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Original Article

Fast determination of 26 amino acids and their content changes in royal jelly during storage using ultra-performance liquid chromatography

Wu Liming, Zhou Jinhui, Xue Xiaofeng, Li Yi, Zhao Jing *

Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Bee Products Quality Supervision and Testing Center, Beijing, 100093, PR China

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ABSTRACT

A rapid ultra-performance liquid chromatography (UPLC) method was developed for feasible separation and quantification of 26 amino acids in royal jelly. The analysis was performed on Acquity UPLC system with Acquity UPLC AccQ·Tag Ultra Column within 8 min. The correlation coefficient values ($r^2 > 0.9978$) indicated good correlations between the investigated compounds' concentrations and their peak areas within the test ranges. The limits of quantitation and detection of 26 amino acids were 42.7–235.1 ng/mL and 12.9–69.3 ng/mL, respectively. The recoveries ranged from 90.1% to 100.9% and the overall relative standard deviations for intra- and inter-day were lower than 2.8%. The results showed that UPLC was a powerful tool for the analysis of amino acids in royal jelly. The method was also applied to quantitatively determine free amino acid (FAA) and total amino acid (TAA) profiles in RJ samples stored at different temperatures (−18 °C, 4 °C and 25 °C) for different time intervals (1, 3, 6 and 10 months). Results showed that the average contents of FAA and TAA in fresh royal jelly were 9.21 mg/g and 111.27 mg/g, respectively; the major FAAs were Pro, Gln, Lys, Glu, and the most abundant TAAs were Asp, Glu, Lys and Leu. Although the concentration of most FAAs and TAAs showed no significant difference during storage, contents of total Met and free Gln decreased significantly and continuously, and might be a parameter to predict the quality of royal jelly.

1. Introduction

Royal jelly (RJ) is part of the diet of honeybee larvae and is believed to play an important role in the development of the queen honeybee (Haydak, 1970; Osamu et al., 2004). It is a most interesting healthy and functional food because it possesses several health-promoting and pharmacological properties (FAO, 1996; Nagai and Inoue, 2004). And now, it has been widely used in commercial medical products, health foods and cosmetics in many countries.

RJ is a complex matrix that contains water (60–70%), crude protein (12–15%), carbohydrates (10–16%), lipids (3–7%), traces of mineral salts and vitamins (Lercker et al., 1981; Chen and Chen, 1995; Crane, 1990). It may spoil or deteriorate and lose its commercial value eventually when it is stored improperly, so RJ should be stored at 4 °C or less to guarantee a good preservation of its quality (Chen and Chen, 1995). The quality problems of RJ have recently attracted scientists' attention.

Some quality and freshness evaluation indexes, such as furosine (Emanuele et al., 2002; Messia et al., 2005), superoxide dismutase (Chaozhong and Youlu, 1999), glucose oxidase (Cuiwen and Fuxin, 1990), (E)-10-hydroxydec-2-enoic acid (Jean-Francois et al., 2003), 57-kDa protein (Masaki et al., 2001), have been proposed. But up to now, few suitable physicochemical parameters as freshness and quality standards have been accepted worldwide.

It was reported that there was a gradual deterioration of protein components in RJ (Okada et al., 1979), and the concentration of some FAAs also changed throughout the storage (Emanuele et al., 2003). Namely, modifications that affect the protein and amino acid fractions may play an important role in assessing the commercial quality of RJ during the storage of the product. Additionally, the principal nutritional value of proteins food is determined by their amino acid content, and amino acid analysis has been successfully used for the analysis of proteins and peptides (Matloubi et al., 2004). Therefore, determination of the content changes of TAAs and FAAs may be an effective way for accessing the quality of RJ.

Besides the method of gas chromatography–mass spectrometry (GC–MS) (Emanuele et al., 2003), cation-exchange chromatography with spectrophotometric detection (European Norm 12742, 1999), reversed-phase high performance liquid chromatography (Woo, 2001) and capillary electrophoresis (Ummadi and Weimer, 2002) detection have been extensively used for the analysis of amino acids. However, these methods suffered from either long analysis time of more than 35 min or expensive equipment.

* Corresponding author. Tel.: +86 10 82595649; fax: +86 10 82595649.
E-mail address: apiswu@126.com (Z. Jing).

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Ideally, an analytical method for amino acids should comply with the following requirements: short time of analysis, high sensitivity, linear response, stable and rapidly forming derivatives without any interfering artefact (Ana et al., 2006). Ultra-performance liquid chromatography (UPLC) makes it possible to perform very high-resolution separations in short periods of time with little organic solvent consumption (Swartz, 2005), which has attracted wide attention of pharmaceutical and biochemical analysts (Stephen et al., 2006).

In this paper, a UPLC method for the simultaneous determination of 26 amino acids in RJ was developed. The validated method was also applied to quantitatively determine the FAAs and TAAs of RJ samples stored at different temperatures (−18 °C, 4 °C and 25 °C) for different time intervals (1, 3, 6 and 10 months).

2. Materials and methods

2.1. Reagents and standards

The 26 individual amino acid standards, including histidine (His), serine (Ser), arginine (Arg), glycine (Gly), aspartic acid (Asp), glutamic acid (Glu), threonine (Thr), alanine (Ala), hydroxylysine (Hylys), proline (Pro), cysteine (Cys), lysine (Lys), tyrosine (Tyr), methionine (Met), valine (Val), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), taurine (Tau), γ-aminobutyric (GABA), aminoisobutyric acid (AABA), ornithine (Orn), glutamine (Gln), asparagines (Asn), hydroxyproline (Hypro) and tryptophan (Trp), were purchased from Sigma. They were dissolved in deionized water individually at the desired concentration as the stock standard solutions. Working standard solution including all amino acids was prepared by mixing stock standard solutions, and the final concentration was 5 pmoles/μL of all amino acids except cystine, which had a concentration of 2.5 pmoles/μL.

AccQ·Tag Ultra borate buffer and AccQ·Tag Ultra reagent were all included in the UPLC amino acid analysis application solution. Water was purified with a Milli-Q system (Millipore, Billerica, MA).

2.2. Samples

RJ was harvested at 10 different apiaries in different regions of Zhejiang province during the flowering period of *Camellia sinensis* with the high royal jelly producing Pinghu Italian breed line bees.

The harvested RJ was immediately stored at low temperature (below 4 °C) and sent to the laboratory. After homogenization, RJ was divided in 60 aliquots (10 g for each sample). Each aliquot was placed in a glass vial and caped. 24 vials were used to establish and verify the analysis method. The remaining 36 samples were divided into three groups. The groups were stored at −18 °C, 4 °C, and 25 °C individually for 1, 3, 6 and 10 months in three replications. The samples were analyzed when the programmed storage time was reached.

2.3. Sample preparation

2.3.1. Extraction of FAAs

RJ (1 g) was dissolved with 25 mL of 90% ethanol, dispersed for 1 min with the help of the sonicator and homogenized with the Polytron for 2 min. The homogeneous solution was centrifuged for 5 min at 5000 rpm and the supernatant decanted. Then, the sediment was extracted twice more with 25 mL of 90% ethanol. The extracted solutions were combined and dried with a rotary vacuum evaporator at 40 °C, redissolved and filled up to 50 mL with deionized water. The extracts were filtered through a 0.22 μm syringe filter (Millipore, MA, USA) prior to injecting into the UPLC system.

2.3.2. Extraction of TAAs

0.5 g RJ was accurately weighed and transferred into a Pyrex screw-cap tube, 3 mL of deionized water and 3 mL of 12 M HCl were added and then homogenized for 1 min with the help of the Polytron. A moderate stream of purified nitrogen was blown into the liquid to remove the air in the test tube, and then the tightly sealed tube was kept upright in an oven at 110 °C for 24 h. After removing the tube from the oven and cooling to room temperature, the acid hydrolysate was filtered through a common filter paper and neutralized to pH 4.8–5.2 using 6 M NaOH, redissolved and filled up to 50 mL with deionized water. And then, 2.0 mL extracts were pipetted and made up to 10.0 mL in a volumetric flask with deionized water. The extracts were filtered through a 0.22 μm syringe filter (Millipore, MA, USA) before injection.

2.4. Derivatization

70 μL AccQ·Tag Ultra borate buffer, 10 μL sample extracts and 20 μL AccQ·Tag Ultra reagent were added into a recovery vial sequently. The mixed solution was vortexed immediately for 5 s, and allowed to stand at room temperature for 1 min before being placed in a heating block at 55 °C for 10 min. After 10 min, the mixed solution was removed and analyzed using the Acquity UPLC System. All RJ samples were prepared and analyzed in triplicate.

2.5. UPLC conditions

All analyses were performed on a Waters Acquity UPLC system, including a binary solvent manager, a sample manager fitted with a 2 μL loop, and a Tunable UV (TUV) detector, connected to a Waters Empower™ 2 software. A 2.1 mm × 100 mm Acquity UPLC AccQ·Tag Ultra Column also from Waters was used. The standards or samples were separated using a gradient mobile phase consisting of 5% AccQ·Tag Ultra Eluent A (A) and AccQ·Tag Ultra Eluent B (B). The gradient condition was: 0–0.54 min, 0.1% B; 0.54–5.74 min, 0.1–9.1% B; 5.74–7.74 min, 9.1–21.2% B; and finally, reconditioning the column with 0.1% B isocratic for 0.86 min after washing column with 59.6% B for 0.90 min. The flow rate was set at 0.70 mL/min and the injection volumes for all samples and standards were 1.0 μL. The column temperature was set at 55 °C. The peaks were detected at 260 nm.

2.6. Calibration curves and limits of detection

The stock solution of mixed standards containing 26 amino acids was prepared and diluted to appropriate concentrations for the establishment of calibration curves. Each concentration of the mixed standard solution was injected in triplicate, and then the calibration curves were constructed by plotting the peak areas versus the concentrations of each amino acid.

The stock solution containing 26 amino acids was diluted with deionized water to appropriate concentrations, and an aliquot of the diluted solutions was injected into UPLC for analysis. The limit of detection (LOD) and quantification (LOQ) for each amino acid was determined at a signal-to-noise ratio (S/N) of about 3 and 10, respectively.

2.7. Recovery and precision

The recovery was preformed by adding a known amount of individual amino acid standards into a certain amount (1.0 g for FAAs and 0.5 g for TAAs) of RJ. Three replicates were performed for the test. The mixture was extracted and analyzed using the methods described in Sections 2.3.1 and 2.3.2, respectively. The quantity of each amino acid was subsequently obtained from the corresponding calibration curve.