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Sensory evaluation and antibacterial activity of bee pollen extracts isolated from several stingless bees in two drying methods

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Abstract. *Naibaho NM, Salusu HD, Rudito, Saragih B, Kusuma IW, Fatriasari W, Arung ET. 2023. Sensory evaluation and antibacterial activity of bee pollen extracts isolated from several stingless bees in two drying methods. Biodiversitas 24: 2682-2688.* This study aims to determine six stingless bee pollen extracts' sensory characteristics and antibacterial activity. The samples were dried using a chiller method at 4°C (14-22 days) and a 40°C oven (8-10 days). The sensory qualities of color, scent, taste, texture, and the antibacterial activity of six stingless bees were assessed while the bee pollen was drying. It was revealed for the first time that six bee pollen products from East Kalimantan had characteristics that were very close to what consumers preferred. The findings demonstrated that, compared to a 40°C oven, the 4°C chiller approach offered superior sensory value and antibacterial activity. Bee pollen *Tetragonula reepeni* (Friese, 1918) received the highest sensory score using the 4°C chiller method, scoring 4.81 (very like) for color and 3.71 (like) for taste. Bee pollen *Tetragonula fuscobalteata* (Cameron, 1908) received a score of 3.47 (like) for aroma, while the highest texture value was obtained by bee pollen *Heterotrigona itama* (Cockerell, 1918), scoring 4.19 (like very much). Bee pollen *H. itama* had the greatest sensory scores when using the 40°C oven method, scoring 3.58 (like), 3.22 (like), and 3.54 (like) for color, scent, and taste, and 3.69 (like) for texture. The antibacterial activity of the bee pollen extracts *T. reepeni* and *Tetragonula iridipennis* (Smith, 1854) achieved higher inhibition zone values when using the 4°C chiller method and 40°C oven method, with values of 15 mm, 16.00 mm, 14.33 mm, and 13.26 mm, respectively, for the bacterium *Propionibacterium acnes* NN657. The lowest Minimum Inhibitory Concentration (MIC) for antibacterial activity was 125 µL/mL for *Staphylococcus aureus* ATCC 25932, *Staphylococcus epidermis* NN349, *Propionibacterium acnes* NN357 and *Escherichia coli* ATCC 8742 of 250 µL/mL. Comparing the organoleptic characteristics of bee pollen, the chiller method at 4°C for 14 days retained quality attributes better than the bee pollen oven-drying method at 40°C.

Keywords: Antibacterial, bee pollen, drying methods, sensory test

INTRODUCTION

Bee pollen is a derivative of stingless bee products cultivated in various regions of Indonesia; it is a very important apiculture product because bee pollen contains minerals, fiber, lipids, proteins, and carbohydrates (Dias et al. 2016), and this is a good source of nutrition for the human body. According to some sources, bee pollen is a versatile food that can be eaten alone or in combination with other dishes. Regular consumption of bee pollen by humans can increase immunity, physical and mental activity, and other benefits. However, its water content and activity significantly influence bee pollen's organoleptic qualities and shelf life (Kayacan et al. 2018). Thus, bee pollen must be treated carefully because of its high moisture content, with about 20-30 g water/100 g on a wet basis and 25-42.9 g water/100 g for dry solid bee pollen (De-Melo and Almeida-Muradian 2017), so growth spoilage microbes easily damage this product. Microbial damage will affect sensory properties, such as bee pollen's color, taste, aroma, and texture. In addition, several environmental factors, including temperature, humidity, pressure, and different processing methods, significantly impact the physicochemical characteristics of bee pollen (Dias et al. 2016). Therefore, to maintain the quality of bee pollen, it is necessary to reduce the water content after the harvest process to below 10% or 6-10 g water/100 g for the wet base and 6.4-11.1 g water/100 g for dry solids. Furthermore, the decay process caused by microbial and enzyme metabolic activity degradation will decrease, and harvested bee pollen is more durable and can be stored longer.

Drying is one method of naturally preserving food, such as traditional drying, which uses sunlight. Still, this method is ineffective for drying bee pollen because it depends on sunlight which can result in insects, birds or environmental contaminations, and product loss. The sunlight drying

process also requires floor space, is difficult to control, and has an unpleasant odor that can result in a change to or the loss of some bioactive compounds, such as those found in apigenin (Hostetler et al. 2013). Moreover, various drying processing procedures affect bee pollen's nutritional components differently, such as freezing and hot air drying (Dias et al. 2016). In addition to freeze drying, microwave vacuum drying has also been explored for drying bee pollen. The results showed microwave vacuum drying had fewer antioxidant chemicals (tocopherols) than freeze drying (Conte et al. 2017). However, changes in bee pollen morphology caused by microwave drying do not always have a negative effect. Compared to other microwave settings, drying at 180 watt improved in a better quality of the physicochemical properties and organoleptic characteristics of dried bee pollen grains (Isik et al. 2018). Even though there was no obvious correlation between the vacuum drying parameters and the qualities studied, they significantly impacted bee pollen's antioxidant activity and vitamin C content. Therefore, compared to traditional procedures, microwave drying at a lower power level produces faster-dried bee pollen of acceptable nutritional quality (Kanar and Mazı 2019). For example, the proline, rutin, and total free amino acids content of chestnut pollen can all be preserved during freezing-temperature drying (Ranieri et al. 2017). By freeze-drying bee pollen, its phenolic components, total flavonoids, antioxidant activity, vitamin E content, total phenols, and Total Equivalent Antioxidant Capacities (TEAC) all can be preserved (Dias et al. 2016; Kanar and Mazı 2019).

Even though bee pollen's of better quality, this freezing approach was found ineffective for the drying process because it was shown to be ineffective compared to a drying method utilizing heat (Kanar and Mazı 2019). Kayacan et al. (2018) reported that drying bee pollen at 35-45°C reduced the water content and water activity but not the protein, ash, or fiber content. And a drying temperature of 35°C instead of 45°C maximized vitamin C and carotene concentrations. Therefore, using a 40°C oven and a low temperature of 4°C chiller can simulate a drying process by utilizing heat and a low temperature, which can reduce the water content of bee pollen by up to 12% (Orvalho et al. 2021). Thus, low-processing preservation methods should be applied. Furthermore, different drying methods, extraction techniques, and bee pollen types will affect bee pollen's sensory profile, color, and antibacterial activity. Therefore, to produce high-quality dried bee pollen at a reasonable cost, the stingless bee farming community can benefit from using the findings of this work.

MATERIALS AND METHODS

Preparation of samples

Stingless bee farmers in the Lempake district of Samarinda City, East Kalimantan Province, provided pollen samples from the following species: *Heterotrigona itama* (Cockerell, 1918), *Tetragonula reepeni* (Friese, 1918), *Tetragonula pagdeni* (Schwarz, 1939), *Tetragonula iridipennis* (Smith, 1854), *Tetragonula fuscobalteata* (Cameron, 1908), and *Tetragonula testaceitarsis* (Cameron, 1901). These samples were obtained in June 2022. A stainless steel spoon was used to collect the bee pollen, which was then placed in polyethylene plastic and kept in a fridge for one day before drying. The following day, the bee pollen and propolis were separated, weighed, and then dried in an oven at 40°C for 8-10 days or in a chiller at 4°C for 15-22 days.

Extraction

At room temperature, dried bee pollen was macerated with 96% ethanol solvent for 72 hours. The extraction results were filtered using a 0.1 mm Whatman filter paper to separate the residue and bee pollen filtrate. The extract was concentrated using an evaporator at a temperature of 40°C, then dried in an oven at 40°C for 24 hours. Finally, it was put in a small vial flask and stored in a refrigerator in a closed condition before further testing.

Sensory analysis

Sensory analysis was performed according to Meilgaard et al. (2016), with slight modifications; next, a preference test used 25 trained panelists, followed by a food test after 20 hours. Panelists aged 20-25 expressed their likes or dislikes using a hedonic rating scale. A verbal hedonic scale consisting of 5 points (0-1.0=dislike very much, 1.1-2.0=dislike, 2.1-3.0=neutral, 3.1-4.0=like 4.1-5.0=like very much) was used to measure the affective dimension of consumers' perceptions of dried bee pollen samples. Panelists evaluated sensory attributes, including the appearance of color, aroma, taste, texture, and overall reception in individually partitioned booths in an environmentally controlled room (23±2°C and 50±5% RH) under white fluorescent lighting. Single-use, white plastic plates were used to hold the samples, which weighed about 5 g. They were given to the panelists randomly and labeled with five random digits. Each sample was given to each participant individually, followed by an appropriate questionnaire, with a 3-minute gap between samples. Scores between 5 and 4 are acceptable, meaning that potential customers will likely have unfavorable impressions of the product if the score for each analyzed feature falls below this level.

Color analysis

Using CIELAB and CIELCH color systems, a Miniscan EZ color reader was used to determine the color. Bee pollen extracts were dissolved in 98% ethanol and adjusted to 2,000 ppm concentrations. Using CIE L*, a*, and b* color characteristics, a Hunterlab small scanEZ Color Reader worked with D65 (daylight) and a measuring cell with a 30 mm opening; a* and b* are the chromatic coordinates, while L* is the brightness variable. Furthermore, the device was calibrated using a standard white reflector plate (L* = 100). L*, a* (from [-] greenness to [+] redness), and b* (from [-] blueness to [+] yellowness) were obtained for each sample after three measurements were made at random locations.

Antibacterial activity test

Antibacterial testing using the agar disk diffusion method is described in Naibaho et al. (2012). Appropriate broth cultures of S*taphylococcus aureus* ATCC 25932,

Escherichia coli ATCC 8742, S*taphylococcus epidermis* NN349 and *Propionibacterium acnes* NN357 were swabbed onto Muller Hinton Agar (MHA) plates and allowed to absorb for a certain period. The infected plates were filled with sterile 6 mm filter paper (Whatman No. 1) discs that had been impregnated with 550 µL/mL of extracts of dried bee pollen that had been dissolved in sterile Dimethyl Sulfoxide (DMSO). DMSO was utilized to prepare a negative control. The positive control was chloramphenicol (5g/mL). The plates were incubated at 37°C overnight. Antibacterial activity was assessed by measuring the inhibitory zones surrounding the disc in diameter (mm).

Minimum Inhibition Concentration (MIC) value

MIC values were measured in a disc diffusion test. Inoculated bacteria were prepared from a 24-hour nutrient broth culture and suspension adjusted to 0.5 McFarland turbidity standard. Dry bee pollen extract from two different methods was dissolved in DMSO and first diluted to the highest concentration (550µL/mL) to be tested, then double dilutions were made in a concentration range 125µL/mL up to 550µL/mL. The concentration of bee pollen extract that showed the least obvious inhibition was taken as the MIC level.

Statistical analysis

Triplicate (n=3) sensory evaluation findings, physicochemical analysis, and antibacterial activity tests are presented as mean and standard deviation.

RESULTS AND DISCUSSION

Sensory analysis

Table 1 shows the results of organoleptic tests on six types of dried bee pollen using a 4°C chiller method and a 40°C oven method. In the 4°C chiller method, the color acceptability of bee pollens *T. reepeni* and *H. itama*, respectively, was 4.81 and 4.43 (like very much range), which was preferred compared to bee pollens *T. pagdeni*, *T. iridipennis*, *T. fuscobalteata*, and *T. testaceitarsis*. The panelists preferred the aroma of the six types of stingless bee pollen with a range of 3.29-3.49 (like). As for the taste of the six types of bee pollen, the highest score of 3.71 (like) was for bee pollen *T. reepeni* and the lowest for bee pollen *T. fuscobalteata* at 2.12 (rather like). Meanwhile, for bee pollen texture, *H. itama* got the highest score of 4.19 (like very much), with the lowest of 3.55 (like it) being for bee pollen *T. testaceitarsis*. Meanwhile, bee pollen *T. iridipennis* had the lowest color score of 2.34, and bee pollen *H. itama* was the highest at 3.58 while dried in an oven at 40°C (neither like nor dislike). Bee pollen *T. iridipennis* scored 2.34 (rather like it), whereas aroma *H. itama* received a rating of 3.58 (like it). For texture, *T. reepeni* was rated at 3.69 (like) and *T. iridipennis* at 2.64 (like very much). The results of the two drying techniques indicated that, on average, the panelists appreciated bee pollen in terms of its color, scent, taste, and texture.

A food product's sensory quality affects whether consumers will accept it. As a result, the panelists assessed the dried bee pollen samples' sensory quality based on their visual preferences for color, scent, taste, and texture. The 4°C chiller method received a higher score than the average dry bee pollen score with the 40°C oven method, according to the average sensory score value related to the panelists' acceptability. It is probable that the panelists found the 4°C chiller method to be more appealing and favored it over the 40°C oven drying method in terms of the visual appearance of color, scent, taste, and texture. The differences in drying methods are thought to significantly affect the visual quality of bee pollen. A low temperature of 4°C maintained the original color, aroma, taste, and texture of the six types of bee pollen. At the same time, the 40°C oven drying method did not affect the panelists' preference levels for the bee pollen produced. The color produced at the highest temperature of 4°C is shown for the bee pollen type *T. reepeni* as orange, while the colors of other bee pollens such as *H. itama* and *T. iridipennis* are pale yellow and brown, respectively. The level of panelists' preference for color was observed as the dominant orange color was preferred compared to the other five types of bee pollen.

The orange bee pollen shown visually to the panelists may have been influenced by the cream, orange, or yellow *Tetraqonula laeviceps* (Smith, 1857) plant source collected by the bees (Agus et al. 2019). Of the various bee pollen colors, it is suspected that polyphenolic or flavonoid compounds are important in influencing the value of antibacterial activity (Spulber et al. 2018). In addition, the color of *T. iridipennis* bee pollen is dark brown, so the color produced in bee pollen using these two drying methods is less intense than that of other bee pollens. Silva et al. (2006) reported that 89.84% of brown bee pollen comes from the *Fabaceae* family. The high percentage of brown bee pollen is thought to have affected the sensory response of the panelists to the bee pollen produced; the darker the color of the bee pollen, the lower the level of preference of the panelists. The highest color value was found for bee pollen type *T. reepeni*, which received the highest scores of 4.81 and 3.27; based on color testing, rather pale yellow and brown had the lowest scores of 2.99 and 2.34 for *T. Iridipennis* bee pollen. The low value of the sensory score for bee pollen *T. Iridipennis* might have been influenced by panelists who did not like the color yellow. Silva et al. (2006) reported that 98.95% of yellow bee pollen comes from the *Mimosa qemmulata* Barneby plant (Mimosaceae), which the panelists may not have liked. According to Naibaho et al. (2021), color is an important determining factor for food quality because it is the first thing consumers perceive when other quality factors are considered. Color strongly influenced the panelists' levels of preference and was also attractive from a visual standpoint; the more attractive the color of bee pollen, the greater the panelists' interest in choosing the product.

The aroma of bee pollen from both drying methods showed that bee pollen types *T. reepeni* and *T. pagdeni* got the lowest scores, respectively, of 3.29 and 2.76. The low values for aroma in the two types of bee pollen might be due to the bee pollen having a very strong scent, such as containing a-methyl alcohol and ketone groups. These components function to repel insects, so when the panelists were presented with these bee pollens visually, they may not have liked the aroma compared to other bee pollen scents. Each panelist has a unique and different sensitivity to and reception of the

aroma produced by an object with a distinctive aroma. Each panelist has a unique feeling of sensitivity, despite being able to detect it (Meilgaard et al. 2016); hence, the sensory data collected show disparities in the grading given by the panelists. Taste is one of the most important organoleptic tests; even if other evaluation criteria are favorable, a product may still be rejected if the taste is unappealing. When consumers buy items, the taste is one of the crucial factors. The taste scores for bee pollen using the two drying methods were low and could not reach the expected level. It is suspected that the taste of bee pollen was not preferred because of its sour taste, thus reducing the taste scores result. Naibaho et al. (2021) reported that a sour taste gives an unpleasant taste sensation to food products, potentially damaging the panelists' preference level. In addition, taste sensitivity was different for each panelist due to their habit and cultural factors. The trying-new foods factor is primordial nature (fear or food restriction), so the panelists did not taste bee pollen using good luminal receptors. The taste of bee pollen is also affected by anatomical differences, with women having more fungiform papillae and taste buds than men. The sensory test scores for the six types of bee pollen did not show large differences; this may have been influenced by the low temperature maintaining an attractive appearance. A low temperature can also reduce respiration and transpiration levels and, over time, will cause the appearance of the six types of bee pollen to change and become less attractive. Likewise, a temperature of 40°C is thought to maintain the quality of bee pollen and does not result in high sensory assessment change. Isik et al. (2018) reported that the sensory evaluation attributes of dry bee pollen samples at 40°C remained higher but better than dried bee pollen at 45, 50, 55, and 60°C. Therefore, drying bee pollen at 4°C and 40°C in this study can be considered as drying that can maintain the quality of bee pollen, which will later be related to the nature of the antibacterial activity of each bee pollen extract produced.

The panelists' preference levels for all bee pollens depended on each individual who tested them. Sensory profiling describes a sample's sensory characteristics, often based on evaluating those characteristics by giving each one an intensity value. One of the most popular methods for thoroughly describing products is quantitative profile analysis. The benefit of employing multivariate statistical approaches to link sensory profiling to other instrumental, chemical, or physical attributes is that it is simple. The mapping of products can be completed, and the instrumental values of the sensory properties examined are calculated in this method (Sipos et al. 2020).

Color analysis

Color measurement was done by diluting bee pollen extract in 98% ethanol at a concentration of 2,000 ppm. All types of bee pollen that were dried by a chiller method at 4°C and in an oven at 40°C showed different L and hue^o values, but for the samples of all types of dried bee pollen, a* values were lower than the values of L, b*, C and hue^o (Table 2). The L* parameter indicates a color's darkness or brightness; dried bee pollen samples generally have L* values higher than 20°C. The L* values of bee pollen samples dried using the 4°C chiller method are not much different from the L* values of bee pollen dried in an oven at 40°C. All types of bee pollen dried in an oven at 40°C showed the highest L* values compared to the lower 4°C chiller drying temperature. The lightness value of bee pollen, which was dried in a 4°C chiller, ranged from 26.20 to 63.00 (*T. testaceitarsis and T. fuscobalteata*). *T. iridipennis* and *H. itama* dry bee pollens are lighter in color than *T. reepeni* bee pollen. The hue^o of the dry bee pollen angle with the 4°C chiller method was from 73.00-93.33 (ocher brown color). Meanwhile, the color of the lightest bee pollen dried by the oven method at 40°C ranged from 27.33-72.77 (*T. testaceitarsis and T. fuscobalteata*), with olive and honey yellow colors. And the corner hue of bee pollen using the oven method of 40°C ranged from 76.00-101.00 (*H. itama and T. fuscobalteata*), with an ocher brown color. Both drying methods showed a decrease in a* values for all types of bee pollen. The decrease in a* values was due to bee pollen originating from fruit or plant flowers. In contrast, L* and a* values were associated with color changes due to enzymatic browning for fruit tissue. The L* values decreased, and the a* values increased with increasing browning; this explains the L* and a* values of the bee pollen samples after drying. The lowest a* value was in the chiller drying method at a temperature of 4°C. Therefore, a 4°C chiller can inhibit the Maillard reaction on dried bee pollen (Kayacan et al. 2018). A decrease in the value of a* is also interpreted as a decrease in yellow intensity. The color of dry bee pollen is light yellow, light green, brown, or orange. On sight, the colors of bee pollen are *H. itama* (light yellow), *T. reepeni* (orange), *T. pagdeni* (yellow), *T. Iridipennis* (dark brown), *T. fuscobalteata* (brown yellow), and *T. testaceitarsis* (light brown). The botanical origin of pollen influences differences in its color appearance and its ability to absorb and reflect various colors of light. In addition, it is influenced by the level of solubility of tested bee pollens with differences in physical properties (solubility) and the type or characteristics of each source of bee pollen from plants.

Table 1. Effect of bee pollen dried using two methods on sensory evaluation

Types of bee pollen	Chiller method 4°C				Oven method 40°C			
	Color	Aroma	Flavor	Texture	Color	Aroma	Flavor	Texture
H. itama	4.43±0.49	3.38±0.06	3.53±0.11	4.19±0.23	3.58±0.57	3.20±0.04	3.54±0.38	3.45±0.10
T. reepeni	4.81±0.18	3.29±0.10	3.71±0.11	4.14±0.08	3.27±0.06	3.18±0.02	3.29±0.68	3.69±0.08
T. pagdeni	3.53±0.12	3.39±0.13	3.32±0.07	4.01±0.24	3.04±0.22	2.76±0.62	3.05±0.50	3.55±0.16
T. iridipennis	2.99±0.13	3.40±0.18	2.77±0.34	3.39±0.67	2.34±0.18	3.20±0.24	2.63±0.52	2.64±0.22
T. fuscobalteata	3.45±0.05	3.47±0.17	3.12±0.05	3.75±0.14	2.98±0.80	2.77±0.45	2.86±0.50	3.63±0.26
T. testaceitarsis	3.65± 0.02	3.49±0.05	2.98±0.10	3.55±0.17	3.00±0.24	2.92±0.55	2.92±0.21	3.37±0.30

Note: *Three replications of data are used to calculate the standard deviation

Antibacterial test

Table 3 shows antibacterial activity in six types of stingless bee pollen extract which were dried using a chiller method at 4°C and an oven at 40°C. This can inhibit the growth of *S. aureus* ATCC 25932, *E.coli* ATCC 8742, *S. epidermis* NN349, and *P. acnes* NN357. Measurement values in the inhibition zone of bacteria with six bee pollen extracts in two different drying methods varied with each concentration used. Antibacterial testing and bacterial inhibition zones were determined using a disc diffusion method and Minimum Inhibitory Concentration (MIC) with five concentrations to determine the capability of six types of bee pollen extract to inhibit the growth of *S. aureus* ATCC 25932, *E.coli* ATCC 8742, *S. epidermis* NN349 and *P. acnes* NN657 on all prepared media. There were five test concentrations: 550 ppm, 250 ppm, 125 ppm, 62.5 ppm, and 25 ppm. The concentration of 550 ppm, while compared to other concentrations, produced the biggest inhibitory zone of the five concentrations tested. The probability of supporting antibacterial activity decreased with the concentration of the six stingless bee pollen extracts using the chiller drying method at 4°C and an oven at 40°C. *P. acnes* NN657, *S. aureus* ATCC 25932, and *S. epidermis* NN349 displayed a larger inhibitory zone in the 4°C chiller than in the 40°C oven method or with *E. coli* ATCC 8742. The 4°C chiller method showed that bee pollens *T. iridipennis*, *T. reepeni*, and *H. itama* produced the largest inhibition zones for all types of bacteria. For *T. iridipennis* and *T. reepeni*, *P. acnes* NN657 bacteria had the biggest inhibitory zones, measuring 16.00 mm (53.33%) and 15.00 mm, respectively. Bee pollen from *T. reepeni* and *H. itama* had *S. epidermis* NN349 bacteria zones with diameters of 14.33 mm (50%) and 14.41 mm (48.03%). Bee pollens *H. itama* and *T. pagdeni*, *S. aureus* ATCC 25932 had inhibitory zones of 12.43 mm (41.43%) and 11.34 mm (37.80%). *P. acnes* NN657 gave bee pollen *T. reepeni* and *T. iridipennis* bacterial inhibition values of 14.33 mm (47.78%) and 13.26 mm (44.20%), respectively, according to the value of the bacterial inhibition zone in the 40°C oven technique. *S. epidermis* NN349 bacteria had n inhibition zones of 13.33 mm (44.44%) and 12.75 mm (43.89%) for bee pollens *T. pagdeni* and *H. itama*. The inhibition zone of *S. aureus* ATCC 25932 was 9.75 mm (32.49%) and 9.47 mm (31.58%) for bee pollens *T. reepeni* and *T. testaceitarisis*. While the inhibition zone of *E.coli* ATCC 8742 in both the 4°C chiller and 40°C oven method gave the lowest values compared to the other three types of bacteria. The differences in the inhibition zones produced for each type of bacterium are thought to be influenced by the drying method, the type of bee pollen, and the chemical components present in each type of bee pollen. The quality or components of dried bee pollen may be maintained at lower temperatures. In contrast, with the 40°C oven method, there is a larger risk of harming the bee pollen's quality and constituent parts with a higher drying temperature. According to Abhay et al. (2016), the drying process encourages the breakdown of polyphenols through the oxidation of phenolic components (both enzymatic and non-enzymatic), which causes the product to turn brown. Therefore, during the drying process, polyphenol-oxidase enzymes will be activated (Anjos et al. 2019). Bee pollen contains flavonoids and phenolic acids responsible for antibacterial activity (Komosinska-Vassev et al. 2015; Gonçalves et al. 2018). A freeze-drying process can retain these components (Dias et al. 2016). Moreover, due to their high polarity, flavonoids can easily penetrate bacterial cell walls and damage the permeability of the cytoplasmic membrane, making it difficult for bacteria to access the nutrients they need to survive. Additionally, because of the damaged cytoplasm's permeability, the proteins that make up a cell can escape independently. Flavonoids have antibacterial activity (Komosinska-Vassev et al. 2015). In addition, the gram-positive bacteria with one bilayer membrane include *S. epidermis* NN349, *P. acnes* NN657, and *S. aureus* ATCC 25932, while gram-negative bacteria with two bilayer membranes include *E. coli* ATCC 8742. Variations are believed to influence the permeability of the extract penetrating the cell wall in the cell walls of gram-positive and gram-negative bacteria. The ability of bacteria to grow depends on the presence of chemical compounds in bee pollen. These compounds affect membrane permeability and cell material loss, which deactivates or destroys the genetic material of bacterial cells and reduces bacterial inhibitory zones (De-Melo and Almeida-Muradian 2017). The study by Akhir et al. (2017) used ethanol extract and *H. itama* bread bee extract against *Bacillus cereus*, *S. aureus*, *E. coli*, and *Salmonella* sp. Gram-positive bacteria were more sensitive to bee bread extract, while ethanolic bacteria extract showed stronger antibacterial activity than hexane extract. In their explanation, Sulbarán-Mora et al. (2018) claim that the ethanol extract from bee pollen has potent antibiotic effects against gram-positive and gram-negative bacterial infections and pathogenic fungi. Because it relates to the chemical components produced, the conditions of a bee pollen plant's origin (Kalaycıoğlu et al. 2017; Spulber et al. 2018), conservation practices, and the type of bee pollen (even though it was collected from the same location) (Anjos et al. 2019), all had a significant impact on antibacterial activity test results. In the formation of complexes in the bacterial cell wall by surface-exposed adhesins and polypeptides and/or cell membrane enzymes, the polyphenols and flavonoids found in bee pollen caused damage to the integrity of the cell wall, a blockade of ion channels, and inhibition of electron flow in the transport chain, enticing electrons which control the synthesis of Adenosine Triphosphate (ATP) (Sulbarán-Mora et al. 2018); when compared with chloramphenicol as a positive control, in a test it produced an inhibition zone of ±30 mm compared to the six types of stingless bee pollen extracts. The large inhibition zone of chloramphenicol is because it is a pure antibacterial substance that works to inhibit protein synthesis and is bacteriostatic. Sensitivity test results showed that the bacteria *S. epidermis* NN349, *P. acnes* NN657, *S. aureus* ATCC 25932, and *E.coli* ATCC 8742 in the six bee pollen drying methods used were sensitive to the antibiotic chloramphenicol. Sensitivity to chloramphenicol can occur through the ribosome of the antibiotic, producing an inactivator in the form of the enzyme chloramphenicol acetyltransferase and the mechanism of the antibiotic entering continuously through the membrane and pumping the antibiotic into the cytoplasm. Unlike a negative control which uses DMSO to ensure that it does not have an antibacterial

inhibition zone against *S. epidermis* NN349, *P. acnes* NN657, *S. aureus* ATCC 25932 and *E. coli* ATCC 8742.

The Minimum Inhibitory Concentration (MIC) was determined to obtain the lowest concentration of the six types of *stingless bee* extracts that could inhibit the growth of *S. epidermis* NN349, *P. acnes* NN657, and *S. aureus* ATCC 25932 and *E. coli* ATCC 8742. MIC was determined using liquid dilution with various concentrations of stingless bee pollen dry extract, the same as in the disc diffusion method. MIC test results in the chiller and oven methods for *S. epidermis* NN349, *P. acnes* NN657, and *S. aureus* ATCC 25932 can be inhibited at a concentration of 125 µl/mL. Meanwhile, *E. coli* ATCC 8742 in both drying methods showed a MIC concentration value of 250 µl/mL. According to research (Milanda et al. 2021), the MIC value of bee pollen is 0.78 mg/mL for *S. aureus* bacteria and 1 mg/mL for *E. coli* bacteria. MIC values differ from those. The type of bee pollen, the treatment technique, the bee pollen's botanical origin (Mauriello et al. 2017), and other factors all impacted the variations in MIC findings from bee pollen. In contrast to earlier investigations, which used bee pollen powder that was instantly dissolved with DMSO, the bee pollen used in this work was extracted with organic solvents to produce crude extracts. Because the chemical components in bee pollen vary due to varied feed sources, variations in a pollen's botanical origin also affect the MIC of bacteria (Kalaycıoğlu et al. 2017; Spulber et al. 2018).

Table 2. Color values of dried bee pollen from two methods

Drying method	Color values				
	L*	a*	b*	C	Hue(°)
Chiller method 4°C					
H. itama	37.17±1.03	-13.97±1.82	49.10±3.06	47.87±1.73	73.00±0.82
T. reepeni	44.50±2.24	-12.57±0.97	53.07±3.66	53.23±3.66	76.67±1.70
T. pagdeni	38.53±0.82	-12.13±0.47	41.97±0.88	43.67±0.80	74.00±0.82
T. iridipennis	26.23±2.94	-7.10±0.64	18.03±1.39	16.01±3.26	76.67±1.70
T. fuscobalteata	63.00±1.84	-2.0±0.22	42.0±3.19	44.0±3.19	92.0±1.25
T. testaceitarsis	26.20±2.97	-1.63±0.29	15.70±1.20	16.90±1.53	93.33±2.36
Oven method 40°C					
H. itama	27.27±1.02	-10.60±0.92	35.67±1.71	42.13±0.60	76.00±1.63
T. reepeni	34.50±2.24	-11.23±0.82	43.07±3.66	43.23±1.82	84.00±0.82
T. pagdeni	39.20±0.67	-13.47±0.25	44.63±1.23	48.67±0.42	87.00±1.63
T. iridipennis	46.50±2.73	-9.00±0.57	25.97±4.03	24.61±2.91	85.00±1.41
T. fuscobalteata	72.77±1.84	-3.07±0.26	54.93±2.47	53.70±1.27	101.00±0.82
T. testaceitarsis	27.23±2.19	-1.80±0.26	15.47±1.37	17.03±1.64	96.33±1.70

Note: *Three replications of data are used to calculate the standard deviation

Table 3. Antibacterial activity of extracts from stingless bees at 550 ppm

Sample	Inhibition zone (mm) (chiller 4°C)				Inhibition zone (mm) (Oven 40°C)			
	S. aureus	*E. coli*	*S.epidermis*	*P. acne*	*S. aureus*	*E. coli*	*S.epidermis*	*P. acne*
H. itama	12.43±0.23	10.58±0.24	14.33±0.94	15.33±1.25	9.37±0.30	7.93±0.32	11.67±0.47	13.33±0.94
T. reepeni	10.75±0.05	10.04±0.26	15±2.94	5.33±0.47	9.78±0.04	7.88±0.33	16.67±3.30	6.67±0.47
T. pagdeni	11.34±0.13	10.01±0.25	14.00±1.63	12.75±1.77	8.27±0.08	7.64±0.50	13.17±0.24	12.75±1.77
T. iridipennis	8.91±0.47	9.35±0.88	16.00±1.63	9.35±0.88	5.68±0.17	6.86±1.42	17.00±1.41	9.64±0.51
T. fuscobalteata	7.32±0.09	8.98±1.40	12.33±1.25	8.98±1.40	7.52±0.33	7.20±0.24	13.00±0.82	10.09±0.82
T. testaceitarsis	11.27±0.19	9.92±0.24	13.00±0.82	10.81±0.81	9.47±0.26	8.17±0.56	12.67±0.47	10.15±0.90
Kloramfenikol	30.00±0.00	30.00±0.00	30.00±0.00	30.00±0.00	30.00±0.00	30.00±0.00	30.00±0.00	30.00±0.00
DMSO	0	0	0	0	0	0	0	0

Table 4. Minimum Inhibitory Concentration (MIC) of extracts from stingless bees at 550 ppm

Sample	MIC (chiller 4°C)				MIC (Oven 40°C)			
	S. aureus	*E. coli*	*S.epidermis*	*P. acne*	*S. aureus*	*E. coli*	*S. epidermis*	*P. acne*
T. itama	125	250	125	125	125	250	125	125
T. reepeni	125	250	125	125	125	250	125	125
T. pagdeni	125	250	125	125	125	250	125	125
T. iridipennis	125	250	125	125	125	250	125	125
T. fuscobalteata	125	250	125	125	125	250	125	125
T. testaceitarsis	125	250	125	125	125	250	125	125
Kloramfenikol	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5
DMSO	0	0	0	0	0	0	0	0

In conclusion, all samples of bee pollen, which were dried by a chiller method at 4°C and an oven at 40°C, showed a* values were lower than b* values, while L* values were higher than b* and a* values in both drying methods. In addition, the values of C and Hue^o showed higher values than those of L*, a*, b*, and C for all types of dry bee pollen. However, the values of antibacterial activity in all samples of the 4°C chiller method showed greater inhibition values than in the 40°C oven method. The overall analysis of the drying evaluation of physical properties via an organoleptic test of dried bee pollen samples showed that the panelists preferred the 4°C chiller method, which could maintain the quality of its bioactivity compared to the 40°C oven method. Therefore, drying at a temperature of 4°C with a chiller is recommended for drying fresh bee pollen to preserve other bioactivity components.

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