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Variations in composition and characteristics of honey from different nectar sources

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Abstract

Honeybees gather nectar from flowers and store it in hives as honey, which has varying compositions and characteristics depending on the floral source. The properties of honey derived from different nectar origins are described by measuring the total polyphenol content, antioxidant capacity, glucose and fructose content, and amylase activity of each honey sample. A comprehensive study of the characteristics of honey based on the nectar source was conducted. The findings indicated that buckwheat honey had higher total polyphenols and antioxidant capacity, whereas vitex and acacia honey had lower levels. Glucose concentrations were higher in buckwheat and lower in manuka honey. Conversely, fructose was higher in manuka and lower in lotus root honey. The amylase activity was higher in acacia and lower in oranges. The findings of this study can be applied to honey processing and other processed foods.

1. Introduction

Bees produce honey, a sweet liquid from nectar secreted by the nectar glands of flowers and store it in their hives. Honey is a liquid primarily composed of glucose, fructose, and various polyphenols. The composition and characteristics of honey vary according to the nectar source. For example, lavender flowers contain 0.35±0.17 mg quercetin/100 g honey, whereas quercetin is absent in chestnut nectar (Petretto *et al*., 2015). Similarly, orange and pine flowers contain 2.85 and 0.23 mg quercetin/kg honey, respectively (Karabagias *et al*., 2014). The glucose and fructose contents also differ considerably among the nectar sources. *Rhododendron* nectar comprises 18.9% glucose and 43.4% fructose, whereas acacia nectar contains 18.0% glucose and 46.7% fructose (Tedesco *et al*., 2022). Honey also contains amylase, a starch-degrading enzyme derived from honeybee saliva. Amylase activity varies among honeybee species, with approximately 32.5 U/mL and 13.0 U/mL in the thoracic saliva of Egyptian and Carniolan honeybees, respectively (Al-Sherif *et al*., 2017). These studies indicate that the characteristics of honey are strongly influenced by nectar source and bee species. Therefore, the current study aimed to investigate the characteristics of honey samples from various sources, including honey from lotus and buckwheat, for which the polyphenol content, glucose and fructose contents, antioxidant activity, and amylase activity have rarely been reported. The ultimate goal was to elucidate the quality attributes of honey from different floral sources.

2. Materials and methods

2.1 Materials

Various types of honey, including hundred-flower honey (a blend of vitex, acacia, and rapeseed; made in China), lotus (made in China), orange (made in Mexico), acacia (made in China and Hungary), manuka (made in Australia), and buckwheat (made in Japan), were purchased from local retailers for use in this study.

2.2 Evaluation of total polyphenol content and antioxidant activity of honey samples

Total polyphenol content was determined using the Folin–Ciocalteu method (Kasai *et al*., 2024). Each honey sample (0.5 g) was diluted two-fold (w/w) using a 1% HCl–methanol solution (Fujifilm Wako Pure Chemicals

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Corporation, Japan). To a 0.125 mL aliquot of this solution, Folin–Ciocalteu (0.5 mL) (Nacalai Tesque, Inc., Japan) was added, stirred, and allowed to stand for 3 min. Subsequently, 0.325 mL of a 1% sodium carbonate solution (Fujifilm Wako Pure Chemicals Corporation) was added and stirred thoroughly. The absorbance was measured at 750 nm on a plate reader (Infinite F50, Tecan Japan Co., Ltd., Japan). Gallic acid (Kanto Chemical Co., Inc., Tokyo, Japan) was used as the reference standard, and the results were expressed as gallic acid equivalents.

Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (Kasai *et al.*, 2024). The honey sample (0.1 mL) was mixed with 0.9 mL of DPPH solution (160 mg/L) (Tokyo Chemical Industry Co., Ltd., Japan) in 50% ethanol, stirred, and allowed to stand for 30 min before measuring the absorbance at 540 nm (Infinite F50, Tecan Japan Co., Ltd.) (B). Similarly, the absorbance of a distilled water control sample solution (C) and a sample solution to which ethanol was added instead of DPPH (blank A) was measured after 30 min.

DPPH radical scavenging activity was calculated by substituting the absorbance values in the following equation:

$$\text{DPPH radical scavenging activity (\%)} = (C-(A-B)/C) \times 100 \quad (1)$$

2.3 Sugar content of honey samples

Each honey sample (0.5 g) was accurately weighed and vortexed with 5 mL of pure water. The volume of the honey–water mixture was made up to 10 mL with acetonitrile (Fujifilm Wako Pure Chemicals Corporation) and mixed thoroughly. Subsequently, the sample solution was filtered through a 0.22-μm membrane filter (Hangzhou Cobetter Filtration Equipment Co., Ltd., China) and analyzed using high-performance liquid chromatography (pump: LC-10AS, Shimadzu Corporation, Japan; column oven: CTO-10Asvp, Shimadzu Corporation; detector: RI-2031 Plus, JASCO Corporation, Tokyo, Japan). The high-performance liquid chromatography conditions were: column: KUMA -SAN NH2-5 (Lab Company Co. Ltd., Japan), mobile phase: acetonitrile: ultrapure water (8:2), flow rate: 1 mL/min, temperature: 50°C, detector: RI, injection volume: 20 μL.

2.4 Amylase activity of honey samples

The honey sample was diluted two-fold with pure water and dialyzed at 5°C for 2 days. The dialyzed liquid was used as the crude enzyme solution. Then, 0.1 mL of crude enzyme solution was added to 1 mL of 0.1 g/100 mL starch solution (Fujifilm Wako Pure Chemicals Corporation), and the mixture was allowed to react at 35°C. After 17 h, 10 μL of 1% potassium iodide solution (Fujifilm Wako Pure Chemicals Corporation) was added, stirred well, and the absorbance was measured at 570 nm using a plate reader (Infinite F50, Tecan Japan Co., Ltd.).

2.5 Statistical analysis

Each sample was analyzed in triplicate, adhering to Fisher's three principles. Statistical comparisons were conducted using an analysis of variance. All statistical tests were performed using two-tailed tests at the 5% significance level in Microsoft Excel 2019.

3. Results and discussion

3.1 Evaluation of total polyphenol content and antioxidant activity of honey samples

The polyphenol content of the honey samples was highest at 434 mg/100 g in buckwheat to lowest at 28.2 mg/100 g in acacia (p = 8.7E-11; Table 1). A study of 60 samples from 19 nectar plants across Turkey reported that thyme, *Pinus*, carob, *Eucalyptus*, and *Rhododendron* contained the highest phenol concentrations in that order, with levels varying based on the nectar source (Kivrak and Kivrak, 2017). Research has also shown that the botanical origin of honey influences its polyphenolic compounds, including phenolic acids and flavonoids (Majid *et al.*, 2020), as well as its polyphenol content. Various studies have reported differing amounts of gallic acid in different honey types: 18.42 mg/100 g in vitex (Imtara *et al.*, 2019), 0.02 mg/100 g in acacia (Scripcă *et al.*, 2019), 1.89 mg/100 g in orange (Guldas *et al.*, 2022), and 11.55 mg/100 g in manuka (Afrin *et al.*, 2018). Moreover, variations in gallic acid content were observed even within the same floral source. Both plant source and geographical location influence the composition and quantity of polyphenols in honey (Moniruzzaman *et al.*, 2014), leading to regional differences in the polyphenol content of honey from the same floral nectar origin. For instance, the ferulic acid content in acacia honey varies by location: 0.020 mg/100 g of ferulic acid in Romania (Scripcă *et al.*, 2019), 0.034 mg/100 g in Johor (Shamsudin *et al.*, 2022). Ferulic acid in Polish acacia honey was reported to be 1.28 mg/kg (Halagarda *et al.*, 2020) and 1.34 mg/kg in Polish buckwheat honey (Kedzierska-Matysek *et al.*, 2021), suggesting that polyphenol levels can differ even when the origin and source of honey are the same. Although the results of our study differed from previously published values, this discrepancy may be attributed to differences in production areas.

The antioxidant activity was highest in honey from

Table 1. Composition of honey samples.

Types of Honey	Total polyphenols (mg/100 g)	Antioxidant activity (%)	Glucose (g/100 g)	Fructose (g/100 g)	Amylase activity (mg/g/h)
Vitex	64.7±2.6	26.6±1.0	28.1±0.13	42.2±0.36	0.370±0.0073
Lotus	67.9±3.4	37.4±1.5	28.3±0.10	34.5±0.28	0.711±0.0088
Acacia	28.2±1.6	22.0±0.25	28.9±0.43	44.2±0.88	0.767±0.0031
Orange	69.6±3.8	61.4±1.4	31.0±0.58	45.5±0.20	0.0290±0.00069
Manuka	103±6.3	80.0±0.69	26.5±0.53	45.6±1.4	0.229±0.0077
Buckwheat	434±39	93.1±2.6	33.5±0.10	41.3±1.5	0.607±0.0090

Values are presented as mean±SD, n = 3. The total polyphenol content is expressed as gallic acid equivalents.

buckwheat at 93.1% to lowest in acacia at 22.0% (p = 8.5E-15). The ferric reducing antioxidant power of Polish buckwheat honey and acacia honey is reportedly 2144 μMFe(II)/kg and 289 μMFe(II)/kg, respectively (Kedzierska-Matysek *et al.*, 2021). Similarly, our study found higher antioxidant activity in buckwheat than in acacia. The elevated antioxidant activity of buckwheat honey is attributed to its higher polyphenol content (Kedzierska-Matysek *et al.*, 2021), which is also evident in our results. Given that antioxidant activity is generally strongly correlated with total polyphenols (Yayinie *et al.*, 2022), polyphenols were identified as one of the factors affecting antioxidant activity in the current study. Maillard reaction products, which are pigment components, have been identified as reducing substances (Cao *et al.*, 2022). As buckwheat honey has a higher concentration of Maillard reaction products than acacia honey (Starowicz *et al.*, 2021), it is probable that these compounds also influenced the determination of total polyphenols in this study.

3.2 Sugar content of honey samples

Glucose was highest in buckwheat honey at 33.5 g/100 g and lowest in manuka honey at 26.5 g/100 g (p = 4.8E-9). Fructose was highest in manuka honey at 45.6 g/100 g and lowest in lotus honey at 34.5 g/100 g (p = 4.9E-7; Table 1). Fresh nectar collected from flowers always contains more fructose than glucose (Polak-Sliwinska *et al.*, 2021), which is reflected in honey derived from nectar. Lotus honey contains more glucose (Enomoto, 2019), and manuka honey contains more fructose (Nguyen *et al.*, 2018), which was also observed in this study. A previous study found approximately 35.0 g/100 g and 38.0 g/100 g glucose and fructose, respectively, in lotus (Enomoto, 2019), which was similar in this study. Given that the glucose and fructose contents of honey vary based on the production region (Karabagias *et al.*, 2017; Mahmoodi-Khaledi *et al.*, 2017), it is possible that environmental differences, such as geographical region, may influence sugar content, and as previously reported, honey is more prone to crystallization when the glucose-to-fructose ratio is high (Nguyen *et al.*, 2018; Polak-Sliwinska *et al.*, 2021). Based on the results of the current study, it can be inferred that lotus and buckwheat honey are more likely to crystallize. In contrast, manuka honey is less likely to crystallize. High-quality honey is characterized by a combined glucose and fructose content exceeding 60 g/100 g (Nordin *et al.*, 2018). In this study, the total glucose and fructose values were above 60 g/100 g for all examined honey samples, indicating a satisfactory quality.

3.3 Amylase activity of honey samples

The amylase activity of honey samples was highest in acacia at 0.767 mg/g/h and lowest in orange at 0.0290 mg/g/h (p = 4.1E-16; Table 1). This result aligns with those of a previous study that found that buckwheat honey had approximately 10 times higher amylase activity than orange honey (Babacan *et al.*, 2002), and orange honey had lower amylase activity. Additionally, mixed honey containing manuka had lower amylase activity than other mixed honey types (Torley *et al.*, 2004), a trend also observed in the current study. The majority of amylase in honey is derived from honeybees, with Egyptian and Carniolan honeybee thoracic saliva showing amylase activities of approximately 32.5 U/mL and 13.0 U/mL, respectively (Al-Sherif *et al.*, 2017). This suggests that various honey types contain different amylases, with multiple amylases potentially coexisting in a single honey type. Most commercially available honey samples undergo heat treatment during processing, which diminishes amylase activity. Studies on buckwheat, Chinese, and clover honey have shown that the amylase activity of buckwheat honey decreases more rapidly than other species when exposed to heat (Babacan *et al.*, 2002), suggesting that heat resistance varies among amylase types.

4. Conclusion

In the current study, the total polyphenol content, antioxidant activity, glucose and fructose contents, and amylase activity were investigated in various honey samples. The findings indicated that buckwheat honey had the highest total polyphenol content and antioxidant activity, whereas vitex and acacia showed lower levels.

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The sugar content analysis demonstrated variations in glucose and fructose levels among different nectar sources. Buckwheat honey had higher glucose levels than manuka honey. The fructose content was higher in manuka than in lotus. Amylase activity varied depending on the nectar source and was higher in acacia and lower in oranges. The findings of this study can be applied to honey processing and other processed foods. The quality of honey is said to be influenced by honeybees and the local environment. In the future, it will be necessary to compare honey from the same nectar source in different regions and examine regional differences.

Conflict of interest

The author declares no conflict of interest.

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