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Research article

Effects of freeze-drying and vacuum-drying on the quality, total phenolic contents, and antioxidant activities of bee honey in northern Thailand

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Abstract: We aimed to evaluate the effect of freeze-drying and vacuum-drying on moisture, water activities (a_w), total soluble solid (TSS), hydroxymethylfurfural (HMF), diastase activity, total phenolic content (TPCs), and antioxidant activities (ABTS, DPPH, FRAP, ORAC) of longan (*Dimocarpus longan* Lour.) blossom honey and Siam weed (*Chromolaena odorata* Lour.) honey. The fresh longan blossom honey and Siam weed honey were collected from a local apiarist in Northern Thailand. Freeze-drying at 0.013 kPa, −54°C for 72 h or vacuum-drying at 2.5 kPa at 60 °C for 12 h was applied to dehydrate fresh honey. The moisture of freeze-dried samples was 10.10% for longan blossom honey and 11.50% for Siam weed honey, and the a_w of both freeze-dried honeys was 0.43. Freeze- and vacuum-dried produced more TSS than fresh honey did ($p \leq 0.05$). However, the amounts of TSS derived freeze-dry and vacuum-dry were not significantly different. Freeze-dried honey contained the highest HMF and the lowest diastase activity regardless of honey origins ($p \leq 0.05$). Drying processes significantly increased the TPCs levels of honey (approximately 2 folds by freeze-drying method). The antioxidant activity of dried honey was significantly higher than that of fresh honey. It was observed that the freeze-drying method tended to better preserve the antioxidant activity of honey compared to vacuum-drying methods in both types of honey. The results indicated that drying processes significantly affect the quality of dried honey, including TSS, HMF, diastase activity, TPCs,

and antioxidant activity. In this study, freeze-drying emerged as the relatively low temperature drying method that can preserve the quality of honey, especially in terms of TPCs and antioxidant activity.

Keywords: honey; longan blossom; Siam weed; vacuum-drying; freeze-drying; hydroxymethylfurfural; diastase activity; antioxidants

1. Introduction

Honey is a natural sweetener produced by honey bees from the nectar of flowers or honeydew [1]. It is generally composed of a complex mixture of carbohydrates, amino acids, proteins, lipids, minerals, vitamins, and other substances such as aroma compounds, flavonoids, and phytochemicals [2]. Honey is known also for possessing antimicrobial, antibiotic, anti-inflammatory, antioxidant, antimutagenic, and antitumor effects [3,4]. Nevertheless, honey has high viscosity, stickiness, and a tendency to crystallize, which pose challenges in applications and storage [5]. Therefore, the development of dried honey products addresses some of these issues by providing a more convenient form of honey that is easier to handle and incorporate into various products including food, beverage, food supplement, and cosmetics [6]. The global dried honey market was valued at USD 731.02 million in 2021 and is projected to grow at a compound annual growth rate (CAGR) of 8.11% from 2022 to 2030. It is anticipated to reach USD 1474.76 million in 2030. [7]. The North America region holds the largest market share in the global dried honey market, accounting for 34.17% of the market revenue, while the Asia region is the leading producer and exporter of dried honey [7]. Thailand is one of the significant producers of honey in Southeast Asia, approximately 12,310 metric tons (ranking 24th worldwide) [8]. The country benefits from diverse flora and climates that support the production of various types of honey. Honey from longan blossom (*Dimocarpus longan* Lour.) and Siam weed (*Chromolaena odorata* Lour.) is honey that can be usually harvested in northern Thailand [9]. A previous report indicated that longan blossom honey contains 41.02% fructose (w/w), 34.91% glucose (w/w), 1.91% sucrose (w/w), 20.11% moisture, and has a pH level of 4.28. In contrast, Siam weed honey comprises 35.09% fructose (w/w), 28.87% glucose (w/w), 1.04% sucrose (w/w), 19.73% moisture, and has a pH level of 3.89 [9]. In general, the chemical composition and quality of honey varies depending on the source of the nectar ripening of honey harvesting method climate as well as processing conditions and storage form [10]. Therefore, it would be interesting to study the drying process of these honeys to preserve the nutritional value, phytochemical compounds, and increase the market value of longan and Siam weed honey.

Generally, dried honey has been produced by removing excessive moisture from honey through low-pressure dehydration processes [11]. Spray drying is a common method for processing liquid products to powders because of its cost-efficiency. However, because of the high glucose and fructose solid content, spray drying often results in the stickiness of syrup to the drying chamber walls [12]. Therefore, the relatively low temperature drying methods with the addition of carriers having high glass transition temperatures is an alternative that has garnered increasing interest for the production of dried honey, concentrated syrup, or juice that rich in sugars and organic acids [13,14]. Freeze-drying and vacuum-drying are also known the relatively low temperature drying techniques in industrial production [15,16]. It was found that freeze-drying provides products with better qualities than spray-drying and oven-drying methods [17]. This was attributed to losses in phytochemical and antioxidant

compounds as well as other nutrients as they were exposed to hot air during the drying methods [18]. Freeze-drying and vacuum-drying proceeded under a reduced pressure condition so that water molecules could be eliminated without exposure to high temperatures and high oxygen levels [18]. In this way, nutrients and phytochemical compounds were more conservative by freeze-drying and vacuum-drying, thus they could be more useful techniques for food preservation. However, studies on the effects of relatively low-temperature drying techniques (including freeze-drying and vacuum-drying) on the quality and bioactivity of honey are limited. Therefore, to preserve the quality, bioactivity and extend the shelf life of longan and Siam weed honey, freeze-drying and vacuum-drying were chosen to assess their effects on the qualities of dried honey. The quality parameters evaluated in this study include total soluble solids (TSS), water content, water activity (a_w), diastase activity, total phenolic content (TPCs), hydroxymethylfurfural (HMF), and antioxidant capacity (including 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid, ABTS; 2,2-diphenyl-1-picrylhydrazyl, DPPH; ferric ion reducing antioxidant potential, FRAP; and oxygen radical absorbance capacity, ORAC).

2. Materials and methods

2.1. Chemicals and Raw materials

The fresh longan (Dimocarpus longan Lour.) and Siam weed (Chromolaena odorata Lour.) honey were collected from a local apiarist in Northern Thailand. The samples were kept in airtight plastic containers and stored at 4 °C in the dark until it underwent the drying process or until study on its quality, TPCs, and antioxidant activity.

Folin-Ciocalteu's reagent, methanol, sodium nitrite, hydrochloric acid, formic acid, sodium thiosulphate, aluminium chloride, iron (III) chloride, and vanillin were purchased from Merck (Darmstadt, Germany). 2,2-diphenyl-1-picrylhydrazyl (DPPH), trolox, and gallic acid were obtained from Sigma-Aldrich (Sydney, Australia). Anhydrous sodium carbonate and sodium hydroxide were from Labco-Chemicals (Queensland, Australia). All chemicals used in this study were of analytical grade.

2.2. Honey tablets preparation

In this study, two types of fresh honey were thoroughly mixed with magnesium stearate at the concentration of 5, 10, and 15% (w/w) of TSS content of honey. The magnesium stearate was added in water at a ratio of 1:2 and dissolved at 50 °C for 30 min in a water bath (Memmert Waterbath WNE 29, Schwabach, Memmert, Germany). Dissolved magnesium stearate was added to the honey (25 °C) and then mixed at 200 rpm for 3 min with a stirrer (Wisestir HS-30D, Daihan Scientific, Korea). The temperature of mixture was measured at room temperature (25 °C) between 30 and 35 °C because of pre-heat treatment of magnesium stearate dissolved at 50 °C in the water bath. The mixture samples were placed in a silicone mold. The honey tablets were kept in an air-tight container before drying.

2.3. Drying methods

The drying method was adapted from previous study. For freeze-dried, the frozen honey tablets were placed in a freeze dryer at a pressure of 0.013 kPa, −54 °C for 72 h. [11]. For vacuum-drying, honey tablets were dried in a vacuum-oven at a pressure of 2.5 kPa, 60 °C for 12 h. [19]. During the

processing of honey, heating of honey for 72 h in freeze drying and 12 h for vacuum drying motivates the loss of thermo-labile, aromatic substances. These properties are used together as their values are indicative of the heating intensity to which honey has been subjected. According to composition criteria of honey, the maximum limit for HMF in honey is 40 mg/kg and the diastase activity number should be not less than 8. All dried honey tablet were taken out from the silicone molds and kept in an air-tight container at a 25 °C for further analysis.

2.4. Physicochemical analysis

2.4.1. Moisture content and water activity (a_w)

Moisture content was determined according to AOAC method 969.38 [20]. The water activity (a_w) was performed using a HygroLabC1 water activity meter (Rotronic, Switzerland). A saturated salt solution with an aw range of 0.43–0.75 was used to calibrate the equipment.

2.4.2. Total soluble solid content (TSS)

The calculation of total soluble solid content (TSS) content of each sample followed previous study [21] with some modifications. Mixed 2 g of samples with 8 mL of distilled water and homogenized for 2 min. A refractometer was calibrated with distilled water at 20 °C of 1.333 before being used [22]. Then, the solution was dropped on the space between the focused prisms and the refractive index. Measuring results were reported as °Brix.

2.5. Hydroxymethylfurfural content (HMF)

The analysis of hydroxymethylfurfural (HMF) content in honey tablet samples was carried out according to the International Honey Commission [23]. Briefly, mixed 5 g of samples with 0.5 mL of both Carraz I and II solution followed by dilution with distilled water. A spectrophotometer with absorbance values at 284 nm and 336 nm was used as the reference values. The equation for the calculation of HMF content of each sample is shown below:

$$\text{HMF} = A284nm - A336nm \text{ x } 149.7 \text{ x } 5 \text{ x } \frac{Dilution\ factor}{Weight\ (g)\ of\ the\ sample} \quad (1)$$

where A284 nm= absorbance at 284 nm; A336 nm = absorbance at 336 nm; 149.7 = constant values; 5 = nominal sample weight. The results were expressed as mg per kg (mg/kg).

2.6. Diastase activity

Diastase activity was determined according to the International Honey Commission [23] with some adaptations. 10 g of sample was dissolved in 20 mL of distilled water. The volume was filled up with 0.1M acetate buffer (pH 5.2) in 100 mL volumetric flask. Then, 5 mL of solution was placed in a water bath at 40 °C. Phadebas was added to the solution 15 min later. The solution was then mixed in a water bath for 30 min. After, 1 mL of 0.5M NaOH was added. The solution was then filtered through a filter paper with a diameter of 70 mm. A Specord 200 spectrophotometer (Analytic Jena,

Germany) with an absorbance of 620 nm was used to analyze the enzyme activity of the honey samples.

2.7. Total phenolic content (TPCs)

The analysis of TPCs in honey tablets was detected using the Folin-Ciocalteu colorimetric method [24] with a minor modification. A 10 g honey tablet sample was dissolved with 1000 mL of distilled water and diluted to 1 mg/mL solution. The 1.5 mL of Folin-Ciocalteu agent and 1.5 mL of 7.5% Na_2CO_3(w/v) was added to the diluted honey samples. The solution was filtrated and incubated at room temperature for 60 min in the darkness. A UV spectrophotometer was used to measure the intensity of the blue color at 765 nm. A gallic acid (0–0.1 mg/mL) standard curve was conducted to quantify the results and expressed as mg gallic acid equivalent per kg of dry weight (mg GAE/kg DW).

2.8. Determination of antioxidant activities

2.8.1. ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay

ABTS activity was measured using the procedure from previous study [25]. Briefly, a 1.9 mL ABTS solution was added to 100 µL diluted honey samples. An equal volume of ethanol (100 µL) was used as a control sample (blank). After thoroughly mixing, the mixtures were incubated at room temperature for 20 min. The absorbance was read at 734 nm. Percentage inhibitions for the DPPH and ABTS assays were calculated according to the following formula:

$$\text{ABTS radical scavenging activity (\%)} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100 \quad (2)$$

2.8.2. 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

Antioxidant capacity was calculated by the DPPH assay method as described from previous studies [24,26,27] with minor modifications. A solution of DPPH was first prepared by dissolving 6 mg of DPPH with 100 mL of methanol. After that, the 2 mL DPPH solution was added to 1 mL of each diluted honey solution, shaken, and incubated for 30 min in the dark. The absorbance was at 517 nm using a spectrophotometer. For the standard calibration curve, 1 mL honey mixed with 2.7 methanol was used as a control and Trolox was set as a standard (between 25 and 800 µM Trolox). Results were reported in milligrams of Trolox equivalents per 100 g (mg TE/100g).

2.8.3. Ferric ion reducing antioxidant potential (FRAP) assay

FRAP assay was performed following the method [28,29] with minor changes. The FRAP reagent solution was first made containing 300 mM acetate buffer (pH 3.6), 8 mM 2,4,6-tri(2-pyridyl)-striazine (TPTZ) (in 8 mM HCl), and 20 mM $FeCl_3$ (in water) in the ratio 10:1:1 (v/v). Dried honey samples were extracted with methanol. 0.5 mL of the extract was mixed with 3.0 mL of FRAP reagent and incubated in a water bath at 37 °C for 10 min. After 4 min of dark incubation, the absorbance was read at 593 nm with UV-Visible 1800 Spectrophotometer (Shimadzu, Japan). A calibration curve was set between 200 and 1000 µmol/L $FeSO_4$ solution. The results were expressed as µmol of Fe (II)/g dry honey sample with reference to a dose-response curve for $FeSO_4$.

2.8.4. Oxygen radical absorbance capacity, ORAC assay

ORAC assay was carried out following the methods [6] with slight changes, using a fluorometer Varioskan LUX microplate reader (Thermo Fisher, USA). Briefly, 187 µL of 1 mg/mL of honey sample was first diluted in 75 mmol Na_3PO_4 buffer (pH 7.4). After that, 3 µL of 4.1 µmol fluoresceine disodium was added to the prepared honey samples, 187 µL buffer (blank), or to a mixture of 181 µL buffer and 6 µL 0.02 M Trolox solution in a 96-well plate and the plate kept at 37°C for 5 min. Then, 10 µL 0.37 M of 2,2′-azobis (2-amidopropane) dihydrochloride (ABAP) solution was added and measured at 37 °C every 5 min throughout 90 min. The results were reported in µmol Trolox/g dry honey powder using the following equation:

$$\text{ORAC (µmol Trolox/g)} = \frac{\text{(Area sample}-\text{Area blank)}}{\text{(Area Trolox}-\text{Area blank)}} \times \frac{1}{\text{µmol Trolox}} \quad (3)$$

2.9. Statistical Analysis

All data were reported as mean ± standard deviation form triplicate experiments. SPSS software version 17 (Chicago, Illinois, USA) was used for statistical analysis. The variances of processes were separated for analyzing the sample data significantly different among means tested using Duncan's new multiple range test ($p \leq 0.05$).

3. Results

3.1. Physicochemical properties

3.1.1. Moisture content and water activity (a_w)

The moisture content and water activity (a_w) of fresh and dried honey from two types was reported in Table 1. Fresh longan blossom honey and Siam weed honey did not significantly different, with a mean moisture content of 17.50% for both types. However, fresh longan blossom honey had a significantly lower a_w value than fresh Siam weed honey did. After freeze-drying or vacuum-drying, dried honey showed significantly lower moisture content and a_w for both types compared to fresh honey. The moisture content of honey was similar between freeze-dried and vacuum-dried honey. Interestingly, we observed that dried longan blossom honey had statistically lower moisture content than Siam weed honey in both drying methods, despite having the same initial moisture content (fresh honey)

Table 1. Moisture content and water activity (a_w) of fresh and dried honey.

	Longan blossom honey			Siam weed honey		
	Fresh	Freeze-drying	Vacuum-drying	Fresh	Freeze-drying	Vacuum-drying
Moisture (%)	$17.50^{A} \pm 0.80$	$10.10^{C} \pm 0.30$	$10.50^{C} \pm 0.70$	$17.50^{A} \pm 0.20$	$11.50^{B} \pm 0.80$	$11.70^{B} \pm 0.70$
a_w	$0.55^{B} \pm 0.00$	$0.43^{C} \pm 0.00$	$0.405^{E} \pm 0.00$	$0.60^{A} \pm 0.00$	$0.43^{C} \pm 0.01$	$0.42^{D} \pm 0.00$

Values are expressed as mean ± S.D. $^{A–E}$ Different uppercase letters in the different columns in the same row indicate significant differences between treatments ($p \leq 0.05$).

3.1.2. Total soluble solid content (TSS)

The total soluble solid content (TSS) contents of fresh and dried honey was shown in Table 2. For fresh honey, Siam weed type was significantly higher in TSS than longan type. The amount of TSS of dried honey for both types was detected to be statistically greater than the fresh honey. However, there were no significant differences in TSS content between freeze-dried and vacuum-dried honey for both types.

Table 2. The total soluble solids (TSS) content of fresh and dried honey.

	Longan blossom honey			Siam weed honey		
	Fresh	Freeze-drying	Vacuum-drying	Fresh	Freeze-drying	Vacuum-drying
TSS (°Brix)	$80.20^{C} \pm 0.90$	$88.90^{A} \pm 0.50$	$89.50^{A} \pm 0.80$	$82.50^{B} \pm 0.30$	$88.50^{A} \pm 0.90$	$88.50^{A} \pm 0.70$

Values are expressed as mean ± S.D. $^{A–C}$ Different uppercase letters in the different columns in the same row indicate significant differences between treatments ($p \leq 0.05$).

3.2. Hydroxymethylfurfural (HMF) content and diastase activity

Table 3 shows hydroxymethylfurfural (HMF) content and diastase activity of fresh and dried honey. Both types, fresh honey presented a similar content of HMF and diastase activity as provided in Table 3. However, drying procees (freeze-drying and vacuum-drying) significantly increased HMF contents and decreased diastase activity in both honey types compared to fresh honey. Freeze-drying statistically resulted in produced more HMF compounds than vacuum-drying, regardless of honey type. Similarly, concerning diastase activity, it was found that freeze-dried honey had a lower activity when compared to the vacuum-dried honey regardless of honey type as well. Interestingly, dried Siam weed honey demonstrated greater diastase activity than dried longan blossom honey (both drying method), despite having similar diastase activity in both fresh honey types.

Table 3. HMF content and diastase activity of fresh and dried honey.

	Longan blossom honey			Siam weed honey		
	Fresh	Freeze-drying	Vacuum-drying	Fresh	Freeze-drying	Vacuum-drying
HMF (mg/kg)	$3.26^{D} \pm 0.25$	$15.26^{B} \pm 0.64$	$9.56^{C} \pm 0.78$	$3.28^{D} \pm 0.45$	$16.57^{A} \pm 0.65$	$10.25^{C} \pm 0.44$
Diastase activity	$19.20^{A} \pm 0.80$	$12.10^{E} \pm 0.30$	$13.80^{D} \pm 0.50$	$19.60^{A} \pm 0.70$	$15.90^{C} \pm 0.60$	$16.80^{B} \pm 0.80$

Values are expressed as mean ± S.D. $^{A–E}$ Different uppercase letters in the different columns in the same row indicate significant differences between treatments ($p \leq 0.05$).

3.3. Total phenolic content (TPCs)

The total phenolic contents (TPCs) of fresh and dried honey shown in Table 4. We found that fresh longan blossom honey contained a significantly higher level of TPCs compared to fresh Siam weed honey, at 958 mg GAE/kg and 758 mg GAE/kg, respectively. Compared to fresh honey, drying processes significantly increased the TPCs levels of honey, especially through the freeze-drying method. Freeze-drying significantly increased the TPCs level of both types of honey (approximately 2.1–2.2 folds). For vacuum-dried honey, it was observed that the TPCs level increased by around 2 folds in longan blossom honey (significant; $p \leq 0.05$), but only by around 1.2 folds in Siam weed honey (not significant; $p > 0.05$).

Table 4. The total phenolic contents (TPCs) of fresh and dried honey.

	Longan blossom honey			Siam weed honey		
	Fresh	Freeze-drying	Vacuum-drying	Fresh	Freeze-drying	Vacuum-drying
TPCs (mg GAE/kg)	$958.0^{C} \pm 21.0$	$2010.0^{A} \pm 36.0$	$1958.0^{AB} \pm 25.0$	$758.0^{D} \pm 45.0$	$1682.0^{B} \pm 35.0$	$895.0^{CD} \pm 25.0$

Values are expressed as mean ± S.D. $^{A–D}$ Different uppercase letters in the different columns in the same row indicate significant differences between treatments ($p \leq 0.05$).

3.4. Antioxidant activities

The in vitro abilities to resist oxidative stress (antioxidant activity) of fresh and dried honey were investigated by ABTS, DPPH, FRAP, and ORAC assay. The results of antioxidant activity were shown in table 5. Overall, dried honey exhibited higher antioxidant activity than fresh honey by weight. This suggests that both freeze-drying and vacuum-drying significantly preserved the antioxidant activity of honey, resulting in higher levels compared to fresh honey. However, freeze-drying tended to better preserve antioxidant activity than vacuum-drying, regardless of honey type. Our results showed that when compared with the antioxidant activity of fresh honey, freeze-dried longan blossom honey exhibited approximately a 1.2-fold increase in antioxidant activity for ORAC, 1.5-fold for ABTS, and 2-fold for both DPPH and FRAP assays. Similarly, freeze-dried Siam weed honey exhibited approximately a 1.5-fold increase in antioxidant activity for ORAC, 1.4-fold for ABTS, 2.2-fold for DPPH, and 2.5-fold for FRAP assays.

Table 5. Antioxidant activity of fresh and dried honey.

	Longan blossom honey			Siam weed honey		
	Fresh	Freeze-drying	Vacuum-drying	Fresh	Freeze-drying	Vacuum-drying
ABTS (%)	$60.50^{C} \pm 1.20$	$90.40^{A} \pm 1.30$	$72.30^{B} \pm 2.10$	$62.20^{C} \pm 2.50$	$89.50^{AB} \pm 1.60$	$75.60^{B} \pm 1.10$
DPPH (mg TE/100 g)	$958.00^{C} \pm 21.00$	$2010.00^{A} \pm 36.00$	$1958.00^{AB} \pm 25.00$	$758.00^{D} \pm 45.00$	$1682.00^{B} \pm 35.00$	$895.00^{CD} \pm 25.00$
FRAP (μmmol Fe (II)/g)	$380.00^{D} \pm 25.00$	$784.00^{A} \pm 45.00$	$556.00^{B} \pm 22.00$	$273.00^{E} \pm 25.00$	$689.00^{A} \pm 33.00$	$457.00^{C} \pm 44.00$
ORAC (μmmol Trolox/g)	$22.20^{C} \pm 0.57$	$26.50^{B} \pm 0.77$	$25.80^{BC} \pm 0.68$	$18.60^{D} \pm 0.89$	$28.90^{A} \pm 0.68$	$22.10^{C} \pm 0.47$

Values are expressed as mean ± S.D. $^{A–E}$ Different uppercase letters in the different columns in the same row indicate significant differences between treatments ($p \leq 0.05$).

4. Discussion

The composition of honey consists of a complex of carbohydrates including fructose, glucose, sucrose, and other disaccharides [2,30]. In addition, honey also contains other elements such as water, proteins, minerals, vitamins, enzymes, organic acids, phenols and pigments [2,30,31]. These different elements make it complex and unique to each type of honey. In general, the major causes of quality deterioration include high temperatures, high moisture content, poor packaging and poor storage conditions [31]. To solved on the quality of honey and preserved health promoting activity, food processing techniques (including drying processes) are therefore crucial and require thorough study and development to fulfill these requirements [6,32,33]. Moisture is one of the most important parameter of honey quality. For fresh honey, according to quality criteria by the Council of the European Union, honey should contain less than 20% moisture content [34]. Our longan blossom

honey and Siam weed honey complied with this limit (17.50%). After the drying process, the moisture content was significantly decreased. This is consistent with a previous study on sunflower and acacia honey, which reported that freeze-drying and vacuum-drying processes can reduce the moisture content from 17-18% to 10–12% [35]. In addition, we detected the moisture content was similar between freeze-dried and vacuum-dried honey. This was similar to a study that found insignificant differences in the moisture content of freeze-dried and vacuum-dried stink bean powder [18]. However, some previous studies reported that moisture content of sugar-concentrated products after freeze-drying without any carrier agents was higher than what we had observed. For instance, freeze-dried date powders detected that moisture content in a range of 11.5–12.5% [36]. The a_w of their product between freeze-dried and vacuum-dried in that study was insignificantly different. Consistent with previous studies, which reported that there were insignificant differences of a_w between freeze-dried and vacuum-dried sunflower and acacia honey [35]. In contrast, a significant reduction of moisture content was observed in vacuum-dried mushroom as compared with freeze-dried mushrooms [37]. Therefore, discrepancies in moisture content and aw may be due to differences in sample materials including the food matrix or food components, especially sugars. Since sugar is the main portion of honey composition that influences soluble solids dissolved in honey, it could also affect moisture content and a_w [38]. Consistent with the TSS results, it was found that dried longan blossom honey had a slightly higher TSS content than the other samples (not significant). Previous studies indicated the TTS content in the mixture increased and simultaneously decreased the moisture content during an extension of processing time. The increasing temperature fastened the evaporation of water molecules creating less moisture content in the products, consequently increasing the TTS [39,40]

HMF is a furanic compound that is derived from fructose decomposition through the Maillard reaction (non-enzymatic browning reaction) during overheat food processing or long-term storage [34,41]. It has been reported to have negative effects on human health, such as cytotoxicity (mucous membranes, skin and respiratory), mutagenicity, (chromosomal aberrations and DNA adduct), and carcinogenicity (not classified by the IARC), which may contribute to safety of honey consumer [42–44]. Diastase is a starch-digesting enzyme that use to quality factor, influenced by honey storage and heating [45,46]. HMF and are honey quality criteria. According to the Honey Quality, international Regulatory Standards and legislation, it has been demonstrated that less than 40 mg/kg of HMF and greater than 8 diastase numbers [46,47]. In this study, all samples complied with this criterion. However, the HMF content of freeze-dried honey significantly increases compared to vacuum-dried honey. This is consistent with a study stating that the HMF content of freeze-dried sunflower and acacia honey greater than vacuum dried honey [35]. Interestingly, comparing the HMF content and diastase activity with other honey from previous studies [35], longan blossom honey and Siam weed honey are of good quality and have the potential to compete in the market. Moreover, the HMF content of honey (fresh and vacuum-dried) was lower than the criteria that some European bee federations (Germany, Belgium, Italy, Austria, Spain) set to allow and permission to market using the term "quality honey" (HMF < 15 mg/kg) [46].

Honey also contains a wide range of phenolic acids and flavonoids such as caffeic acid, chlogenic acid, gallic acid, *p*-coumaric acid, apigenin, kaempferol, galangin, quercetin, etc. [48]. Compared with other honey, the TPC levels of fresh honey in our study was within the previously reported range (127.2–2053.5 mg GAE/kg) [49–52]. There are many factors that affect the TPC of honey, including the type of honey, origin, season, etc. Interestingly, our results suggest that freeze-drying significantly increases the TPC levels, regardless of honey type, potentially doubling them. Previous studies demonstrating that freeze-drying is the superior technology for maximizing the retention of antioxidant

compounds in dried fruits and vegetables. Due to the very low temperatures applied, freeze-drying prevents the degradation of TPCs without exposure to heat and oxygen [53–55]. This finding is consistent with a previous study that indicated freeze-drying processes enhanced the TPCs content of pure jam pulp powder (26.53mg GAE/g) as compared to before drying (14.45 mg GAE/g) [56]. However, their study found that vacuum-drying processes can enhanced the TPCs content more than freeze-drying [56]. This result may be due to the breakdown of esterified and glycosylated bonds influenced by heat, causing more phenolic compounds to be released from the food matrix after drying [57]. In contrast, in this study, vacuum-dried honey had a significantly lower TPCs content than freeze-dried honey. This difference may be due to variations in the food matrices of the different samples. In addition, hypoxic condition that effectively decreased polyphenol oxidase and enzyme activities, leading to lower phenol degradation and extraction[58]. Another possible reason for a lower detection of TPCs levels of samples under VD conditions was that a shorter drying time and lower oxygen concentration caused little collapse and contraction strength of the solid matrix, which exhibited a small proportion of pores [59].

The antioxidant properties of honey are considered an important quality criterion [60]. In this study, we investigated the ability to resist oxidative stress (antioxidant activity) using both electron transfer (SET) and hydrogen atom transfer (HAT) mechanisms through the ABTS (SET), FRAP (SET), DPPH (SET and HAT), and ORAC (HAT) assays [61]. Our results are consistent with a study reporting that honey powders had higher antioxidant activities than fresh honey [6]. The antioxidant activity using the ABTS assay observed in this study was in line with previous study that demonstrated the ABTS value of cacao pod husks (CPH) was greatest after freeze-drying [62]. They indicated the increases in the antioxidant activities of dried CPH were influenced by the inactivation of the oxidative and hydrolytic enzymes during drying [62]. Furthermore, we discovered that the freeze-drying method yields higher ORAC values than vacuum dried honey, which is consistent with the result of TPCs of dried honey. A previous study has reported a strong positive relationship between TPCs and ORAC, as phenolic compounds possess the ability to donate protons to free radicals [63]. Additionally, this mechanism is highly correlated with the DPPH assay [64]. Comparing it with other honeys from a previous study, longan blossom honey and Siam weed honey showed good antioxidant activity in the DPPH and FRAP assays [65]. Considering all the results in this study, dried honey obtained from longan blossom and Siamese weed meets the required quality criteria and has good potential for market competitiveness as a functional ingredient for application in healthy food, beverages, or dietary supplements. Vacuum drying proved to be a good method for preserving the quality of honey (HMF and diastase activity), while freeze-drying was effective method for preserving the bioactive compounds and their activities (TPCs and antioxidants).

5. Conclusions

Honey is a good source of TPCs and has high moisture and antioxidant activities. The conditions of drying affected the quality of dehydrated honey. Freeze-drying was an efficient method for reducing moisture content and aw of honey regardless of honey origins. Both types of honey after vacuum-drying detected the lowest aw levels. The TSS of dehydrated honey with different methods was significantly higher than the fresh one; however, neither Freeze-drying nor vacuum-drying produced significant differences in TSS contents. Compared to vacuum-drying, freeze-drying increased HMF, TPCs, and antioxidant activities using the ABTS and FRAP assays, while decreasing the diastase activity of longan blossom honey at a statistically significant level. Similarly, freeze-drying more

efficiently elevated HMF, TPCs, and antioxidant capacities using the DPPH, FRAP, and ORAC assays, whilst lowering the diastase activity of Siam weed honey. Freeze-drying is an efficient drying method for dehydrated honey production.

Use of AI tools declaration

The authors declare that they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare no conflicts of interest or personal relationships with other people or organizations that can inappropriately influence this work.

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