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Original Research Article

Development of a simple analytical method using capillary electrophoresis-tandem mass spectrometry for product identification and simultaneous determination of free amino acids in dietary supplements containing royal jelly

Shigeki Akamatsu *, Takao Mitsuhashi

Hyogo Prefectural Institute of Public Health and Consumer Sciences, 2-1-29, Arata-cho, Hyogo-ku, Kobe 652-0032, Japan

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ABSTRACT

A simple capillary electrophoresis-tandem mass spectrometry (CE-MS/MS) method was developed for the analysis of free amino acids in commercial royal jelly (RJ) products containing various kinds of matrices. This method required no concentration step for sample preparation, and all 16 amino acids were determined without derivatization. The CE separation was achieved in an uncoated fused-silica capillary using a 1 M formic acid solution (pH 1.8) as the electrolyte, followed by MS/MS detection after mixing with a sheath liquid comprising 50% (v/v) methanol. The limits of detection (LODs) ranged from 0.61 to 10.5 μg (dry weight)/g for each amino acid. The recoveries for tablets, liquid drinks, and raw materials ranged from 88.3 to 108.6%, and the relative standard deviations (RSDs) were within 10%. The method was applied to 17 commercial RJ products, and the results were compared to those for honey. The relative proportions of free amino acids were specific for each RJ product, and the method was found to be useful in distinguishing not only among the different RJ products but also between RJ and honey.

1. Introduction

Royal jelly (RJ) is a whitish cream secreted from the pharyngeal and mandibular glands of young worker honeybees (Munstedt and Von Georgi, 2003). RJ is essential for the growth of queen honeybees and has been used worldwide in dietary supplements, medical products, and cosmetics.

RJ consists mainly of water, proteins, carbohydrates, fatty acids, and vitamins. The main fatty acid in RJ is trans-10-hydroxy-2-decenoic acid (10-HDA), the contents of which have been used to differentiate and assess the quality of RJ. Although many methods have been described for determining 10-HDA content (Ohta et al., 1993; Zhou et al., 2007; Akamatsu et al., 2010), the amino acid composition has not been extensively investigated in spite of the important role of these components in the quality and taste of RJ and as protein constituents. Compositional analysis of free amino acids, especially in the food science field, is receiving plenty of attention. Several studies have reported that the profiling of free amino acids can be used to discriminate the origin of a substance and its shelf life (Iglesias et al., 2006; Shen et al., 2011). Therefore, the development of a simple analytical method for evaluating free amino acids is important in order to assess the quality of commercial RJ products.

High-performance liquid chromatography (HPLC) (Versari et al., 2008; Liming et al., 2009; Kelly et al., 2010) and gas chromatography–mass spectrometry (GC–MS) (Starke et al., 2001; Nozal et al., 2004) are often used for analyzing amino acids; however, these methods require cumbersome and complicated derivatization procedures to separate or detect them. Therefore, an HPLC–mass spectrometry (HPLC–MS) that eliminates the need for derivatization procedures has also been described using an ion-pairing reagent and a hydrophilic interaction chromatography (HILIC) column (Armstrong et al., 2007; Conventz et al., 2007; Tang et al., 2008; Soto et al., 2011). However, this method requires a long time for instrument equilibration, leading to an increase in the total run time. In such cases, a problem arises concerning the robustness of the column. Meanwhile, capillary electrophoresis (CE) is more preferable because of its high resolution, low cost, and short preconditioning time. In addition, CE, wherein the derivatization procedure is not necessary, is suitable for the analysis of highly polar, ionizable compounds such as amino acids (Soga and Imaizumi, 2001; Jiang et al., 2009). The main drawback of CE is the low detection capability incurred as a consequence of the small

* Corresponding author. Tel.: +81 78 511 6640; fax: +81 78 531 7080.
E-mail addresses: shigeki_akamatsu@pref.hyogo.lg.jp (S. Akamatsu), takao_mitsuhashi@pref.hyogo.lg.jp (T. Mitsuhashi).

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sample volume; however, the utilization of hyphenated MS detection offers high sensitivity and the possibility of structurally characterizing the analyte (Soga et al., 2004).

It has also been reported that the free amino acid content in RJ, as measured by HPLC analysis using a derivatization reagent, changes during storage of the raw materials (Liming et al., 2009). However, this method contains a derivatization step and is thus time-consuming and impractical. In addition, because RJ products are marketed not only as raw materials but also as pharmaceutical formulations containing additives, this method suffers from detrimental effects due to the complex sample matrices in these commercial formulations.

In this study, we developed a simple CE-tandem mass spectrometry (CE-MS/MS) method for simultaneously determining the free amino acids in RJ. Furthermore, the proposed method was applied to several types of RJ products, and the free amino acid content was compared to that in honey samples to evaluate the differences between the two products.

2. Materials and methods

2.1. Chemicals

Amino acid standards L-alanine (Ala), L-arginine (Arg) hydrochloride, L-aspartic acid (Asp), L-glutamic acid (Glu), glycine (Gly), L-histidine (His) hydrochloride, L-isoleucine (Ile), L-leucine (Leu), L-lysine (Lys) hydrochloride, L-methionine (Met), L-phenylalanine (Phe), L-proline (Pro), L-threonine (Thr), L-tyrosine (Tyr), and L-valine (Val) were purchased from Kyowa Hakko Kogyo (Tokyo, Japan). L-Cysteine (Cys), L-serine (Ser), and γ-aminobutyric acid (GABA) were purchased from Wako Pure Chemical (Osaka, Japan). All other reagents and solvents were purchased from Wako Pure Chemical. Methanol and ethanol were of liquid chromatography grade, and all amino acid standards, hydrochloric acid (HCl), and formic acid were of analytical grade. Water was purified using a Milli-Q Water Purification System (Millipore, Billerica, MA, USA).

Stock standard solutions of each amino acid (50 mM) were prepared in 0.1 M HCl. Working standard solutions were also prepared by mixing the stock solutions and diluting with 0.1 M HCl.

The internal standard (IS) stock solution (1 mg/mL) was prepared by dissolving 10 mg of Cys in 10 mL of 0.1 M HCl.

2.2. Samples

Seventeen RJ products were purchased on the market in Japan. Eight samples were tablets (31.5–99.0 g), five were capsules (26.5–123.6 g), one was a powder (45 g), one was a liquid drink (100 g), and two were raw materials (100 g each). Three honey products (500 g each) were also purchased on the market in Japan.

2.3. Instrumentation

The analyses were carried out on an Agilent 7100 CE apparatus (Waldbronn, Germany) coupled with an Agilent 6410 triple quadrupole tandem mass spectrometer. Separation was performed on an uncoated fused-silica capillary (50 μm, I.D., 100 cm in length) at 20 °C, and a 1 M formic acid solution (pH 1.8) was used as the electrolyte. Samples were applied to the capillary using hydrodynamic injection (50 mbar) for 5 s. The capillary was conditioned with water for 2 min, 0.1 M NaOH for 3 min, water for 2 min, and then the operating buffer for 3 min between runs. The applied voltage was set at 30 kV. Electrical contact at the electrospray needle tip was established via a sheath liquid comprising 50% (v/v) methanol delivered at a flow rate of 8 μL/min by an Agilent 1260 binary pump.

Table 1
Optimized MRM parameters for each amino acid in positive ion mode.

Amino acid	Transition (*m*/*z*)	Fragmentor voltage (V)	Collision energy (eV)
Ala[a]	90.1	50	–
Arg	175.2 → 70.1	100	25
Asp	134.1 → 74.1	70	10
GABA	104.1 → 87.2	70	5
Glu	148.1 → 84.1	100	10
Gly[a]	76.1	70	–
His	156.2 → 110.1	50	10
Leu, Ile	132.2 → 86.1	50	5
Lys	147.2 → 84.1	50	25
Met	150.2 → 133.1	70	5
Phe	166.2 → 120.1	70	10
Pro	116.1 → 70.1	100	10
Ser	106.1 → 60.2	70	5
Thr	120.1 → 103.1	150	15
Tyr	182.2 → 136.0	70	5
Val	118.2 → 71.9	50	5
Cys (IS)	122.1 → 75.9	70	15

[a] SIM by single MS mode.

The mass spectrometer was operated in the positive-ion mode with multiple reaction monitoring (MRM) and selective ion monitoring (SIM). A capillary voltage of 4 kV was applied after sample injection. The nebulizing and drying gas conditions were 10 psi N_2 and 10 L/min N_2 at 300 °C, respectively. Quantitative optimizations of the fragmentor voltage and collision energy were achieved by infusing an amino acid standard. Each parameter was selected to obtain the highest intensity. The optimized parameters are listed in Table 1.

2.4. Sample preparation

The tablets were ground using a Millser IFM-700G (Iwatani, Tokyo, Japan), and the capsules were opened to release the contents. A 1.0 g aliquot was accurately weighed in a 15 mL test tube, and 9 mL of 75% (v/v) ethanol was added. The sample was extracted in an ultrasonic bath (VS-25; VELVO-CLEAR, Osaka, Japan) for 5 min at room temperature. A 0.9 mL aliquot of the supernatant, obtained by centrifugation at 3000 rpm (1200 × *g*) for 5 min (Kubota 8700, Tokyo, Japan), was mixed with 0.1 mL of 1 M HCl (equivalent to 0.1 g/mL). A 0.1 mL portion of the IS (1 mg/mL Cys) was added prior to CE-MS/MS analysis.

2.5. Accuracy and precision

Three kinds of typical RJ samples (tablet, liquid drink, and raw material) were spiked with all amino acid reference standards and equilibrated for 30 min prior to extraction. The spiked levels were 10 μmol/g for Pro and Lys, 2 μmol/g for Glu and Asp, and 0.5 μmol/g for the other amino acids. In a 15 mL test tube, 1 g of each spiked sample was prepared and analyzed in quintuplicate on three consecutive days according to the method described in Section 2.4. The recovery was calculated by comparison of the pre- and post-spiked samples. The intraday and interday precisions were determined using one-way variance analysis.

3. Results and discussion

3.1. Optimization of the CE-MS/MS conditions

Separation by CE is based on differences in solute velocities in an electric field, and the solute velocity depends on the charge and size of the electric field. To achieve simultaneous determination of free amino acids, all solutes must acquire an electrical charge. Fortunately, the isoelectric points (pI) of all 18 amino acids are