Original Article
Food Science and Technology
ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)
DOI: https://doi.org/10.5327/fst.23023

Analysis of physicochemical components and antioxidants of four kinds of Guizhou honey

Min LIU[1,2], Weixi DENG[3], Hongli ZHANG[3*], Yihua WU[3], Lunxin WANG[3], Ying FAN[4]

Abstract

The physicochemical profile composition of four different types of honey samples and their antioxidant activity were analyzed. The physicochemical properties, including fructose, glucose, sucrose, total phenols (TP), flavonoids, insoluble matter, 5-hydroxymethylfurfural (5-HMF), amylase, sucrase, and 17 amino acids, and antioxidant power, were determined in four kinds of honey from Guizhou. The results showed significant differences in the physicochemical composition and antioxidant power of different honey samples. Among them, compared with other honey samples, *Coptis chinensis* Franc honey showed more TP and total phenolic content and a stronger antioxidant capacity. The correlation analysis showed that 5-HMF was negatively correlated with antioxidant indicators, TP, flavonoids, and multiple amino acids, implying that 5-HMF may affect the quality and biological activity of honey. Various amino acids were significantly correlated with antioxidant activity, TP, and flavonoids. Principal component analysis revealed that multiple amino acids played a major role in the first principal component (PC1), which may suggest that amino acids are an important factor in the quality of honey. Taken together, these findings provide a reference for further research on honey quality from different sources and its associated health effects.

Keywords: honey; physicochemical properties; antioxidant; correlation analysis; principal component analysis.

Practical Application: Guizhou province is a highland region in China, there are so rich in floral reserves that it can provide thousands of tonnes of honey. Biodiversity, natural riches and a large variety of flora and climate gives honeys with many different colors, aromas and flavors. Therefore, analyzing the physicochemical composition of honey in Guizhou will provide a theoretical basis for the processing and preservation of honey, as well as contribute to study its effect on health.

1 INTRODUCTION

Honey is a naturally sweet food produced by honeybees from the nectar of flowers, which bees pollinate and combine with their special components, and it is stored and brewed into honeycombs (da Costa & Toro, 2021). Honey contains complex compounds, which include more than 200 substances. Water, fructose, and glucose are the major food elements in honey (da Silva et al., 2016). In addition, monosaccharides and disaccharides account for 90% of the total sugars in honey. These sugars greatly influence honey's physical and chemical properties, such as energy value, viscosity, morphology, hygroscopicity, and granulation. The other 10% of the compounds, including proteins, minerals, phenolic compounds, amino acids, enzymes, organic acids, carotenoids, vitamins, minerals, and other aromatic substances, affect organoleptic features such as color, flavor, aroma, and texture (Pereira et al., 2020). The physicochemical composition of honey greatly affects its biological activity.

Total flavonoids and polyphenols are considerable, vital active ingredients in honey and are regarded as potential indicators of the botanical source of honey (Muhammad & Sarbon, 2023). In addition, these compounds also dramatically affect the color, flavor, and aroma of honey and exert beneficial health effects on humans through anti-inflammatory, antimicrobial, and antioxidative activities in vitro and in vivo (Pauliuc et al., 2020). For example, a study showed that honey intake increased the antioxidant capacity of athletes compared to alternative sweetener intake by increasing total antioxidant capacity, superoxide dismutase, and catalase (CAT) activities, and reducing active oxygen and malondialdehyde levels (Tartibian & Maleki, 2012). In New Zealand, Manuka honey with a deeply dark color and high level of phenolic substances is a useful treatment for people with wounds and burns (Stephens et al., 2010). In Turkey, light-colored honey had lower ferric ion-reducing antioxidant power (FRAP) values than dark-colored honey, and the higher total phenolic (TP) content indicated that the honey had a higher antioxidant capacity and FRAP value (Can et al., 2015).

The protein content In honey is usually low, between 0.1 and 0.5%, and its content varies depending on the type of honey. The proteins in honey are mainly enzymatic compounds such as cellulase, glucosidase, CAT, acid phosphatase, amylase, and glucose oxidase (Sak-Bosnar & Sakac, 2012). The changes in protein content, both quantitative and qualitative, in honey

Received 11 May, 2023.
Accepted 29 Jun., 2023.
[1]*Huazhong Agricultural University, College of Food Science and Technology, Wuhan, China.*
[2]*Wuhan Engineering Research Center of Bee Products on Quality and Safety Control, Wuhan, China.*
[3]*Zunyi Vocational and Technical College, Department of Modern Agriculture, Zunyi, China.*
[4]*Guizhou Provincial Animal and Poultry Genetic Resources Management Station, Guiyang, China.*
*Corresponding author: 930360784@qq.com

were associated with polyphenolic substances (Brudzynski et al., 2013). Diastases are a group of amylolytic enzymes, including α-amylase and β-amylase. α-Amylase hydrolyzes α-D-(1-4)-linked starch chains to produce dextrins. β-Amylases hydrolyze the terminal starch chains, leading to the formation of maltose. Diastase content in honey depends on bee age, the physiological stage of the colony, the floral and geographical origins, and the large amount of nectar and its sugar content, which can lead to lower enzyme levels. Moreover, glucose oxidase in honey has antibacterial properties by producing hydrogen peroxide and gluconic acid derived from glucose.

Amino acids are one of the compounds in honey, originating mostly from bees but occasionally from plant pollen sources (Archilia et al., 2021). More than 20 amino acids were detected in honey, and their type and relative amount depended on the flower nectar or honeydew (Hermosín et al., 2003). Among them, proline was predominant, accounting for 50–85% of all amino acids, followed by aspartate, glutamate, lysine, and some other types of amino acids (Archilia et al., 2021). Since amino acids rely on the pollen in honey, the profile of amino acids in honey may be a feature of its geographical or botanical origin. In addition, proline has been used as a criterion for evaluating the maturity of honey (da Silva et al., 2016).

The Maillard reaction is an important nonenzymatic reaction for color formation in honey. The reaction is generated by the carboxyl group on the reducing end of the sugar with the free amino group of amino acids and proteins, which produces many intermediate products. 5-Hydroxymethylfurfural (5-HMF) is a cyclic aldehyde formed via the existence of glucose, fructose, and many acids in honey (Lee and Nagy, 1990). 5-HMF is one of the main intermediates of the Maillard reaction and is formed when honey is heated or stored for long periods of time (Yücel & Sultanoğlu, 2013). In addition, 5-HMF can also be produced by the dehydration of honey in an acidic environment (Islam et al., 2014). In general, fresh honey has no or low levels of 5-HMF, while it has a high concentration of 5-HMF when aged and/or stored at relatively high or moderate temperatures. The use of metal containers for honey storage is positively associated with the level of 5-HMF in honey (Shapla et al., 2018). Therefore, 5-HMF is often used as an essential indicator of poor storage conditions and poor honey quality. 5-HMF has been reported to have negative effects on human mucous membranes, skin, and eyes, as well as causing cytotoxicity in the upper respiratory tract, chromosomal aberrations, and carcinogenic effects.

Different types and amounts of compounds in honey depend on bee species, flower source, climate, region, season, harvest mode, environmental conditions, processing techniques, and storage (Tornuk et al., 2013), and these factors may impact the antioxidant activity of honey. The objective of this study was to quantify the chemical compositions of four Guizhou honey samples from different nectar sources, including amylase and sucrase, insoluble matter, TP, 5-HMF, amino acids, glucose, fructose, and sucrose. Furthermore, the antioxidant activity was analyzed via 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,"-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) assays. The correlation between chemical parameters and antioxidant power was further evaluated through Pearson's correlation analysis.

2 MATERIALS AND METHODS

2.1 Materials

Coptis chinensis Franc honey (sample 1), Baihua honey (sample 2), *Sapium sebiferum* (L.) Roxb honey (sample 3), and *Rhus chinensis* Mill honey (sample 4) were kindly provided by the apiary of ZhenZhen (Zunyi, China). All the honey samples were stored in tightly closed containers at 4°C until further use. The sucrase kit was purchased from Suzhou Michy Biomedical Technology Co., Ltd. (Suzhou, China). DPPH and ABTS were purchased from Sigma Chemical, Co. (Sigma, USA). All chemicals and reagents used were of analytical grade.

2.2 Measurement of fructose, glucose, and sucrose

Glucose, fructose, and sucrose were determined by HPLC (Waters 2695, Massachusetts, USA) and an Althena NH2-RP column (250 mm*4.6 mm, 5 μm). The mobile phase used was 80% acetonitrile water at a flow rate of 1 mL/min, the column temperature was maintained at 40°C, and the injection volume was 10 μL. The values are shown as mg/g of honey. The ratio of fructose to glucose (F/G) was measured by dividing the fructose content by the glucose content.

2.3 Analysis of total phenols and flavonoids, 5-HMF, and Insoluble matter of the four types of honey

2.3.1 Total phenols and flavonoids

TPs in all honey samples were analyzed by the Folin-Ciocalteu method (Guo et al., 2019). Briefly, a 1 g honey sample was added to 20 mL of ultrapure water. A 20-μL honey solution was mixed with 20 μL of Folin-Ciocalteu reagent, 30 μL of 20% sodium carbonate, and a fixed volume of 200 μL of ultrapure water. After 30 min of incubation in the dark at room temperature, the absorbance of the mixture was recorded at 760 nm using a microplate reader (Multiskan GO, Thermo-Fisher, USA). The amounts of flavonoids were measured by a colorimetric method at 415 nm. Briefly, honey (0.5 g) was diluted to 25 mL with 95% ethanol, shaken at 65°C for 45 min, and then centrifuged at 5,000 rpm for 10 min; 200 μL of supernatant was added to 4 μL of 0.5 mol/L aluminum nitrate solution and 4 μL of 1 mol/L potassium acetate solution, mixed well, and then left at 25°C for 30 min. The absorbance of the mixture was determined at 415 nm by a microplate reader. Rutin was used as the standard to set up a validation curve.

2.3.2 Determination of 5-HMF content

5-HMF was determined by HPLC equipped with UV detection. Briefly, 1 g of honey was weighed, added to 10 mL of ultrapure water, dissolved by shaking, leached overnight after sonication for 1 h, centrifuged to remove the supernatant, and filtered for testing. Separation was performed on an HP-C18 reversed-phase column (250 mm ´ 4.6 mm, 5 μm). The signals were compared with those from standards of known concentration.

2.3.3 Assessment of the insoluble matter

The insoluble matter was determined according to the method of Pereira et al. (2020). Briefly, honey samples (5 g) were weighed, dissolved in 10 mL ultrapure water, shaken, and centrifuged at 10,000 × g for 5 min. The supernatant was collected, 2 mL of ultrapure water was added, and the mixture was heated to 60°C and filtered using Whatman filter paper. The sediment was dried at 80°C until its weight remained constant. The percentage of insoluble matter was calculated using the Equation 1:

$$Insoluble\ matter(\%) = \frac{M1 - M2}{M} \times \% \quad (1)$$

Where:

$M1$: the mass of the precipitate;

$M2$: the weight of the paper filter before filtration;

M: the weight of the honey.

Measurements of amylase and sucrase

The amylase in honey samples was measured using a previously described method (Ajlouni & Sujirapinyokul, 2010). We dissolved 5 g of the honey sample in 15 mL of ultrapure water and 5 mL of acetate buffer solution (pH 5.0), added 3 mL of sodium chloride solution, and made the volume up to 50 mL. Notably, 10 mL of the solution were transferred to a 50-mL flask and placed in a water bath at 40°C together with another flask containing 10 mL of the 1% starch solution. After 15 min, 5 mL of the starch solution was dissolved in the honey solution and mixed. At regular intervals, the first time after 5 min, 0.5 mL of the mixture was mixed with 5 mL of diluted iodine solution and 22 mL ultrapure water, vortexed, and immediately measured against a water blank at 660 nm. Sucrase was determined according to the manufacturer's instructions with a commercial assay kit (Suzhou, China).

2.5 Detection of amino acids

Amino acid compositions were determined as described in previous studies (Sommano et al., 2020) with minor modifications. One gram of honey was dissolved in 10 mL of an extraction solution consisting of 6 mol/L HCl and 0.1% phenol, digested for 2 h, and cooled at room temperature. Then, 1 mL of hydrolysis solution was placed into a 10 mL volumetric flask, 1 mL of 6 mol/L NaOH was added to neutralize the solution, and the volume was brought up to 10 mL with ultrapure water, passed through a 0.22 μm filter membrane, and then put into the machine. HPLC analysis was carried out using a RIGOL L3000 HPLC system (Dongke, China) with a Sepax Amethyst C18-H (250 mm × 4.6 mm, 5 μm). Detection was conducted at 254 nm with an injection volume of 10 μL. Next, 7.6 g of anhydrous sodium acetate was added to 925 mL of ultrapure water. After ultrasonic dissolution, the pH was set to 6.5 with glacial acetic acid, and then 70 mL of acetonitrile was added (A) along with 80% acetonitrile (B) with a flow rate of 1 mL/min. The injected sample volume was 10 μL. The gradient of mobile phase B was as follows: 0% at 0 min, 0% at 2 min, 10% at 15 min, 30% at 25 min, 45% at 33 min, 100% at 33.1 min, 100% at 38 min, 0% at 38.1 min, and 0% at 45 min. The peak areas were applied for the quantification of amino acids. The results for each amino acid are shown in μg/mL.

2.6 Analysis of antioxidant activity in honey: DPPH and ABTS assays

The DPPH free radical scavenging rate was measured by the Leon-Ruiz method with some modifications (Leon-Ruiz et al., 2013). Honey samples were dissolved in ultrapure water at a ratio of 1:1 (m/v) and then diluted 10 times. A volume of 100 μL of the solution were mixed with 100 μL of 0.2 mmol/L DPPH-ethanol solution. The mixture was incubated in the dark at room temperature for 30 min, and then the absorbance of the remaining DPPH was measured at 517 nm against a blank. In addition, the sample control group was set up without DPPH-ethanol solution and without diluted solution as a blank control group, and the clearance was calculated according to the Equation 2:

$$Clearance = \left[1 - \frac{(\text{sample assay OD value} - \text{sample control OD value})}{\text{blank control OD value}}\right] \times 100\% \quad (2)$$

The ABTS scavenging capacities of honey samples were measured following a previously described procedure with slight modification (Dudonné et al., 2009). The honey samples were dissolved in water at a ratio of 1:1 (m/v) and then diluted 10 times. A volume of 40 mL of diluted solution was mixed with 160 μL of ABTS working solution (2.6 mmol/L potassium persulfate and 7.4 mmol/L ABTS were mixed in equal volumes, and the mixture was allowed to stand in the dark at room temperature for 12 h before use). Then, the mixture was diluted in deionized water to an absorbance of 0.7 (0.02) at 734 nm and placed in the dark for 8 min, and the absorbance values were analyzed at 734 nm using a microplate reader. In addition, the sample control group was set up by not adding ABTS working solution and without diluted solution as a blank control group, and the clearance was calculated according to the Equation 3:

$$Clearance = \left[1 - \frac{(\text{sample assay OD value} - \text{sample control OD value})}{\text{blank control OD value}}\right] \times 100\% \quad (3)$$

2.7 Statistical analysis

The results are shown as the mean±standard deviation (SD). Data analysis was carried out using the SPSS 24.0 statistical software (SPSS Inc., Chicago, IL, USA) with Tukey's HSD test. Probability values of $p<0.05$ were accepted as statistically significant. Pearson correlation analysis was performed to evaluate the correlation between the effects of different honey samples on antioxidants and their chemical compositions.

3 RESULTS AND DISCUSSION

3.1 Determination of sugar content in four types of honey

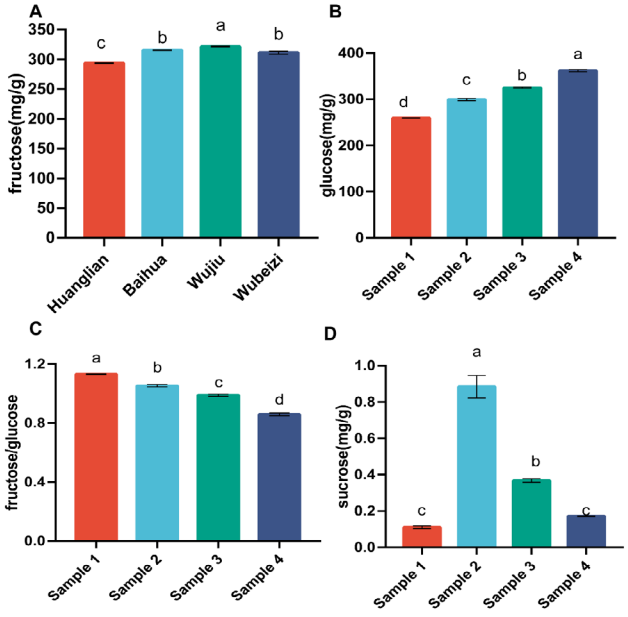
Fructose, glucose, and sucrose could be taken as indicators to estimate the physicochemical properties and nutritional value of honey. Fructose is related to the sweetness of honey, while the glucose content determines the nectar source (Aljohar et al., 2018). The sucrose content can be used to evaluate the maturity and freshness of honey. The lower the sucrose content, the more mature the honey, and the better the collection time (Pereira et al., 2020).

In this study, the results showed that fructose, glucose, sucrose, and the ratio of F/G were significantly different among the four honey samples with different nectar sources ($p<0.05$; Figure 1). The fructose content of sample 3 was the highest, while that of sample 1 was the lowest. The glucose content in sample 4 was the highest, while the glucose content in sample 1 was the lowest, which led to an increase in the F/G ratio in sample 4 and a decrease in the F/G ratio in sample 1. A previous study found that honey from wild blossom plants showed higher glucose values (Pereira et al., 2020). The fructose and glucose contents of any particular honey largely depend on the composition of the plant secretions. Some studies reported that the F/G ratio of honey was between 1.10 and 1.36, which can reduce the crystallization of honey and allow it to remain liquid for longer periods (Ouchemoukh et al., 2010). In this study, the lowest amount of sucrose was found in sample 1, while the highest content of sucrose was found in sample 2.

3.2 Total phenols, flavonoids, 5-HMF, and insoluble matter in the four types of honey

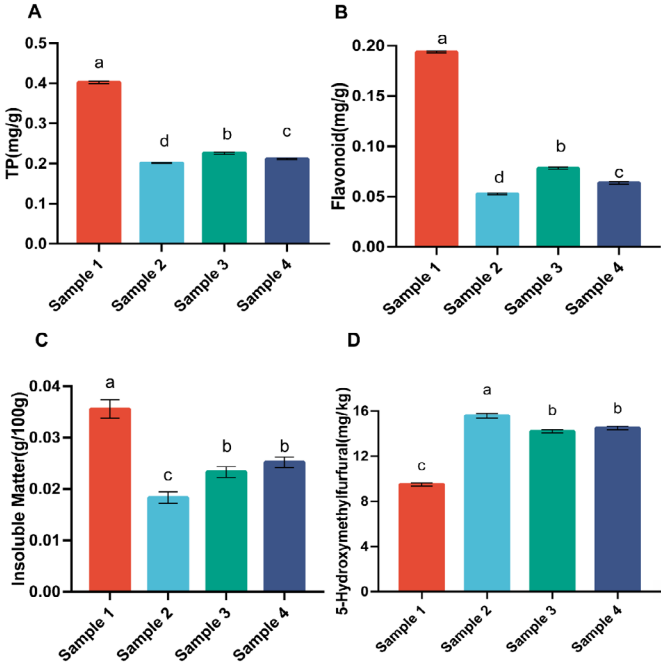
The biological characteristics of honey depend on the amount of native antioxidants in the pollen and other nectar collected by the bees (Schramm et al., 2003). The antioxidant activity of honey is not only related to the existence of phenolics, flavonoids, and CAT but also associated with the reaction products of the macrobiotic reaction in honey (Gheldof & Engeseth, 2002; Sergiel et al., 2014). The TP and flavonoid contents were analyzed in four honey samples, and there were significant differences in the TP and total flavonoid contents of the four honey samples (Figures 2A and 2B; $p<0.05$). The highest TP (0.402 mg/g) and flavonoid (0.194 mg/g) contents were identified in sample 1, followed by sample 3, sample 4, and sample 2. Sample 1 (*Coptidis rhizome*) is one of the most widely used traditional Chinese medicine herbs in the field. There are also a variety of active ingredients in sample 1, such as berberine, epiberberine, phenylpropanoids, flavonoids, and phenolic compounds (Han et al., 2019). These components may provide a reference for a wide range of bioactive functions in sample 1.

The quantification of 5-HMF is important to verify the deterioration of honey, which indicates elevated temperatures for heat treatment and/or unsuitable storage Conditions (Tornuk et al., 2013). Therefore, honey with low levels of 5-HMF can be considered fresh or high-quality honey and can be stored in better conditions. Values between 5.91 and 15.60 mg/kg were shown in four types of honey (Figure 2D; $p<0.05$). The highest content of 5-HMF was found in sample 1 (15.60 mg/kg). However, the 5-HMF values of sample 1 were lower than the Chinese national standard (China, 2003), which is 20–40 mg/kg, and much lower than the HMF value of 80 mg/kg for tropical countries according to the Codex Alimentarius standard (Commission, 2000). Studies have shown that excessive 5-HMF may cause potential carcinogenic risk by inducing gene mutations



*Means with different letters differ significantly in each subfigure ($p<0.05$).

Figure 1. Analysis of fructose, glucose, and sucrose contents in honey. (A) fructose; (B) glucose; (C) fructose/glucose (F/G); (D) sucrose*.



*Means with different letters differ significantly in each subfigure ($p<0.05$).

Figure 2. Analysis of TP, flavonoids, insoluble matter, and 5-HMF content in honey. (A) TP; (B) flavonoid; (C) insoluble matter; (D) 5-HMF*.

and DNA strand breaks (Feridoun & Hiroyuki, 2007), while some scholars have claimed that 5-HMF itself is not toxic and it can form sulfoxymethylfurfural and 5-chloromethylfurfural in vivo and in vitro, respectively, which are highly carcinogenic and genotoxic (Guo et al., 2011).

Insoluble matter was produced by processing failures, and it was used to evaluate the cleanliness and content of physical impurities in honey. In this study, the insoluble matter values in all honey samples were significantly lower than the Brazilian legislation (Brazil, 2000) (Figure 2C), which is a maximum of ≤0.10 g/100 g. The values of water-insoluble solids were consistent with the results in Brazilian honey and Turkish honey (Can et al., 2015; Pereira et al., 2020), and all the honey samples showed values within the criteria.

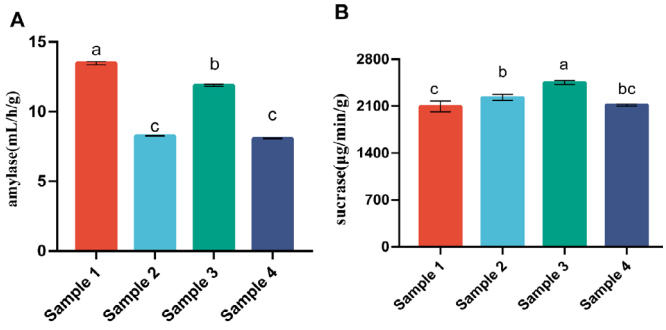
3.3 Amylase and sucrose activities in four types of honey

Amylase was used to assess the freshness of honey, which plays a role in the digestion of starch and is secreted from the honeybee. Among the four types of honey, the highest amylase activity was found in sample 1 (13.47±0.19 mL/h/g), followed by sample 3 (11.89±0.15 mL/h/g), sample 2 (8.27±0.07 mL/h/g), and sample 4 (8.07±0.07 mL/h/g).

In honey samples, sucrose transferase is more sensitive to temperature changes than amylase. Both honey and nectar contain sucrase, which is mainly produced by the subpharyngeal glands of bees and then flows into the mouth, where it is most likely to be added to the nectar or honey (Żoltowska et al., 2012). In the present work, sucrose transferase was determined in four honey samples, and the results are shown in Figure 3. Sucrose transferase activity showed obvious differences in all honey samples, with values ranging from 2,117.45±80.10 to 2,095.22±25.85 μg/min/g. The differences in amylase activity in honey samples are influenced by storage conditions, harvesting methods, and honeybee species. In this research, the activities of both enzymes showed significant differences in four honey samples, which may be related to the nectar source.

3.4 Amino acid content in four types of honey

Amino acids are a class of small-molecule compounds in honey that mainly depend on the pollen of flowering plants, climate, environment, and bee species. In this study, 17 amino acids were identified in the four honey samples, and the results are shown in Table 1. Honey from different floral origins possessed the same type of amino acids in different amounts. The highest amino acid content was found in sample 1, followed by sample 3, sample 2, and sample 4. The amino acid content of sample 1 is 2.43 times higher than that of sample 4, and it is 1.95 times higher than that of sample 2. Glutamic acid (620.06±12.44 μg/g) was the most abundant amino acid in sample 1, followed by proline (461.74±40.25 μg/g). The other three types of honey contained the most phenylalanine, followed by proline. In contrast to other studies (da Silva et al., 2016; Hermosín et al., 2003), proline was not the most abundant amino acid in these honey samples, but glutamic acid and phenylalanine were followed by proline. This difference may be due to differences in honeybee species, geography, and nectar plants. Many studies have noted that the amount of proline in honey is a marker of purity, and the proline content is noticeably reduced in adulterated honey (Can et al., 2015; Cavrar et al., 2013; Cotte et al., 2004). According to the Honey Code, the desired level of proline in honey is 250 mg/kg, but the higher the quality of the honey, the more proline It contains.



*Means with different letters differ significantly in each subfigure ($p<0.05$).

Figure 3. Determination of amylase and sucrase activities. (A) amylase; (B) sucrase*.

3.5 Antioxidant power of the four types of honey

Oxidative stress can be characterized as an imbalance between oxidants and antioxidants in favor of oxidants. Oxidative stress results in oxidative damage that can impact various physiological functions. Free radicals and reactive oxygen species are the main oxidants in the cellular system, and they are involved in aging and in the development of many kinds of diseases. Many honey flavonoids and phenolic acids have antioxidant activity.

DPPH and ABTS possess excellent reproducibility and stability, and they were used to assess the in vitro antioxidant levels in honey. The results are shown in Figures 4A and 4B, indicating that the scavenging activity of DPPH and ABTS followed a similar pattern as that of TP and flavonoids. Sample 1 exhibited the highest ABTS scavenging activity, which was 2.65±0.02 μmol Trolox/g, followed by sample 4 (1.86±0.03 μmol Trolox/g), sample 2 (1.81±0.01 μmol Trolox/g), and sample 3 (1.68±0.02 μmol Trolox/g). A similar result was obtained for the DPPH free radical scavenging capacity, with the highest DPPH value found in sample 1. These results demonstrated that sample 1 had the highest antioxidation capacities, which may be associated with its bioactive ingredients, such as phenolic acids, flavonoids, and berberine, and that antioxidant activity showed differences in honey from different sources.

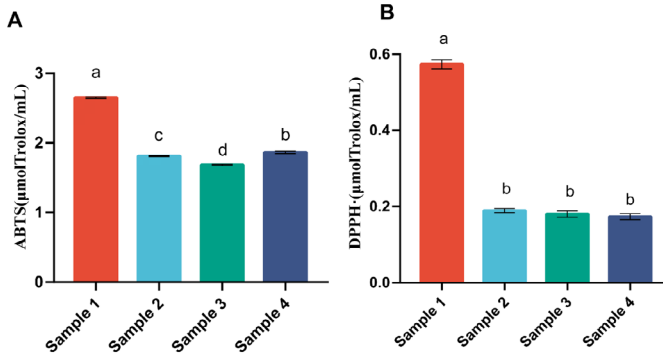
3.6 Correlation analysis

There were significant differences in the physicochemical composition of the honey samples as well as in their antioxidant activity. To verify the correlation between physicochemical indicators and antioxidant activity or the physicochemical indicators themselves, Spearman's correlation analysis was performed in this study. Based on the heatmap (Figure 5), 5-HMF was negatively correlated with sucrose, TP, flavonoids, DPPH,

Table 1. Amino acid analysis of honey samples*.

μg/g	Sample 1	Sample 2	Sample 3	Sample 4
Asp	192.54±5.78a	36.07±2.36b	36.55±3.47b	33.70±4.76b
Glu	620.06±12.44a	18.44±0.37b	16.19±0.68b	18.47±1.02b
Ser	118.99±5.31a	21.39±1.47b	8.92±0.29c	8.28±0.34c
Gly	24.54±2.40a	4.054±0.24b	2.94±0.15b	2.67±0.15b
His	43.26±2.09a	7.045±1.04b	6.60±0.60b	8.70±0.99b
Arg	ND	ND	ND	ND
Thr	21.36±4.14a	8.20±0.37b	5.17±0.83b	6.16±0.90b
Ala	62.07±4.11a	9.53±0.30b	10.18±0.32b	9.78±0.74b
Pro	461.74±40.25a	184.05±2.55c	231.68±2.61b	159.72±6.57c
Tyr	31.31±2.36a	11.99±0.48b	10.47±0.42b	12.77±0.79b
Val	26.43±2.61a	19.69±0.48b	19.21±0.76b	19.52±1.29b
Met	ND	ND	ND	ND
Cys	ND	ND	ND	ND
Ile	91.39±5.92a	45.72±0.25c	38.67±1.10d	51.64±1.07b
Leu	7.29±1.38a	4.92±0.50b	5.27±0.29b	7.09±0.75a
Phe	135.14±3.50d	560.31±3.67b	1331.12±5.92a	412.22±17.30c
Lys	12.01±1.80a	12.45±0.20a	9.98±0.19b	12.04±0.59a
Sum	1848.14	943.88	1732.95	762.74
Mean value	132.01	67.42	123.78	54.48

*Data are expressed as the mean±SD; means with different letters within a row differ significantly ($p<0.05$); ND: not detected.



*Means with different letters differ significantly in each subfigure ($p<0.05$).

Figure 4. Analysis of free radical scavenging rates in honey. (A) ABTS; (B) DPPH*.

and a variety of amino acids (Pro, Try, Val, Ala, His, Glu, Gly, and Asp). There was a significant positive correlation among multiple amino acids. Furthermore, DPPH was significantly and positively correlated with Pro, TP, flavonoids, Ile, ABTS, Try, Val, Ala, His, Gly, and Glu, and ABTS was significantly and positively correlated with sucrose and TP. In addition, TP and flavonoids showed a significant positive correlation with Pro. In summary, our results indicated that 5-HMF was significantly and negatively associated with multiple physical and chemical indicators, which may suggest that 5-HFM has a strong influence on honey quality. A variety of amino acids were significantly correlated with antioxidant activity (ABTS and DPPH), TP, and flavonoids, which may suggest that the effects of amino acids on the quality and biological activity of honey samples may be of greater interest.

3.7 Principal component analysis

PCA is a method of statistical procedure analysis that is usually used to compare the results of analytical methods for a group of samples. In this study, PCA was performed to evaluate four different types of honey samples from various nectars in Guizhou and identify honey samples with similar characteristics.

The first principal component (PC1) contributed to 41.7% of the variance, while the second principal component (PC2) represented 23.6% of the variance; the first two principal components accounted for a total of 65.3% of the original variances. As shown in Figure 6A, there was a clear difference in the four honey samples, and they were clearly separated from each other. Specifically, samples 2 and 4 were closer together, which indicated that the fingerprint profiles of the physicochemical indicators of these two types of honey were more similar, while sample 1 was far from sample 3, indicating that these honey samples had different physicochemical fingerprint profiles. All physiochemical parameters are normalized to ensure that all parameters have equal weight in the outcome. According to Figure 6B, PC1 was closely and positively correlated with the values of DPPH, ABTS, TP, flavonoids, and multiple amino acids (Glu, Ala, His, Tyr, Ser, and Gly) but negatively correlated with 5-HMF and fructose. PC2 was closely related to the physiochemical components of sucrase, amylase, Phe, Lys, and

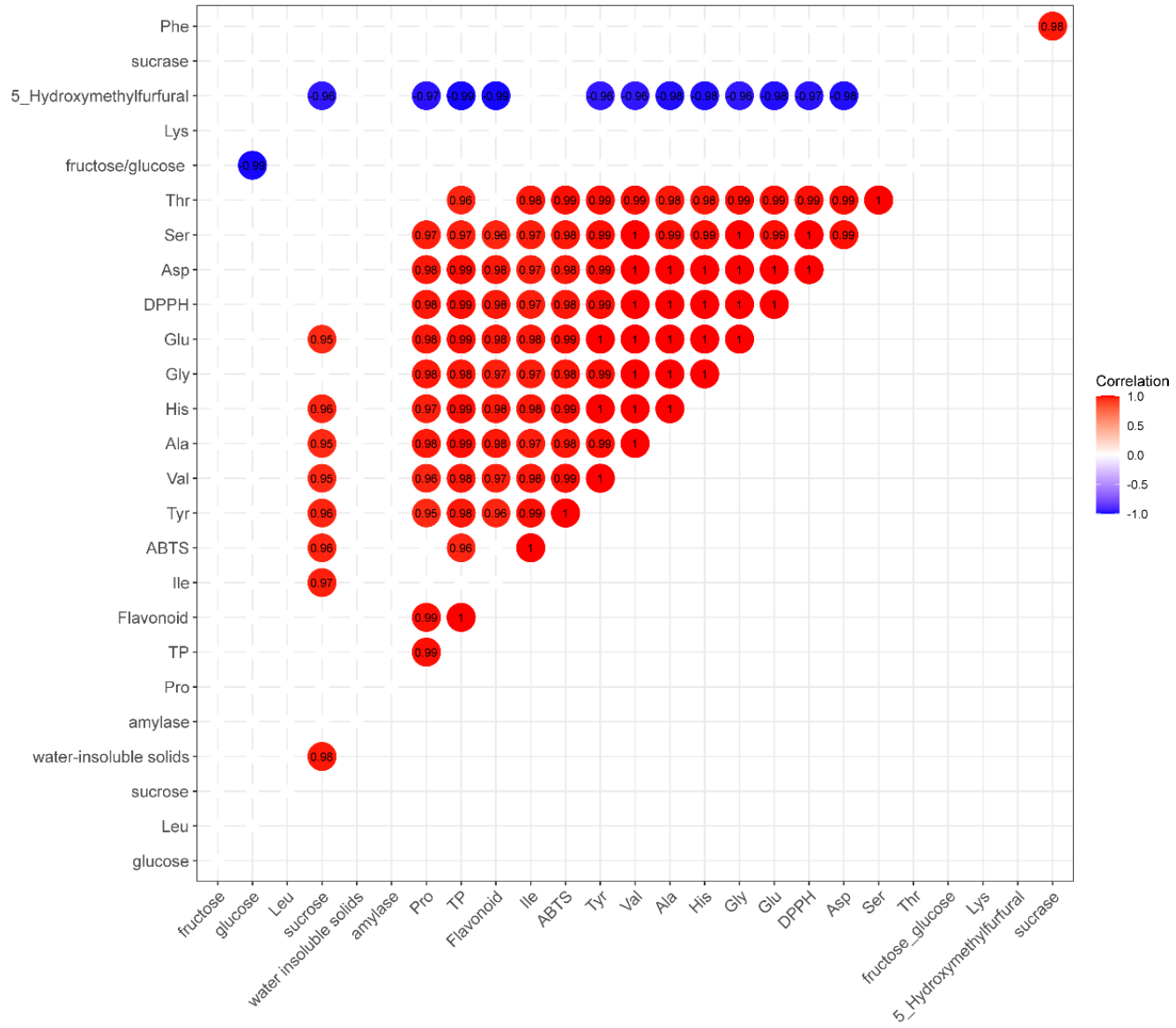


Figure 5. Spearman correlation analysis.

F/G. The main variables for PC3 were sucrose, glucose, and Leu. The results showed that the physicochemical parameters, including TP, flavonoids, Pro, F/G, 5-HMF, and amylase, were important indicators that may affect the quality of the four honey samples.

4 CONCLUSION

In this study, a number of physicochemical parameters were measured in four different types of honey with different nectars from Guizhou. The findings demonstrated that there were significant differences in the physicochemical parameters of honey samples from different nectar sources. In particular, compared with the other groups, Franc honey had lower 5-FMH and sucrose contents but the highest F/G value, total phenols, and total flavonoids, and the strongest ability to scavenge free radicals. Additionally, glutamic acid was the most abundant amino acid in Franc honey (sample 1), while the other honey samples were rich in phenylalanine. The correlation analysis showed that 5-HMF was strongly and negatively associated with multiple physical and chemical indicators, which may suggest that 5-HFM has a strong influence on honey quality. PCA showed that physiochemical components such as TP, flavonoids, Pro, F/G ratio, 5-HMF, and amylase could be important factors affecting the quality of honey.

ACKNOWLEDGMENTS

The present study was supported by the project of Science and Technology Bureau of Zunyi City Cooperative Research Funds (HZ word (2020) No. 174). The authors are grateful to Zogona Daniel, PhD, for language polishing of the whole manuscript.

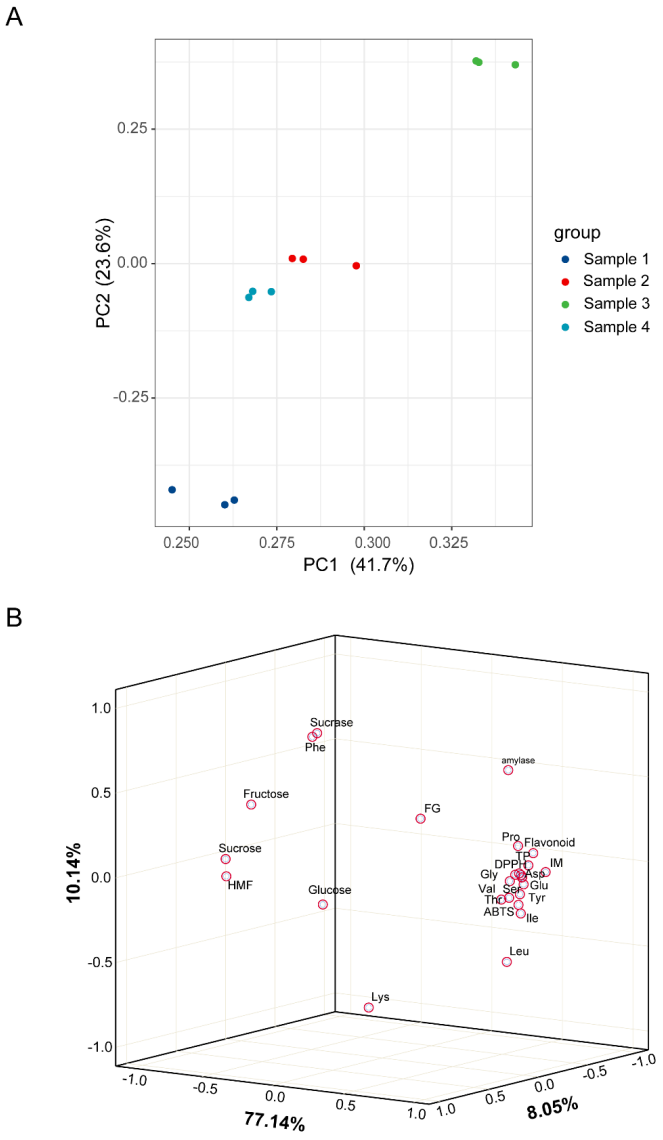


Figure 6. Principal component analysis. (A) Distribution of honey samples on a score plot. (B) Distribution of variables on the loading plot.

REFERENCES

Ajlouni, S., & Sujirapinyokul, P. (2010). Hydroxymethylfurfuraldehyde and amylase contents in Australian honey. *Food Chemistry*, *119*(3), 1000-1005.

Aljohar, H. I., Maher, H. M., Albaqami, J., Al-Mehaizie, M., Orfali, R., Orfali, R., & Alrubia, S. (2018). Physical and chemical screening of honey samples available in the Saudi market: An important aspect in the authentication process and quality assessment. *Saudi Pharm Journal*, *26*(7), 932-942. https://doi.org/10.1016/j.jsps.2018.04.013

Archilia, M. D., Neto, A. A. L., Marcucci, M. C., Alonso, R. C. B., Camargo, T. C. de, Camargo, R. C., & Sawaya, A. C. H. F. (2021). Characterization of Brazilian monofloral and polyfloral honey by UHPLC-MS and classic physical-chemical analyses. *Journal of Apicultural Research*, *62*(3), 578-589. https://doi.org/10.1080/00218839.2021.1886747

Brazil (2000). *Technical regulation of honey identity and quality*.

Brudzynski, K., Sjaarda, C., & Maldonado-Alvarez, L. (2013). A New Look on Protein-Polyphenol Complexation during Honey Storage: Is This a Random or Organized Event with the Help of Dirigent-Like Proteins? *Plos One*, *8*(8), e72897. https://doi.org/10.1371/journal.pone.0072897

Can, Z., Yildiz, O., Sahin, H., Akyuz Turumtay, E., Silici, S., & Kolayli, S. (2015). An investigation of Turkish honeys: their physico-chemical properties, antioxidant capacities and phenolic profiles. *Food Chemistry*, *180*, 133-141. https://doi.org/10.1016/j.foodchem.2015.02.024

Commission, C. A. (2000). *The draft revised Codex standard for honey*. Food and Agriculture Organization of the United Nations.

Cotte, J. F., Casabianca, H., Giroud, B., Albert, M., Lheritier, J., & Grenier-Loustalot, M. F. (2004). Characterization of honey amino acid profiles using high-pressure liquid chromatography to control authenticity. *Analytical and Bioanalytical Chemistry*, *378*(5), 1342-1350. https://doi.org/10.1007/s00216-003-2430-z

Cavrar, S., Yildiz, O., Sahin, H., Karahalil, F., & Kolayli, S. (2013). Comparison of physical and biochemical characteristics of different quality of Turkish honey. *Uludag Bee Journal November*, *13*(2), 55-62.

da Costa, I. F., & Toro, M. J. U. (2021). Evaluation of the antioxidant capacity of bioactive compounds and determination of proline in honeys from Para. *Journal of Food Science and Technology*, *58*(5), 1900-1908. https://doi.org/10.1007/s13197-020-04701-1

da Silva, P. M., Gauche, C., Gonzaga, L. V., Costa, A. C., & Fett, R. (2016). Honey: Chemical composition, stability and authenticity. *Food Chemistry*, *196*, 309-323. https://doi.org/10.1016/j.foodchem.2015.09.051

Dudonné, S., Vitrac, X., Cutière, P., Woillez, M., Mérillon, J.-M. (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assay. *Journal of Agriculture and Food Chemistry*, *57*(5), 1768-1774. https://doi.org/10.1021/jf803011r

Feridoun, S., & Hiroyuki, Y. (2007). Kinetics of the Decomposition of Fructose Catalyzed by Hydrochloric Acid in Subcritical Water: Formation of 5-Hydroxymethylfurfural, Levulinic, and Formic Acids. *Industrial & Engineering Chemistry Research*, *46*(23), 7703-7710. https://doi.org/10.1021/ie061673e

Gheldof, N. & Engeseth, N. J. (2002). Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. *Journal of Agriculture and Food Chemistry*, *50*(10), 3050-3055. https://doi.org/10.1021/jf0114637

Guo, M., Xing-Tao, X. U., & Zhi-Wu, W. U. (2011). Binding mechanism of rhaponticin and human serum albumin. *Acta Pharmaceutica Sinica*, (12), 1084-1092.

Guo, P., Deng, Q., & Lu, Q. (2019). Anti-alcoholic effects of honeys from different floral origins and their correlation with honey chemical compositions. *Food Chemistry*, *286*, 608-615. https://doi.org/10.1016/j.foodchem.2019.02.058

Han, L., Wang, R., Zhang, X., Yu, X., Zhou, L., Song, T., Deng, X., Zhang, Y., Zhang, L., & Bai, C. (2019). Advances in Processing and Quality Control of Traditional Chinese Medicine Coptidis rhizome (Huanglian): A Review. *Journal of AOAC International*, *102*(3), 699-707. https://doi.org/10.5740/jaoacint.18-0303

Hermosín, I., Chicón, R. M., & Dolores Cabezudo, M. (2003). Free amino acid composition and botanical origin of honey. *Food Chemistry*, *83*(2), 263-268. https://doi.org/10.1016/S0308-8146(03)00089-X

Islam, M. N., Khalil, M. I., Islam, M. A., & Gan, S. H. (2014). Toxic compounds in honey. *Journal of Applied Toxicology*, *34*(7), 733-742. https://doi.org/10.1002/jat.2952

Lee, H. S., & Nagy, S. (1990). Relative reactivities of sugars in the formation of 5-hydroxymethylfurfural in sugar-catalyst model systems. *Journal of Food Processing and Preservation*, *14*(3), 171-178. https://doi.org/10.1111/j.1745-4549.1990.tb00126.x

Leon-Ruiz, V., Gonzalez-Porto, A. V., Al-Habsi, N., Vera, S., San Andres, M. P., & Jauregi, P. (2013). Antioxidant, antibacterial and ACE-inhibitory activity of four monofloral honeys in relation to their chemical composition. *Food & Function*, *4*(11), 1617-1624. https://doi.org/10.1039/C3FO60221D

Muhammad, N. I. I., & Sarbon, N. M. (2023). Physicochemical profile, antioxidant activity and mineral contents of honey from stingless bee and honey bee species. *Journal of Apicultural Research*, 62(2), 394-401. https://doi.org/10.1080/00218839.2021.1896214

Ouchemoukh, S., Schweitzer, P., Bachir Bey, M., Djoudad-Kadji, H., & Louaileche, H. (2010). HPLC sugar profiles of Algerian honeys. *Food Chemistry*, *121*(2), 561-568. https://doi.org/10.1016/j.foodchem.2009.12.047

Pauliuc, D., Dranca, F., & Oroian, M. (2020). Antioxidant activity, total phenolic content, individual phenolics and physicochemical parameters suitability for romanian honey authentication. *Foods*, *9*(3), 306. https://doi.org/10.3390/foods9030306

Pereira, J. R., Campos, A. N. da R., Oliveira, F. C. de, Silva, V. R. O., David, G. F., Silva, J. G. Da, Nascimento, W. W. G., Silva, M. H. L., & Denadai, Â. M. L. (2020). Physical-chemical characterization of commercial honeys from Minas Gerais, Brazil. *Food Bioscience*, *36*, 100644. https://doi.org/10.1016/j.fbio.2020.100644

Sak-Bosnar, M., & Sakac, N. (2012). Direct potentiometric determination of diastase activity in honey. *Food Chemistry*, *135*(2), 827-831. https://doi.org/10.1016/j.foodchem.2012.05.006

Schramm, D. D., Karim, M., Schrader, H. R., Holt, R. R., Cardetti, M., & Keen, C. L. (2003). Honey with high levels of antioxidants can provide protection to healthy human subjects. *Journal of Agriculture Food Chemistry*, *51*(6), 1732-1735. https://doi.org/10.1021/jf025928k

Sergiel, I., Pohl, P., & Biesaga, M. (2014). Characterisation of honeys according to their content of phenolic compounds using high performance liquid chromatography/tandem mass spectrometry. *Food Chemistry*, *145*, 404-408. https://doi.org/10.1016/j.foodchem.2013.08.068

Shapla, U. M., Solayman, M., Alam, N., Khalil, M. I., & Gan, S. H. (2018). 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: effects on bees and human health. *Chem Cent Journal*, *12*, 35. https://doi.org/10.1186/s13065-018-0408-3

Sommano, S. R., Bhat, F. M., Wongkeaw, M., Sriwichai, T., Sunanta, P., Chuttong, B., & Burgett, M. (2020). Amino Acid Profiling and Chemometric Relations of Black Dwarf Honey and Bee Pollen. *Frontiers Nutrition*, *7*, 558579. https://doi.org/10.3389%2Ffnut.2020.558579

Stephens, J. M., Schlothauer, R. C., Morris, B. D., Yang, D., Fearnley, L., Greenwood, D. R., & Loomes, K. M. (2010). Phenolic compounds and methylglyoxal in some New Zealand manuka and kanuka honeys. *Food Chemistry*, *120*(1), 78-86. https://doi.org/10.1016/j.foodchem.2009.09.074

Tartibian, B., & Maleki, B. H. (2012). Correlation Between Seminal Oxidative Stress Biomarkers and Antioxidants with Sperm DNA Damage in Elite Athletes and Recreationally Active Men. *Clinical Journal of Sport Medicine*, *22*(2), 132-139. https://doi.org/10.1097/jsm.0b013e31823f310a

Tornuk, F., Karaman, S., Ozturk, I., Toker, O. S., Tastemur, B., Sagdic, O., Dogan, M., & Kayacier, A. (2013). Quality characterization of artisanal and retail Turkish blossom honeys: Determination of physicochemical, microbiological, bioactive properties and aroma profile. *Industrial Crops and Products*, *46*, 124-131. https://doi.org/10.1016/j.indcrop.2012.12.042

Yücel, Y., & Sultanog˘lu, P. (2013). Characterization of honeys from Hatay Region by their physicochemical properties combined with chemometrics. *Food Bioscience*, *1*, 16-25. https://doi.org/10.1016/j.fbio.2013.02.001

Żoltowska, K., Lipinski, Z., Łopieńska-Biernat, E., Farjan, M., & Dmitryjuk, M. (2012). The activity of carbohydrate-degrading enzymes in the development of brood and newly emerged workers and drones of the Carniolan honeybee, Apis mellifera carnica. *Journal of Insect Science*, *12*, 22. https://doi.org/10.1673/031.012.2201