Determination of (2*E*)-10-hydroxydec-2-enoic acid in Croatian royal jelly by high-performance liquid chromatography

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Summary

Although health-promoting properties of royal jelly are known for many years, the absence of quality standards and methods prescribed for their determination leads to royal jelly adulteration. Royal jelly is a source of unique unsaturated fatty acid, (2*E*)-10-hydroxydec-2-enoic acid (10-HDA), and its content is considered as one of the freshness and authenticity parameter. In this study, high performance liquid chromatographic method (HPLC) with UV detection was validated and 10-HDA content in fresh royal jelly samples produced in Eastern Croatia was determined. The data about Croatian royal jelly are not available, therefore the results presented in this study could be considered as pioneer results for Croatian royal jelly characterization in respect of the 10-HDA. Method performance characteristics showed that the used method is fit for purpose. The content of 10-HDA in analysed samples varied from 1.56% up to 3.78%. According to the international recommendations for royal jelly quality, samples were fresh and authentic regarding 10-HDA content. Furthermore, the effect of packaging material on 10-HDA content was observed. Based on the obtained results, the conclusion was reached that if the samples were collected at the same time and frozen after collection, collection and storage of fresh royal jelly in glass and plastic containers had no effect on 10-HDA content.

Keywords: royal jelly, 10-HDA, HPLC method, authenticity, packaging material

Introduction

Royal jelly is produced by the young nurse worker bees to feed larvae of worker bees, drone and queen for the first three days of life, and afterwards only the queen larvae and queen bee are fed with royal jelly. Feeding exclusively with royal jelly is considered to be the major cause of morphological differences, development period and life span between queen and worker bees (Krell, 1996; Ferioli et al., 2007; Bogdanov, 2016).

Royal jelly is yellowish, viscous jelly substance with slightly astringent, sour odour of sour and sweet taste (Bogdanov, 2016). Main constituents are water (60 - 70 %), proteins (27 - 41 % of the dry matter), carbohydrates (up to 30% of the dry matter) and lipids (3 - 8 % of the dry matter), while vitamins and minerals can be found in lower concentrations (Sabatini et al., 2009). Due to its high nutritive value, royal jelly usage is increasing, both in human nutrition in native form, and as active compound in other products, like dietary supplements, medicine and cosmetic products (Bărnuţiu et al., 2011).

Demanding and time consuming production technology and consequently high marketing price, often lead to royal jelly adulteration with cheaper products like starch corn slurry, yoghurt, egg white, condensed milk, unripe banana and water. Adulteration results in changes of sensory characteristics and some physical properties which can be used as one of the criteria for royal jelly authenticity. Garcia-Amoedo and Almeida-Muradian (2007) showed that adulteration can cause turbidity of samples in alkaline medium.

Royal jelly quality is dependent on storage conditions and time, and several studies showed that quality degradation is a result of Maillard reaction products formation, decrease of enzyme activity and interactions between protein and lipid fractions (Antinelli et al., 2003; Garcia-Amoedo and Almeida-Muradian, 2007; Isidorov et al., 2012). In order to minimise quality degradation, royal jelly should be stored in dark containers in the freezer (Antinelli et al., 2003; Sabatini et al., 2009; Bogdanov, 2016).

Most of the health-promoting properties of royal jelly are related to the presence of (2*E*)-10-hydroxydec-2-enoic acid (10-HDA). 10-HDA is an unsaturated fatty acid present only in royal jelly and its content is considered as one of the authenticity and quality parameters (Sabatini et al., 2009). Till today, only several countries have defined national quality

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standards or guidance for royal jelly quality determination (Kanelis et al., 2015). Royal jelly working group of the International Honey Commission (IHC) proposed the guidance for royal jelly standardisation based on the collected data, while International Organisation of Standardisation (ISO) issued a draft of international standards for royal jelly specifications (Sabatini et al., 2009). Recently, Kanelis et al. (2015) proposed revised guidelines for royal jelly quality standards. Fresh and genuine royal jelly should contain minimum 1.4% of 10-HDA, according to the ISO and IHC. Antinelli et al. (2003) suggested 1.8% as minimum content, while Kanelis et al. (2015) suggested that minimum content should be 1.0% based on the latest research of 10-HDA content in authentic royal jelly samples.

Since the official method for 10-HDA determination in royal jelly is not prescribed, a wide number of various techniques and methods are developed and available in the literature, with high performance liquid chromatography (HPLC) with UV detection as the preferred technique, due to the high sensitivity and precision, fast analysis and relatively simple sample preparation (Ferioli et al., 2007; Zhou et al., 2007a, 2007b; Kim and Lee, 2010; Muñoz et al., 2011; Isidorov et al., 2012).

The aims of this study were the evaluation of performance characteristics of the used HPLC method, and the determination of 10-HDA content in fresh Croatian royal jelly samples. Additionally, the effect of packaging material on 10-HDA content was evaluated.

Materials and methods

Royal jelly samples

The beekeepers from Osijek-Baranja County, Republic of Croatia were kindly asked to collect fresh royal jelly into 1.5 mL dark glass containers and 1.5 mL blurry plastic containers, and freeze the samples immediately after collection. The samples were transported to the laboratory and were not defrosted until the analysis. Four samples were gathered in both plastic and glass containers, while one sample (Sample 4) was provided only in a glass container.

Method

The original method used in this study was HPLC method, described by Kim and Lee (2010). The chromatographic conditions (mobile phase composition, column temperature and injection volume) were modified and optimized for the applied chromatographic column and instrument. Afterwards, the method performance characteristics were determined. The analysis was performed on liquid chromatographic system consisting of Shimadzu LC-20AD solvent delivery module, Shimadzu CTO-20AC column oven, Shimadzu autosampler SIL-10AF and Shimadzu SPD-M20A photodiode array detector coupled to a computer with LabSolution Lite software (Release 5.52). HPLC column Chrompack Inertsil 5 ODS-3 (Varian, USA, 4.6 mm IDx150 mm) was used for the separation. Mobile phase was composed of HPLC grade methanol (J. T. Baker, Netherland) and ultrapure water (50:50, v/v); the final pH was adjusted to 2.5 with HPLC grade phosphoric acid (Fluka, USA). Mobile phase flow rate was 1 mL/min, injection volume 10 μL.

The column temperature was set at 35 °C. Monitoring wavelength range was 190-400 nm, while the detection wavelength was set at 215 nm. The identification of 10-HDA was achieved based on the retention time, and comparison of the 10-HDA absorbance spectrum of royal jelly with pure component spectrum. Internal standard method was used for quantification, with methy l-4-hydroxybenzoate (MHB; ≥ 99%, Sigma Aldrich, USA) as internal standard. The internal standard (100 μg/mL) was prepared by weighing 0.0050 g of MHB and dilution in solvent (methanol:water, 50:50, v/v) to final volume of 50 mL. Based on the expected 10-HDA concentrations in royal jelly, a range of standard 10-HDA solutions (0.5 – 80 μg/mL) was prepared using standard of 10-HDA (≥ 99%, Cayman chemicals, USA) for standard solutions preparation. The standard solutions and internal standard solution were filtered through a 0.2 μm membrane nylon filter. For HPLC analysis, 750 μL of standard solution and 250 μL of MHB solution were mixed into autosampler vial. Royal jelly solutions were prepared by weighing the 0.0100 g of fresh royal jelly and dilution in solvent (methanol:water, 50:50, v/v) to final volume of 10 mL. Sample solutions were vortexed for 1 minute, and sonicated until complete dissolution. Subsequently, sample solutions were filtered, first through a 0.45 μm, and after through a 0.2 μm membrane nylon filter. Finally, 750 μL of sample solution and 250 μL of MHB solution were mixed into autosampler vial for HPLC analysis. All analyses were done in duplicate, and each sample solution was injected twice. The results were expressed as % of 10-HDA in fresh royal jelly.

The method performance characteristics estimated for validation were: linearity, accuracy and precision. Linearity was evaluated by the preparation of 10-HDA standard solutions (0.5 – 80 μg/mL), and creation of the calibration curve. Precision was tested as measurement repeatability and sample preparation repeatability. For precision and accuracy measurement,

the Sample 5 collected in a glass container was used. Measurement repeatability was performed by the repeated measurement of the same royal jelly solution 5 times, while for sample preparation repeatability the same royal jelly sample was weighed 5 times and each solution was measured twice. The method precision was expressed as relative standard deviation (RSD, %) of the obtained data set for each method. Accuracy was tested by the determination of 10-HDA content in pure royal jelly and fortified royal jelly solutions with the known amount of standard 10-HDA. The accuracy of method was expressed as % recovery of known, added amount of 10-HDA.

Data analysis

Average values, standard deviations for each parameter, and additionally, relative standard deviations for precision were calculated using Microsoft Excel 2010 (Microsoft Corp.) software.

Results and discussion

After chromatographic conditions were optimized to applied chromatographic column and instrument, performance characteristics of the HPLC method were evaluated. Methods' linearity was estimated in concentration range from 0.5 μg/mL to 80 μg/mL (0.05 - 8 % of 10-HDA in royal jelly), which is a range of the expected 10-HDA concentrations in royal jelly. As shown in Fig. 1, method was linear in the whole evaluated concentration range with the correlation coefficient 0.999. Measurement repeatability was estimated by the multiple injection of the same sample solution, and the obtained relative standard deviation (RSD) was 0.22% (Table 1), while the sample preparation repeatability RSD was 6.56%. According to the guidance for validation of chromatographic methods (CDER, 1994), RSD of injection repeatability should be < 1%. Ferioli et al. (2007), and Caparica-Santos and Marcucci (2007) both reported intraday precision RSD of 1.0%, which is considerably higher that the RSD obtained in this study. Recovery was used to evaluate methods' accuracy and based on the obtained results presented in Table 2, the used HPLC method is accurate. Similar recovery values were reported by Zhou et al. (2007a; 2007b), Genç and Aslan (1999) and Kim and Lee (2010), while Ferioli et al. (2007) obtained recovery values (%) of 90.5 ± 3.8. Overall performance characteristics imply that the used HPLC method is fit for purpose.

Table 1. Precision of the used HPLC method estimated through measurement repeatability and sample preparation repeatability

Method performance characteristic	10-HDA (%)
Precision	
Measurement repeatability	
1	3.68
2	3.66
3	3.67
4	3.66
5	3.66
Average	3.66
Standard deviation	0.01
Relative standard deviation (%)	0.22
Sample preparation repeatability	
1	3.68
	3.66
2	3.90
	3.90
3	3.86
	3.86
4	3.42
	3.38
5	3.36
	3.35
Average	3.34
Standard deviation	0.24
Relative standard deviation (%)	6.56

Concentrations of 10-HDA in analyses of royal jelly samples are presented in Table 3. 10-HDA content in collected royal jelly samples ranged between 1.56% and 3.78%, and according to the recommendations given by Sabatini et al. (2009) and Kanelis et al. (2015), all analysed samples were fresh and authentic. Genç and Aslan (1999) reported 0.33 – 2.54 % of 10-HDA in Turkish royal jelly samples. Mureşan et al. (2016) reported that commercial royal jelly from Romania had 10-HDA content from 1.35% to 2.03%. Based on the results of Kanelis et al. (2015), Greek royal jelly had 10-HDA content from 0.8% to 6.5% (n=97), while Kim and Lee (2010) obtained 10-HDA content from 1.85% to 2.18% in three samples of pure royal jelly from the U.S. market. Though Ferioli et al. (2007) reported that Italian royal jelly has higher 10-HDA concentrations than extra-European one, Zhou et al. (2007a) obtained 10-HDA content for Chinese royal jelly (1.26% - 2.25%) similar to our results and other European samples mentioned above.

Table 2. Accuracy of the used method estimated as recovery (%)

Sample	Added standard concentration (μg/mL)	Calculated value (μg/mL)	Measured value (μg/mL)	Recovery (%)
RJ			20.643	
RJ+std1 (1:1)	13.720	17.182	16.387	95
RJ+std2 (1:1)	6.860	13.752	13.285	97
RJ+std3 (1:1)	1.372	11.008	10.897	99

RJ-royal jelly, std-10-HDA standard

Table 3. Concentrations of 10-HDA (%) in royal jelly samples collected in different packaging materials

Sample	Packaging material	10-HDA (%)	Average ± SD (%)
1	Dark glass	2.95	2.98 ± 0.05
		2.92	
		3.02	
		3.02	
	Blurry plastic	2.08	2.06 ± 0.02
		2.07	
		2.06	
		2.05	
2	Dark glass	1.56	1.56 ± 0.01
		1.55	
		1.57	
		1.55	
	Blurry plastic	1.57	1.56 ± 0.01
		1.56	
		1.56	
		1.56	
	Dark glass	1.80	1.80 ± 0.01
		1.80	
		1.80	
		1.81	
	Blurry plastic	1.77	1.78 ± 0.01
		1.79	
		1.78	
		1.79	
4	Dark glass	1.66	1.65 ± 0.01
		1.67	
		1.64	
		1.65	
5	Dark glass	3.68	3.78 ± 0.13
		3.66	
		3.90	
		3.90	
	Blurry plastic	3.63	3.61 ± 0.02
		3.62	
		3.60	
		3.60	

SD-standard deviation

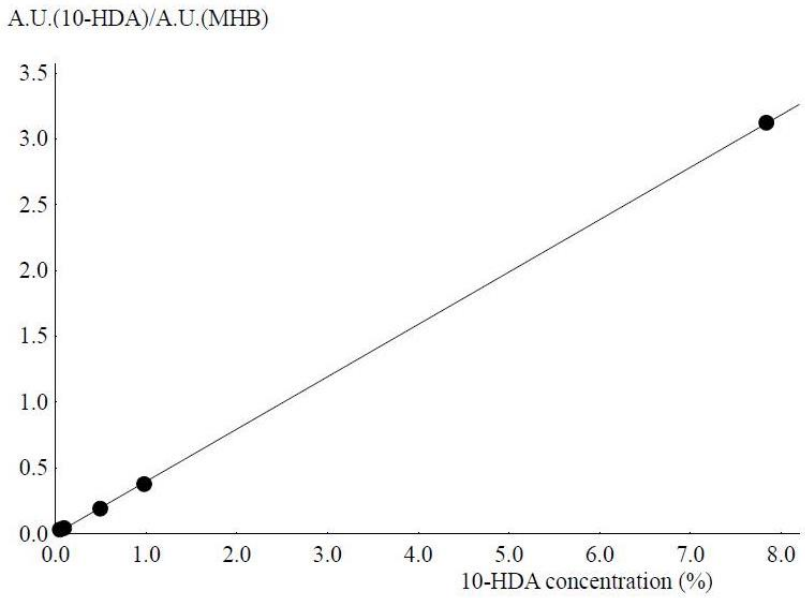

Fig. 1. Calibration curve for 10-HDA determination in royal jelly

Time of royal jelly collection after grafting is very important for 10-HDA content. Namely, most of the producers collect royal jelly 72 hours after grafting, because, according to Zheng et al. (2011), that is the time when 10-HDA content is maximal. Earlier royal jelly collection causes incompliance of some major components (e.g. water and carbohydrate) with the international recommendations, as reported by Zheng et al. (2011). The effect of collection time on 10-HDA content was also observed in this study. According to the beekeepers' information, sample 1 was collected in dark glass and blurry plastic containers with detachment of a few days. As shown in Table 3, only the Sample 1 had slightly different 10-HDA content when different packaging materials are compared, which can be attributed to the different collection time rather than different packaging material. All other analysed samples had similar 10-HDA content regardless of the packaging material (Table 3). The beekeepers usually collect fresh royal jelly in small, dark glass containers with 10, 15 or 20 g of royal jelly. Sometimes, cheaper and more attractive plastic containers can also be used (Krell, 1996; Bogdanov, 2016). According to the results obtained in this study, packaging material has no effect on 10-HDA content.

Conclusions

The used HPLC method was found to be fit for purpose taking into account the evaluated performance characteristics. 10-HDA content in collected royal jelly samples ranged between 1.56% and 3.78%. The data obtained in this study present a pioneer contribution to the Croatian royal jelly characterization. Further research should include more samples from other regions of the Republic of Croatia in order to provide more information and set the limits of 10-HDA content in Croatian fresh royal jelly.

References

Antinelli, J. F., Zeggane, S., Davico, R., Rognone, C., Faucon, J. P., Lizzani, L. (2003): Evaluation of (E)-10-hydroxydec-2-enoic acid as a freshness parameter for royal jelly. *Food Chem.* 80 (1), 85-89. https://doi.org/10.1016/S0308-8146(02)00243-1.

Bărnuţiu, L. I., Mărghitaş, L., Dezmirean, D. S., Mihai, C.M., Bobiş, O. (2011): Chemical Composition and Antimicrobial Activity of Royal Jelly-Review. *Sci. Papers: Anim. Sci. Biotech.* 44 (2), 67-72.

Bogdanov, S. (2016): *The Royal Jelly, Bee Product Science.* http://www.bee-hexagon.net/. Accessed May 20, 2016.

Caparica-Santos, C., Marcucci, M. C. (2007): Quantitative determination of *trans*-10-hydroxy-2-decenoic acid (10-HDA) in Brazilian royal jelly and commercial products containing royal jelly. *J. Apic. Res.* 46 (3), 149-153. https://doi.org/10.1080/00218839.2007.11101386.

CDER, Center for Drug Evaluation and Research (1994): *Validation of Chromatographic Methods.* CMC 3. https://www.fda.gov/downloads/Drugs/Guidances/UCM134409.pdf. Accessed May 20, 2016.

Ferioli, F., Marcazzan, G. L., Caboni, M. F. (2007): Determination of (*E*)-10-hydroxy-2-decenoic acid content in pure royal jelly: A comparison between a new CZE method and HPLC. *J. Sep. Sci.* 30, 1061-1069. https://doi.org/10.1002/jssc.200600416.

Garcia-Amoedo, L. H., Almeida-Muradian, L. B. (2007): Physcochemical composition of pure and adultered royal jelly. *Quim. Nova* 30, 257-259. https://doi.org/10.1590/S0100-40422007000200002.

Genç, M., Aslan, A. (1999): Determination of *trans*-10-hydroxy-2-decenoic acid content in pure royal jelly and royal jelly products by column liquid chromatography. *J. Chromatogr. A* 839, 265-268. https://doi.org/10.1016/S0021-9673(99)00151-X.

Isidorov, V. A., Bakier, S., Grzech, I. (2012): Gas chromatographic–mass spectrometric investigation of volatile and extractable compounds of crude royal jelly. *J. Chrom. B* 885-886, 109-116. https://doi.org/10.1016/j.jchromb.2011.12.025.

Kanelis, D., Tananaki, C., Liolios, V., Dimou, M., Goras, G., Rodopoulou, M. A., Karazafiris, E., Thrasyvoulou, A. (2015): A suggestion for royal jelly specifications. *Arch. Hig. Rada Toksikol.* 66, 275-284. https://doi.org/10.1515/aiht-2015-66-2651.

Kim, J., Lee, J. (2010): Quantitative analysis of trans-10-hydroxy-2-decenoic acid in royal jelly products purchased in USA by high performance liquid chromatography. *J. Apic. Sci.* 54 (1), 77-84.

Krell, R. (1996): Royal jelly. In: Value-added products from beekeeping, FAO (ed.), Rome, Italy, pp. 187-214.

Muñoz, O., Decap, S., Ruiz, F., Arbildua, J., Monasterio, O. (2011): Determination of 10-hydroxy-2-decenoic acid in royal jelly by capillary electrophoresis. *J. Chil. Chem. Soc.* 56 (3), 738-740. https://doi.org/10.4067/S0717-97072011000300004.

Mureşan, I. C., Mărghitaş, A. L., Dezmirean, S. D., Bobiş, O., Bonta, V., Zacharias, I., Mărgăoan, R., Paşca, C. (2016): Quality Parameters for commercialized Royal Jelly. *Bulletin UASVM Anim. Sci. Biotechn.* 73 (1), 1-8. https://doi.org/10.15835/buasvmcn-asb:11630.

Sabatini, A. G., Marcazzan, G. L., Caboni, M. F., Bogdanov, S., Almeida-Muradian, L. B. (2009): Quality and standardisation of Royal Jelly. *J. ApiProd. ApiMed. Sci.* 1 (1), 1-6. https://doi.org/10.3896/IBRA.4.01.1.04.

Zheng, H. Q., Hu, F. L., Dietemann, V. (2011): Changes in composition of royal jelly harvested at different times: consequences for quality standards. *Apidologie* 42 (1), 39-47. https://doi.org/10.1051/apido/2010033.

Zhou, J., Xue, X., Li, Y., Zhang, J., Zhao, J. (2007a): Optimized Determination Method for *trans*-10-Hydroxy-2-Decenoic Acid Content in Royal Jelly by High-Performance Liquid Chromatography with an Internal Standard. *J. AOAC Int.* 90 (1), 244-249.

Zhou, J., Zhao, J., Yuan, H., Meng, Y., Li, Y., Wu, L., Xue, X. (2007b): Comparison of UPLC and HPLC for Determination of trans-10-Hydroxy- 2-Decenoic Acid Content in Royal Jelly by Ultrasound-Assisted Extraction with Internal Standard. *Chromatographia* 66 (3), 185-190. https://doi.org/10.1365/s10337-007-0305-8.

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