows, the best treatment was P3 with the addition of 15% bee pollen, yielding a total antioxidant activity of 92.962 mg/ml.

Organoleptic

Table 8. Average Organoleptic Values

Treatments	Organoleptic				
	Color	Taste	Texture	Aroma	Acceptance
P_0	$1,4^{a}$±0,723	$3,3^{a}$±0,802	4,0±0,639	$2,0^{a}$±1,201	$4,2^{a}$±0,639
P_1	$2,5^{b}$±0,900	$3,1^{ab}$±0,912	4,2±0,610	$2,7^{ab}$±1,135	$4,0^{a}$±0,639
P_2	$3,1^{bc}$±0,758	$3,0^{bc}$±0,982	4,4±0,498	$3,0^{b}$±0,980	$3,6^{a}$±0,718
P_3	$3,5^{c}$±0,727	$2,8^{c}$±0,805	4,3±0,466	$3,2^{b}$±1,104	$3,7^{a}$±0,651

Description: Different superscripts in the column indicate a highly significant effect ($P<0.01$) on color, aroma, and acceptance, while for taste, the effect is significant ($P<0.05$), and texture shows no significant effect ($P>0.05$).

Color

The analysis of variance results indicate that the addition of bee pollen to marshmallows has a highly significant effect ($P<0.01$) on color. The average color scores range from 1.4 to 3.5 (from strongly dislike to like). The color of marshmallows is generally influenced by the main ingredients used, such as sugar, gelatin, and water. Marshmallows have a naturally white base color because their ingredients do not contain significant pigments. Meanwhile, bee pollen contains natural pigments such as carotenoids and flavonoids (Rahmawati and Junus, 2024). This is supported by Saras (2023), who stated that bee pollen exhibits different colors depending on the type of plant pollen collected by bees. However, most bee pollen has yellow, orange, or yellow-brown hues, which can significantly alter the color of marshmallows that originally have a white base.

According to Wandita and Rosida (2023), marshmallows achieved an organoleptic color score of 3.16 (moderately like) in their study on the effects of the proportion of chayote and butterfly pea flower extract, as well as the addition of gelatin, on marshmallow characteristics. The addition of other food ingredients containing pigments can affect the color of the marshmallows. Bee pollen contains beta-carotene, one of its pigments, which can influence the color score in the organoleptic evaluations by the panelists. Based on these findings, the best treatment was P3 with 15% bee pollen addition, which achieved an organoleptic color score of 3.5 (like). This is because the higher the percentage of bee pollen added to the marshmallows, the greater its impact on the color and the acceptance by the panelists.

Aroma

The analysis shows that the addition of bee pollen to marshmallows has a highly significant effect ($P < 0.01$) on aroma. The average aroma scores ranged from 2.0 to 3.2 (dislike-moderately like). According to Hardiayanti (2016), aroma testing is essential for evaluating consumer perception of the product. Bee pollen has a distinctive aroma, typically described as a combination of floral, herbal, and citrus notes. Starowicz et al. (2021), characterized bee pollen aroma as floral, herbal, grassy, and citrus-like. The chemical composition of bee pollen, particularly its volatile compounds, plays a crucial role in determining its aroma. These compounds may vary depending on the flower source, geographical location, and environmental conditions where the bee pollen is collected, thus influencing the characteristic aroma and its impact on marshmallow sensory quality.

According to Wandita and Rosida (2023), marshmallows obtained an organoleptic aroma score of 3.28 (moderately liked) in their study on the effects of chayote proportion, butterfly pea flower extract, and gelatin addition. The incorporation of other food ingredients with a stronger aroma can influence the overall aroma of marshmallows. Bee pollen contains volatile compounds that play a crucial role in determining aroma, thereby affecing the sensory evaluation of marshmallows with bee pollen addition by panelists. Based on the research findings, the best treatment was P3, with the addition of 15% bee pollen, which received an organoleptic aroma score of 3.2 (moderately like). This suggest that a higher percentage of bee pollen provides a distinctive aroma, enhancing product acceptance among panelists.

Taste

The analysis of variance results indicate that the addition of bee pollen to marshmallows had a significant effect ($P<0.05$) on taste. The average taste scores ranged from 2.8 to 3.3 (moderately like). The reduction in taste score with increasing bee pollen may be related to its idstinct and complex flavor profile. Suarti et al. (2016) described the taste of bee pollen as nutty, earthy, floral, and herbal. Furthermore, Starowicz et al. (2021) stated that while bee pollen flavor is generally acceptable at low concetrations, higher levels intensify its taste and aroma, which may reduce acceptance, particularly amoong consumers unfamiliar with its unique sensory characteritics.

According to Wandita and Rosida (2023), marshmallows have an organoleptic taste score of 3.6 (like) in their study on the effects of chayote proportion, butterfly pea flower extract, and gelatin addition on marshmallow characteristics. However, Aldarf et al. (2023) stated that a high level of bee pollen addition can reduce panelists' acceptance of taste due to the emergence of a dominant bitter flavor. The decline in organoleptic taste scores in marshmallows with bee pollen addition is likely influenced by several factors; nevertheless, the product’s taste remains acceptable to panelists. Based on the study results, the best treatment was P1, with the addition of 5% bee pollen, which achieved an organoleptic taste score of 3.1 (moderately like). This is because, as the percentage of bee pollen increases in marshmallows, the taste becomes increasingly similar to that of bee pollen itself, thereby affecting panelists' acceptance.

Acceptance

The analysis of variance results indicate that the addition of bee pollen to marshmallows has a highly significant effect ($P<0.01$) on acceptance. The average acceptance scores ranged from 3.7 (accepted) to 4.2 (accepted). Although the mean acceptance scores decreased slightly from P0 to P3, they remain within the same acceptance range, from 4.2 (accepted) to 3.7 (accepted).

The decline in acceptance scores with increasing levels of bee pollen in marshmallows may be attributed to several factors, including chenges in color, aroma, and taste. Saras (2023), who stated that bee pollen can impart a yellow to brown coloration to marshmallows, which may be perceived as less appealing by panelists who are more accustomed to white or soft pastel colored marshmallows. Such color changes could reduce the visual acceptance of the product. In addition, Starowicz et al. (2021) reported that bee pollen has a distinct and intense aroma and flavor profile, characterized as floral, herbal, and slightly bitter. The increased intensity of these sensory attributes at higher concentrations may lower panelists' acceptance, particularly among those unfamiliar with these characteristics.

Based on the study by Suryani and Nisa (2015) on the modification of cassava starch (*Manihot esculenta*) using α-amylase enzyme as a foaming agent and its application in marshmallow production, the acceptance test results showed that 6 panelists rejected the marshmallow from the best maltodextrin treatment, while 14 panelists accepted it. This indicates that 70% of the panelists considered the marshmallow from the best maltodextrin treatment acceptable in terms of taste, texture, and color. Based on these findings, the most accepted treatment by panelists was P1, with the addition of 5% bee pollen, which achieved an organoleptic color score of 4.0 (like). Although no significant differences were found between treatments, all variations remained highly acceptable to the panelists.

CONCLUSION

Based on the research findings, it can be concluded that the addition of bee pollen at different concentrations in marshmallow candy decreases water activity (Aw), enhances texture (making it chewier), improves color, increases ash content, reducing sugar levels, and increases antioxidant activity, as well as affecting organoleptic attributes (color, aroma, taste, and overall acceptance). The best results were obtained in treatment P3, with a bee pollen concetration of 15% which recorded an Aw 0.74, texture 1.04 N, and lightness (L*) 79.70, redness (a*) 3.38, and yellowness (b*) 33.27, ash content of 2.404%, reducing sugar content 6.677%, antioxidant activity 92.962 mg/ml, and organoleptic scores of 3.5 (like) for color, 2.8 (moderately like) for taste, 3.2 (moderately like) for aroma, and (3.7, accepted) for overall acceptance.

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