

Research Article

Effects of Dehydration and Roselle Powder Addition on Physicochemical Characteristics, Antioxidant Properties and Hydroxymethylfurfural Formation in Stingless Bee Honey

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ABSTRACT

High moisture in stingless bee honey increases the risk of fermentation, which may adversely affect the quality. This study evaluates the effects of dehydration and roselle powder addition on the physicochemical characteristics, hydroxymethylfurfural (HMF) formation, antioxidant properties, and sensory acceptance of stingless bee honey. The study was conducted using a 4 × 2 factorial design. The factors investigated were dehydration treatment (food dehydrator at 55°C for 18 h) and roselle powder addition at four levels (0%, 5%, 10%, & 20%). Physicochemical analyses included moisture content, colour intensity, pH, total soluble solids, hygroscopicity, and HMF content. Total phenolic and flavonoid contents were determined, and antioxidant activity was evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay. Sensory evaluation was conducted with 30 panelists to assess multiple sensory attributes. All data were analysed using two-way ANOVA. Both factors had significant effects on the physicochemical characteristics ($p < 0.05$). HMF content increased significantly following dehydration and roselle powder addition ($p < 0.05$). Antioxidant activity, phenolic and flavonoid contents increased significantly with both dehydration and roselle powder addition ($p < 0.05$). Sensory evaluation revealed that overall acceptance remained at a moderate liking level, with no significant differences ($p > 0.05$) observed among the samples. In conclusion, controlled dehydration combined with roselle powder addition improved the physicochemical properties and antioxidant characteristics of stingless bee honey while maintaining comparable sensory acceptance to the control samples. However, dehydration also increased HMF formation, indicating that further optimisation of the dehydration process is required.

Key words: Antioxidant, hydroxymethylfurfural (HMF), moisture, physicochemical, roselle powder, stingless bee honey

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INTRODUCTION

Stingless bee honey, also known as kelulut honey, is produced by stingless bees of the genus *Trigona* spp. and is characterised by a unique sweet taste combined with sour and acidic notes (Yegge *et al.* 2020). Stingless bee honey possesses high nutritional value and strong antioxidant activity (Pote *et al.* 2025). However, its high moisture content leads to microbiological instability and increases the risk of fermentation, thereby affecting sensory quality, safety, and shelf life (Ikhsan *et al.* 2022). According to Yap *et al.* (2019), stingless bee honey faces serious storage challenges as it is highly susceptible to alcoholic fermentation immediately after harvesting due to moisture content exceeding 30% and the presence of osmotolerant yeasts. The naturally high moisture content of stingless bee honey increases its susceptibility to microbial growth and alcoholic fermentation, which may reduce shelf stability and adversely affect product quality. Therefore, reducing the moisture content through

dehydration is important to improve the stability and storage quality of stingless bee honey.

One of the proposed approaches to improve the stability of stingless bee honey is dehydration. Dehydration is a preservation method in food processing technology that removes moisture to inhibit microbial growth (Riaz *et al.* 2022). Although dehydration has been identified as an effective method to reduce moisture content, this process may alter the physicochemical properties of honey and promote the formation of hydroxymethylfurfural (HMF), which serves as an indicator of sugar degradation resulting from heat treatment (Yap *et al.* 2019). Therefore, a comprehensive evaluation of the effects of dehydration is necessary to ensure the quality and safety of stingless bee honey.

In addition, the incorporation of natural products such as roselle powder into honey formulations has been proposed as a strategy to

enhance flavor and health benefits, owing to the well-documented antioxidant properties of roselle, including anthocyanins, phenolic acids, flavonoids, and organic acids (Sapian *et al.* 2023). Previous studies have primarily focused on the effects of dehydration on basic physicochemical parameters of stingless bee honey, with limited attention given to the use of natural additives, such as roselle powder, to enhance its functional value through the incorporation of natural antioxidant compounds. Furthermore, research on the combined effects of dehydration and natural bioactive ingredients, such as roselle powder, on antioxidant activity, phenolic and flavonoid contents, and HMF formation remains limited. Sensory acceptance of stingless bee honey processed using such approaches has also received insufficient attention, despite its importance for consumer acceptance and commercialisation potential.

This study aimed to evaluate the effects of dehydration treatment and roselle powder addition, as well as their interaction, on moisture content, colour intensity, pH, total soluble solids, hygroscopicity, HMF content, antioxidant characteristics and sensory acceptance of stingless bee honey. The findings are expected to contribute to the development of stingless bee honey as a more stable, safe, and consumer-acceptable functional food product.

MATERIALS AND METHODS

Materials

Stingless bee honey used in this study was obtained from HM Mega Bee Farm. The authenticity of the stingless bee honey was ensured, as no processing was applied before analysis. Roselle powder was purchased from A&T Ingredients Sdn. Bhd. and was certified as 100% pure without any additives or adulterants.

Experimental design and sample preparation

The study was conducted using a 4×2 factorial design. The factors investigated were dehydration treatment and roselle powder addition at four levels (0%, 5%, 10% & 20%), involving two main factors with predefined treatment levels. The combination of these factors resulted in eight different sample groups (4 levels $\times$ 2 treatments = 8 samples), including control samples. Each honey sample was standardised to a fixed weight of 90 g, while roselle powder was added at four different ratios: 0% (0 g), 5% (4.5 g), 10% (9.0 g), and 20% (18.0 g). Four samples underwent dehydration using a food dehydrator (DRM, China) at 55°C for 18 h (Yap *et al.* 2019), while the remaining four samples were left undehydrated. All treatments were prepared independently in triplicate, and the reported values represent the mean of three independent sample preparations.

Physicochemical characteristics

Moisture content

Moisture content was determined by placing the samples on flat aluminium plates and drying them overnight in a hot-air oven at 100–110°C. Moisture content (%) was calculated based on the loss in sample weight according to the method of Ogidi and Otenep (2019), with a minor modification in which a flat aluminium plate was used in place of the flat-bottom dish.

Colour intensity

Colour intensity was measured by preparing a 5% (w/v) honey solution using warm water (45–50°C) and filtering to remove coarse particles (Beretta *et al.*, 2005). Absorbance was measured, and net absorbance was calculated as the difference between absorbance values at 450 nm and 720 nm. Measurements were conducted in triplicate and expressed as milli-absorbance units (mAU).

pH Value

pH values were determined according to AOAC method 962.19 (AOAC, 2000). Honey solutions were prepared by dissolving 10 g of honey in 75 mL of deionised water. pH values were measured using a digital benchtop pH meter (Peak Instrument).

Total Soluble Solids (TSS)

Total soluble solids (TSS) were determined using a refractometer (Atago Pocket Refractometer). About 1–2 drops of sample were placed on the prism surface, and the prism cover was carefully

closed to avoid the formation of air bubbles. The °Brix reading was recorded once the value stabilised. The TSS value for each sample was expressed in °Brix (Kamal *et al.*, 2019).

Hygroscopicity

One gram of each honey sample was placed at room temperature in an airtight plastic container containing saturated sodium chloride (Sigma-Aldrich) solution (75.29% relative humidity). After one week, samples were weighed, and hygroscopicity was expressed as grams of moisture absorbed per 100 g of dry solids (Yap *et al.* 2019).

Hydroxymethylfurfural (HMF)

The HMF content was determined using the method described by Maeda *et al.* (2023). Five grams of the honey sample were dissolved in 25 mL of distilled water and transferred into a 50 mL volumetric flask. Carrez I and Carrez II solutions (0.5 mL each) were added, followed by filtration using filter paper, with the first 10 mL of filtrate discarded. Five milliliters of the filtrate were transferred into two separate test tubes. One tube was mixed with 5 mL of distilled water, while the second tube was mixed with 0.2% sodium bisulfite solution as the reference. Absorbance was recorded at 284 nm and 336 nm.

Antioxidant properties

Total Phenolic Content (TPC)

Total phenolic content (TPC) was determined spectrophotometrically using the Folin–Ciocalteu method (Kek *et al.* 2014). Honey samples were diluted to 0.05 g/mL with distilled water. One milliliter of honey solution was mixed with 5 mL of 0.2 N Folin–Ciocalteu (Sigma-Aldrich) reagent. After 5 min, 4 mL of 7.5% (w/v) sodium carbonate (Sigma-Aldrich) solution was added. The mixture was incubated in the dark at room temperature for 2 h, and absorbance was measured at 765 nm. Gallic acid (Macklin) (10–100 µg/mL) was used to generate the calibration curve. Results were expressed as mg gallic acid equivalents (GAE) per kg of honey.

Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was determined according to Ibrahim and Hajdari (2020) with slight modifications. Approximately 150 µL of 10% (w/v) $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (Bendosen) in methanol was mixed with 100 µL of honey extract (2.5 g honey/25 mL water), followed by the addition of 75 µL of 5% (w/v) NaNO_2 (Bendosen) for 5 min. Subsequently, 500 µL of 1 M NaOH (Sigma-Aldrich) was added, and the final volume was adjusted to 2 mL with distilled water. Quercetin (Macklin) (0–100 mg/mL) was used as the standard. Absorbance was measured at 510 nm, and results were expressed as mg quercetin equivalents (QE) per 100 g of honey.

Antioxidant activity

Antioxidant activity was determined using the Ferric Reducing Antioxidant Power (FRAP) assay following the method of Hazirah, Yasmin Anum, and Norwahidah (2019) with slight modifications. FRAP reagent was prepared by mixing 1 mL of 10 mM TPTZ (Macklin) solution in 40 mM HCl, 1 mL of 20 mM FeCl_3 , and 10 mL of 0.3 M acetate buffer (pH 3.6). The TPTZ solution was freshly prepared and dissolved in a water bath at 50°C before use. Forty microliters of honey solution were mixed with 1.2 mL of FRAP reagent, and absorbance was measured at 593 nm after 10 min of incubation. Ferrous sulfate (Sigma-Aldrich) (FeSO_4 0–2.5 mM) was used as the standard. Results were expressed as mean values of triplicate analyses and reported as mM ferrous equivalents (FE) per kg of honey.

Sensory evaluation (hedonic analysis)

Each sample was assigned a random code to minimise bias. A hedonic evaluation form in English using a nine-point scale (1 = dislike extremely, 9 = like extremely) was used to assess taste, aroma, texture, colour, viscosity, and overall acceptability. A total of 30 panelists participated in the evaluation and were instructed on the use of the evaluation form and the importance of following the prescribed evaluation sequence. Drinking water was provided for palate cleansing between samples. Samples were presented in

random order to minimize order bias, and panelists evaluated each attribute objectively based on their individual perception.

Statistical analysis

All analyses were performed in three replications. Data analysis was performed using Minitab software version 18.0 at a confidence level of 95%. Data were analysed using two-way analysis of variance (ANOVA) to evaluate the main effects of dehydration treatment, roselle powder concentration, and their interaction. When significant differences were detected ($p < 0.05$), Tukey's multiple comparison test was used to compare treatment means.

RESULTS AND DISCUSSION

Physicochemical characteristics

Physicochemical characteristics are key indicators of stingless bee honey quality, stability, and safety, particularly due to its high moisture content and susceptibility to fermentation. Parameters including moisture content, colour intensity, pH, TSS, hygroscopicity, and HMF are widely used to assess the effects of processing on honey quality. Results are shown in Table 1.

Moisture content

Results indicated that the addition of roselle powder had a significant effect on the moisture content of stingless bee honey samples ($p < 0.05$). Increasing the level of roselle powder significantly influenced the changes in moisture content, suggesting that the composition of roselle powder plays an important role in modifying the sample's ability to retain or lose water. Additionally, the dehydration treatment factor also significantly affected the moisture content ($p < 0.05$), with dehydrated samples exhibiting lower moisture content compared to non-dehydrated ones. Overall, the interaction between roselle powder addition and dehydration treatment was also significant ($p < 0.05$).

Non-dehydrated control without roselle had the highest moisture ($30.48 \pm 0.02\%$), while dehydrated samples without roselle showed the lowest ($16.90 \pm 0.03\%$). These observations highlight the effectiveness of dehydration in reducing moisture content, as dehydrated samples consistently showed lower moisture than non-dehydrated samples. Dehydration typically involves the application of heat, which removes water from the food matrix (Michel *et al.* 2024). These results align with Ikhsan *et al.* (2022), who reported

that conventional drying at $40\text{--}70^\circ\text{C}$ for 18–36 hr reduces moisture below 17%, and with Yap *et al.* (2019), who found that dehydration of stingless bee honey at 40°C for 36 hr, or $55\text{--}70^\circ\text{C}$ for 18 hr, yields moisture below 8% (dry basis) and water activity under 0.6.

Colour intensity

Roselle powder addition significantly increased the colour intensity of stingless bee honey ($p < 0.05$), with higher levels of roselle producing a more intense colour. Dehydration treatment also significantly enhanced colour intensity ($p < 0.05$), and the interaction between roselle addition and dehydration was significant ($p < 0.05$), indicating an interaction effect. The highest colour intensity (531.00 ± 2.65 mAU) was observed in dehydrated samples with 20% roselle, while the lowest (143.33 ± 1.53 mAU) was in non-dehydrated controls without roselle powder addition. The increase in colour intensity is attributed to the high anthocyanin content in roselle powder (Larasati *et al.* 2024). Additionally, dehydration can enhance colour intensity through biochemical mechanisms such as the Maillard reaction, a series of complex reactions between reducing sugars and amino acids that produce pigments responsible for browning (El Hosry *et al.* 2025). Previous studies reported honey colour intensities ranging from 211.3 to 1480 mAU, with higher intensities linked to antioxidant potential (Kek *et al.* 2014), and dehydration at $40\text{--}70^\circ\text{C}$ significantly increased colour intensity by 23–215% (Yap *et al.* 2019).

pH Value

Roselle powder addition significantly affected the pH of stingless bee honey samples ($p < 0.05$), with higher levels of roselle lowering the pH. Dehydration treatment also significantly reduced pH ($p < 0.05$), and the interaction between roselle addition and dehydration was significant ($p < 0.05$), indicating a complementary effect of both factors on the final acidity. In this study, the lowest pH values were observed in dehydrated samples with 0%, 5%, and 10% roselle powder, with pH values of 2.82 ± 0.01 , 2.81 ± 0.01 , and 2.83 ± 0.01 , respectively. The slight decrease in pH caused by dehydration may be attributed to the concentration of organic acids and other components as water is removed (Degwale *et al.* 2022). Generally, stingless bee honey is naturally acidic, with a pH range of 3.2 to 4.5, due to the presence of organic acids, esters, and higher concentrations of inorganic ions, such as phosphate, chloride, sulfate, and

Table 1. Effects of dehydration and roselle powder addition on the physicochemical characteristics of samples.

Treatment	Roselle Powder (%)	Moisture Content (%)	Colour Intensity (mAU)	pH	TSS (°Brix)	Hygroscopicity (%)	HMF (mg/kg)
Non-dehydrated	0	30.48 ± 0.02^a	143.33 ± 1.53^h	2.95 ± 0.02^a	68.83 ± 0.17^g	3.45 ± 0.56^d	52.61 ± 0.35^f
Non-dehydrated	5	29.83 ± 0.08^b	231.00 ± 1.00^f	2.93 ± 0.04^a	70.17 ± 0.00^f	6.53 ± 0.51^c	55.95 ± 0.95^e
Non-dehydrated	10	28.89 ± 0.06^c	330.00 ± 0.00^d	2.88 ± 0.01^b	71.89 ± 0.10^e	7.91 ± 0.37^c	61.24 ± 0.40^d
Non-dehydrated	20	27.35 ± 0.35^d	519.33 ± 0.33^b	2.86 ± 0.02^c	74.17 ± 0.17^d	10.99 ± 0.86^{ab}	68.08 ± 0.48^a
Dehydrated	0	16.90 ± 0.03^g	180.00 ± 1.73^g	2.82 ± 0.01^{cd}	82.45 ± 0.25^a	8.13 ± 0.76^c	55.60 ± 0.17^e
Dehydrated	5	21.46 ± 0.32^f	293.00 ± 1.73^e	2.81 ± 0.01^d	78.28 ± 0.19^c	10.30 ± 0.75^b	57.20 ± 0.79^c
Dehydrated	10	22.80 ± 0.07^e	367.33 ± 0.58^c	2.83 ± 0.01^{bcd}	78.00 ± 0.00^c	11.74 ± 1.17^{ab}	63.79 ± 0.69^c
Dehydrated	20	22.34 ± 0.32^e	531.00 ± 2.65^a	2.88 ± 0.01^b	78.94 ± 0.10^b	12.46 ± 0.65^a	66.24 ± 0.38^b

TSS: Total Soluble Solid; HMF: Hydroxymethylfurfural. ^{a-h} Different letters indicate significant differences ($p < 0.05$), within the same column.

nitrate (Gadge *et al.* 2024). A previous study reported pH values of raw stingless bee honey ranging from 3.15 to 6.64, which decrease slightly after dehydration to between 3.11 and 5.14 (Ikhsan *et al.* 2022). These observations are consistent with the findings of Purbowati *et al.* (2020), who reported that the addition of higher levels of roselle powder further reduces pH in beverages, emphasizing the combined effect of roselle's acidic compounds and dehydration in lowering the acidity of honey.

Total Soluble Solid (TSS)

Roselle powder addition significantly influenced the TSS of stingless bee honey ($p < 0.05$), with higher levels of roselle increasing TSS. Dehydration treatment also significantly affected TSS ($p < 0.05$), as dehydrated samples exhibited higher TSS than non-dehydrated ones. The interaction between roselle powder and dehydration was significant ($p < 0.05$), indicating an interaction effect on final TSS values. Based on Table 1, the highest TSS ($82.45 \pm 0.25^\circ\text{Brix}$) was observed in dehydrated samples without roselle powder (0%), whereas the lowest TSS ($68.83 \pm 0.17^\circ\text{Brix}$) was recorded in non-dehydrated samples without roselle powder. This pattern demonstrates that moisture content is a primary factor influencing TSS, as higher moisture levels dilute soluble solids, leading to lower TSS values (Ikhsan *et al.* 2022). During dehydration, water is removed from the honey matrix, concentrating dissolved components such as sugars, organic acids, and phenolic compounds present in roselle, which consequently increases TSS. A previous study reported TSS values for raw stingless bee honey ranging from 64.5 to 75.8 $^\circ\text{Brix}$ (Ikhsan *et al.* 2022), indicating that pre-dehydration samples have lower TSS due to higher moisture content. After dehydration, TSS values increased, ranging from 69 to 86.93 $^\circ\text{Brix}$. Similar findings were reported by Yegge *et al.* (2020), where dehydration via microwave heating and dehumidification significantly increased TSS from 67.9 to 86.93 $^\circ\text{Brix}$ and 82.69 $^\circ\text{Brix}$, respectively. This increase in TSS demonstrates the direct effect of moisture reduction through dehydration, leading to a higher concentration of soluble solids in the samples. Additionally, the effect of added flower powders on TSS has been observed in other food matrices. Pamungkaningtyas *et al.* (2024) reported that increasing the amount of butterfly pea and roselle flower powders in white chocolate ganache led to higher TSS, as more soluble compounds, including pigments, sugars, and other water-soluble components, were released from the added powders. This aligns with this study, where increasing roselle powder content, particularly in non-dehydrated samples, contributed to higher TSS values due to the release of additional soluble compounds from the powder.

Hygroscopicity

Roselle powder addition significantly affected the hygroscopic properties of stingless bee honey ($p < 0.05$). Dehydration treatment also had a significant effect ($p < 0.05$), with dehydrated samples exhibiting higher hygroscopicity than non-dehydrated samples. A significant interaction between roselle powder addition and dehydration ($p < 0.05$) indicates that the influence of roselle powder on moisture absorption depends on the dehydration process, suggesting a complementary action of both factors. The increased hygroscopicity may be attributed to the spray-dried roselle powder, which is highly susceptible to environmental moisture uptake, as well as the formation of amorphous structures during rapid moisture removal (Farooq *et al.* 2025; Minh 2020). Dehydration further intensifies this effect, as dried honey predominantly exists in an amorphous sugar state, which is known to exhibit high hygroscopicity (Samborska 2019; Yap *et al.* 2019). These findings are consistent with Silva-Espinoza *et al.* (2021), who reported increased hygroscopicity in dehydrated fruit powders due to the concentration of moisture-absorbing compounds. Similarly, Yap *et al.* (2019) observed higher hygroscopic values in stingless bee honey dehydrated at elevated temperatures (hygroscopicity values of 20.15 ± 0.41 , 27.23 ± 0.42 ,

and 29.70 ± 0.29 g absorbed water per 100 g dry solids for stingless bee honey dehydrated at 40, 55, and 70 $^\circ\text{C}$, respectively), where greater moisture gradients between the sample and surrounding environment promoted moisture transfer.

Hydroxymethylfurfural (HMF)

Based on statistical analysis results and Table 1, results revealed that roselle powder addition significantly influenced HMF formation in honey ($p < 0.05$), with increasing roselle levels resulting in higher HMF content. Dehydration treatment also had a significant effect ($p < 0.05$), resulting in higher HMF content, and a significant interaction between roselle powder addition and dehydration ($p < 0.05$) was observed. The highest HMF value (68.08 ± 0.48 mg/kg) was observed in dehydrated samples containing 20% roselle powder, while the lowest value (52.61 ± 0.35 mg/kg) occurred in non-dehydrated control samples without roselle. HMF formation is strongly influenced by pH and temperature, where lower pH and elevated temperatures accelerate sugar dehydration reactions (Yang *et al.* 2019). In this study, roselle powder addition likely reduced the pH of the honey matrix, enhancing HMF formation when combined with heat during dehydration. HMF generation is typically slow during storage but increases substantially under thermal exposure, explaining the higher HMF levels in dehydrated samples (Ng *et al.* 2021). Loh *et al.* (2018) reported lower average HMF content in Malaysian stingless bee honey (*Heterotrigona itama*) at 23.42 ± 17.79 mg/kg, suggesting that processing conditions in the present study contributed to higher values. Variations in HMF content may also be influenced by factors such as heating intensity, storage duration, sugar composition, acidity, mineral content, floral origin, and environmental conditions (Zaldivar-Ortega *et al.* 2024). Furthermore, Rababah *et al.* (2024) demonstrated that HMF levels remained below 40 mg/kg during the first 5 hr at 50–85 $^\circ\text{C}$ but increased sharply thereafter, reaching 55.11 mg/kg at 90 $^\circ\text{C}$, confirming temperature as a dominant factor in HMF formation. Although dehydration effectively reduced moisture content and enhanced several physicochemical and antioxidant properties, the accompanying increase in HMF content indicates that excessive thermal processing may compromise honey quality.

Antioxidant properties

Table 2 presents the antioxidant properties of stingless bee honey that were evaluated through the determination of TPC, TFC, and FRAP, as these parameters collectively reflect the contribution of phenolic and flavonoid compounds to overall antioxidant capacity.

Total Phenolic Content (TPC)

Results showed that roselle powder addition significantly affected the TPC of stingless bee honey samples ($p < 0.05$), with increasing roselle powder levels leading to a significant increase in TPC. Dehydration treatment also had a significant effect ($p < 0.05$), as dehydrated samples exhibited higher TPC values than non-dehydrated samples. A significant interaction between roselle powder addition and dehydration ($p < 0.05$) indicates an interaction effect of both factors on the overall phenolic content of the honey. The highest TPC value was observed in dehydrated samples with 20% roselle powder (828.01 ± 2.94 mg GAE/kg), while the lowest TPC was recorded in non-dehydrated control samples without roselle powder (253.01 ± 4.00 mg GAE/kg). The increase in TPC following dehydration may be attributed to the release of phenolic compounds upon heating and the formation of Maillard reaction products (MRPs), such as melanoidins, which contribute to measured phenolic content (Chong *et al.* 2017; Ikhsan *et al.* 2022). Additionally, the incorporation of roselle powder enhances TPC due to its high phenolic content, particularly anthocyanins (Mohd-Nasir *et al.* 2021). These findings are consistent with previous studies reporting TPC values of approximately 250.60 ± 3.98 mg GAE/kg in raw stingless bee honey (Yusof *et al.* 2021) and increased TPC following dehydration at elevated temperatures (Yap *et al.* 2019). Furthermore, Purbowati *et al.* (2020) reported that higher proportions of roselle calyx are

associated with increased phenolic content. The increase in TPC observed in the present study may also be attributed to the presence of phenolic compounds naturally found in both stingless bee honey and roselle powder. Previous studies have reported that stingless bee honey contains various phenolic acids, including caffeic acid, *p*-coumaric acid, and ferulic acid, which contribute to its antioxidant properties (Biluca *et al.* 2020). Similarly, roselle contains phenolic compounds and anthocyanins (Sapian *et al.* 2023), and the incorporation of roselle powder likely contributed additional phenolics to the honey matrix, resulting in the higher TPC observed.

Total Flavonoid Content (TFC)

Roselle powder addition significantly affected the TFC of stingless bee honey samples ($p < 0.05$). Increasing levels of roselle powder resulted in a significant increase in TFC, which is consistent with the high flavonoid content of roselle that directly enhances the antioxidant potential of the honey. Dehydration treatment also had a significant effect on TFC ($p < 0.05$), with dehydrated samples exhibiting higher flavonoid content than non-dehydrated samples. Furthermore, a significant interaction between roselle powder addition and dehydration ($p < 0.05$), suggesting an interaction effect of both factors in enhancing total flavonoid content. The highest TFC value (159.67 ± 1.67 mg QE/100 g) was observed in dehydrated samples with 20% roselle powder, while the lowest TFC value (36.33 ± 1.67 mg QE/100 g) was recorded in non-dehydrated control samples without roselle powder. Dehydration reduces moisture content, thereby concentrating solids in the final product. Additionally, heating during dehydration may release flavonoids from bound forms, contributing to increased TFC (Ikhsan *et al.* 2022). The observed increase in TFC is further attributed to the rich flavonoid composition of roselle, particularly anthocyanins responsible for its intense colour (Almajid *et al.* 2023). These findings are consistent with a previous study reporting TFC values of 0.2–32.3 mg QE/g in raw stingless bee honey and increased TFC values ranging from 0.2–36.43 mg QE/g after dehydration (Ikhsan *et al.* 2022). The higher TFC observed may also be attributed to the flavonoids naturally present in both stingless bee honey and roselle. Stingless bee honey has been reported to contain flavonoids such as quercetin, isoquercetin and taxifolin (Biluca *et al.* 2020), while roselle is also rich in flavonoids and anthocyanins (Sapian *et al.* 2023). The addition of roselle powder therefore likely contributed to the increased TFC.

Antioxidant activity

Results showed that roselle powder addition significantly influenced the antioxidant activity of stingless bee honey as measured by FRAP ($p < 0.05$), with higher roselle levels leading to increased FRAP values due to its rich antioxidant content. Dehydration treatment also significantly increased FRAP values ($p < 0.05$), and a significant interaction between roselle addition and dehydration

($p < 0.05$), highlighting the combined role of both factors in determining overall antioxidant capacity. The highest FRAP value (23.60 ± 0.13 mM FE/kg) was observed in dehydrated samples containing 20% roselle powder, while the lowest FRAP value (3.84 ± 0.41 mM FE/kg) was recorded in non-dehydrated control samples without roselle powder. The increase in FRAP values was primarily influenced by dehydration treatment and higher roselle powder levels, with the highest antioxidant activity associated with greater roselle concentration combined with dehydration. The enhancement of FRAP values can be directly attributed to the high phenolic and flavonoid contents of roselle, which possess strong electron-donating capacity (Hapsari *et al.* 2021; Yusoff *et al.* 2024). Additionally, dehydration, particularly involving heat application, has been reported to enhance antioxidant activity through the formation of Maillard reaction products with strong antioxidant properties (Chaipoot *et al.* 2025). These findings are consistent with a previous study by Chen *et al.* (2021), who reported a significant increase in FRAP values ($p < 0.05$) in vacuum-dried honey, especially at higher temperatures (60°C), with the highest FRAP values of 327.4 and 344.2 $\mu\text{mol Fe}^{2+}/100$ g observed in honey samples with moisture contents of 11% and 5%, respectively.

Sensory evaluation

A sensory hedonic test was conducted to evaluate consumer acceptance of honey samples formulated with different dehydration treatments and levels of roselle powder addition. The sensory evaluation covered seven key attributes: colour, aroma, viscosity, taste, sweetness, sourness, and overall acceptance, all assessed using a 9-point hedonic scale. The scores for each attribute are presented in Table 3.

Colour

The results showed roselle powder addition had a significant effect on colour hedonic scores ($p < 0.05$), whereas dehydration treatment showed no significant effect ($p > 0.05$), indicating that dehydration did not directly influence colour perception. The interaction between roselle powder addition and dehydration was also not significant ($p > 0.05$), suggesting that the effect of roselle on colour acceptance was consistent regardless of dehydration treatment. Samples containing moderate to high levels of roselle powder showed higher and comparable colour acceptance. This improvement is attributed to water-soluble anthocyanins from roselle, which disperse within the honey matrix and increase in concentration with roselle addition and dehydration-induced pigment concentration. These findings are consistent with a previous study reporting enhanced colour acceptance in functional foods enriched with roselle or anthocyanin-rich ingredients (Căta *et al.* 2025).

Table 2. Effects of dehydration and roselle powder addition on the antioxidant properties of samples.

Treatment	Roselle Powder (%)	TPC (mg GAE/kg)	TFC (mg QE/100 g)	FRAP (mM FE/kg)
Non-dehydrated	0	$253.01 \pm 4.00^{\text{h}}$	$36.33 \pm 1.67^{\text{h}}$	$3.84 \pm 0.41^{\text{h}}$
Non-dehydrated	5	$390.83 \pm 4.00^{\text{f}}$	$58.56 \pm 1.93^{\text{f}}$	$8.66 \pm 0.17^{\text{f}}$
Non-dehydrated	10	$551.09 \pm 1.11^{\text{d}}$	$83.00 \pm 1.67^{\text{d}}$	$14.53 \pm 0.17^{\text{d}}$
Non-dehydrated	20	$794.68 \pm 2.94^{\text{b}}$	$151.89 \pm 0.96^{\text{b}}$	$20.28 \pm 0.10^{\text{b}}$
Dehydrated	0	$286.99 \pm 1.11^{\text{g}}$	$46.33 \pm 1.67^{\text{g}}$	$6.46 \pm 0.21^{\text{g}}$
Dehydrated	5	$454.94 \pm 4.00^{\text{e}}$	$68.57 \pm 2.55^{\text{e}}$	$10.45 \pm 0.17^{\text{e}}$
Dehydrated	10	$595.96 \pm 3.85^{\text{c}}$	$98.00 \pm 1.67^{\text{c}}$	$17.26 \pm 0.13^{\text{c}}$
Dehydrated	20	$828.01 \pm 2.94^{\text{a}}$	$159.67 \pm 1.67^{\text{a}}$	$23.60 \pm 0.13^{\text{a}}$

TPC: Total Phenolic Content; TFC: Total Flavonoid Content; FRAP: Ferric Reducing Antioxidant Power; GAE: gallic acid equivalents; QE: quercetin equivalents; FE: ferrous equivalents. ^{a-h} Different letters indicate significant differences ($p < 0.05$), within the same column.

Aroma

For the aroma attribute, the results showed that roselle powder addition, dehydration treatment, and their interaction had no significant effect on aroma hedonic scores ($p>0.05$), indicating that aroma perception remained consistent across all treatments and roselle levels. The table showed no clear increasing or decreasing trend, with panelist acceptance remaining neutral. Honey aroma is largely determined by naturally occurring volatile compounds, which vary with honey type and origin (Mahmoud *et al.* 2024). The stability of aroma observed in this study may be attributed to the mild dehydration conditions, which were insufficient to induce the formation of new volatile compounds through Maillard or Strecker degradation reactions, typically associated with higher temperatures or prolonged heating (Machado *et al.* 2020). Consequently, the characteristic aroma of stingless bee honey remained dominant despite roselle addition or moderate dehydration. In addition, roselle-derived aroma compounds may have been present at low concentrations or partially lost during processing, making aroma differences difficult for panelists to detect.

Viscosity

Two-way ANOVA confirmed that dehydration treatment had a significant effect on viscosity hedonic scores ($p<0.05$), indicating that dehydration is the primary factor influencing the perceived viscosity of stingless bee honey. In contrast, roselle powder addition alone had no significant effect ($p>0.05$). However, a significant interaction between dehydration and roselle addition ($p<0.05$) suggests that the impact of dehydration on viscosity depends on specific roselle levels. Mechanistically, honey viscosity is closely related to water content, sugars, and total soluble solids. Dehydration reduces moisture, leading to higher sugar concentration and increased flow resistance, resulting in a thicker mouthfeel (Ikhsan *et al.* 2022). Similar findings were reported by Yegge *et al.* (2020), who observed increased honey viscosity following moisture reduction through drying. The significant interaction may be attributed to the hygroscopic nature of roselle powder, which can absorb water and modulate viscosity under dehydrated conditions, making its effect less predictable compared to dehydration alone (Farooq *et al.* 2025; Minh 2020).

Taste

Roselle powder addition significantly affected taste hedonic scores ($p<0.05$), indicating that variations in roselle levels influenced overall taste perception of stingless bee honey. In contrast, dehydration treatment had no significant effect on taste acceptance ($p>0.05$), and no significant interaction was observed between roselle addition and dehydration ($p>0.05$), suggesting that the effect of roselle on taste was consistent regardless of dehydration. Across all samples, taste scores ranged from 5 to 6, reflecting neutral to slightly liked acceptance. The relatively stable scores indicate that stingless bee honey formulations enriched with roselle powder remained sensorially acceptable to panelists. Stingless bee honey is characterised by its distinctive sweet-sour taste and lower viscosity compared to *Apis mellifera* honey (Abu Bakar 2024), while roselle is appreciated for its berry-like and naturally tart flavor (Juhari & Petersen, 2018). The combination of these two ingredients likely produced a more balanced and appealing sensory profile, where the natural sweetness of honey was complemented by the fruity acidity of roselle.

Sweetness

Roselle powder addition significantly affected sweetness hedonic scores ($p<0.05$), indicating that varying roselle levels influenced perceived sweetness of stingless bee honey. In contrast, dehydration treatment alone had no significant effect ($p>0.05$), while a significant interaction was observed between roselle addition and dehydration ($p<0.05$), suggesting that the effect of roselle on sweetness depends on the type of treatment applied. Overall, sweetness scores remained in the moderate range, indicating that stingless bee honey enriched with roselle powder maintained an acceptable level of sweetness for panelists. Mechanistically, the presence of organic acids can suppress sweetness perception through sensory interactions between sweet and sour tastes. This aligns with a previous sensory study showing that increased acidity can reduce perceived sweetness in food products (Mao *et al.* 2025).

Sourness

Results showed that roselle powder addition and dehydration treatment did not have a significant effect on the hedonic scores for sourness ($p>0.05$), including their interaction. This indicates that the perception of acidity in stingless bee honey was consistent across all treatment combinations and levels of roselle addition, with panelist acceptance being neutral. Mechanistically, stingless

Table 3. Sensory evaluation of samples.

Treatment	Roselle Powder (%)	Colour	Aroma	Viscosity	Taste	Sourness	Sweetness	Overall acceptance
Non-dehydrated	0	5.10 ± 2.38 ^c	5.67 ± 1.97 ^a	3.33 ± 2.04 ^c	5.03 ± 1.97 ^b	5.23 ± 1.87 ^{ab}	5.17 ± 1.82 ^a	5.17 ± 2.02 ^b
Non-dehydrated	5	4.73 ± 2.08 ^c	5.20 ± 1.58 ^a	3.80 ± 1.86 ^{bc}	5.70 ± 1.62 ^{ab}	5.77 ± 1.78 ^{ab}	5.60 ± 1.73 ^a	5.80 ± 1.56 ^{ab}
Non-dehydrated	10	5.77 ± 1.96 ^{abc}	5.03 ± 1.97 ^a	4.63 ± 2.13 ^{abc}	6.03 ± 1.69 ^{ab}	5.40 ± 1.50 ^{ab}	5.97 ± 1.71 ^a	5.87 ± 1.80 ^{ab}
Non-dehydrated	20	7.10 ± 1.86 ^a	5.87 ± 1.74 ^a	5.33 ± 2.09 ^{ab}	5.80 ± 1.96 ^{ab}	5.60 ± 1.98 ^{ab}	5.60 ± 1.65 ^a	6.07 ± 1.70 ^{ab}
Dehydrated	0	5.43 ± 2.37 ^{bc}	5.57 ± 2.08 ^a	5.97 ± 1.99 ^a	5.57 ± 2.42 ^{ab}	5.73 ± 1.87 ^{ab}	5.40 ± 2.09 ^a	5.63 ± 2.44 ^{ab}
Dehydrated	5	4.63 ± 1.83 ^c	5.33 ± 1.09 ^a	5.70 ± 1.64 ^a	6.33 ± 1.85 ^{ab}	6.43 ± 1.83 ^a	6.03 ± 1.88 ^a	6.17 ± 1.74 ^{ab}
Dehydrated	10	6.20 ± 1.86 ^{abc}	5.80 ± 1.83 ^a	6.27 ± 1.84 ^a	6.63 ± 1.59 ^a	6.43 ± 1.22 ^a	6.27 ± 1.55 ^a	6.87 ± 1.41 ^a
Dehydrated	20	6.97 ± 2.24 ^{ab}	5.90 ± 1.88 ^a	4.63 ± 2.90 ^{abc}	5.80 ± 2.30 ^{ab}	4.77 ± 2.10 ^b	5.77 ± 2.25 ^a	5.93 ± 2.32 ^{ab}

^{a-c} Different letters indicate significant differences ($p<0.05$), within the same column.

bee honey naturally exhibits a unique balance of sweetness and acidity and has a less viscous texture compared to *Apis mellifera* honey, where the natural acidity contributes to the fresh taste characteristic of stingless bee honey (Abu Bakar 2024). Roselle also provides a pleasant sour taste due to its high content of bioactive compounds, including organic acids, phenolics, and anthocyanins (Shruthi & Ramachandra, 2019). However, excessively high acidity could negatively affect consumer acceptance, highlighting the need for formulation optimisation to ensure that acidity remains within an acceptable range for panelists (Purbowati *et al.* 2020).

Overall acceptance

Referring to statistical analysis results and Table 3, roselle powder addition and dehydration treatment did not have a significant effect on the overall acceptance scores of stingless bee honey ($p>0.05$), including their interaction. This indicates that overall acceptance remained consistent across all treatment combinations and roselle addition levels, with no clear trend of increase or decrease. Panelist responses suggest that the honey formulations, even with roselle enrichment and dehydration, were generally neutral to moderately liked. Although the sensory scores were generally within the moderate liking range, no significant reduction in overall sensory acceptance was observed compared with the control sample. This suggests that the incorporation of roselle powder and dehydration treatment did not adversely affect consumer acceptability under the conditions evaluated.

CONCLUSION

This study demonstrated that controlled dehydration combined with roselle powder addition improved the physicochemical properties and antioxidant characteristics of stingless bee honey. Although dehydration increased HMF formation, the levels remained within safe Codex Alimentarius limits. The treatments maintained overall sensory acceptance comparable to the control samples. These findings demonstrate the potential of this approach for developing value-added stingless bee honey products. Future studies should optimise dehydration conditions and roselle powder concentration to further improve product quality while minimising HMF formation.

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ETHICAL STATEMENT

The sensory evaluation was carried out following the Institute of Food Science & Technology (IFST) Guidelines for Ethical and Professional Practices for the Sensory Analysis of Foods, including appropriate risk assessment, food safety, and participant welfare considerations. The study involved a low-risk sensory evaluation of food products without invasive procedures or significant pain, distress, or discomfort.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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