# Evaluation of Propolis as a Natural Preservative: Effects on The Nutritional Quality of Milk Jelly Candy

Faradisa Zul Alfi Husna[1], Dedes Amertaningtyas[1*], Aris Sri Widati[2], Agus Susilo[2]

[1]Magister Student of Animal Science Faculty, Universitas Brawijaya, Malang 65145, Indonesia
[2]Faculty of Animal Science, Universitas Brawijaya, Malang 65145, Indonesia

**Abstract.** Propolis is a bee product that bees collect from resin plant then secreted by bee saliva. Propolis has antioxidant, antifungal and antimicrobial activity so it is generally used as a natural preservative. The aim of this study was to determine and evaluate the nutritional content of milk jelly candy with added propolis at different concentrations. This study used a Completely Randomized Design (CRD) with 5 treatments and 5 replications. Data analysis used the ANOVA test and DMRT follow-up test. The treatments in this study were the levels of propolis addition P0 (0%), P1 (0.3%), P2 (0.5%), P3 (1%), and P4 (1.5%). The parameters included protein content, water content, fat content, ash content and carbohydrate content. The results showed that, addition of propolis at various concentrations had highly significantly (P<0.01) in protein content (17.51-18.48%), water content (37.67-49.5%), fat content (0.12-0.38%), ash content (0.75-0.83%), and carbohydrate content (31.14-43.09%). Increasing propolis levels improved fat content, ash content, carbohydrate content, but reduced in water content and protein content. The conclusion showed that the addition propolis as a natural preservative has almost the same effect as the use of synthetic preservatives, in some parameters has a better effect.
**Keywords:** bee, processing, product, proximate

## 1 Introduction

Milk jelly candy is a semi wet-food candy that has a soft texture and a sweet taste. Milk jelly candy can be made from fresh cow's or goat's milk mixed with gel-forming ingredients such as gelatin and sweeteners such as sugar or honey, cooked for 10-20 minutes [1, 2]. The relatively short cooking time and the absence of preservatives make milk jelly candy susceptible to microbial contamination. Husna and Amertaningtyas [1] showed that milk jelly candy made from 60% fresh cow's milk, 25% honey, 10% gelatin, and 5% glucose syrup with 10 minutes of cooking produced a water activity of 0.84-0.87. This Aw value is still susceptible to contamination, because mold and yeast can grow in products with an Aw of

* Corresponding author: dedesfpt@ub.ac.id

0.6-0.7. The addition of preservatives is very necessary to inhibit microbial growth and extend the shelf life of milk jelly candy.

Preservatives are substances used to extend the shelf life of products by inhibiting the growth of microorganisms. Various synthetic preservative compounds, such as sodium benzoate, sorbic acid, and sodium nitrite, are widely used to inhibit microbial growth and extend the shelf life of products [3]. Based on SNI No. 01-0222-1995 and PERMENKES RI No. 033 of 2012 concerning Food Additives, the permitted preservatives for jelly candies are sodium benzoate, potassium sorbate, and sulfur dioxide. However, traces of chemical residues remaining in food and the potential for long-term side effects such as disruption of the intestinal microbiota balance, allergic reactions, and the formation of carcinogenic compounds have raised consumer concerns [4]. Today's consumers are increasingly demanding transparency in composition and the use of additives recognized as natural. Based on this, the search for safe and effective alternative natural preservatives has become a necessity in food product innovation. One natural ingredient that functions as a preservative is propolis.

Propolis is a bee product collected from buds, shoots, pollen to leaves which are then secreted by bee saliva [5]. The main compounds found in propolis include resin and balsam (45–55%), wax (8–35%), essential and aromatic oils (5–10%), fatty acids (5%), pollen (5%) and organic and mineral substances [6]. Propolis has antioxidant, antitumor, anticancer, antifungal, antiprotozoal, antibacterial activity. Gniewosz et al. [7] made edible films by adding propolis at various concentrations (3%, 5%, and 10%). The addition of propolis slightly reduced the water content of the edible film.

The use of preservatives in food products must also consider their impact on the product's nutritional content, such as protein, fat, water, ash, and carbohydrate content. Research on the use of propolis as a natural preservative in milk jelly candies to determine its effect on nutritional content has not been conducted; several previous studies have focused on product storage. Based on this, the use of propolis as a natural preservative in milk jelly candies needs to be studied specifically to determine its effect on protein, fat, water, ash, and carbohydrate content.

# 2 Materials and Methods

## 2.1 Materials

The material used in this study was milk jelly candy with added propolis. The ingredients for making milk jelly candy include fresh cow's milk, gelatin, kapok honey, and propolis.

## 2.2 Methods

This research was conducted as a laboratory experiment. The research method used was a Completely Randomized Design (CRD) with 5 treatments and 5 replications. The treatments were P0 = 0% propolis; P1 = 0.3% propolis; P2 = 0.5% propolis; P3 = 1% propolis and P4 = 1.5% propolis. The milk jelly candy formulation can be seen in Table 1.

**Table 1.** Milk Jelly Candy Formulation.

| The Ingredients (%) | P0 | P1 | P2 | P3 | P4 |
|---|---|---|---|---|---|
| Fresh Milk | 65 | 65 | 65 | 65 | 65 |
| Gelatin | 25 | 25 | 25 | 25 | 25 |
| Randu Honey | 10 | 10 | 10 | 10 | 10 |
| Propolis | 0 | 0.3 | 0.5 | 1 | 1.5 |

### 2.3 Milk Jelly Candy Preparation

Prepare the tools and ingredients. First, the stopwatch is turned on while cooking the milk jelly candy. Fresh cow's milk is put into a pan and cooked over medium heat for approximately 2 minutes (until a temperature of 70°C) while the milk is stirred. When the temperature has reached 70°C the stove is turned down, then the gelatin is added and immediately stirred so that the gelatin does not clump. Cooking is continued at a temperature of 60-70C until the 7th minute on the stopwatch. Then the kapok honey is added to the mixture and cooked until the 11th minute. Next, propolis is added according to treatment P1 (0%), P1 (0.3%), P2 (0.5%), P3 (1%) and P4 (1.5%) cooked until the 13th minute. The total cooking time is 13 minutes, during cooking the mixture must be continuously stirred to avoid clumping of gelatin and milk during cooking. Next, put the jelly candy into a silicone mold and chill it in the refrigerator for 16 hours.

### 2.4 Parameters

The protein content of the samples was measured using the Kjeldahl method [8]. The water content was measured using the gravimetric method [8]. The fat content was determined using the Soxhlet extraction method [8]. The ash content was measured using the ashing/gravimetric method [8]. The carbohydrate content was calculated using the by different method [8].

### 2.5 Data Analysis

Statistical data analysis was conducted using one-way analysis of variance (ANOVA). If significant or highly significant differences was observed, further analysis was performed using Duncan's Multiple Range Test (DMRT) to determine specific pairwise differences.

## 3 Result and Discussion

### 3.1 Protein Content

Based on the results of statistical calculations in Table 2, it shows that the addition of propolis as a natural preservative with various concentrations (0%; 0.3%; 0.5%; 1% and 1.5%) has a highly significant effect ($P < 0.01$) on the protein content of milk jelly candy. The average protein content in each treatment was 17.51 - 18.48%. A highly significant effect occurred in the P2 treatment (0.5% propolis), there was a decrease in protein content of 17.51%. This decrease may be caused by the chemical interaction of propolis and milk protein content. Zhao et al. [9] explained that phenolic compounds can interact with proteins through hydrophobic interactions, these hydrophobic interactions can affect protein solubility. In the manufacture of milk jelly candy, the interaction of active compounds in propolis such as flavonoids and phenolics can bind with milk proteins and form insoluble complexes, thereby inhibiting protein detection during analysis.

Milk jelly candy P0 (0% propolis) has a higher protein content of 18.48% compared to milk jelly candy with added propolis of 17.51-18.25%. The decrease in protein content in milk jelly candy with added propolis also occurs in drinks using sodium benzoat as a preservative. Research by Dari et al. [10] used sodium benzoat as a preservative in pedada (*Sonneratia sp.*) fruit juice drinks with different concentrations (0.0% and 0.04%). The higher concentration resulted in a decrease protein content of 3.45% - 2.15%. This decrease is thought to occur due to the protein denaturation process that can be caused by temperature, pressure and chemical interactions in the ingredients used. This shows that the use of preservatives at increasing concentrations can reduce the protein content of a product. When the preservatives is compared, propolis as a natural preservative is superior because at several concentrations propolis can still maintain the protein content in milk jelly candy, the decrease only occurs at one concentration.

**Table 2.** The average value of milk jelly candy with the addition propolis.

| | **Protein Content (%) ± SD** | **Water Content (%) ± SD** | **Fat Content (%) ± SD** | **Ash Content (%) ± SD** | **Carbohydrate Content (%) ± SD** |
|---|---|---|---|---|---|
| P0 (propolis 0%) | $18.48 \pm 0.43^{b}$ | $49.51 \pm 1.54^{c}$ | $0.12 \pm 0.04^{a}$ | $0.75 \pm 0.07^{a}$ | $31.14 \pm 0.79^{a}$ |
| P1 (propolis 0,3%) | $18.09 \pm 0.38^{b}$ | $48.82 \pm 1.92^{c}$ | $0.16 \pm 0.02^{a}$ | $0.83 \pm 0.04^{b}$ | $32.10 \pm 0.82^{b}$ |
| P2 (propolis 0,5%) | $17.51 \pm 0.26^{a}$ | $41.30 \pm 1.47^{b}$ | $0.38 \pm 0.03^{c}$ | $0.82 \pm 0.05^{b}$ | $39.99 \pm 0.56^{c}$ |
| P3 (propolis 1%) | $18.25 \pm 0.42^{b}$ | $39.76 \pm 0.99^{ab}$ | $0.29 \pm 0.03^{b}$ | $0.83 \pm 0.03^{b}$ | $40.95 \pm 0.53^{d}$ |
| P4 (propolis 1,5%) | $18.16 \pm 0.20^{b}$ | $37.67 \pm 1.11^{a}$ | $0.25 \pm 0.04^{b}$ | $0.83 \pm 0.03^{b}$ | $43.09 \pm 0.83^{e}$ |

Description:

- The addition propolis has a highly significant effect ($P < 0.01$) on the protein content, fat content, water content, ash content and carbohydrate content of milk jelly candy
- Notations ($^{a,b,c,d,e}$) in the columns show different highly significant effect ($P < 0.01$)

### 3.2 Water Content

The addition of propolis at various concentrations in the milk jelly candy had a highly significant effect ($P<0.01$) on the water content. The average water content in milk jelly candy with the addition of propolis was 37.67–48.82%. The water content of milk jelly candy was still relatively high and did not meet the SNI 3547.2-2008 standard for soft candy [11], which is a maximum water content of 20%. The high water content may be caused by the cooking time and cooking temperature. The milk jelly candy was cooked for 13 minutes at a temperature not exceeding 70°C. The cooking time in making the jelly candy was still quite short, so the resulting water content was quite high. Majidah et al. [2] showed that the longer the cooking time (15, 20, 25, 30, 35, 40 minutes) the water content of goat milk jelly candy decreased by 11.28–5.55%. A 20-minute cooking time resulted in a moisture content of 11.28%, while a 40-minute cooking time resulted in a lower moisture content of 5.55%.

Cooking too quickly results in insufficient free water evaporation, while slow cooking can help reduce water evaporation. Although milk jelly candies still have a relatively high moisture content, the use of propolis at increasing concentrations can help reduce the product's moisture content.

The addition of propolis at increasing concentrations to milk jelly candies can reduce the water content. Similar to Gniewosz et al. [7] who created edible films by adding propolis at various concentrations (3%, 5%, and 10%), the addition of propolis can reduce the moisture content of edible films. Propolis contains various bioactive compounds such as flavonoids, polyphenols, phenolic acids, resins, and waxes that are hydrophobic, meaning they are insoluble in water [12]. This hydrophobic nature prevents these compounds from interacting directly with water, but rather with other components. As a result, water can evaporate during the heating process. In the production of milk jelly candy, the phenolic compounds in propolis can form hydrogen bonds and hydrophobic interactions with protein and gelatin molecules, resulting in a denser and more compact gel structure. This dense structure has a lower capacity to retain free water, allowing water to evaporate more easily during cooking.

### 3.3 Fat Content

The addition of propolis as a natural preservative at different concentrations (0%, 0.3%, 0.5%, 1%, and 1.5%) had a highly significant effect ($P<0.01$) on the fat content of milk jelly candy. Further testing showed that the addition of propolis concentrations of 0.3%, 0.5%, 1%, and 1.5% significantly increased the fat content compared to jelly candy without propolis (P0). This contrasts with Dari et al. [10] who used sodium benzoate as a preservative in Sonneratia sp. fruit juice drinks at different concentrations (0.0% and 0.04%), which showed a decrease in fat content of 3.45-3.36%. The higher fat content of milk jelly candy with propolis compared to P0 (0% propolis) may be due to the relatively high fat content of propolis, particularly wax and fatty acids, at 5% [6].

In treatment P1 (0.3% propolis) has a low fat content (0.16) compared to the fat content of other propolis milk jelly candies, this may be due to the low concentration of propolis used so that the fat content from the addition of propolis is still less detectable. An increase in fat content occurred in treatment P2 (0.5% propolis) which was 0.38% but decreased in P3 (1% propolis) by 0.29% and P4 (1.5% propolis) by 0.25%. The decrease in fat content along with the increase in propolis concentration is in line with the research of Yerlikaya and Sarıçoban [13] which showed that increasing propolis concentration (1%, 2%, and 3%) reduced the fat content by 29.76-11.88% in freeze-dried beef powder. This decrease is likely caused by propolis forming a complex with fat, thereby reducing the detected fat content. In the manufacture of milk jelly candy, it is possible that propolis with a higher concentration can spread the compound distribution more widely which can cause the fat in the propolis to bind with other milk components, so that the fat becomes hidden and the detectable value decreases.

### 3.4 Ash Content

The addition of propolis at various concentrations in cow's milk jelly candy formulations had a highly significant effect ($P<0.01$) on ash content. The ash content of cow's milk jelly candy with propolis added was 0.75-0.83%. This ash content complies with the SNI 3547-2-2008 soft candy quality standard [11] which indicates a maximum ash content of 3%. Further testing showed that the jelly candy with propolis added (P1, P2, P3) produced a higher ash content than the one without propolis added (P0). The higher ash content of the cow's milk jelly candy with propolis added compared to the one without propolis (P0) is in line with the use of sodium benzoate as a preservative in making drinks that have increasing ash content.

Dari et al. [10] in the production of pedada (*Sonneratia sp.*) fruit juice drinks using sodium benzoate as a preservative resulted in an increase in ash content of 1.55-2.16% along with increasing sodium benzoate concentration (0% and 2%).

Table 2 shows that milk jelly candies with some propolis additions have a higher ash content (0.82-0.83%) compared to jelly candies without propolis (0.75%). The higher ash content in the propolis-added treatment compared to P0 (0% propolis) may be due to the mineral content of propolis. Kurek-Górecka [14] explain that propolis contains 5% of the most well-known minerals, namely Fe and Zn. The ash content of jelly candies is greatly influenced by the mineral content of the food ingredients used.

### 3.5 Carbohydrate Content

Statistical calculations showed that the addition of propolis at various concentrations had a highly significant effect ($P<0.01$). The average carbohydrate content in milk jelly candies was 31.14-43.09%. The highest carbohydrate content was found in P4 (1.5% propolis) at 43.09%, and the lowest in P0 (0% propolis) at 31.14%. The increase in carbohydrate content occurred with increasing propolis concentration. This increase in carbohydrate content is suspected not to originate directly from the addition of propolis, but rather due to changes in the distribution of proximate components that affected the calculation results using the by-difference method. The carbohydrate content of milk jelly candies was obtained using the by-difference method, which calculates the carbohydrate content by subtracting the other components (100% – [protein + fat + water + ash]). Based on the average results, the study showed that the addition of propolis significantly reduced the water content. This reduction in water content mathematically contributed to an increase in the calculated carbohydrate value.

The carbohydrate content of milk jelly candies increased with increasing propolis concentration (32.01-43.09%). This increase is comparable to research by Dari et al. [10] which showed that the higher the sodium benzoate content in pedada fruit juice, the higher the carbohydrate content, from 69.09 to 70.61%. The increase in carbohydrate content in the drink is thought to be due to the absence of a fermentation process by microorganisms, as carbohydrates are typically the primary substrate for the fermentation process [15]. The presence of sodium benzoate functions to inhibit the growth of microorganisms. The increase in carbohydrate content in milk jelly candy occurs along with the increase in propolis concentration. Propolis in milk jelly candy has the same function as sodium benzoate, namely as a preservative that can inhibit the growth of microorganisms. Based on this, the possibility of an increase in carbohydrate content.

## 4 Conclusion

The use of propolis as a natural preservative has almost the same effect as the use of synthetic preservatives, and in some parameters, it has a better effect. Regarding protein levels, the use of propolis at various concentrations can better maintain protein content. The use of propolis can also help reduce the water content of milk jelly candy. The fat and ash content in milk jelly candy increases with the addition of propolis because propolis contains fat and minerals. The increase in carbohydrate content in milk jelly candy with the addition of propolis indicates the absence of microbial activity in the product.

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