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The difference in composition and nutritional potency of honey extracted by centrifugation and pressed processes

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Abstract

In traditional beekeeping, the two most important methods for extracting honey are centrifugation and honeycomb-pressing. In this study, the physicochemical composition of honey samples extracted using two distinct procedures was compared, as well as the impact of antioxidant capacity and nutritional potency on the lifespan and learning memory of worker bees. Honey samples were collected from ten colonies of *Apis cerana*: five samples via centrifugation and five via honeycomb-pressing. Our results showed that honey extraction methods influence the nutritional composition and potency of honey. Most parameters were superior in pressed honey, and the amylase activity in centrifuged honey was higher. The effects of antioxidant capacity and nutritional potency on worker bees' lifespans and learning memory were also superior in pressed honey. Pressed honey had higher nutritional composition and potency. However, whether pressed honey, which is rich in pollen, spoils more easily requires further investigation.

Keywords: Honey extraction methods; physicochemical analyses; survival; learning memory ability; antioxidant activity.

Introduction

Honey bees are essential for pollination and play an important role in plant diversity in nature (Requier *et al.*, 2019). Many plants have evolved through the synergistic evolution of bees, nectar plants, and natural selection. Plants improve the yield and quality of their fruits or seeds through pollen dispersal by bees; the growth and development of seeds pollinated by bees increase the yield and quality of the next generation of plants (Seeley, 2014).

Honey is a pure, natural, and sweet substance created by honey bees fully brewing nectar or honeydew from the flowers of nectar plants, and is a delicacy provided by nature (Codex Alimentarius Commission, 2001). Honey is the most commonly used bee product, which has been widely used in everyday life and is a traditional medicinal product. It has high nutritional value as it is rich in protein, amino acids, glucose, fructose, vitamins, and phenols, which are beneficial to human health (da Silva *et al.*, 2016). Several studies have shown that honey has unique and effective physiological effects on the human body in terms of its antibacterial, immune system, antioxidant, anti-mutagenic, and anti-inflammatory properties (Almasaudi, 2021). The dietary nutrition level affects the development of worker bees (Wright *et al.*, 2018). Nutrient imbalances in honey affect the lifespan, immunity, and learning and memory abilities of bees. Honey is rich in nutrients such as pollen and protein, which are essential for brood-rearing activities and colony development (Bouchebti *et al.*, 2022; Topal *et al.*, 2022). Honey extracted by pressing contains more pollen and protein (Homrani *et al.*, 2020). The lifespan of bees affects the colony population and the learning memory ability of worker bees affects their foraging ability (Tait *et al.*, 2019).

Currently, honey is the main source of income for most beekeepers and some beekeepers earn additional household income by using bees to pollinate their crops (Sawe *et al.*, 2020). The nutritional contents of honey differ depending on factors such as the type of honey, year of harvest, apiary climate, and production season (Juan-Borrás *et al.*, 2015). *Apis cerana* is the most important indigenous Chinese species and is widely reared in the mountainous areas of China (Yue *et al.*, 2018). In modern beekeeping apiculture, honey extraction methods mainly include centrifugation and pressing (Codex Alimentarius Commission, 2001). Honey extraction, a vital component of beekeeping, entails obtaining honey out of combs. For centrifuged honey, an entire wooden frame filled with honey is removed from the apiary and placed into a honey extraction centrifuge; the honey is then separated from wax cells using centrifugal force. For honey pressing, a comb filled with honey is chopped into pieces and filtered to extract the pressed honey using pressure applied through various means (Maradun and Sanusi, 2013).

There are fundamental differences between the two extraction methods; pressed honey contains more minerals and beneficial characteristics (Kadri *et al.*, 2017). As a result, it is logical to expect that pressed honey is more nutritious. However, such studies lack experiments with *Apis cerana*,

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and the antioxidant capacity and nutritional potency of both types of honey have not yet been investigated. The quality and nutritional potency of pressed honey may be better than that of centrifuged honey. Accordingly, the present study aimed to evaluate and compare honey extracted using centrifugation and pressing methods based on the physicochemical contents and the effects of its antioxidant capacity and nutritional potency on the lifespan and learning memory of worker bees, with *Apis cerana* as test subjects.

Materials and Methods

Sampling

The experiment was carried out at a beekeeping farm in Shuiyuan Township, Xunwu County, Ganzhou City, Jiangxi Province, China, at 115°646° E and 25°133° N, which is located in a humid subtropical monsoonal climate at an average elevation of 240 m above sea level.

The experimental design of the nutritional potency of honey on the lifespan and learning memory of worker bees, as shown in Figure 1. The experiment was conducted using ten colonies of *A. cerana* with similar population sizes (approximately 10 000 bees). In July 2019, the colonies were bred and raised according to standard apicultural practices until the end of the flowering period (June 2020). After the uncapped portion of the honey was removed, the honey was extracted from each colony.

After removing the honeycomb cappings from five colonies with a clean knife, the uncapped honeycombs were placed in a normal stainless steel honey extraction centrifuge to obtain the honey. Honeycombs from the other five colonies were cut off with a clean knife, and the honey was extracted by squeezing and filtering using a honey press. All steps were conducted in sanitary facilities using disinfected equipment, and the operation met the hygiene requirements set by the Codex Alimentarius Commission (Codex Alimentarius Commission, 2003). The extracted honey was kept at –20 °C until analysis.

Methods

Physicochemical analysis

The moisture content and total acidity of honey were determined using the Harmonised methods of the International Honey Commission (Bogdanov *et al.*, 2002). The 5-hydroxymethylfurfuraldehyde (HMF) content in the honey was measured using the Harun Kurtagi-described high-performance liquid chromatography (HPLC) technique (Kurtagić *et al.*, 2021). The amylase value in the sample was determined via the same method used to determine the diastase number in the honey-spectrophotometric method (Sakač and Sak-Bosnar, 2012). The total protein content in the honey samples was assayed using the Coomassie Brilliant Blue stain method (Miłek *et al.*, 2021). Fructose, glucose, and sucrose levels were determined using a novel, rapid, and robust HPLC method (Agilent, Palo Alto, CA, USA) (Soyseven *et al.*, 2022).

Antioxidant activity

Total flavonoid contents

According to the aluminum chloride colorimetric method, rutin (Weiye, Beijing, China) was used as a reference standard to evaluate the total flavonoid content of honey samples (Hernández-Fuentes *et al.*, 2021). Fifteen grams of honey was dissolved in 15 mL of ultrapure water and mixed well as a sample. After centrifugation, 7 mL of sample supernatant was added to 2 mL of 1% $AlCl_3$ (Xilong Scientific, Shantou, China) ethanol solution and volumized to 10 mL with 95% ethanol. The mixture was placed into a 96-well plate (Thermo Fisher Scientific, Waltham, MA, USA) after 10 min of incubation at 25 °C. A microplate reader (BioTek, Winooski, VT, USA) was used to measure the absorbance of the samples at 405 nm. Each group of samples was measured four times in parallel. A standard curve was plotted according to the rutin concentration as the horizontal coordinate and absorbance values as the vertical coordinate to calculate the total flavonoid content of the samples in the centrifuged (CeH) and pressed honey (PrH) groups, and the regression equation was y=11.5103x–0.0337 (R^2=0.9996).

Total phenolic content

Using gallic acid (Weiye, Beijing, China) as a reference standard, the Folin–Ciocalteu spectrophotometric method was used to assess the total phenolic content of honey samples (Adaškevičiūtė *et al.*, 2019). Five grams of honey was dissolved in 15 mL of ultrapure water and mixed well. After centrifugation, 2 mL of sample supernatant was added to 6 mL of ultrapure water, 0.5 mL of Folin–Ciocalteu color developer (Yuanye, Shanghai, China), and 1.5 mL of 20% Na_2CO_3 (Xilong Scientific, Shantou, China), and volumized to 10 mL with ultrapure water. The mixture was incubated for 30 min in the dark at 25 °C and then transferred into a 96-well plate. The absorbance of the samples was measured at 763 nm using a microplate reader. Each group of samples was measured four times, in parallel. A standard curve was plotted according to the concentration gradient as the horizontal coordinate and absorbance value of gallic acid as the vertical coordinate to calculate the total phenolic acid content of the samples, and the regression equation was y=4.6433x+0.2109 (R^2=0.9989).

DPPH assay (radical scavenging activity)

The antioxidant activity of honey samples was measured by 2,2-diphenyl-1picrylhydrazyl (DPPH) radical scavenging assay (Dżugan *et al.*, 2018). A 50 μL portion of extract at different concentrations (3.3–333 μg/mL) was mixed with 100 μL of DPPH ethanol solution (8 mg/100 mL) (Phygene, Fuzhou, China) in a 96-well plate. Simultaneously, 100 μL of ethanol was mixed with 100 μL of DPPH ethanol solution (8 mg/100 mL) as a blank, and 100 μL of ethanol was mixed with 100 μL of honey as a control. The samples were maintained for 30 min in the dark at 25 °C. The absorbance of the resulting mixture was measured at 517 nm using a microplate reader. Radical scavenging activity (A) was calculated using the following equation:

$$A = [1 - (A_1\ A_0)\ /A_2] \times 100\%$$

where A_1 is the absorbance of the studied sample, A_2 is the absorbance of the control sample, and A_0 is the absorbance of the solvent control.

The IC_{50}-DPPH was calculated using a calibration curve obtained from the antioxidant activity of each sample solution at various concentrations.

ABTS assay (radical scavenging activity)

Antioxidant activities via 2,2-biazo-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) free radical scavenging activity of honey samples were measured (Nenadis *et al.*, 2004). Experiments were conducted using the Total

Antioxidant Capacity (T-AOC) Assay Kit (ABTS microplate method) (Yuanye, Shanghai, China). The ABTS working master mix was prepared by mixing equal volumes of ABTS solution and oxidant the day before the experiment and storing it at room temperature in the dark for 12–16 h before use. A 7-μL portion of the extract at different concentrations (3.3–333 μg/mL) was mixed with 280 μL of ABTS working solution and placed in a 96-well plate. Simultaneously, 7 μL of ultrapure water was mixed with 280 μL of ABTS working solution as a control group. The samples were then maintained for 5 min at 25 °C. The absorbance of the resulting mixture was measured at 734 nm using a microplate reader. Radical scavenging activity (A) was calculated using the following equation:

$$A = [(A_0 - A_1)/A_0] \times 100\%$$

where A_1 is the absorbance of the sample to be measured and A_0 is the absorbance of the control sample.

The method described in the Section of 'DPPH assay (radical scavenging activity)' was used to calculate IC_{50}-ABTS.

Nutritional potency of honey on the lifespan and learning memory of worker bees

The experimental design of the nutritional potency of honey on the lifespan and learning memory of worker bees is shown in Figure 1. The queens of three colonies of *A. cerana* with similar population sizes were confined to a comb without any eggs or larvae for 24 h to lay eggs in the apiaries of Jiangxi Agricultural University (28.46°N, 115.49°E). Frames with capped broods were chosen from the colonies after 19 d and transferred into a chamber with controlled humidity and temperature (T, 34 °C; RH, 75%, AIKANE, Shanghai, China). Three groups of approximately 100 worker bees that newly emerged from the comb were randomly selected and placed into special wooden boxes with wire mesh on the top and bottom. The worker bees in the boxes were kept in a chamber with controlled temperature and humidity (AIKANE, Shanghai, China) and fed daily with enough centrifuged honey, pressed honey, and sucrose solutions (total sugar content was 50% in all solutions). To perform the proboscis extension response (PER) conditioning experiment and gene expression analysis, three groups of approximately 60 newly emerged worker bees from the comb were randomly selected and maintained for 7 d in the conditions described above. With newly emerged bees from the three initial colonies, this experiment had three independent biological replicates.

Nutritional potency of honey on the lifespan of worker bees

The environment in the special wooden boxes was observed over time, and the sugar-water solutions with different honey samples were replaced every day. After the worker bees had been fed for three days, the dead bees were recorded and removed every day until all the worker bees had died.

Nutritional potency of honey on the learning memory of worker bees

According to the above feeding conditions, 30 7-day-old worker bees captured from each group were used for the PER conditioning experiment. The bees were stunned with CO_2 and placed on ice for a brief freeze-stun. Each worker bee was quickly fixed with thin slices in a U-shaped metal tube (not too loose nor too tight). They were then placed in a controlled temperature and humidity chamber (T, 35 °C; RH, 75%) for 2 h of starvation. Worker bees that were in poor condition and did not respond to the sugar water were eliminated, and the remaining worker bees were trained and their memory and ability to learn were tested (Baracchi *et al.*, 2018).

Nutritional potency of honey on the expression of genes related to learning and memory

Three heads of 7-day-old worker bees were collected together as one sample, and each group included three samples. The total RNA of samples was extracted using the TransZol Up Plus RNA Kit (TransGen, Beijing, China), and the experimental method was described by Qin *et al.* (2012); five samples were included in each treatment group in a single colony. PrimeScript™ RT reagent Kit was used to synthesize the cDNA, and a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) was used to measure the concentration of cDNA in each sample. The cDNA was stored at −80 °C in a refrigerator.

Primers were designed using Primer 5.0 with *GAPDH* as the internal reference gene, based on the learning memory-related genes (*AcCREB*, *Acdop2*, and *Acdop3*) of *A. cerana* (Liao *et al.*, 2016), and synthesized by Sangon Biotech (Shanghai, China), as shown in Table 1.

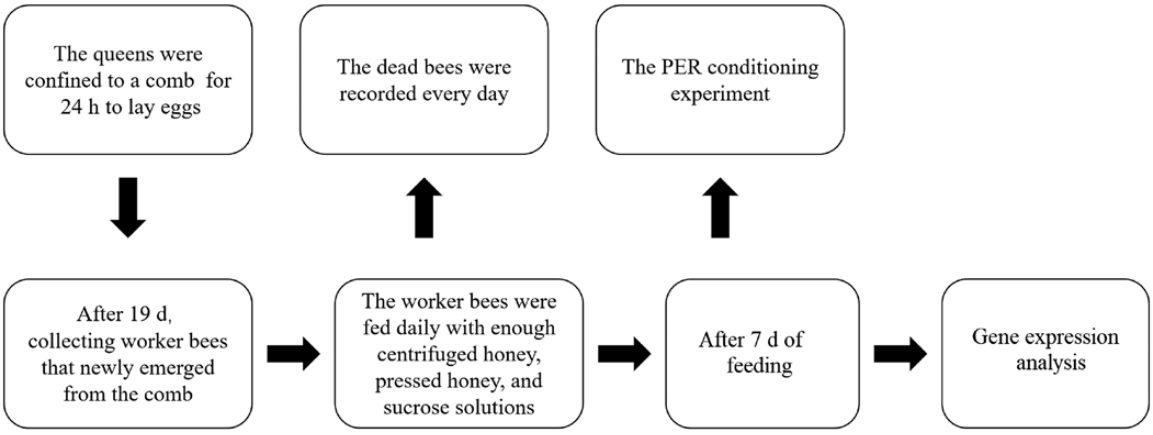

Figure 1. Experimental designs of the nutritional potency of honey on the lifespan and learning memory of worker bees.

The fluorescence quantitative PCR mixture (10 μL) contained 1 μL of cDNA, 5 μL of TB Green® Premix ExTaq™ II (TaKaRa, Beijing, China), 0.4 μL each of forward and reverse primers, 0.2 μL of ROX Reference Dye, and 3 μL of ultrapure sterile water. The reaction conditions were 95 °C for 30 s and 60 °C for 1 min for 40 cycles (Liao *et al.*, 2018). Each reaction was performed in four technical replicates. Cycle threshold (Ct) values of reference and target genes were collected using Bio-Rad CFX software (version 2.1; Hercules, CA, USA), and the method for calculating the relative expression of each target gene was based on the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Statistical analysis

StatView and GraphPad Prism (version 9.0; La Jolla, CA, USA) were used for data analysis. Data are presented as mean±standard deviation (SD). The strength of the linear relationship between the variables was determined by Pearson's correlation coefficient test (r). Analysis of variance (ANOVA) and Tukey's test ($P<0.05$) were used to compare means.

Results

Physicochemical analysis

The results of the physicochemical analyses (moisture, total acidity, fructose, glucose, sucrose, qualitative presence of HMF, amylase activity, and total proteins) of the centrifuged and pressed honey are summarized in Table 2.

There was no significant difference in the moisture, fructose, or glucose contents of the centrifuged and pressed samples ($P=0.996$, $P=0.1254$, and $P=0.2364$, respectively). The total acidity, amylase activity, sucrose, and total protein contents were significantly higher in the centrifuged honey group than in the pressed honey group. HMF was not detected in either the centrifuged or pressed honey ($P<0.001$, $P=0.0146$, $P<0.001$, and $P<0.001$, respectively).

Antioxidant activity

Total flavonoid and total phenolic contents

The total flavonoid and phenolic contents in the pressed honey (PrH) group were significantly higher than those in the centrifuged honey (CeH) group ($P<0.0001$; $P<0.0001$) (Figures 2A and 2B).

Radical scavenging activity

The IC_{50} represents the concentration needed to scavenge 50% of the free radicals. Lower IC_{50} values indicate a better ability to scavenge free radicals and greater antioxidant capacity.

The IC_{50}-DPPH and IC_{50}-ABTS of the CeH group were all significantly higher than those of the PrH group ($P=0.0013$ and $P=0.0039$, respectively) (Figure 2C).

Lifespan analysis

As shown in Table 3, the average lifespan of workers in the CeH and PrH groups was significantly higher than that of worker bees fed the sucrose solution (control group) ($P=0.0006$ and $P<0.001$, respectively). The average lifespan of worker bees fed with centrifuged honey was significantly lower than that of worker bees fed with pressed honey ($P=0.0272$). The median lifespan of worker bees fed with centrifuged and pressed honey was significantly higher than that of worker bees fed with the sucrose solution ($P=0.0021$ and $P<0.001$, respectively). There was no significant difference ($P=0.1214$) in the median lifespan of worker bees between the CeH and PrH groups.

Analysis of memory and learning abilities

The learning and memory abilities at 6 h in the CeH and PrH groups were significantly higher than those in the control

Table 1. Gene primers used in the fluorescence quantitative PCR

Gene name	Forward primer (5′—3′)	Reverse primer (5′—3′)
AcCREB	TGAAAATCCAGTTTGATCATTCGAT	TTCAAATAATCAGCAAATCATGCAC
Acdop2	TTGGTTCTCCCTCTCTCCGA	CCAAGAGGTCACTATGAATGCG
Acdop3	AGAAGGACAAGAAAAATGCCG	CCAAGAGGTCACTATGAATGCG
GAPDH	GCTGGTTTCATCGATGGTTT	ACGATTTCGACCACCGTAA

Table 2. Results of physicochemical analysis of the centrifuged and pressed honey

Process	Group	
	Centrifuged honey	Pressed honey
Moisture (%)	21.66±0.337	21.360±0.178
Total acidity (mL/kg)	24.594±0.675a	20.894±0.606b
Fructose (%)	33.426±0.609	32.377±0.571
Glucose (%)	43.807±1.654	43.027±1.351
Sucrose (%)	1.526±0.044a	1.151±0.076b
HMF (mL/kg)	ND	ND
Amylase activity (mL/(g·h))	28.139±1.27a	27.149±0.742b
Total proteins (mg/100 g)	39.725±3.421a	50.580±3.468b

Data are presented as mean±standard deviation (SD), based on four measurements ($n=5$ honey samples/methods). According to Tukey's test, different letters in the same row denote significant differences between values ($P<0.05$). ND, not detected.

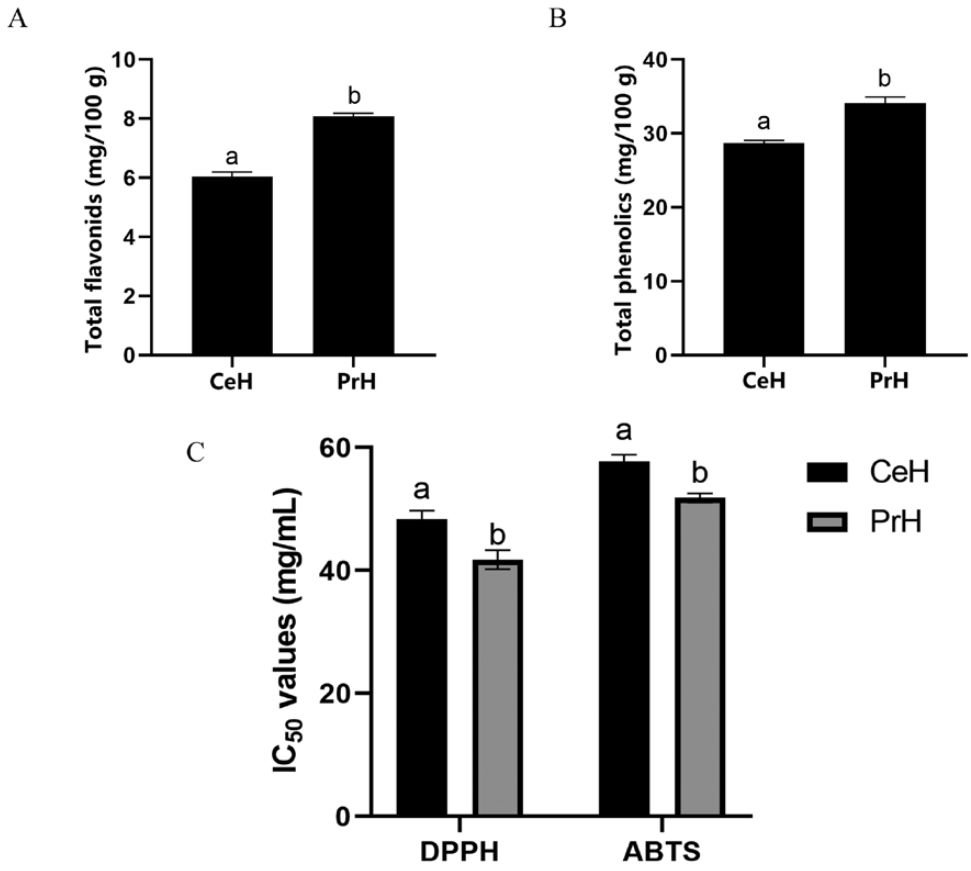

Figure 2. Effects of centrifuged honey and pressed honey on total flavonoid content (A), total phenolic content (B), and radical scavenging activity (C). Boxplots with different letters above them show substantial group differences (ANOVA test, $P<0.05$).

Table 3. Effects of different types of honey on the average lifespan of worker bees

Group	Average lifespan (d)	Median (d)	Sample size
Control	20.257±1.059a	20.00±2.07a	356
CeH	24.201±1.176b	24.33±2.08b	236
PrH	26.743±1.707c	26.50±0.50b	266

According to Tukey's test, different letters in the same column denote significant differences between values ($P<0.05$), whereas the same letters denote no significant difference ($P>0.05$).

group ($P=0.0054$ and $P=0.0006$, respectively). There was no significant difference ($P=0.0587$) in learning and memory abilities at 6 h between the CeH and PrH groups. Learning and memory abilities at 24 h were significantly higher in the CeH and PrH groups than in the control group ($P=0.0141$ and $P=0.0005$, respectively). The learning and memory abilities at 24 h in the PrH group were significantly higher than those in the CeH group ($P=0.0132$) (Figure 3).

Gene expression analysis

The relative expression of *Acdop3* and *AcCREB* in the CeH and PrH groups was significantly higher than that in the control group ($P=0.0054$ and $P=0.0006$, respectively). There was no significant difference in the relative expression of *Acdop3* and *AcCREB* between the CeH and PrH groups ($P=0.0001$ and $P=0.0022$, respectively), or *Acdop2* between the control, CeH, and PrH groups ($P=0.6441$) (Figure 4).

Discussion

In traditional beekeeping in the countryside and deep mountains of China, the two most important methods of extracting honey are centrifugation and pressing. Few studies on how honey extraction methods affect honey quality have been conducted; therefore, this study evaluated the differences in nutritional composition and potency of honey using two extraction methods.

Moisture content is one of the most important measures of honey quality, as moisture in honey indirectly affects its stability (Singh and Singh, 2018). Our results showed that the moisture content of both types of honey was similar because the honeycombs were both completely capped. However, the moisture contents for all the samples were slightly above the range required by the European Quality Regulation (no more than 20%) (European Council, 2002). Honey typically shows some differences in moisture content based on honey production

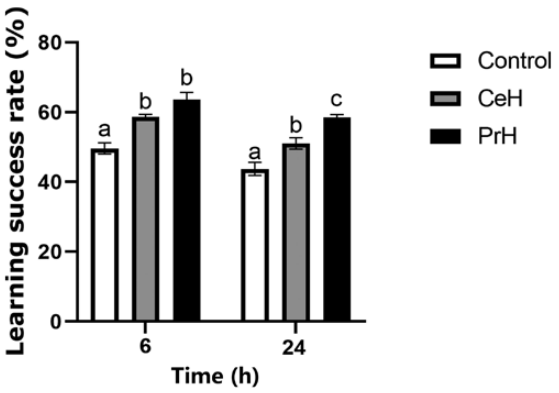

Figure 3. Effects of different doses of honey on the learning and memory abilities of worker bees (*A. cerana*). Different letters above the bars indicate significant differences between groups (ANOVA test, $P<0.05$), whereas the same letters indicate no significant difference ($P>0.05$).

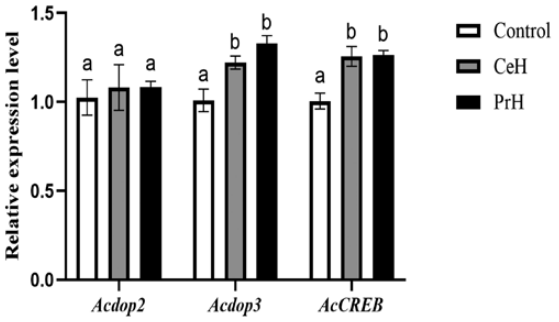

Figure 4. Relative expression levels of genes involved in the learning and memory of worker bees and the effects of different honeys (centrifuged and pressed honeys) on the relative expression levels of worker bees. The internal control gene in each group was *GAPDH*. Different letters above bars indicate significant differences between groups (ANOVA test, $P<0.05$), whereas the same letters indicate no significant difference ($P>0.05$).

districts and seasons, and the honey of *A. cerana* has a high moisture content, especially for honey produced in southern China (Engidaw *et al.*, 2020).

HMF content, total acidity, and amylase activity are widely recognized as important parameters for determining the freshness of honey (Silva *et al.*, 2009; Pasias *et al.*, 2017). Our results showed that HMF was not detected in either honey group, because the honey samples were freshly harvested and HMF was practically absent (Juan-Borrás *et al.*, 2015). Furthermore, the centrifuged honey's total acidity and amylase activity were higher. These results are inconsistent with the conclusions of a study by Kadri *et al.* (2017). We believe that the main reason for this is the presence of pollen in the pressed honey from *A. cerana*. Bee pollen has higher pH values than other bee products (Adaškevičiūtė *et al.*, 2019), and has inhibitory properties against α-amylase (Keskin and Özkök, 2020). Both honey types had similar levels of fructose and glucose; however, the pressed honey had a lower sucrose content and a higher protein content. We suspect that impurities (such as beeswax and bee pollen, which are rich in protein), were mixed in the extraction process of the pressed honey (Giampieri *et al.*, 2018; Thakur and Nanda, 2020).

Moreover, many studies have shown that honey has anti-inflammatory and antibacterial properties (Almasaudi, 2021). In this study, we investigated the differences in the antioxidant capacity of centrifuged and pressed honey by measuring their flavonoid and phenolic acid contents as well as their free radical scavenging capacity. Our results show that pressed honey has higher total flavonoid and phenolic acid contents and higher free radical scavenging capacity than centrifuged honey, indicating that pressed honey has a greater antioxidant capacity. We hypothesize that this is due to the presence of pollen in pressed honey, which contains significant amounts of polyphenolic substances, mainly flavonoids (Li *et al.*, 2018). Honey flavonoids have been confirmed to reduce oxidative damage to human red blood cells (Silva *et al.*, 2021). Several studies on the nutrition of honey point to the fact that honey is the only insect-derived natural product with nutritional, therapeutic, spiritual, cosmetic, and industrial value, and that honey polyphenols have neuroprotective and memory-improving effects on human health (El-Seedi *et al.*, 2020).

Honey bees are social insects that perform a complex set of social behaviors and possess excellent learning and memory abilities (Tsvetkov *et al.*, 2019). The nutrition of the food they collect is crucial for bees to grow and reproduce normally. Olfactory learning and memory training are often used to assess the learning and memory abilities of honey bees (Bitterman *et al.*, 1983). In the lifespan experiments, it was found that worker bees in both the CeH and PrH groups had significantly higher survivability than those in the control group, and survivability was highest in the PrH group. The results of the proboscis extension reflex experiment showed that the learning and memory abilities of worker bees in the CeH and PrH groups were better than those in the control group. Because honey samples from the CeH and PrH groups are made of a mixture of D-glucose, D-fructose, and sucrose (Siddiqui, 1970), the nutritional content is superior to that of the sucrose solutions used in the control group. Furthermore, the long-term memory (24 h) of worker bees in the PrH group was better, indicating that pressed honey is of relatively good quality. The reason may be that pressed honey has good antioxidant activity, which could improve the expression of memory-related genes and long-term memory. The brain is an organ with learning and memory functions. The antioxidant ability of honey may inhibit either the death or the functional aberration of neural cells (El-Seedi *et al.*, 2020).

The main functions of *AcCREB* are to regulate the transcription of specific genes, enhance intercellular linkages, influence neuronal development and regeneration, and participate in memory functions in the body (Yin *et al.*, 1995). Dopamine is an important neurotransmitter in the central nervous system of animals and is involved in the regulation of a variety of behavioral and physiological processes in insects, including learning recall. *Acdop2* is involved in the locomotor behavior of honey bees (Mustard *et al.*, 2010). *Acdop3* is activated by homovanillyl alcohol, a major component of the queen mandibular pheromone, and causes changes in cAMP in the brain, leading to memory production (Baracchi *et al.*, 2020). In this research, we evaluated the relative expression levels of three memory-related genes (*AcCREB*, *Acdop2*, and *Acdop3*) in 7-day-old worker bees fed different honey samples. Our study showed that the relative expression of *Acdop3* and *AcCREB* was significantly higher in the CeH and PrH groups than in the control group; however, there was no significant difference between the CeH and PrH groups. The

nutritional composition of honey has been suggested to affect the expression of genes related to memory learning in honey bees, but the two methods of extracting honey do not affect the expression of genes related to memory and learning in honey bees. In addition, the relative expression of *Acdop2* in the two experimental groups was not significantly different from that in the control group, probably because the expression of this gene is mainly related to the locomotory behavior of bees. According to the results of the PER experiment, honey extraction methods influence the effects of the nutritional potency of honey on the learning and memory abilities of worker bees. This shows that different kinds of honey regulate other learning- and memory-related genes in worker bees, but further research is required to confirm this. Our results support the hypothesis that pressed honey is more nutritious, both in terms of composition and potency.

To eliminate unknown variables, all honey samples analyzed in this study were produced in the same apiary using standard handling procedures, and the honey bees collected nectar from the same range of nectar plants. Therefore, the observed differences were due to the honey extraction process. Our experimental design had two limitations. First, all experiments were conducted in the laboratory and were not combined with production farming. Second, there is a limitation in that we do not know the nutritional value or impact on human health.

Conclusions

This study compared centrifuged and pressed honey based on the physicochemical contents and the effects of antioxidant capacity and nutritional potency on the lifespan and learning memory of worker bees. Our results showed that honey extraction methods influence the nutritional composition and potency of honey. Most parameters (total acidity, sucrose, and total protein content) in pressed honey were superior, and amylase activity in centrifuged honey was superior ($P<0.05$). The effects of antioxidant capacity and nutritional potency on the lifespans and learning memory of worker bees were also superior in pressed honey. Pressed honey is a differentiated product with a higher nutritional composition and potency. However, whether pressed honey, which is rich in pollen, spoils more easily requires further investigation.

Author Contributions

In this work, Xiaobo Wu conceived the research and designed the experiments. Yueyang Hu carried out the laboratory work. Yueyang Hu and Xiaobo Wu wrote the manuscript. Zhen Li and Shoucheng Wang contributed to the laboratory work. All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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