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Minireview

Methodologies and Characterization of Physico-chemical Properties of *Apis cerana* Honey: A Short Review

Sampat Ghosh[1] and Chuleui Jung[1,2,*]

[1]Agriculture Science and Technology Research Institute, Andong National University, Andong 36729, Republic of Korea
[2]Department of Plant Medical, Andong National University, Andong 36729, Republic of Korea

Abstract Besides the diverse factors associated with ecology, environment, and botanical origin, honey quality may also vary according to honey bee species. In this present review, we present important standard methods of assessing honey quality and functional properties, as well as some properties of *Apis cerana* honey. Despite being significantly present in Asia and having long-standing recognition for its medicinal values, *A. cerana* honey receives comparatively less scientific attention than *A. mellifera* honey. Although scarce, we have compiled some of the available data on physico-chemical properties of *A. cerana* honey from the scientific literature and examined them according to standards set by the Codex Alimentarius, and the International Honey Commission. In order to promote *A. cerana* honey, standardization of *A. cerana* honey is of utmost importance.

Keywords Honey authenticity, Functional properties, Moisture, Sugar composition, Proline, Antioxidant

INTRODUCTION

Honey, a natural product produced by honey bees, has been appreciated for its nutritional and functional properties since ancient civilizations (Ediriweera and Premarathna, 2012; Eteraf-Oskouei and Najafi, 2013; Khan *et al*., 2018; Sandhya and Pai, 2022). The diverse composition of honey is influenced by various factors, including botanical origin of nectar, and climatic conditions. Additionally, manipulation during the honey production, storage, and packaging poses a significant concern. In order to prevent the adulteration and maintain the quality of honey, several legislation provisions have been adopted by different countries, and there even exists an international standard of honey (Thrasyvoulou *et al*., 2018). However, these regulations often fail to account for the complexities of honey variation. For instance, the Codex Alimentarius Commission (2001) defines honey as 'natural sweet substance produced by honey bees from nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects'. Codex has taken into consideration that the different chemical composition of honey corresponding to different botanical origin. On the other hand, European Council Directive defines honey as 'the natural sweet substance produced by *Apis mellifera* bees', explicitly distinguishing it from honey produced by other *Apis* species.

Despite significant beekeeping activities in Asia involving bees other than *A. mellifera*, such as *A. cerana*, there is an insufficiency of literature on the quality of honey produced by these bees. For nearly two thousand years, the Asian honey bee *A. cerana* has been raised in indigenous cultures across Asia (Crane, 1995 *cf*. Theisen-Jones and Bienefeld, 2016). However, in the late 19th and early 20th centuray, there was a shift towards importing the European honey bee, *A. mellifera*, which gradually replaced the traditional beekeeping practices involving *A. cerana*. The large colonies of *A. mellifera*, with worker numbers ranging from 30000 to 50000,

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*Corresponding author. E-mail: cjung@andong.ac.kr

compared to *A. cerana* colonies with 2000 to 20000 workers, along with its higher honey production capacity, were particularly appealing (Abrol, 2013; Jung and Cho, 2015). Although Korea has a long tradition of beekeeping practices dating back to early reference in time of Great king Dongmyeongsung of Goguryeo between 37–19 BC (Jung, 2014), notably, *A. mellifera* was introduced in the country during early 1900s (Jung and Cho, 2015). In India, the earliest mention of honey (referred to as *Madhu*) can be traced back to the Vedic period around 1200 BC and its medicinal qualities are extensively documented in various Ayurvedic texts, forming an integral part of the Indian cultural heritage (Sandhya and Pai, 2022). However, when it comes to *A. mellifera*, the initial attempts to introduce this species in India during the 1880s proved to be unsuccessful. Nonetheless, it was eventually accomplished between 1962 and 1968 in the northern region of the country, marking its successful establishment (Theisen-Jones and Bienefeld, 2016). Therefore, *A. mellifera* in India is relatively young.

Despite its significant presence in Asia and long-standing recognition for its medicinal properties, there have been limited efforts to establish standardized quality for *A. cerana* honey. Therefore, the present review attempts to compile the chemical characteristics of *A. cerana* honey based on the available literature and briefly exploring the commonly employed methodologies for assessing honey quality.

METHODOLOGICAL CONCERNS OF PHYSICO-CHEMICAL PROPERTIES

Regarding the assessment of honey quality, the analysis of its physico-chemical properties holds significant importance. Few properties like moisture, electric conductivity, sugar compositions, and hydroxymethylfurfural (HMF) content receives more attention in order to determine the quality and authentication of honey while others are used to understand the functional properties of honey. However, it is worth mentioning that the methodology concerning the chemical analyses of *Apis cerana* honey is largely similar to that of *Apis mellifera* honey. The widely practiced methods typically adhere to the established protocols of AOAC (1990) and other standardized methods endorsed by the International Honey Commission (2009).

1. Moisture content determination

Method

Codex Standard for Honey (2001): Refractometric method

Refractometric method, AOAC 969.38B

Principle

Moisture refers to the water content of the honey. This can be determined using the refractometric method, which is based on the fact that the refractive index (the speed at which light travels through the material) increases with solid content. The International Honey Commission (2009) provides the table that allows the conversion of the refractive index obtained by the refractometer of a honey sample to its moisture content.

Procedure

To conduct the test, the surface of the clean prism of the refractometer is covered with honey sample and the refractive index can be read. The moisture content corresponding to the obtained refractive index can be found in the table provided (relationship of water content of honey to refractive index table). If the honey sample contains granulated crystals, it should be placed in a water bath or incubator at 50°C in an airtight flask until the crystals completely dissolve before estimating the moisture content.

Other methods

Karl Fisher method

Fourier Transform Infrared Spectroscopy (FTIR)

2. Electrical conductivity determination

Method

Harmonised methods of European Honey commission (Bogdanov *et al*., 1997)

Principle

Electrical conductivity is determined through the measurement of the electrical resistance. It is defined as the voltage required to induce a certain amount of electric current to flow.

Procedure

Electrical conductivity is measured with the help of conductometer.

3. Determination of sugar content

Method

Codex Standard for Honey, AOAC 977.20: HPLC-RI method

Procedure

5 g of honey sample is dissolved in 40 mL distilled water (HPLC grade). Then, 25 mL of methanol is taken into a volumetric flask and the dissolved honey solution is transferred to the flask. The flask is then filled with distilled water (HPLC grade) until it reaches a total volume of 100 mL. The solution is filtered through a 0.45 µ membrane and the filtered solution is transferred into sample vials for estimation using HPLC-RI. The quantity of each sugar (glucose, fructose, sucrose etc.) can be estimated by comparing them to known quantities of standards run under the same conditions.

The mass percentage of the sugars (W) is calculated following the formula below:

$$W = \frac{A_1 \times V_1 \times m_1 \times 100}{A_2 \times V_2 \times m_0}$$

W = Mass percentage of sugars (g/100 g)
A_1 = Peak area of the given sugar compound in the sample solution
A_2 = Peak area of the given sugar compound in the standard solution
V_1 = Total volume of the sample solution (mL)
V_2 = Total volume of the standard solution (mL)
m_1 = Mass amount of sugar in grams in the total volume of the standard (V_2)
m_0 = Sample weight (g)

Chromatographic condition

Flow rate: 1.3 mL/min
Mobile phase: acetonitrile: water (80 : 20, v/v)
Column detector temperature: 30°C
Sample volume: 10 µL

Other methods

Enzymatic determination
HPLC-PAD (pulsed amperometric detector) and anion exchange column
UPLC-ELSD (evaporative light scattering detector)
Electrochemical determination
Raman spectroscopy
Fourier Transform Infrared Spectroscopy (FTIR)

4. Determination of Hydroxymethylfurfural (HMF) content

Method

AOAC 980.23: Spectrophotometric method

Principle

Hydroxymethylfurfural (HMF) is absorbed at 284 nm.

Procedure

5 g of honey is dissolved in 25 mL of distilled water and placed into 50 mL beaker. Next, 0.5 mL of Carrez solution I (15 g of ferrocyanide dissolved in 100 mL of distilled water) is added, followed by vortexing to ensure thorough mixing. Similarly, 0.5 mL of Carrez solution II (30 g of zinc acetate dissolved in 100 mL of distilled water) is added into it, followed by additional vortexing to ensure thorough blending. Distilled water is then added until the total volume reaches 100 mL. The solution is filtered through filter paper. 5 mL of filtered liquid is transferred into each of two test tubes. 5 mL of distilled water is to be added in one test tube and mixed using vortexing. Likewise, another test tube is filled with 5 mL of a 0.2% sodium bisulfite solution, followed by vortexing. The absorbance of the sample solution, comprising 5 mL of the initial honey solution and 5 mL of distilled water, is measured against the reference solution, which contains 5 mL of the initial honey solution and 5 mL of a 0.2% sodium bisulfite solution, at wavelengths of 284 nm and 336 nm, within duration of one hour. The HMF content is calculated by the following formula:

$$HMF\ (mg/kg) = (A_{284} - A_{336}) \times 149.7 \times 5 \times D/W$$

A_{284} = absorbance at 284 nm
A_{336} = absorbance at 336 nm
D = Dilution factor (in case dilution is necessary)
W = Weight of the honey sample (g)

5. Determination of protein content and proline content

Protein content estimation method

AOAC (1990), Kjeldahl method

Principle

Total nitrogen content of the honey sample is quantified using the Kjeldahl method. The Kjeldahl process involves converting nitrogen present in the sample into

ammonium sulfate through digestion with sulfuric acid. Subsequently, the solution is alkalized, and the resulting ammonia is estimated by distilling it into a known volume of standard acid. The excess of which is determined either through titration or a colorimetric method. Furthermore, the nitrogen content is multiplied by a conversion factor, typically 6.25, to obtain the protein content.

Procedure

In the Kjeldahl method for protein estimation, a known weight of honey sample is digested with concentrated sulfuric acid at 420°C. This digestion process converts the nitrogen in the sample into ammonium sulfate. The digested sample is then mixed with 40% sodium hydroxide, which converts the ammonium sulfate into ammonia.

To separate the ammonia, steam distillation is used, and the liberated ammonia is collected in a receiving solution containing boric acid. The collected ammonia is subsequently titrated with a standardized acid solution, such as hydrochloric acid. The titration endpoint is determined by the colour change of the boric acid complex formed with the ammonia.

By measuring the volume of the acid solution required to neutralize the ammonia, the nitrogen content of the sample can be determined. The protein content is then calculated by multiplying the nitrogen content by the conversion factor of 6.25

$$\text{Kjeldahl nitrogen (\%)} = [(V_s - V_b) \times M \times 14.01] / [W \times 10]$$

V_s = volume (mL) of standardized acid used to titrate a test
V_b = volume (mL) of standardized acid to titrate reagent blank
M = molarity of standard HCl
14.01 = atomic weight of N
W = weight (g) of sample (honey)
10 = factor to convert mg/g to percentage
Crude protein (%) = Kjeldahl nitrogen (%) × 6.25

Proline content estimation method

Harmonised Methods of the International Honey Commission (2009), Proline content by Spectrophotometric method.

Principle

Proline forms coloured complex with ninhydrin. This is measured by the spectrophotometer against the reference standard concentration of proline and quantifies the proline content of the sample.

Procedure

5 g of honey is dissolved in 50 mL of distilled water and transferred to a 100 mL volumetric flask. Using an accurate syringe, pipette 0.5 mL of the sample solution into one tube. Similarly, pipette 0.5 mL of distilled water (for blank test) into a second tube, and pipette 0.5 mL of proline standard solution into three other tubes. Add 1 mL of formic acid and 1 mL of ninhydrin solution to each tube. Cap the tubes and shake them vigorously for 15 minutes. Subsequently, the tubes should be placed in a boiling water bath for 15 minutes, followed by transferring them to water bath at 70°C. Add 5 mL of a 2-propanol-water solution to each tube and cap them immediately. The tubes are allowed to cool for 45 minutes, then the absorbance should be measured at 510 nm.

Proline content has been calculated following the method below:

$$\text{Proline (mg/kg)} = \frac{E_s \times E_1 \times 80}{E_a \times E_2}$$

E_s = Absorbance of sample solution
E_a = Absorbance of the proline standard solution
E_1 = Proline taken for the standard solution (mg)
E_2 = Weight of honey (g)
80 = Dilution factor

6. Determination of total polyphenol content

Method

Spectrophotometric method (Socha *et al*., 2009; Ferreira *et al*., 2009).

Procedure

4 g of honey is dissolved in 100 mL distilled water. Then, 0.5 mL of the solution is mixed with 0.3 mL of Folin-Ciocalteu reagent, followed by the addition of 2 mL of sodium carbonate solution (15% w/v). 5 mL of distilled water is added to the mixture and thoroughly mixed. The resulting mixture is then incubated for 2 hours, and absorbance is measured at 798 nm. A standard calibration curve of gallic acid is used to quantify the total polyphenol content of honey sample. The results are expressed as mg gallic acid equivalent (GAE)

per kg of honey.

7. Determination of total flavonoid content

Method

Spectrophotometric method (Zhishen *et al*., 1999)

Procedure

20 g of honey is dissolved in 100 mL of distilled water. Then, 1 mL of honey solution is mixed with 4 mL of distilled water. To this mixture, 0.3 mL of sodium nitrite solution (5% w/v) is added. After 5 minutes, 0.3 mL of aluminium chloride solution (10% w/v) is added, followed by the addition of 2 mL of sodium hydroxide solution (1 M) after 6 minutes. The volume is then made up to 10 mL with distilled water. Absorbance is measured at 510 nm, and total flavonoid content is quantified using a standard calibration curve of catechin. The results are expressed as mg catechin equivalent per kg of honey.

8. Estimation of Antioxidant potential

DPPH and ABTS assays are two significant methods used to assess the antioxidant potential of a substance, among various other approaches available for estimation.

1) DPPH assay

Method

Spectrophotometric method (Sung *et al*., 2018)

Principle

$DPPH^{\bullet+}$(2,2-diphenyl-1-picrylhydrazyl hydrate radical) is a coloured and stable radical cation with purple colour, exhibiting maximum absorbance at 517 nm. When antioxidant compound transfers an electron to $DPPH^{\bullet+}$, it causes discolouration of the solution. This reaction is rapid and proportional to the antioxidant capacity of the sample.

Procedure

The honey samples are diluted in distilled water at concentrations ranging from 0.05 g/mL (5 g honey mixed in 100 mL of distilled water) to 0.25 g/mL (25 g of honey mixed with 100 mL of distilled water). From each dilution, 0.3 mL solution is mixed with 2.7 mL of DPPH (0.002 g/100 mL methanol). The mixtures are thoroughly vortexed, then left at room temperature in a dark room for 1 hour. The absorbance is subsequently measured at 517 nm. The Radical Scavenging Activity (RSA) is calculated as a percentage of DPPH using the following equation:

$$\% \text{ RSA} = (\text{Abs. DPPH} - \text{Abs. sample} / \text{Abs. DPPH}) \times 100$$

2) ABTS assay

Method

Spectrophotometric method (Sung *et al*., 2018)

Principle

ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate)), at neutral pH and in the presence of a suitable solution, can form a stable and coloured radical cation ($ABTS^{\bullet+}$), which exhibits maximum of absorbance at 734 nm. Antioxidant compounds quench the colour, resulting in a discoloration of the solution. The degree of discolouration is proportional to the antioxidant capacity of the sample.

Procedure

A known concentration of honey sample is prepared using ethanol as the solvent. Then, 5 µL of sample is transferred into tube, followed by the addition of 200 µL of ABTS solution. The solution is thoroughly mixed by continuous stirring for 5 minutes, and the absorbance is measured at 734 nm at 27°C. The antioxidant property (%) is calculated as a percentage of ABTS using the following equation:

$$\text{Inhibition of } A_{734\text{ nm}}(\%) = [1 - (A_f/A_0)] \times 100$$

A_0 = absorbance of uninhibited radical cation

A_f = absorbance measured 5 minutes after the addition of antioxidant sample

CHEMICAL CHARACTERISTICS OF *A. CERANA* HONEY

1. Physico-chemical characteristics of *Apis cerana* honey

Compared to *A. mellifera* honey, there is scarcity of data concerning the chemical composition of *A. cerana* honey. Nevertheless, this article endeavours to gather and analyse the available scientific literature data to

Table 1. Physico-chemical characteristics of *Apis cerana* honey

Location	Moisture (%)	pH	Electrical conductance (mS/cM)	Carbohydrate (%)			HMF (mg/kg)	Reference
				Fructose	Glucose	Sucrose		
Korea (Odaesan Kangwon)	18.62 ± 0.19	4.31 ± 0.02	0.70 ± 0.02	36.66	26.72	5.51	–	Unpublished data from CJ
Korea (Namwon)	17.42 ± 0.08	3.72 ± 0.02	0.12 ± 0.02	30.54	26.07	16.23	–	
Korea (Jiri)	20.32 ± 0.08	3.8 ± 0.02	0.12 ± 0.01	35.27	30.64	6.41	–	
Korea (Jiri)	17.56 ± 0.19	4.25 ± 0.01	0.43 ± 0.02	41.66	25.17	5.01	–	
Korea (Bonghwa)	19.34 ± 0.26	3.94 ± 0.05	0.58 ± 0.10	39.77	29.21	3.52	–	
China (Qinling Mountains, Shaanxi)	17.03 ± 0.02	6.66	–	34.76	28.69	2.08	–	Wang *et al*., 2021
	18.44 ± 0.06	5.97	–	42.85	35.28	2.71	1.69	
	17.82 ± 0.00	5.61	–	36.33	32.04	1.41	–	
	17.74 ± 0.08	4.56	–	33.92	29.22	1.87	–	
Thailand (Chiangmai)	21.2 ± 1.0	–	–	–	–	–	–	Buawangpong and Burgett, 2019
India (Uttarkashi, Uttarakhand)	23.0	–	–	38.25	37.04	0.49	26.10	Gairola *et al*., 2013
	22.6	–	–	37.27	36.01	0.35	23.12	
	20.1	–	–	39.51	37.30	0.84	16.89	
	21.0	–	–	37.93	35.37	0.67	36.86	
	25.0	–	–	37.88	35.12	1.02	13.80	
	19.0	–	–	39.15	37.14	0.19	24.60	
	19.5	–	–	40.08	37.18	0.86	25.72	
	21.8	–	–	39.41	38.04	0.45	21.50	
	21.0	–	–	40.13	37.82	0.27	22.00	
	20.8	–	–	40.51	37.58	0.41	20.12	
	22.4	–	–	37.93	35.37	0.74	21.50	
Borneo	16.99 ± 0.31	4.03 ± 0.06	0.75 ± 0.006	63.06 ± 0.54*		3.74 ± 1.03	28.50 ± 1.05	Moniruzzaman *et al*., 2013
India (Uttara Kannada of Western Ghats of Karnataka)	20.87	4.85	–	39.75	33.90	2.70	–	Balasubramanyam, 2011
India (Madikeri of Western Ghats of Karnataka)	21.07	3.83	–	39.15	32.97	3.14	–	
India (Dakshina Kannada of Western Ghats of Karnataka)	19.87	3.90	–	39.71	33.12	3.07	–	

Table 1. Continued

Location	Moisture (%)	pH	Electrical conductance (mS/cM)	Carbohydrate (%)			HMF (mg/kg)	Reference
				Fructose	Glucose	Sucrose		
Pakistan	20.06	3.59	0.59	73.9†		7.15	23.62	Iftikhar *et al*., 2011
Nepal (Chitwan)	20.12 ± 2.66	3.62 ± 0.40	0.65 ± 0.45	48.25 ± 1.62	44.02 ± 4.54	1.39 ± 1.71	–	Joshi *et al*., 2000

*Expressed as reducing sugar
†Calculated from the data provided as total sugar deducted sucrose content

determine if they fall within the conventional honey quality standards. Table 1 showcases the compilation of physico-chemical properties, ehich are typically employed for authentication purposes, of *A. cerana* honey while Table 2 displays the chemical composition relevant to the functional properties of honey.

1) Moisture content

Moisture content of *Apis cerana* honey samples ranged from 16.9 to 25.0% (Table 1). According to CODEX standard, the permissible value for moisture is 20%. While honey samples from Korea, China, and Borneo satisfy this recommended level, honey from some parts of India, Thailand were found to have higher moisture content than the recommended value. Bees regurgitate nectar over time and deposit it in the honeycomb once its moisture content reaches about 20%. To accelerate evaporation, bees employ their wings to fan the nectar and facilitates its condensation. The less moisture content minimizes the scope of microbial contamination and honey can be preserved well. High moisture content increases the scope of fermentation of honey (Singh and Singh, 2018). Therefore, honey having higher moisture content is often subjected to thermal treatment which prevents the fermentation. Various systems have been devised to facilitate the reduction of moisture content in honey, such as the ulitization of rotating discs, cones, passage through holes, or wire mesh to increase the surface area involved in the process (Singh and Singh, 2018). However, the moisture content can be largely dependent on the geographical origin, and climatic conditions (Azonwade *et al*., 2018). The initial moisture content of nectar could be one determinant factor (Azonwade *et al*., 2018). Tropical countries with high humidity particularly during the honey flow time may have effect on the moisture content of honey and therefore moisture content higher than 20% (up to 24%) is often observed. The National Bee Board of India also brought up the matter to the authorities, such as Apimondia, in 2019.

2) Electric conductivity

Electrical conductivity (EC) is an indication of ionisable acids and compounds in an aqueous solution. EC value (0.12 to 0.75 mS/cM, Table 1) was found within the value of 0.8 mS/cM, recommended for honey by CODEX standard. In principle, a higher ash and acid content in honey results in a higher EC. The pH range of the *A. cerana* honey samples falls within 3.59 to 6.66 (Table 1). The acidity of honey is caused by organic acids like citric, oxalic, acetic acid etc., either derived from nectar or secreted by bees.

3) Carbohydrate composition

Carbohydrate composition of the honey samples followed the general trend viz. fructose was found to be predominant, followed by glucose and sucrose. Fructose ranged from 30.54 to 48.25 g/100 g honey, glucose ranged from 25.17 to 44.02 and sucrose ranged 0.19 to 16.23 g/100 g (Table 1). Forager honey bees collect nectar through proboscis and the nectar undergoes chemical changes. This process primarily involves three different enzymes. Invertase breaks down sucrose into fructose and glucose in the honey stomach (crop), glucose oxidase further breaks glucose and stabilizes pH of honey, and lastly, catalase is responsible for converting hydrogen peroxide into water and oxygen. However, the carbohydrate composition varies with the different floral origin of honey, and environment also has an impact on it. The higher amount of fructose and the least amount of sucrose content make honey beneficial for human health, as fructose has a minimal effect on blood sugar elevation. On the other

Table 2. Total phenolics, flavonoids, protein content (mg/100 g) and Antioxidative activity (%) of *Apis cerana* honey

Location	Total polyphenol	Total flavonoid	Protein	Antioxidative activity (%)				Reference
				DPPH	ABTS	Nitrite	Reducing power	
Korea	74.025	10.220	26.95	9.97	31.57	21.01	0.106	Sung *et al*., 2018
	18.795	1.460	7.15	7.32	9.78	1.53	0.037	
	56.965	11.568	16.86	−1.66	25.29	1.69	0.065	
	96.580	6.851	37.42	0.10	24.51	16.10	0.073	
	23.422	4.155	6.58	4.76	11.62	−6.90	0.031	
China	49.4	9.0	–	–	–	–	–	Wang *et al*., 2021
	47.0	9.8	–	–	–	–	–	
	37.8	10.9	–	–	–	–	–	
	47.6	6.7	–	–	–	–	–	
Borneo	20.6	2.6	0.22	–	–	–	–	Moniruzzaman *et al*., 2013

hand, sucrose is responsible for elevating blood sugar level, leading to diabetes. In order to comply with the CODEX standard for honey, most of the honey samples contained a combined amount of fructose and glucose exceeding 60 g per 100 g honey or approached the limit. However, in contrast, sucrose level was found to be higher than the recommended level in the CODEX standard in most cases.

4) HMF content

The amount of HMF (Hydroxymethylfurfural) is used as an effective indicator of honey freshness and overheating. Fresh honey does not contain HMF, but its level increases with storage and temperature. The HMF content of *A. cerana* honey varied within a range of 1.69 to 36.86 mg per kg of honey (Table 1). According to the Codex Alimentarius the permissible range of HMF content is a maximum of 80 mg per kg of honey (from tropical region). However, as per some European bee federations, namely Germany, Belgium, Italy, Austria consider the maximum HMF content as 15 mg per kg honey to be 'quality honey'. In international trade, up to 40 mg of HMF per kg of honey is permitted.

5) Protein content

The compilation of protein content data for *A. cerana* honey reveals a considerable range, from 0.22 to 37.42 mg per 100 g of honey. The proline content is commonly utilized as an indicator of honey ripeness and can also indicate the presence of sugar adulteration (Von der Ohe *et al*., 1991).

6) Total polyphenol and total flavonoid content

The total polyphenol content of *A. cerana* honey samples ranged from 18.80 to 96.58 mg GAE per 100 g of honey (Table 2). Although phosphotungstic acid and phosphomolibdic acid often reacts with non-phenolic reducing compounds such as ascorbic acid, some sugars, and amino acids, leading to overestimation, the Folin-Ciocalteu method remains useful and commonly used to evaluate relative content of polyphenolic contents. Generally, dark coloured honey contains higher levels of polyphenols (Al-Farsi *et al*., 2018). Total flavonoid content ranged from 1.46 to 11.57 mg per 100 g of honey (Table 2).

7) Antioxidant assay

Data of antioxidant value of *A. cerana* honey is very limited, except from Korea (Sung *et al*., 2018). In order to understand the functional properties, antioxidative activity serves as an effective parameter. Antioxidant capacity is defined as the ability of food to scavenge free radicals and prevent their harmful effects. Antioxidative agents include antioxidative enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase), non-enzymatic substrates like vitamins (L-ascorbic acid, retinol, tocopherol), and other biomolecules (glutathione, uric acid, lipoic acid, bilirubin, coenzyme Q, polyphenols, flavonoids, carotenoids, etc.). The antioxidant capacity of honey often depends on the floral origin. Al-Farsi *et al*. (2018) reported higher antioxidant values (IC_{50} in mg/mL) for multifloral honey compared to monofloral, such as

Acacia or *Ziziphus* honey. Climatic conditions, geography, ecology, and seasons have their own impact on the chemical composition of honey (Anklam, 1998; Soares *et al*., 2017), especially polyphenolics, and therefore, their antioxidant activity (Lachman *et al*., 2010). In a recent study reported by Nicewicz *et al*. (2021), rural honey was found to have higher protein, phenolics and antioxidant capacities than the honey from urban areas.

2. Attempts to discriminate entomological origin of honey with emphasis on *A. cerana* and *A. mellifera* honey

Authentication of honey has been a persistent challenge, prompting the exploration of various scientific approaches, as discussed in a recent paper by Mohamadzade Namin *et al*. (2023). In order to provide a brief outline relevant to this discussion, Zhang *et al*. (2019a) highlighted the utility of SDS-PAGE protein pattern in distinguishing *A. mellifera* and *A. cerana* honey. The researchers discovered three distinct protein bands, ranging in molecular weight from 15 and 29.4 KDa in case of *A. cerana* honey, while *A. mellifera* honey exhibited six specific bands between 13.8 and 33.1 KDa (Zhang *et al*., 2019a). Another approach involved the development of species-specific primers targeting the tRNAleu-cox2 intergenic region for detecting *A. cerana* DNA via polymerase chain reaction, as demonstrated by Soares *et al*. (2018). Additionally, Zhang *et al*. (2019b) proposed the use of species-specific primers based on the Major Royal Jelly protein 2 gene as a valuable tool for discriminating between *A. cerana* and *A. mellifera* honey. Further investigations by Zhang *et al*. (2019a) identified 17-Pentatriacontene and Hentriacontane as characteristic constituents of *A. cerana* and *A. mellifera* honey. Several methods, such as protein-based techniques, chemical profiling, and DNA-based approaches, exist with their respective advantages and disadvantages, making them suitable for specific types of honey or particular situations.

CONCLUSION

Although *A. cerana* honey is widely produced and consumed in Asia, the data on their chemical properties is very limited. *A. mellifera* is often preferred bee species for commercial honey production, resulting in limited scientific attention given to honey quality assessment from other bee species. It is crucial to prioritize the accomplishment of quality assessment and standardization for *A. cerana* honey in order to promote its recognition and quality assurance.

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