

## Proximate Analysis of Milk-Based Jelly Candy with Different Bee Pollen Concentrations

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**Abstract.** This study aimed to evaluate the effect of bee pollen addition on the chemical composition of cow's milk-based jelly candy. Bee pollen is a natural product rich in proteins, minerals, and bioactive compounds, which may enhance the nutritional value of food products. The research was conducted at the Dairy Technology Laboratory, Department of Animal Product Technology, Faculty of Animal Science, Universitas Brawijaya, from June to August 2025. The research method used was a laboratory experiment using Complete Randomized Design (CRD) ANOVA with 4 treatments and 4 replications. If different results were obtained between treatments, Duncan's Multiple Range Test (DMRT) was continued. Four treatments were applied: T0 (control, without bee pollen), T1 (6%), T2 (12%), and T3 (19%). The results demonstrated that bee pollen supplementation had a highly significant effect ( $P < 0.01$ ) on the protein content (Kjeldahl method), moisture content (Gravimetri method), ash content (Dry Ashing method), and carbohydrate content (By Different method), but showed no significant effect ( $P > 0.05$ ) on fat content (Soxhlet method). Protein content increased from 16.83% to 19.30%, moisture content increased from 29.56% to 34.30%, while ash content exhibited a fluctuating trend. Conversely, carbohydrate content decreased from 52.90% to 45.19%. Overall, the addition of bee pollen improved the nutritional quality and functional potential of milk-based jelly candy, particularly as a natural source of protein and minerals.

**Keywords:** Bee pollen, Functional food, Milk jelly candy

## INTRODUCTION

Milk is defined as a yellowish-white liquid secreted by the mammary glands of healthy dairy animals. It is classified as a perishable animal product containing essential nutrients required by humans, such as proteins, fats, lactose, minerals, and vitamins. In addition to direct consumption, milk also serves as a raw material for various processed food products. It is commonly used in the production of fermented and confectionery products such as ice cream, cheese, yogurt, butter, and candy, which enhance its added value and consumer appeal (Jang et al., 2024). The use of milk as a base ingredient in jelly candy formulation contributes to a higher protein content. However, such products still have certain lacks, including a dominant sugar composition that leads to low nutritional density, limited amounts of healthy milk fats, and relatively low mineral content (Teixeira-Lemos et al., 2021). These limitations have encouraged innovation through the incorporation of functional ingredients derived from natural bee products.

One promising bee product for use in milk-based jelly candy is bee pollen. Bee pollen is a natural substance collected by honeybees that contains bioactive components such as flavonoids, phenolic compounds, proteins, and carbohydrates (Ryntathiang et al., 2025). Previous studies have explored the incorporation of bee pollen into various food products, including yogurt, bread, and biscuits, where it has been shown to enhance the nutritional and functional properties of the products. In milk-based jelly candy, the addition of bee pollen may help balance the nutritional profile, resulting in a product with improved nutrient density and potential functional benefits.

To date, studies on cow's milk jelly candy enriched with bee pollen remain limited. The incorporation of bee pollen is expected to optimize the chemical composition of the product by improving proximate analysis parameters. Different concentrations of bee pollen may also yield varying effects on these compositional characteristics. Therefore, it is important to determine how varying levels of bee pollen addition influence the nutritional composition of milk-based jelly candy.

## LITERATURE REVIEW

Normal milk typically contains 3–4% fat, 3.5% protein, 5% lactose, and around 1.2% minerals, with casein accounting for approximately 80% of the total protein content (Kolenda & Sitkowska, 2021). Jelly candy is one of the confectionery products made using milk as a base ingredient, combined with hydrocolloids and sugar (Sana &

Manal, 2023). The use of hydrocolloids plays an important role in determining the organoleptic and textural characteristics of the final product. Commonly used hydrocolloids in jelly candy production include gelatin, starch, and pectin (Silveira et al., 2025). This type of candy appeals to a broad range of consumers, from children to adults. However, to enhance its nutritional value, there is a growing need to fortify jelly candies with natural, nutrient-rich ingredients (Ozcan et al., 2024). Previous studies on jelly candy have explored fortification with various additives, such as passion fruit flavoring combined with *Bacillus coagulans*, which demonstrated good compatibility between the probiotic and the flavor compound (Miranda et al., 2020).

One potential natural additive for nutritional enrichment is bee pollen. Bee pollen is a product collected by honeybees from flowering plants and is known for its high nutritional content, consisting of 10–40% protein, 13–55% carbohydrates, 2–6% minerals, and 3–5% polyphenols, along with essential vitamins (Sharma et al., 2025). Another source reports that bee pollen contains approximately 10.6% moisture, 19.4% protein, 2.4% ash, and notable levels of potassium (9,765.6 mg/kg) and calcium (963.4 mg/kg) (Yadeta et al., 2024).

Due to its rich nutritional composition, bee pollen is considered a promising functional food ingredient (Ghouizi et al., 2023). Several studies have investigated its incorporation into various food products. The addition of 1–5% bee pollen to gluten-free bread formulations improved texture, color, and sensory characteristics, with optimal results observed between 3% and 5% (Conte et al., 2018). Similarly, the enrichment of yogurt with bee pollen at concentrations of 10, 25, 50, and 75 g led to an increase in protein content and modifications in both texture and sensory characteristics (Cosme-Alonso et al., 2023). In another study, bee pollen was added to potato snacks at levels of 1–5% as a source of natural antioxidants, with the highest antioxidant activity observed at 5% addition (Nemš et al., 2025).

## **RESEARCH METHODS**

The study was conducted at the Dairy Technology Laboratory, Division of Milk Science, Faculty of Animal Science, Universitas Brawijaya. The research included both the production of milk-based jelly candy and proximate composition analysis. The experiment took place from June to August 2025.

### **Materials**

The ingredients used in the production of milk-based jelly candy included fresh cow's milk obtained from a local dairy farm in Nganjuk, East Java; gelatin powder (Hakiki brand); granulated sugar (Gulaku brand); glucose syrup purchased from a local chemical store; and bee pollen supplied by PT Kembang Joyo Sriwijaya, which served as the treatment variable. The equipment utilized in the process comprised an analytical balance, thermometer, cooking pan, spatula, gas stove, and silicone candy molds.

## Methods

The research method used was a laboratory experiment using Complete Randomized Design (CRD) ANOVA with 4 treatments and 4 replications. If different results were obtained between treatments, Duncan's Multiple Range Test (DMRT) was continued. The production process began by mixing all ingredients in a pan according to the treatment formulation, without initial heating. The mixture was then heated at a temperature of 60–70°C while continuously stirred for approximately 13 minutes until the gelatin was completely dissolved and the jelly mass reached a homogeneous consistency. The cooked jelly mixture was poured into silicone molds and subsequently refrigerated for 16 hours to allow proper setting and texture formation. The experimental treatments were based on varying concentrations of bee pollen addition can be seen in Table 1.

**Table 1.** Varying Concentrations of Bee Pollen Addition

| Composition      | Treatment |            |             |             |
|------------------|-----------|------------|-------------|-------------|
|                  | T0        | T1         | T2          | T3          |
| Cow's milk       | 206 gr    | 206 gr     | 206 gr      | 206 gr      |
| Granulated sugar | 86 gr     | 66 gr      | 46 gr       | 20 gr       |
| Bee pollen       | 0 gr      | 20 gr (6%) | 40 gr (12%) | 66 gr (19%) |
| Gelatin          | 33 gr     | 33 gr      | 33 gr       | 33 gr       |
| Glucose syrup    | 18 gr     | 18 gr      | 18 gr       | 18 gr       |

## Proximate Analysis

### Protein Content

Protein content was determined using the Kjeldahl method according to (AOAC, 2005). The reagents used included concentrated H<sub>2</sub>SO<sub>4</sub>, K<sub>2</sub>SO<sub>4</sub>, CuSO<sub>4</sub>, 40% NaOH, boric acid, mixed indicator solution, and 0.1 N HCl. The equipment utilized consisted of an analytical balance, Kjeldahl digestion and heating apparatus, distillation unit, burette, volumetric pipette, measuring cylinder, and volumetric flask.

The analysis was performed in three main steps: digestion, distillation, and titration. The protein content was calculated using the following formula:

$$\text{Protein content (\%)} = N \times 14.007 \times F \times 6.25$$

where  $N$  is the normality of HCl and  $F$  represents the titration factor (volume of HCl used).

### **Fat Content**

Fat content was analyzed using the Soxhlet extraction method following (AOAC, 2005). Petroleum ether was used as the extraction solvent. The apparatus included an analytical balance, filter paper, cotton, mortar, complete Soxhlet apparatus, pipette, clamp, and measuring cylinder. Samples that had previously been dried for moisture determination were ground, wrapped in filter paper lined with cotton, and placed in the Soxhlet extractor. Extraction was carried out for 6 hours using petroleum ether. After extraction, the residual fat was dried and weighed. Fat content was calculated as follows:

$$\text{Fat content (\%)} = (\text{Weight of extracted fat} / \text{Initial sample weight}) \times 100\%$$

### **Moisture Content**

Moisture content was determined by the gravimetric method according to (AOAC, 2005). The analysis utilized an analytical balance, Petri dishes, tongs, a drying oven set at 105°C, and a desiccator. Petri dishes were pre-dried in the oven, then samples were weighed and oven-dried at 105°C for 12 hours. The dried samples were cooled in a desiccator and reweighed. Moisture content was calculated using the formula:

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100\%$$

$W_1$  = sample weight before drying (g)

$W_2$  = sample weight after drying (g).

### **Ash Content**

Ash content was measured using the dry ashing method (AOAC, 2005). The analysis required distilled water and fiber-free tissue paper. Equipment included an analytical balance, porcelain crucible, furnace (600°C), desiccator, drying oven (105°C), tongs, and a hotplate. Samples were incinerated in a muffle furnace at 600°C for 4–6 hours until a white or gray ash residue was obtained. The crucibles were cooled in a desiccator and weighed. Ash content was calculated as:

$$\text{Ash content (\%)} = \frac{W_2 - W_0}{W_1 - W_0} \times 100\%$$

$W_0$  = weight of empty crucible (g)

$W_1$  = weight of crucible + sample before ashing (g)

$W_2$  = weight of crucible + ash after ashing (g).

### Carbohydrate Content

Carbohydrate content was determined by difference according to (AOAC, 2005), using the following formula:

$$\text{Carbohydrate (\%)} = 100 - (\text{Moisture} + \text{Ash} + \text{Protein} + \text{Fat})$$

## RESULTS AND DISCUSSION

Based on the analyses conducted, the obtained data were statistically evaluated using Analysis of Variance (ANOVA). The results of the proximate composition analysis of milk-based jelly candy with the addition of bee pollen are presented in Table 2.

**Table 2.** Proximate Composition of Milk-Based Jelly Candy with Bee Pollen Addition

| Variable             | T0                        | T1                        | T2                        | T3                        |
|----------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Protein Content (%)  | 16.83 ± 0.10 <sup>a</sup> | 17.05 ± 0.13 <sup>b</sup> | 19.30 ± 0.10 <sup>c</sup> | 19.14 ± 0.05 <sup>c</sup> |
| Fat Content (%)      | 0.09 ± 0.01               | 0.10 ± 0.03               | 0.10 ± 0.03               | 0.13 ± 0.03               |
| Moisture Content (%) | 29.56 ± 0.17 <sup>a</sup> | 30.77 ± 0.17 <sup>b</sup> | 33.33 ± 0.17 <sup>c</sup> | 34.30 ± 0.34 <sup>d</sup> |
| Ash Content (%)      | 0.63 ± 0.17 <sup>a</sup>  | 1.02 ± 0.05 <sup>b</sup>  | 0.74 ± 0.10 <sup>a</sup>  | 1.24 ± 0.13 <sup>b</sup>  |
| Carbohydrate (%)     | 52.90 ± 0.26 <sup>d</sup> | 51.07 ± 0.16 <sup>c</sup> | 46.54 ± 0.30 <sup>b</sup> | 45.19 ± 0.18 <sup>a</sup> |

Note: Different superscript letters (a, b, c, d) within the same column indicate a highly significant difference ( $P < 0.01$ )

### Protein Content

The results of protein analysis (Table 2) showed a highly significant difference ( $P < 0.01$ ) indicating that a higher concentration of bee pollen increased the protein content of milk jelly candy. This occurred because bee pollen contains a considerable amount of protein, which increases the overall protein composition of the product. Bee pollen has been reported to contain about 19.4% protein (Yadeta et al., 2024). The increase in protein content may also improve the essential amino acid profile of the product, as bee pollen is known to contain 34–48% essential amino acids such as valine, lysine, leucine, isoleucine, histidine, phenylalanine, threonine, tyrosine, methionine, and tryptophan (Sharma et al., 2025). The higher protein content may also enhance product shelf life since bee pollen exhibits antimicrobial activity (Candan et al., 2025).

Animal-derived protein from milk and plant-based protein from bee pollen complement each other in forming a balanced amino acid composition in milk jelly candy. Bee pollen proteins mainly exist as peptides and free amino acids, which are more easily absorbed by the human body compared to complex proteins. The digestibility score of bee pollen protein is reported to be around **69%** (Aylanc et al., 2023). The increase in protein content is inversely related to the decrease in carbohydrate content due to proportional composition shifts.

### **Fat Content**

The fat content analysis (Table 2) showed no significant difference ( $P > 0.05$ ) although a slight increase was observed. The insignificant change may be due to the relatively low fat content of bee pollen, which is approximately **10%** (Li et al., 2023). The small addition of bee pollen in the formulation did not significantly influence the fat content of milk-based jelly candy. During heating, oxidation of unsaturated fatty acids might occur, producing volatile compounds that evaporate, leading to no significant variation in total fat. Similar observations were reported where thermal treatment and homogenization of milk reduced unsaturated fatty acids due to oxidation (Fan et al., 2023).

The homogeneity of fat distribution in the candy may also contribute to this result. Continuous stirring during heating ensures even dispersion of fat particles, preventing phase separation and maintaining consistent fat levels throughout the product. It has been reported that homogenization during production results in uniform fat globule size and distribution (Dhal et al., 2023).

### **Moisture Content**

The results for moisture content (Table 2) indicated a highly significant difference ( $P < 0.01$ ). Increasing bee pollen concentration led to higher moisture levels in the product. This is because bee pollen naturally contains water, contributing to total moisture in the jelly candy. The moisture content of bee pollen ranges between 20–30% (Zhang et al., 2025). In addition, proteins and polysaccharides present in bee pollen can bind water molecules through hydroxyl groups, increasing water retention in the product (Haroune et al., 2025).

Higher bee pollen concentrations also made the jelly mixture thicker, reducing free water evaporation during heating. This occurs because soluble fibers and

polysaccharides increase viscosity, as previously reported that polysaccharides in bee pollen enhance product viscosity (Li et al., 2023). Although moisture levels increased, product stability was not negatively affected since bee pollen contains phenolic and flavonoid compounds that exhibit antimicrobial and antioxidant properties (Ryntathiang et al., 2025).

### **Ash Content**

The ash content analysis (Table 2) showed a highly significant difference ( $P < 0.01$ ), with an irregular trend among treatments. The increase and fluctuation of ash content can be explained by the high mineral composition of bee pollen, which contains potassium (9765.6 mg/kg), calcium (963.4 mg/kg), magnesium (960.5 mg/kg), iron (142.8 mg/kg), and phosphorus (126.5 mg/kg) (Yadeta et al., 2024). These variations may occur due to uneven mineral distribution during heating and mixing processes, as reported in milk-based systems (Fan et al., 2023).

Some minerals may also be lost through volatilization during heating. Interactions between milk proteins, gelling polysaccharides, and bee pollen minerals could affect the remaining mineral residue after ashing. It has been observed that proteins and polysaccharides can adsorb minerals, resulting in lower measurable ash content (Kilic-Akyilmaz et al., 2022). Therefore, although ash levels did not increase linearly, significant differences between treatments confirm that bee pollen influences the mineral composition and structure of the product matrix.

### **Carbohydrate Content**

Carbohydrate analysis (Table 1) showed a significant decrease ( $P < 0.01$ ) with increasing bee pollen concentration. This occurred because bee pollen contains lower carbohydrate levels compared to granulated sugar, which mainly consists of sucrose. The total sugar content in bee pollen is around 19–25 g per 100 g, consisting primarily of fructose and glucose (Bertoncelj et al., 2018), and the overall carbohydrate content is about 13% (Giampieri et al., 2022). Hence, substituting part of the sugar with bee pollen reduces total carbohydrate content in the formulation.

Additionally, during heating, reducing sugars in the mixture may undergo caramelization or Maillard reactions with amino acids, leading to decreased measurable carbohydrate levels. These reactions convert simple sugars into brown-colored compounds (Mutlu et al., 2018). Polysaccharides in bee pollen, such as pectin,

cellulose, and hemicellulose, can also degrade into smaller compounds under heat treatment, further reducing carbohydrate levels (Li et al., 2023).

## CONCLUSION

The addition of bee pollen to milk-based jelly candy showed a highly significant effect ( $P < 0.01$ ) on protein, moisture, ash, and carbohydrate contents, but no significant effect on fat content. Overall, the incorporation of 12% bee pollen (P2) improved the nutritional value and functional potential of the milk jelly candy. The addition of bee pollen at an optimal concentration of 12–19% is recommended to produce milk jelly candy with higher protein and mineral content without significantly altering the texture. Further studies are needed to evaluate the physical and sensory characteristics of the product in order to determine consumer acceptability.

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