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Technical note

Determination of *trans*-10-hydroxy-2-decenoic acid content in pure royal jelly and royal jelly products by column liquid chromatography

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Abstract

In this research, several royal jellies and commercial products containing royal jelly were analysed for their *trans*-10-hydroxy-2-decenoic acid (10-HDA) content by using a column liquid chromatography technique. Ten samples claimed to be pure royal jelly, containing 10-HDA between 0.75 and 2.54%. Seven samples claimed to contain royal jelly as an ingredient which ranged from non-detectable to 0.054%. The technique was found to be rapid with high recovery.

Keywords: Royal jelly; *trans*-10-Hydroxy-2-decenoic acid

1. Introduction

Natural food products that undergo the least processing are now popular. Among them royal jelly (RJ) is one of the most attractive products. RJ is a secretion from the hypopharyngeal (cephalic) and mandibular glands of worker bees (*Apis mellifera* L.). This secretion is a yellowish, creamy and acidic material with a slightly pungent odor and taste. RJ is a fed only temporarily (up to and no more than three days) to the brood of workers and drones but it is a sole food of the queen bee for both her larval and adult life [1]. RJ is generally regarded as the major reason of the significant morphological and functional differences between queen and worker bees [2].

RJ has been a commercial product in many countries for more than 30 years, especially used in dietetics and cosmetics [3]. In Turkey, it is sold either as pure RJ or as products containing RJ as an ingredient, which may contain other ingredients such as honey and pollen.

The unique and chemically most interesting feature of RJ is its fatty acids. Unlike fatty acids of most animal and plant materials which consist mainly of triglyceride fatty acids each having 14–20 carbon atoms, RJ fatty acids are short-chained 8–10 carbon free fatty acids that are usually either hydroxy fatty acids or dicarboxylic acids. The major fatty acid in RJ is 10-hydroxy-2-decenoic acid (10-HDA) and the amount of this fatty acid in pure RJ varies depending on the origin of the jelly and characteristics of the bee. No other natural product containing 10-HDA has been reported. Even other bee products do not contain 10-HDA. Thus, the presence of 10-

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HDA can be used as a marker to differentiate RJ from other bee products [4].

Bloodworth et al. [5] reported that only in four countries – Australia, Korea, Japan and Thailand – the presence of 10-HDA is used as an improvement for pure RJ. According to Thai specifications, the amount of 10-HDA should not be less than 1.5% and 0.16% (w/w), respectively for RJ and RJ products. Also, in Turkey [6] the presence of 10-HDA is used as a prequisite for products that claim to be RJ or RJ products since 1989 and the minimum amount of 10-HDA in pure RJ must be 1.4% whereas it must be 0.16% in RJ products.

According to the Turkish regulation for RJ the amount of 10-HDA is suggested to be determined by gas chromatography (GC) [6]. In this technique, the fatty acids have to be extracted from the sample and converted to volatile compounds prior to the analysis. Extraction often possess difficulties because of the stable emulsion formed in the interface, especially for viscous samples. The formation of an emulsion causes loss of 10-HDA and results in a relatively low recovery (40–75%) [5]. 10-HDA can also be determined by column liquid chromatography (LC) [5,7]. This method is more advantageous than GC because it is not necessary to form a volatile derivative of 10-HDA and therefore there are no losses due to the formation of an emulsion. There are only two papers which deal with the determination of 10-HDA by LC in the literature. Aslan [7] analysed 10-HDA by using a C_{18} column with a acetonitrile–tetrahydrofuran–water eluent system and Bloodworth et al. [5] analysed 10-HDA by using a C_{18} column with a methanol–water eluent system.

In this study the levels of 10-HDA in various pure RJ and RJ products were determined by high-performance liquid chromatography (HPLC) by using a C_{18} column and a acetonitrile–tetrahydrofuran–water eluent system.

2. Experimental

2.1. Materials

Ten pure RJ samples (RJ1–RJ9 and RJ17) and seven RJ products (RJ10–RJ16) were analysed. Two of the pure RJs (RJ1 and RJ2) were harvested in the middle regions of Turkey (Ankara), and supplied by the TKV (Turkish Development Foundation) and RJ8 and RJ17 were supplied by beekeepers from the western parts of Turkey (Kemalpasa). The other pure RJs and RJ products were supplied by beekeepers and health food stores from the middle and western part of Turkey and RJs used in these samples were imported from China and used either in pure or ingredient form.

All chemicals were of HPLC grade, obtained from Merck (Darmstadt,Germany) and Carlo Erba (Milan, Italy) and used without further purification. For standard 10-HDA see below.

2.2. Equipment

A Hewlett-Packard (HP) 1050 Series HPLC system equipped with a Waters tunable absorbance detector (Model 486) and a Waters μBondopak C_{18} column (300×39 mm code No: 27324) adjusted to 35°C as column temperature was employed. The maximum absorbance of 10-HDA is 215 nm.

The eluent was acetonitrile–tetrahydrofuran–deionized water (with 0.1% phosphoric acid) (pH 2.5) (50.4:21.6:28, v/v/v) which was degassed before use. The flow-rate was 1 ml/min. The total run time was about 15 min.

2.3. Preparation of standard and samples

The 10-HDA standard was obtained from pure RJ in crystal form by a published purification method [8]. Its purity was checked by determining its melting point (64°C) and its infrared spectrum (peaks at 390, 1710, 1658 and 976 cm^{-1}) It was concluded that 10-HDA found in the standard was in *trans* form. A LC run of 40 min was conducted on a 10-HDA standard solution to ensure that no other peaks were present.

0.008 g 10-HDA was weighed accurately in a 10-ml flask and the volume was made up with the eluent (solution A). A 2-ml volume of solution A was pipetted into another 10-ml flask and the volume was made up (solution B). The final concentration of the solution was 0.16 μg/μl. From this solution, aliquots were injected onto the column and the calibration curve of standards was prepared.

For pure RJs, nearly 200 mg (containing nearly 3 mg 10-HDA) of sample was used. For RJ products sample amount could vary from 5 to 16 g depending

on the RJ content declared on the product label by the producers. An appropriate amount of sample was weighed into a 100-ml volumetric flask. The sample was dissolved in 50 ml of eluent and ultrasonicated for at least 30 min with occasional shaking. Following sonication, the sample solution was made up to the mark with the eluent. A 20-ml aliquot was pipetted and filtered through 1-μm filter paper before analysis.

3. Results and discussion

The retention time of 10-HDA standard was 2.56 min. For recovery evaluation 0.292 g sample (RJ 13) was weighed into a 100-ml flask. After adding 25 ml eluent and ultrasonification, the volume was made up to the mark with the eluent. An 8-ml volume of this sample was added into each of the 10-ml flasks. 0.5; 1.0; 1.5 ml of solution A was added to the 10-ml flask and the volume was made to mark. Five sets of solutions were prepared and used for recovery studies.

Figs. 1 and 2 show typical chromatograms of pure RJ and RJ products. Table 1 shows the levels of 10-HDA found in pure RJ and RJ products. Concentration of 10-HDA in pure RJ ranges from 0.33 to 2.54% (Table 2). The amounts of 10-HDA found in six RJ products varied significantly, depending on the amounts of RJ added to the products. Two

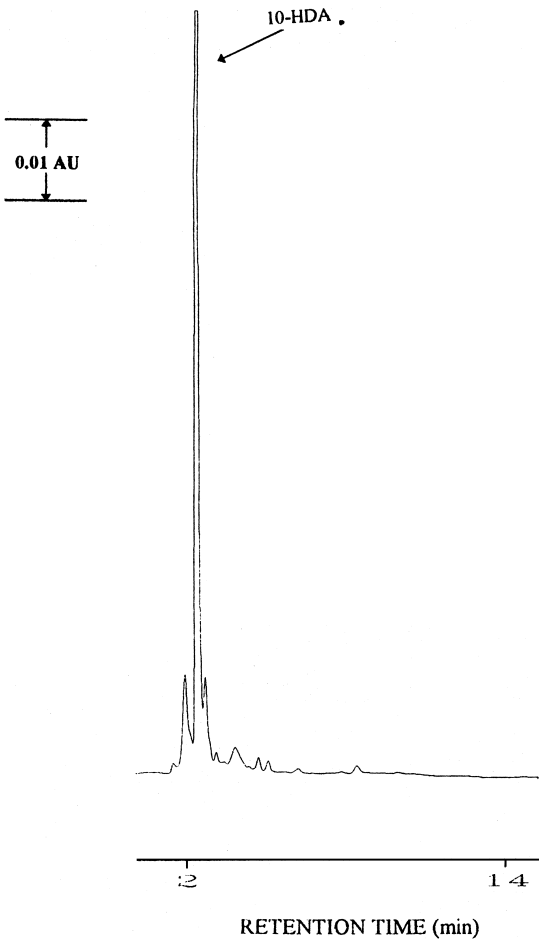

Fig. 1. Typical chromatograms of pure RJ (RJ2).

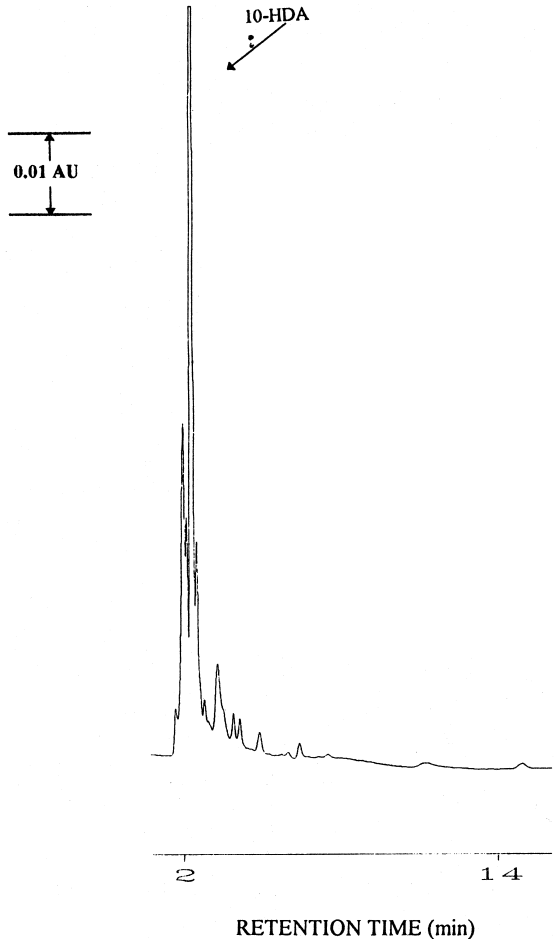

Fig. 2. Typical chromatograms of RJ product (RJ16).

Table 1
The recovery of 10-HDA[a]

Amount of 10-HDA (mg/ml)	Mean recovery±SD (%)
0.042	97.3±2.4
0.084	99.7±1.8
0.126	98.1±4.4

[a] SD=Standard deviation; n=5.

samples did not contain any detectable amount of 10-HDA. Others contain between 0.0093 and 0.054%.

A direct relationship was estimated between the peak areas and the amount of 10-HDA standard. The correlation equation of this relationship was calculated as $y=36.876x$ and the correlation coefficient was found to be $R^2=0.9962$. The minimum recovery determined by the standard addition method was found to be 97.3±2.44% (Table 1).

The results indicate that the method is rapid and accurate for determination of 10-HDA in RJ. Further studies on reproducibility of this technique are suggested.

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Table 2
The levels of 10-HDA found in pure RJ

Royal jelly	10-HDA (%)	RSD[a]	Royal jelly product	10-HDA (%)	RSD[a]
RJ 1 (Turkish RJ)	1.02	1.9	RJ-10	0.046	5.2
RJ-2 (Turkish RJ)	1.85	6.2	RJ-11	0.009	3.2
RJ-3	1.15	5.5	RJ-12	0.014	7.8
RJ-4	0.82	5.1	RJ-13	nd[b]	
RJ-5	0.75	5.8	RJ-14	nd[b]	
RJ-6	1.55	5.4	RJ-15	0.025	3.7
RJ-7	0.33	6.0	RJ-16	0.054	3.5
RJ-8 (Turkish RJ)	1.80	4.5			
RJ-9	2.54	5.4			
RJ-17 (Turkish RJ)	2.38	5.0			

[a] RSD=Relative standard deviation; n=5.
[b] nd=Not detected.