Amino acid composition of royal jelly harvested at different times after larval transfer

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ABSTRACT. The amino acids in royal jelly (RJ) have a wide range of pharmacological and health-promoting functions in humans. Multiple studies on the amino acid quality and composition in RJ have investigated RJ harvested at 72 h after larval transfer. In contrast, the concentration of amino acids in RJ harvested before 72 h remains unknown. In this study, the concentration of free amino acids (FAAs) and total amino acids (TAAs) in RJ harvested at 13 time points between 24 and 72 h after transfer of ten *Apis mellifera* colonies were measured. Our results indicated that the most abundant FAAs were Pro, Phe, Lys, Glu, and Tyr, whereas the most abundant TAAs were Asp, Glu, Leu, Lys, and Val. The total FAA concentration in RJ increased with increasing harvest time, from 4.30 mg/g at 24 h to 9.48 mg/g at 72 h. In contrast, the variation in concentration of TAAs observed was a decrease-increase-decrease trend with 40 h (149.53 mg/g) and 52 h (169.62 mg/g) as inflection

points. The highest and lowest concentrations of TAA were 197.96 and 121.32 mg/g at 24 and 72 h, respectively. To our knowledge, this is the first study to investigate the concentration changes of FAAs and TAAs prior to 72 h after transfer. Our results will provide theoretical support to guide production practices of beekeeping, as well as elucidate the relationship between the harvest time point and RJ content.

Key words: *Apis mellifera*; Royal jelly; Amino acid; Concentration; Harvest time

INTRODUCTION

Royal jelly (RJ) is a yellowish-white cream secreted by the hypopharyngeal and mandibular glands of worker honey bees from 5-15 days of age. RJ is widely recognized as a healthy and functional food for humans (Knecht and Kaatz, 1990). At the same time, RJ is the only diet for the queen throughout her life and for honey bee larvae within three days of age (Townsend and Lucas, 1940; Scarselli et al., 2005). The consumption of RJ affects DNA methylation processes in the queen and promotes the development of gynes (Kucharski et al., 2008). It has been reported that RJ possesses a wide variety of pharmacological activities, including antibacterial, anticancer, antihypertensive, and antioxidative effects, which could serve therapeutic purposes promoting human health (Townsend et al., 1959; Tamura et al., 1987; Bíliková et al., 2001; Tokunaga et al., 2004; Guo et al., 2009; Kim et al., 2010; Karaca et al., 2012; Mihajlovic et al., 2014). Hence, RJ is now widely used in therapeutic products, health foods, and cosmetics (Han et al., 2012).

RJ is a complex compound, which contains 60-70% moisture, 12-15% crude proteins, 10-16% carbohydrates, 3-7% lipids, as well as traces of mineral salts, vitamins, and free amino acids (Nation and Robinson, 1971; Lercker et al., 1981; Howe et al., 1985; Crane, 1990; Chen and Chen, 1995). The RJ proteins have a wide range of pharmacological and health-promoting functions in humans. As a highly protein-rich food, the nutrition of RJ is to a large extent determined by the amino acid concentrations (Matloubi et al., 2004). Thus, determination of the concentrations of total amino acids (TAAs) and free amino acids (FAAs) is an effective method for evaluating RJ quality (Boselli et al., 2003; Wu et al., 2009; Zhao, 2014).

In general, commercial RJ is produced by transferring one-day-old larvae into artificial queen cells to induce nurse bees to secrete RJ in the cells (Chen et al., 2002). The majority of the produced RJ is harvested at three days (72 h) after larval transfer because, at this time, the amount of RJ in the queen cells reaches its peak (Lercker et al., 1981). In recent years, some producers sell RJ that was harvested at two days (48 h) or one day (24 h) after transfer. These earlier harvests shorten the production cycle, but few studies have investigated any difference in the nutritional composition of RJ harvested at different times after transfer. Shen (1991) compared the composition of RJ harvested at 48 and 72 h after transfer, with the aim of identifying freshness indicators. In addition, Zheng et al. (2011) investigated the changes in moisture, protein, 10-HDA, total sugar, acidity value, and superoxide dismutase activity in RJ harvested at 24, 48, and 72 h after transfer. However, to the best of our knowledge, no study has investigated the changes in amino acids in RJ harvested prior to 72 h after transfer.

In this study, the 24-to-72-h harvest interval was subdivided into 13 equidistant time points and the amino acid concentrations were measured at each time point. Our aim was to

provide theoretical support to guide production practices of beekeeping, as well as to elucidate the relationship between harvest time point and RJ content.

MATERIAL AND METHODS

Reagents and standards

The 17 individual amino acid standards, including aspartic acid (Asp), threonine (Thr), serine (Ser), glutamic acid (Glu), glycine (Gly), alanine (Ala), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), lysine (Lys), histidine (His), arginine (Arg), proline (Pro), and cysteine (Cys), were purchased from Sigma (Shanghai, China). All the analytical grade reagents (trisodium citrate, sodium hydrate, sodium chloride, hydrochloric acid, citric acid, ethyl alcohol, phemethylol, thiodiglycol, polyoxyethylene lauryl ether, triketohydrindene hydrate, and octanoic acid) were purchased from Kelong Chemical Regent Company (Chengdu, Sichuan province, China).

Samples

The colony and RJ sample preparation followed the methods of Zheng et al. (2011). Ten colonies of genetically unrelated *Apis mellifera* were randomly chosen to produce fresh RJ in Chongqing, China. To ensure that the RJ production progressed smoothly and to ensure that the experimental conditions were the same, feeding with sugar was conducted throughout the experiment. Worker larvae of approximately 1 day of age were transferred into clean plastic queen cell cups. One frame containing four strips of plastic queen cups (N = 32 per strip) was used for each colony. The frame was placed in the honey compartment of the hives. Subsequently, the queen cell frames were removed from the colonies at 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 68, and 72 h after transfer. At this time, the wax covering the cups and larvae in the cells were removed. The RJ in each cup was collected within 3 min and immediately frozen at -20°C and conserved at this temperature until the amino acid analysis was conducted.

Moisture analysis

The moisture analysis followed the RJ quality standard method published in 2002 by the AQSIQ (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, 2002). Moisture was measured by weight loss upon drying in a vacuum drying oven.

Extraction of FAAs

RJ (0.1000 g) was dissolved in 25 mL deionized water and dispersed for 15 min with the help of a ultrasonic cell disruptor (Xinzhi Company, Ningbo, China) until it was homogenized. To deposit the proteins, 1.5 mL RJ solution was mixed with 8.0% sulfosalicylic acid, adding up to 6.0 mL. The solution was centrifuged for 5 min at 1430 g and the supernatant was then filtered using a 0.45-μm microporous filter, prior to injecting it into the L-8800 amino acid autoanalyzer (Hitachi Ltd, Tokyo, Japan) (González Paramás et al., 2006).

Extraction of TAAs

RJ (0.1000 g) was carefully weighted and transferred into a 20-mL pyrex screw-cap tube. Subsequently, 10 mL 6 M HCl was added and the mix was homogenized for 1 min using a vortex machine (Scientific Industries Company, USA). A moderate stream of purified nitrogen was blown into the liquid to remove the air in the tube. The tightly sealed tube was then kept upright in an oven at $110° \pm 1°C$ for 22 h. Subsequently, the tube was removed from the oven and allowed to cool to room temperature. At this point, the acid hydrolysate was filtered through a qualitative filter paper (Jiaojie Company, Liaoning, China), neutralized to pH 7.0 using 6 M NaOH, and redissolved in deionized water to a total volume of 50 mL. The acid hydrolysate was then filtered using a 0.45-µm microporous filter (Millipore, MA, USA). Before injection into the amino acid autoanalyzer, 1 mL filtrate was mixed with 1 mL hydrochloric acid solution.

Preparation of standard substance and chromatographic conditions

Ten microliters of amino acid standards was carefully measured and transferred into volumetric flasks and filled up to 1 mL using deionized water. All analyses were performed on a L-8800 amino acid autoanalyzer (Hitachi Ltd., Tokyo, Japan), following the manufacturer instructions. A 4.6 x 60 mm ion exchange column was used and the mobile phases comprised a buffer, triketohydrindene hydrate, and water. The flow velocity and detective wavelength of the buffer were 0.400 mL/min and 570 nm, respectively, whereas the flow velocity and the detective wavelength of the triketohydrindene hydrate solution were 0.350 mL/min and 440 nm, respectively. The temperatures of the column and reactor were set to 57° and 153°C, respectively, and the injection volume for all samples and standard solutions was 20 µL.

Data analysis

Microsoft Office Excel 2007 and ANOVA of the SAS 9.2 software were used to deal with data. The concentrations of FAAs were calculated using the square of the difference between the sample FAA and the corresponding standard FAA. Each sample was detected three times and the mean and SD were calculated. The calculation method of TAA concentrations were the same as FAAs. The essential amino acid (EAA) percentages were calculated based on the proportion of each particular amino acid of the total for all the amino acids.

RESULTS

Moisture of RJ harvested at differential times after transfer

The moisture of RJ harvested at the 13 different time points after transfer are shown in Figure 1. In general, the RJ moisture increased with increasing time after transfer. The highest and lowest RJ moisture contents were 50.2 and 67.2%, detected at 24 and 72 h after transfer, respectively. An ANOVA analysis indicated that the RJ moisture harvested at 48 to 72 h was significantly greater than RJ harvested at 24 to 32 h after transfer (P < 0.05). In addition, the RJ harvested at 32 h was significantly greater than RJ harvested at 24 h after transfer (P < 0.05).
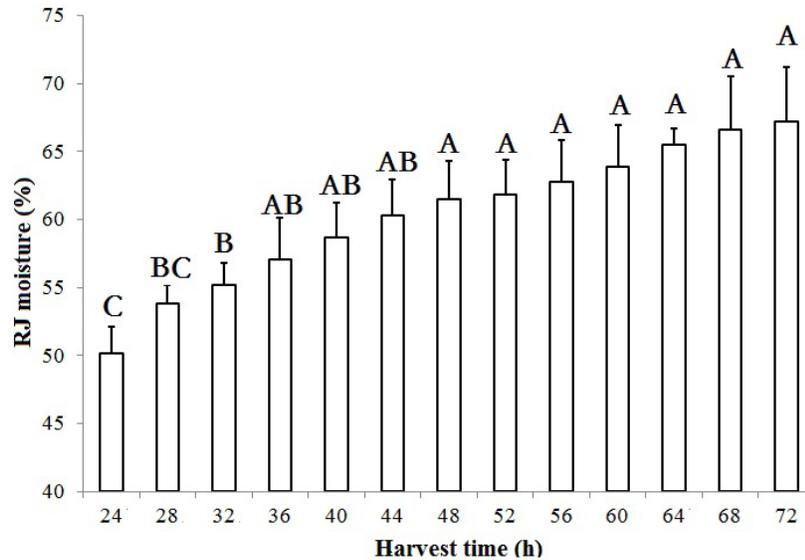
Figure 1. Moisture of royal jelly (RJ) harvested at 13 different time points between 24 and 72 h after transfer. The moisture was measured by weight loss upon drying in a vacuum drying oven. Different letters over each bar indicate significant differences at $P < 0.05$. Error bars represent SD of the mean of 10 samples.

Chromatograms of RJ amino acids

Following the autoanalyzer manufacturer instructions, 20 μL standard amino acid solutions were carefully injected, to obtain standard chromatograms (Figure 2). Subsequently, FAAs and TAAs were injected, to detect the sample FAA and TAA concentrations.
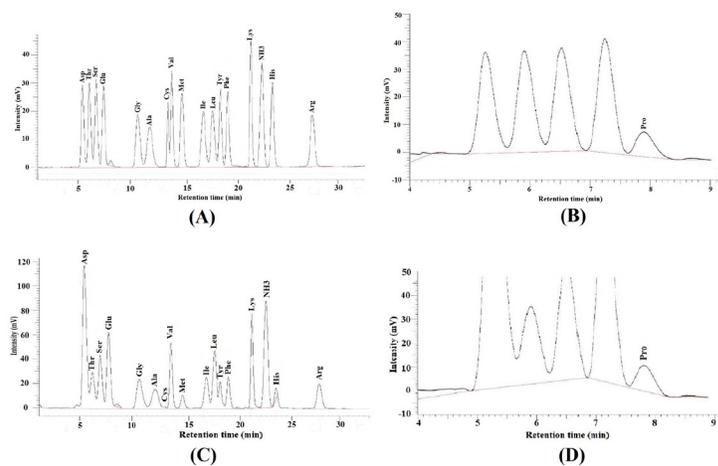

Figure 2. Chromatograms of the 18 FAAs (**A** and **B**) and TAAs (**C** and **D**) in standard solutions. Proline FAA (**B**) and TAA (**D**) are shown separately. The detection instrument used was the L-8800 amino acid autoanalyzer (Hitachi Ltd., Tokyo, Japan) and 20 μL standard solution was injected.

FAA concentrations of RJ harvested at different times after transfer

The concentrations of 17 FAAs, including Asp, Thr, Ser, Glu, Gly, Ala, Cys, Val, Met, Ile, Leu, Tyr, Phe, Lys, His, Arg, and Pro, were calculated were calculated using the square of the difference between the sample FAA and the corresponding standard FAA. Each sample was detected three times and the mean and SD was calculated. The results are presented in Table 1.

Table 1. Amino acid composition of musk deer T2R16 protein.

Amino acids	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
Number	18	12	9	4	5	10	9	7	10	21	50	11	10	27	7	32	18	12	8	21
Percentage	6.00	4.00	3.00	1.30	1.70	3.30	3.00	2.30	3.30	7.00	16.60	3.70	3.30	9.00	2.30	10.60	6.00	4.00	2.70	7.00

The figures represent the total number and percentage of different amino acid residues present in the T2R16 protein.

The main FAA was Pro with the concentration of 5.38 mg/g, which counted for half of the total FAA detected in RJ harvested at 72 h after transferring. Following Pro, the most abundant FAAs were Phe (1.30 mg/g), Lys (0.86 mg/g), Glu (0.34 mg/g), and Tyr (0.33 mg/g).

The total FAA concentration in RJ increased with increasing harvest time. Overall, the lowest (4.30 mg/g) and highest (9.48 mg/g) concentrations were observed in RJ harvested at 24 and 72 h after transfer, respectively. The lowest and highest concentrations of Asp were 0.06 and 0.25 mg/g detected at 24 and 60 h after transfer, respectively. Thr was not detected at all except in RJ harvested at 72 h after transfer (0.06 mg/g). Ser was not detected at 32 and 52 h, and the largest concentration was detected at 72 h after transfer (0.04 mg/g). Ala was not detected before 36 h after transfer and the highest concentration was detected at 40 and 72 h after transfer (0.04 mg/g). Val was not detected before 64 h, with the exceptions of 44 and 56 h. From 0.02 mg/g detected at the 68 h harvest, the concentration of Val rapidly increased to 0.10 mg/g at 72 h. The lowest and highest concentrations of Arg were 0.03 and 0.06 mg/g, harvested at 36 and 72 h after transfer, respectively. The RJ concentrations of Glu, Pro, Gly, Cys, Met, Ile, Leu, Tyr, Phe, Lys, and His were lowest at 24 h and highest at 72 h after transfer, respectively.

TAA concentrations in RJ harvested at different times after transfer

Following the method of Zhao (2014), the concentrations of the 17 TAAs were detected and calculated using the square of the difference between the sample TAA and the corresponding standard TAA. Each sample was detected three times and the mean and SD were calculated. The results are shown in Table 2.

Table 2. Prediction of the amino acid distribution on the transmembrane helices.

Position on the membrane	Distribution of amino acids
Outside	1-9,64-82,152-182,257-260
TM helix	10-32,45-63,83-105,129-151,183-205, 234-256,261-283
Inside	33-44,106-128,206-233,284-301

TM helix, transmembrane helix.

Our results showed that, at 72 h after transfer, the most abundant TAA was Asp, with a concentration of 22.27 mg/g, followed by Glu (16.94 mg/g), Leu (10.65 mg/g), Lys

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(9.10 mg/g), and Val (8.13 mg/g). The concentration variation trend of the TAAs was more consistent compared to the FAAs. As is shown in Table 2, the concentration of all the TAAs combined was highest at 24 h (197.96 mg/g). It then rapidly decreased to 149.53 mg/g at 40 h. Subsequently, an increase to 169.62 mg/g was observed at 52 h, followed by a consistent decrease until 72 h (121.32 mg/g). Consistent with the overall pattern, Asp, Thr, and Ser displayed the decrease-increase-decrease pattern over time, with the intermediate increase occurring between 40 and 52 h. In the other TAAs some variation in the intermediate patterns were observed, but without exception, all TAAs consistently showed the overall high and low occurring at 24 and 72 h, respectively.

Analysis of EAAs in RJ harvested at different times after transfer

The contents and proportions of EAAs play a pivotal role in estimating the nutrition value of food. These days, the amino acid contents recommend by WHO/FAO is widely used to measure the EAAs in food. The EAA concentrations in RJ harvested at different times are presented in Table 3. Our results indicated that, with the exception of Met and Phe, the proportions of Val, Ile, Leu, Thr, and Lys were higher than the standard values recommend by WHO/FAO at all time points. The highest and lowest proportions of total EAAs were 40.10 and 35.33%, occurring at 36 and 64 h, respectively.

Table 3. Identity of T2R16 amino acid sequences between forest musk deer and other species.

Species	GenBank ID	Definition	Identity (%)
Bos taurus	NM_001046628.1	Taste receptor, type 2, member 16 (TAS2R16)	94.48
Ovis aries	XM_004008021.1	Predicted: taste receptor type 2 member 16-like (LOC101105240)	94.37
Tursiops truncatus	KJ524798.1	Taste receptor type 2 member 16 (Tas2r16) pseudogene, complete sequence	85.6
Sus scrofa	XM_003134749.1	Predicted: taste receptor type 2 member 16-like (LOC100513769)	81.95
Camelus bactrianus	XM_010947595.1	Predicted: taste receptor type 2 member 16 (LOC105063129)	79.58
Equus przewalskii	XM_008514411.1	Predicted: taste receptor type 2 member 16 (LOC103547299)	79.58
Camelus dromedarius	XM_010975603.1	Predicted: taste receptor type 2 member 16 (LOC105085339)	79.47
Pteropus vampyrus	XM_011357170.1	Predicted: taste receptor type 2 member 16 (LOC105290162)	79.14
Myotis brandtii	XM_005878870.1	Predicted: taste receptor type 2 member 16-like (LOC102256244)	77.15
Pan troglodytes	NM_001009160.1	Taste receptor, type 2, member 16 (TAS2R16)	76.03
Homo sapiens	NM_016945.2	Taste receptor, type 2, member 16 (TAS2R16)	75.28
Macaca mulatta	NM_001080759.1	Taste receptor, type 2, member 16 (TAS2R16)	75.23
Callithrix jacchus	XM_002752064.2	Predicted: taste receptor, type 2, member 16 (TAS2R16)	72.96

DISCUSSION

RJ is a complex compound containing 60-70% moisture; however, few studies have studied investigated the moisture content in RJ harvested before 72 h after transfer. Zheng et al. (2011) showed that the moisture contents of RJ harvested at 24, 48, and 72 h, were 53.3, 62.8, and 64.3%, respectively. In this study, a total of 13 time points were investigated from 24 to 72 h. Our results indicated that the lowest and highest moisture contents were 50.2 and 67.2% at 24 and 72 h after transfer, respectively. A constant increase in moisture content was observed in RJ between these time points (Figure 1), which was consistent with the observations of Zheng et al. (2011).

In the present study, the total FAA concentration in RJ was found to increase consistently with increasing harvest time. This indicates that the FAA consumption of young larvae decreased over time. The total FAA concentration in RJ was 9.48 mg/g at 72 h. This finding was similar to that of Wu et al. (2009) (9.21 mg/g). The slightly higher value observed

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in the present study may be due to differences in location and food source. The most abundant FAA was Pro, with 5.38 mg/g RJ harvested at 72 h after transfer, which was consistent with the results in previous studies (Takenaka, 1987; Boselli et al., 2003; Wu et al., 2009). Given that RJ is the only food for the queen and young bee larvae, Pro may play an important role in bee growth.

Amino acids are the basic component of proteins, and changes in the TAA concentration are therefore believed to reflect changes in RJ protein contents. Zheng et al. (2011) reported that the crude protein contents in RJ harvested at 24, 48, and 72 h after grafting were 19.6, 16.2, and 15.0%, respectively. In agreement with these changes in RJ protein, our data indicated that the TAA concentrations in RJ harvested at the same time points were 197.96, 157.06, and 121.32 mg/g, respectively. However, the change in concentration observed was not a simple decrease. Following a steady decrease from 24 to 40 h (149.53 mg/g), an increase was observed from 40 to 52 h (169.62 mg/g), followed by a decrease to 72 h. The rapid decrease in TAA concentration in RJ harvested before 40 h after grafting may be caused by a dilution effect driven by the rapid increase in moisture. Together with secretion of TAAs from worker honey bees, a reduction in the moisture increase may have resulted in the observed increase in TAA concentration to 169.62 mg/g at 52 h. The rapid decrease in TAA concentration observed after 52 h may have been caused by a dilution effect due to increased moisture content combined with higher larval consumption as the larvae grew bigger and bigger. The five most abundant amino acids in RJ harvested at 72 h included Asp, Glu, Leu, Lys, and Val. These results are in agreement with the findings of Zheng et al. (2011) and showed only minor differences compared to those of Zhao (2014) who observed that the five most abundant amino acids, including Asp, Glu, Pro, Leu and Lys, in RJ which was purchased in the market. The minor discrepancies found may be due to differences of RJ samples.

The contents and proportions of EAAs play a pivotal role in estimating the nutrition value of food. Previous studies have detected eight EAAs in RJ (Wu et al., 2009). In contrast, only seven EAAs were detected in the present study. Because the samples in this study were treated with acid hydrolysis, all Trp were destroyed. A more appropriate method is needed for a complete amino acid investigation of RJ. Our data indicated that the proportions of all EAAs except Met and Phe were higher than the standard values recommended by WHO/FAO, which was in agreement with the findings by Zhao (2014).

In conclusion, or results indicated that the moisture of RJ increased consistently from 24 to 72 h after transfer. The total FAA concentration in RJ increased with increasing harvest time, from 4.30 to 9.48 mg/g, at 24 and 72 h, respectively. The most abundant FAAs were Pro, Phe, Lys, Glu, and Tyr. The variation in concentration observed in the TAAs was more consistent than that observed in the FAAs. The five most abundant TAAs were Asp, Glu, Leu, Lys, and Val. With the exception of Met and Phe, all the EAA proportions were higher than the standard value recommended by WHO/FAO. To our knowledge, this is the first study on the changes in FAA and TAA concentration prior to 72 h after transfer. This provides theoretical support that may guide the production practices of beekeeping, as well as an in-depth study of the relationships between harvest time point and RJ contents.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Bíliková K, Wu GS and Šimúth J (2001). Isolation of a peptide fraction from honeybee royal jelly as a potential antifoulbrood factor. *Apidologie* 32: 275-283. http://dx.doi.org/10.1051/apido:2001129

Boselli E, Caboni MF, Sabatini AG, Marcazzan GL, et al. (2003). Determination and changes of free amino acids in royal jelly during storage. *Apidologie* 34: 129-137. http://dx.doi.org/10.1051/apido:2003011

Chen C and Chen SY (1995). Changes in protein components and storage stability of royal jelly under various conditions. *Food Chem.* 54: 195-200. http://dx.doi.org/10.1016/0308-8146(95)00031-D

Chen SL, Su SK and Lin XZ (2002). An introduction to high-yielding royal jelly production methods in China. *Bee World* 83: 69-77. http://dx.doi.org/10.1080/0005772X.2002.11099543

Crane E (1990). Bees and beekeeping: science, practice and world resources. Heinemann Newnes, Oxford.

General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (2002). Royal Jelly, GB/T 9697-2002. AQSIQ, Beijing.

González Paramás AM, Gómez Bárez JA, Cordón Marcos C, García-Villanova RJ, et al. (2006). HPLC-fluorimetric method for analysis of amino acids in products of the hive (honey and bee-pollen). *Food Chem.* 95: 148-156. http://dx.doi.org/10.1016/j.foodchem.2005.02.008

Guo H, Kouzuma Y and Yonekura M (2009). Structures and properties of antioxidative peptides derived from royal jelly protein. *Food Chem.* 113: 238-245. http://dx.doi.org/10.1016/j.foodchem.2008.06.081

Han F, Wallberg A and Webster MT (2012). From where did the Western honeybee (*Apis mellifera*) originate? *Ecol. Evol.* 2: 1949-1957. http://dx.doi.org/10.1002/ece3.312

Howe SR, Dimick PS and Benton AW (1985). Composition of freshly harvested and commercial royal jelly. *J. Apic. Res.* 24: 52-61.

Karaca T, Şimşek N, Uslu S, Kalkan Y, et al. (2012). The effect of royal jelly on CD3(+), CD5(+), CD45(+) T-cell and CD68(+) cell distribution in the colon of rats with acetic acid-induced colitis. *Allergol. Immunopathol.* 40: 357-361. http://dx.doi.org/10.1016/j.aller.2011.09.004

Kim J, Kim Y, Yun H, Park H, et al. (2010). Royal jelly enhances migration of human dermal fibroblasts and alters the levels of cholesterol and sphinganine in an *in vitro* wound healing model. *Nutr. Res. Pract.* 4: 362-368. http://dx.doi.org/10.4162/nrp.2010.4.5.362

Knecht D and Kaatz HH (1990). Patterns of larval food production by hypopharyngeal glands in adult worker honey bees. *Apidologie* 21: 457-468. http://dx.doi.org/10.1051/apido:19900507

Kucharski R, Maleszka J, Foret S and Maleszka R (2008). Nutritional control of reproductive status in honeybees via DNA methylation. *Science* 319: 1827-1830. http://dx.doi.org/10.1126/science.1153069

Lercker G, Capella P, Conte LS and Ruini F (1981). Components of royal jelly: I. Identification of the organic acids. *Lipids* 16: 912-919. http://dx.doi.org/10.1007/BF02534997

Matloubi H, Aflaki F and Hadjiezadegan M (2004). Effect of g-irradiation on amino acids concentration of baby food proteins. *J. Food Compos. Anal.* 17: 133-139. http://dx.doi.org/10.1016/j.jfca.2003.09.005

Mihajlovic D, Vucevic D, Chinou I and Colic M (2014). Royal jelly fatty acids modulate proliferation and cytokine production by human peripheral blood mononuclear cells. *Eur. Food Res. Technol.* 238: 881-887. http://dx.doi.org/10.1007/s00217-014-2154-7

Nation JL and Robinson FA (1971). Concentration of some major and trace elements in honeybees, royal jelly and pollens, determined by atomic absorption spectrophotometry. *J. Apic. Res.* 10: 35-43.

Scarselli R, Donadio E, Giuffrida MG, Fortunato D, et al. (2005). Towards royal jelly proteome. *Proteomics* 5: 769-776. http://dx.doi.org/10.1002/pmic.200401149

Shen JK (1991). The effect of harvest period on the yield of royal jelly. *Apic. China* 6: 2-4.

Takenaka T (1987). Nitrogen components and carboxylic acids of royal jelly. In: Chemistry and biology of social insects (Eder J and Rembold H, eds.). Verlag J. Pepemy, Munich, 162-163.

Tamura T, Fujii A and Kuboyama N (1987). Antitumor effects of royal jelly (RJ). *Nippon Yakurigaku Zasshi* 89: 73-80. http://dx.doi.org/10.1254/fpj.89.73

Tokunaga KH, Yoshida C, Suzuki KM, Maruyama H, et al. (2004). Antihypertensive effect of peptides from royal jelly in spontaneously hypertensive rats. *Biol. Pharm. Bull.* 27: 189-192. http://dx.doi.org/10.1248/bpb.27.189

Townsend GF and Lucas CC (1940). The chemical nature of royal jelly. *Biochem. J.* 34: 1155-1162. http://dx.doi.org/10.1042/bj0341155

Townsend GF, Morgan JF and Hazlett B (1959). Activity of 10-hydroxydecenoic acid from royal jelly against experimental leukaemia and ascitic tumours. *Nature* 183: 1270-1271. http://dx.doi.org/10.1038/1831270a0

Wu LM, Zhou JH, Xue XF, Li Y, et al. (2009). Fast determination of 26 amino acids and their content changes in royal jelly during storage using ultra-performance liquid chromatography. *J. Food Compos. Anal.* 22: 242-249. http://dx.doi.org/10.1016/j.jfca.2008.10.022

Zhao L (2014). Amino acids composition and nutritional functions analysis of royal jelly. *Food Res. Dev.* 35: 94-96.

Zheng HQ, Hu FL and Dietemann V (2011). Changes in composition of royal jelly harvested at different times: consequences for quality standards. *Apidologie* 42: 39-47. http://dx.doi.org/10.1051/apido/2010033