Food Chemistry 115 (2009) 715–719

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

HPLC determination of adenosine in royal jelly

Xiao Feng Xue [a], Jin Hui Zhou [a], Li Ming Wu [a,b], Liang Hu Fu [b], Jing Zhao [a,*]

[a] Bee Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100093, China
[b] Bee College of Animal Sciences, Zhejiang University, Hangzhou 310027, China

ARTICLE INFO

Article history:
Received 22 November 2007
Received in revised form 21 October 2008

Keywords:
Adenosine
Royal jelly
HPLC

ABSTRACT

A simple method is described for the determination of adenosine in royal jelly. The adenosine in the sample was extracted using 80% ethanol and analysed by reversed-phase high-performance liquid chromatography (HPLC). Chromatographic separation was performed using a Dionex HPLC system with a Waters Symmetry C18 column and gradient elution with a mixture of two solvents: solvent A, 0.4% phosphoric acid and solvent B, methanol. The effluent was monitored using a UV detector set at 257 nm. The average recoveries were 91.6–98.3% ($n = 6$) with standard deviation below 5.3%. The limits of detection and quantification were 0.017 and 0.048 μg/ml, respectively. The method has been successfully applied to the analysis of royal jelly samples. For 45 royal jelly products, the adenosine content varied from 5.9 to 2057.4 mg/kg.

1. Introduction

Royal jelly (RJ) is a secretion from the hypopharyngeal and mandibular glands of worker bees (*Apis mellifera*) and is involved in the sexual determination of the queen bee (Nagai & Inoue, 2004). Some studies have reported the composition of RJ. The main components are water, proteins, sugars, lipids and other substances (Crane, 1990; Palma, 1992; Piana, 1996a). As a result of the increasing interest in RJ with respect to human health, the authentication of new active ingredients in RJ is becoming the subject of an increasing number of studies. In a recent paper, adenosine monophosphate (AMP) and adenosine monophosphate N1-oxide were found and identified in RJ (Noriko et al., 2006).

Adenosine is a naturally occurring purine nucleoside and is formed by the breakdown of adenosine triphosphate (ATP). ATP is the primary energy source in cells for transport systems and the action of many enzymes. Most of the ATP is hydrolysed to adenosine diphosphate (ADP), which can be further dephosphorylated to AMP. If large concentrations of ATP are hydrolysed, then some of the AMP can be further dephosphorylated to adenosine by the cell membrane associated with enzyme 5′-nucleotidase (Enzo, Maria, & Luciano, 2001). The pathway to adenosine is shown in Fig. 1.

Adenosine acts as a building block for nucleic acids and energy storage molecules, a substrate for multiple enzymes and an extracellular modulator of cellular activity (Alam, Szymusiak, Gong, King, & McGinty, 1999). The endogenous release of adenosine exerts powerful effects in a wide range of organ systems (Olah & Stiles, 1992, 1995). For example, adenosine has a predominantly hyperpolarising effect on the membrane potential of excitable cells, producing inhibition in vascular smooth muscle cells of coronary arteries and neurons in the brain (Basheer, Strecker, Thakkar, & McCarley, 2004). As an endogenous nucleoside, adenosine has been widely investigated in different products. For instance, adenosine is an important index for quality assessment of Lingzhi (*Ganoderma lucidum*) and Cordyceps (Gao et al., 2007; Gong, Li, Lia, Liu, & Wang, 2004). However, up to date, few studies have been reported on the adenosine content of RJ (Piana, 1996b).

The most frequently used procedures for the extraction of adenosine from samples are based on conventional liquid shaking or blending for a few minutes with a mixture of organic solvents or perchloric acid followed by clean-up of the extract and enrichment with SPE (solid-phase extraction). A different approach, using PLE (pressure liquid extraction) and SPE to extract adenosine from natural and cultured Cordyceps has recently been reported (Fan et al., 2006). However, these methods were found to be unsuitable for the extraction of adenosine from RJ due to the fact that it is more complex than other matrixes. Moreover, these extraction methods have certain drawbacks such as the consumption of large amounts of time and noxious solvent, and the necessity for one or more clean-up steps involving liquid–liquid partition or solid-phase extraction. An ideal leaching procedure should be exhaustive with respect to the constituents to be analysed, rapid, simple, inexpensive, environmentally friendly and amenable to automation for routine analysis (Sanchez, Priego, & Luque, 2007). Ultrasound is probably the simplest and most versatile method for sample extraction because the energy imparted facilitates and accelerates some of the steps, such as dissolution, fusion, and leaching amongst others; this method also resolves the problems of solvent and time consumption (Lavillaa, Vilasa, & Bendicho, 2008). In this study, a mixture of ethanol and water was utilised to extract

* Corresponding author. Tel./fax: +86 10 82595649.
E-mail address: zhaojingbqsitc@126.com (J. Zhao).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.12.003

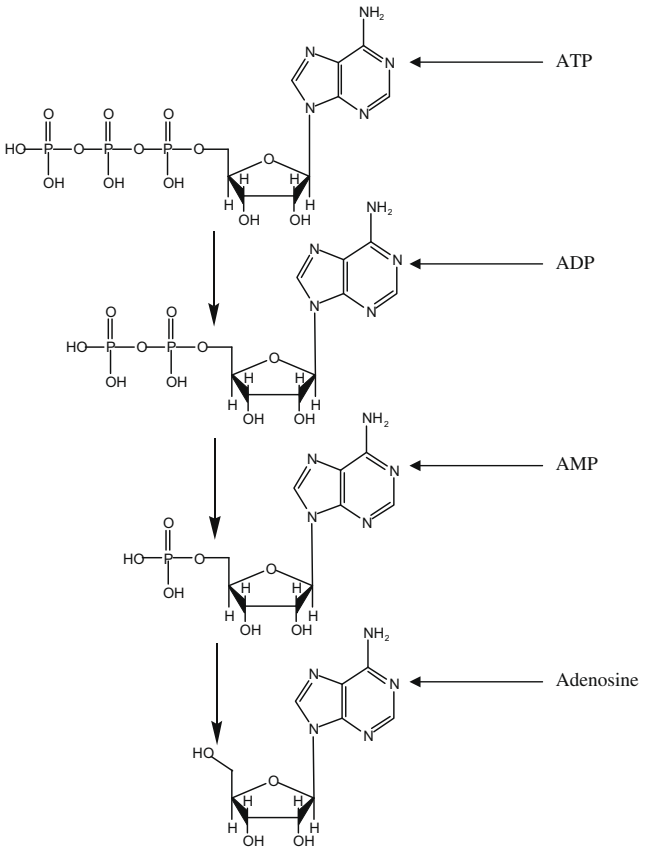

Fig. 1. Pathway to adenosine.

adenosine from RJ using ultrasonic-assisted extraction. The different factors affecting the efficiency of the extraction such as extraction solvent and extraction time were carefully optimised.

Capillary electrophoresis (CE) and high-performance liquid chromatography (HPLC) are currently the most commonly used separation techniques for adenosine in combination with detection by ultraviolet (Gong et al., 2004; Kieszling et al., 2004; Tzeng, Hung, Wang, Chou, & Hung, 2006), diode array and evaporative light-scattering (Yan, Luo, Wang, & Cheng, 2006), and mass spectrometry (Brink, Lutz, Volkel, & Lutz, 2006; Cahours, Dessans, Morin, Dreux, & Agrofoglio, 2000; Fan et al., 2006; Gao et al., 2007). In particular, as a conventional method, HPLC can be carried out using standard equipment in many laboratories and is also simple, sensitive and very suitable for monitoring adenosine. However, there are no detailed studies available concerning the distribution of adenosine in RJ or the analytical methods used for its determination in RJ samples. Development of a simple and rapid HPLC method for the determination of adenosine in RJ is therefore necessary and would be valuable for further study.

The purpose of this study was to develop a simple method for the quantitative analyses of adenosine in RJ. Adenosine was extracted with 80% ethanol and analysed by HPLC–UV. The validated method has been successfully utilised for determining adenosine content and investigating the range of adenosine content in RJ samples.

2. Materials and methods

2.1. Materials and reagents

Twenty-five commercial samples were purchased from supermarkets in Beijing. Twenty commercial samples of known origin were obtained from Pinghu apiary in China's Zhejiang province, a major RJ producing zone. All the samples were kept refrigerated at −18 °C before analysis. Adenosine standard was purchased from Sigma (St. Louis, MO, USA). Methanol (HPLC grade) was purchased from Merck (Darmstadt, Germany). Phosphoric acid and absolute ethanol (analytical grade) were purchased from Beifen (Changping, Beijing). De-ionised water was prepared using a Millipore Milli-Q Plus system (Millipore, Bedford, MA, USA).

2.2. HPLC

HPLC analysis was carried out using a Dionex HPLC system (Dionex, USA), which included a P680 pump, an ASI-100 auto injector, a TCC-100 column oven and a 170UV UV detector, connected to Chromeleon software. A Symmetry C18 column (250 mm × 4.6 mm i.d., 5 μm) from Waters was used. The column temperature was maintained at 30 °C. The standards and samples were separated using a gradient mobile phase consisting of 0.4% phosphoric acid (A) and methanol (B). The linear gradient conditions were: 0–25 min, 90% A; 25–35 min, 20% A; 35–40 min, 90% A and 40–65 min, 90% A. The flow rate was set at 0.9 ml/min and the injection volume was 20 μl. The detection wavelength was set at 257 nm. Identification of adenosine was based on retention time when co-injected with standards.

2.3. Sample extraction procedure

Cold samples were equilibrated at room temperature for 1 h and then homogenised before analysis. A total of 2.0 g of RJ was accurately weighed into a 50 ml volumetric flask. Then 5 ml of ultra-purified water and 40 ml of absolute ethanol were added and the mixture was shaken for 15 min at room temperature using an ultrasonic processor. The volume was made up to the mark with ultra-purified water. A sample from the solution was drawn into a pipette and filtered through a filtering cartridge containing a 0.45 μm nylon membrane using a disposable syringe set before HPLC analysis.

2.4. Preparation of the standard curve

Quantification was based on the external standard method. A stock solution of adenosine standard (1 mg/ml) was prepared by dissolving adenosine in 80% ethanol. The working standard solutions for linear calibration were prepared by diluting the stock solution to produce a concentration sequence of 0.1, 1, 5, 10, 100

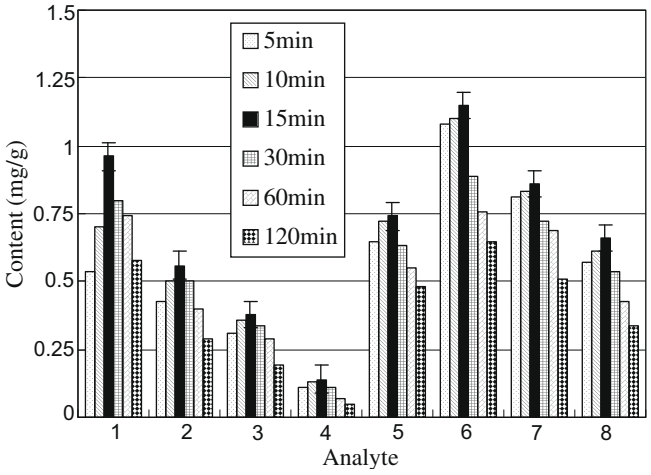

Fig. 2. Effect of time on the extraction of adenosine from RJ.