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STABILITY OF 10-HDA IN LYOPHILIZED ROYAL JELLY AND IN THE FINISHED PRODUCTS

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professional paper

Summary

This paper aims to examine the stability of lyophilized royal jelly by monitoring the content of its biologically active component, 10-hydroxy-2-decenoic acid (10-HDA). Stability was monitored in the lyophilized royal jelly and in the finished products containing it. Analyses were performed using high performance liquid chromatography (HPLC) with diode array detector. Chromatographic conditions for HPLC with diode array detection were as followed: InterSustain® C18 column, 150 x 40 mm, 5 µm; column temperature, 40 °C; mobile phase was a mixture of methanol: ultrapure water: phosphoric acid (250: 250: 1,25) with a flow rate of 1 mL/min. The detection wavelength was UV 210 nm. The amount of 10-HDA decreased regardless of storage conditions (room temperature and light protection), but the content of 10-HDA in any sample of the lyophilized royal jelly did not decline below the declared 3.5% during the shelf life. During the monitoring period, the analysed finished products also shown a decrease in the amount of 10-HDA, but if stored under recommended conditions, all analysed products contain the amount of 10-HDA in accordance with the declared value.

Keywords: 10-HDA, lyophilized royal jelly, stability

Introduction

Royal jelly has a wide range of positive effects on the human body making it one of the most valuable bee products. Bee glands, such as hypopharyngeal, mandibular, venom and wax, secrete numerous volatile and non-volatile components which are integral part of royal jelly, bee venom, and wax, the only three products that bees synthesize *de novo* (Erler and Moritz, 2016; Sabatini et al., 2009). The largest share in fresh royal jelly takes water, making 62.0 - 68.5% while the rest consists of proteins, lipids, carbohydrates, and to lesser extent vitamins and minerals (ISO, 2016). The main representatives of the proteins are the group of proteins called Major royal jelly proteins (MRJP), which make up the largest part of water-soluble proteins and it is assumed that they are responsible for the growth and development of the queen. A total of 26 amino acids were isolated and identified, of which 8 were essential for human organisms. Lipids are very important components of royal jelly of which more than 80% are free fatty acids while the rest are waxes, steroids, phenols and phospholipids. The most important representative of free fatty acids is 10-hydroxy-2-decenoic acid, which is characteristic of royal jelly, and as such is used as an indicator of quality and authenticity. The carbohydrate composition of royal jelly is similar to that of honey and is mostly composed of glucose and fructose, whose share reaches up to 90% of total carbohydrates, while the remaining share consists of ribose, trehalose, maltose and erlose. The most common vitamins are B vitamins, emphasizing pantothenic acid and niacin, whose shares are 0.095%, while the total share of vitamins A, C, D, and E is about 0.008%. The share of minerals ranges from 0.8% to 3%, and amongst them can be found K, Na, P, S, Ca, Al, Mg, Fe, Cu and Mn (Bogdanov, 2017; Xue et al., 2017; Ramadan and Al-Ghamdi, 2012).

Fresh royal jelly is a yellowish-white, viscous substance with a sour-pungent odour and a sweet-sour taste. This highly beneficial blend has numerous biologically valuable components amongst which 10-HDA stands out as one of the most important (Bogdanov, 2017). 10-HDA has an effect on the activation and modulation of the immune system, acts on the production of collagen and has a mild estrogenic effect, antibacterial, anti-tumour, anti-inflammatory, anti-ulcer and anti-rheumatic effects. Due to the reasons above, royal jelly is often used as a dietary supplement and functional food, as well as an ingredient in cosmetic products (Bogdanov, 2017; Oršolić, 2013; Ramadan and Al-Ghamdi, 2012).

Lyophilized royal jelly has the same biological characteristics as the fresh one due to process of lyophilization where the water is removed from the frozen product in the vacuum, which allows the retention of highly valuable components. Therefore, lyophilization is considered the best way to preserve the quality of royal jelly. During storage, it is extremely important to store lyophilized royal jelly in hermetically sealed containers due to its high hygroscopicity (Bogdanov, 2017; Krell, 1996). The chemical composition of lyophilized royal jelly is not yet legally regulated, but there are recommendations given based on the work (Sabatini et al., 2009; Oršolić, 2013). Although Bogdanov (2017) states storing lyophilized royal jelly for a year at

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refrigerator temperature (3 - 5 °C) or two years at freezer temperature (- 18 °C), manufacturers of lyophilized royal jelly recommend keeping it protected from light and high temperatures, with a shelf life of three years from the date of manufacture.

The aim of this study was to examine the stability of lyophilized royal jelly and finished products containing it when stored at room temperature by monitoring the content of 10-HDA.

Materials and methods

Samples of lyophilized royal jelly

Analyses were performed on 4 samples of lyophilized royal jelly. Stability monitoring of 10-HDA in lyophilized royal jelly samples was performed on samples purchased from two manufacturers in the period from 2011 till 2019. All samples originate from China and are submitted with the appropriate documentation or the certificate of analysis. The samples were stored protected from the light in a constant temperature room (22 ± 1.5 °C) with controlled content of the moisture in the air (47 ± 3 %), in accordance with the manufacturer's instructions. Sample 1, purchased from the first manufacturer, was submitted for analysis in 2011 and several analyses were performed, which were compared with the accompanying manufacturer's analysis certificate. The analyses were performed once a year in the period from 2013 till 2016. Another subsequent analysis was made in 2020 to confirm a further reduction in the share of 10-HDA. The remaining three samples were purchased from another manufacturer. They were analysed twice and the analytical reports were compared with the manufacturer's analysis certificate.

Samples of products with lyophilized royal jelly

Samples of 9 finished products containing lyophilized royal jelly from the same manufacturer, which were stored according to the label, i.e. at the temperature up to 25 °C, protected from light and heat sources, were analysed. The declared value of 10-HDA in the samples ranged from 5 to 20 mg of 10-HDA in 10 ml of the sample. The samples were divided into three groups and analysed at half-life, immediately after the expiration date and one year after.

Chemicals

To prepare the mobile phase methanol HPLC grade (Merck, Germany), 85% phosphoric acid (Sigma-Aldrich, USA) and ultra-pure water obtained by the Letzner water purification system (Hückeswagen, Germany) with an electrical conductivity of up to 0.04 μS/cm and a 10-HDA standard (ChromaDex, USA) purity ≥ 97.4% were used.

HPLC analysis

The analysis of lyophilized royal jelly samples and commercial samples containing it was performed on the Shimadzu HPLC system that includes LabSolution software, two quaternary pumps LC-20ADXR, a column chamber CTO-20AC, a diode array detector SPD-M20A and an autosampler SIL20-ACX. The separation of components was achieved on an InertSustain® C18 column from GL Science, measuring 150 x 40 mm, filled with 5 μm particles.

The mobile phase used for the analysis containing methanol, ultrapure water and phosphoric acid, prepared in a ratio of 250: 250: 1.25, was degassed with a vacuum pump before use. The flow of the mobile phase was 1 ml/min at a column temperature of 40 °C with an injection volume of 5 μl. Spectrum recording was performed in the wavelength range from 190 to 370 nm, while detection was performed at a wavelength of 210 nm. The identification of 10-HDA was performed by comparing the retention times of 10-HDA standard solution and sample solution (Fig. 1) and unquestionable identification was confirmed comparing the specific spectrum of 10-HDA standard solution and sample solution.

The method (Garcia-Amoedo and Almeida-Muradian, 2003) was modified to fit the samples for analysis and the device on which it is applied. For method validation, the latter procedures were performed following good laboratory practice (GLP) and good manufacturing practice (GMP). Linearity, which was tested in the working range from 0.13 to 100 μg/mL which proved to be satisfactory for the expected concentrations of 10-HAD in royal jelly as raw material (1.26 - 2.25 % in fresh and 3.01 - 6.26 % in lyophilized royal jelly) and finished products containing it as an integral component. A limit of detection (LOD) was calculated based on signal-to-noise ratio (3.3:1) and it was 0.048 μg/mL and a limit of quantification (LOQ), calculated based on signal-to-noise ratio (10:1) (ICH, 2020), was 0.145 μg/mL. The precision of the method was tested through repeatability by successive measurements of three different concentrations in one day, giving a relative standard deviation (RSD) <0.46%. The average precision was determined by measuring three different concentrations over three days and RSD values obtained for peak area changes were <1.22%. The accuracy of the method was tested through analytical yield by analysis of three concentrations: 12.5, 50 and 100 μg/mL where analytical yields were obtained: 98.8%, 99.2% and 99.6%, respectively. The robustness of the method was tested by changing two experimental parameters (column

temperature and wavelength of detection), meeting the limits of acceptability for the tested changes (≤15%). Statistical analysis was performed using Microsoft Excel 365 (Microsoft Corp.).

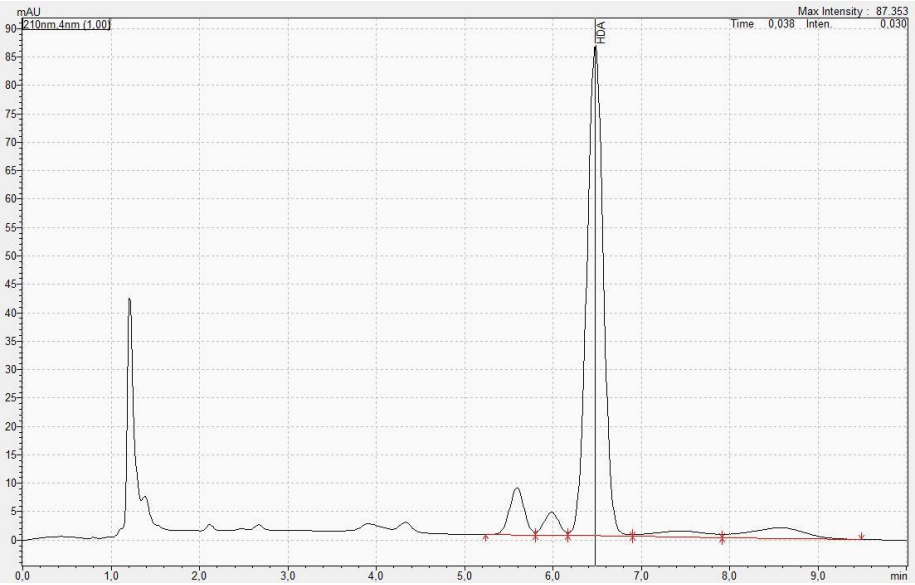

Fig. 1. Chromatogram of the lyophilized royal jelly solution

Results and discussion

Lyophilized royal jelly

The sample of lyophilized royal jelly No. 1 was monitored from the date of production (December 2011) to May 2020 (Fig. 2). This sample was supplied with an analytical certificate stating 4.2% of 10-HDA. During the shelf life, which was declared for 2 years from the date of manufacture, the share of 10-HDA decreased by 2.14%, and in the next year this value falls by 15.95% from the initial value and was at 3.53% of the 10-HDA content, but thus still meets the recommendations for the share of 10-HDA in lyophilized royal jelly. The analysis conducted in the following years showed a further decrease in the share of 10-HDA in the examined sample. Over 10 years, the analyses show a total decrease in the content of 10-HDA in the tested lyophilized royal jelly by 47.62% from the initial value declared on the manufacturer's certificate. Samples 2, 3 and 4 (Fig. 3) were purchased from another manufacturer and supplied with an analysis certificate stating the minimum share of 10-HDA of 5% and the validity period of three years from the date of manufacture. Over 1 year, the share of 10-HDA decreased by <1% and over 2 years to <20%, which is in line with the recommended expiration date.

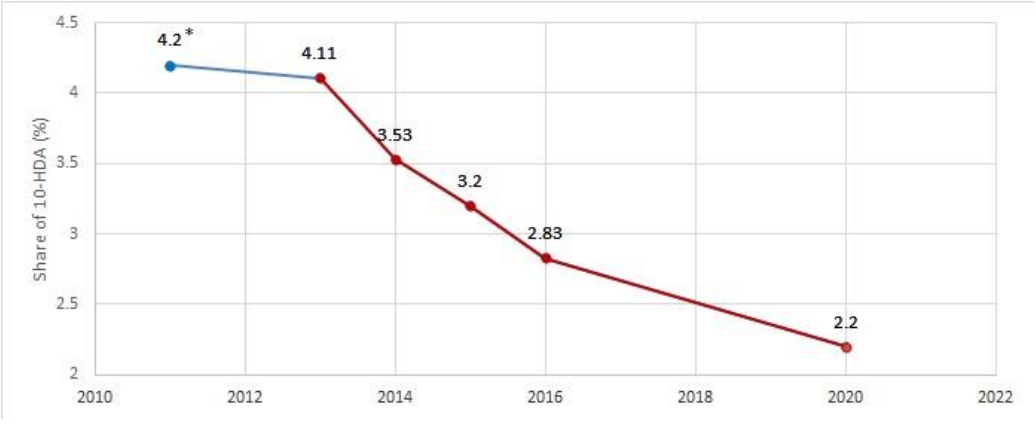

Fig. 2. Monitoring of the share of 10-HDA in sample 1 shown by years (*certified value)

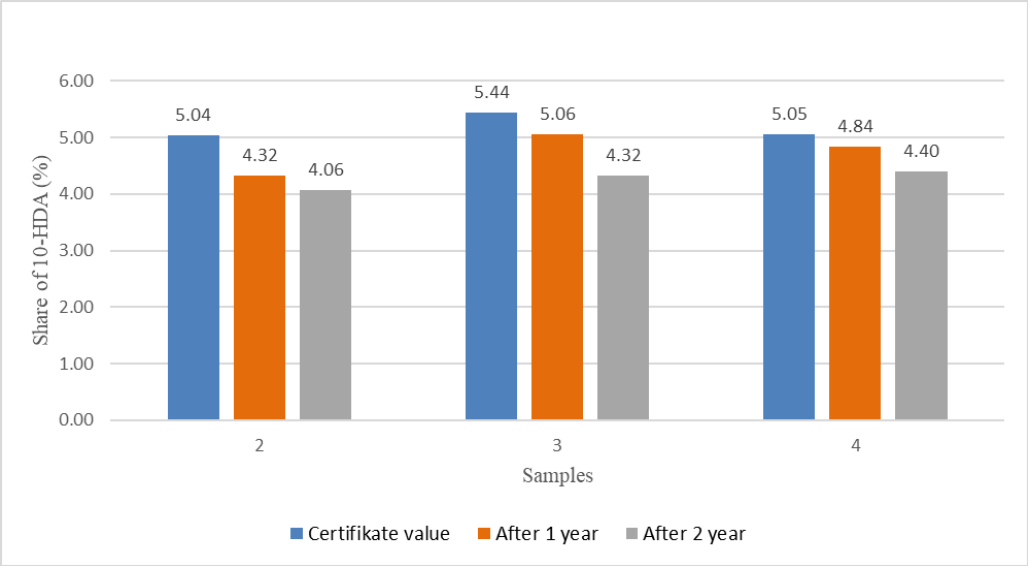

Fig. 3. Demonstration of the reduction of 10-HDA content in lyophilized royal jelly over 2 years

Finished products containing lyophilized royal jelly

Commercially available samples containing lyophilized royal jelly with a declaration stating the value of 10-HDA were analysed. The samples were divided into three groups. The declared value of 10-HDA was compared with the analytically determined share of 10-HDA in the finished product with lyophilized royal jelly one year after production, right after the expiration date and one year after the expiration date. Samples 1, 2 and 3 are the products not yet expired. They show a higher value of 10-HDA than the declared one, on average 33 ± 10.70 %, which is acceptable considering the data indicating a decrease in the value of 10-HDA in the tested samples of lyophilized royal jelly. Products 4, 5 and 6 that are near to the expiration date show the deviation of 11.69 ± 11.94 % from the declared values, which is within legal regulations. Products 7 and 8 expired one year ago show a lower share of 10-HDA than the declared values, 22.89 ± 18.72 %, while product 9 that was also tested one year after the expiration date does not show a significant decrease in the share of 10-HDA (Fig. 4).

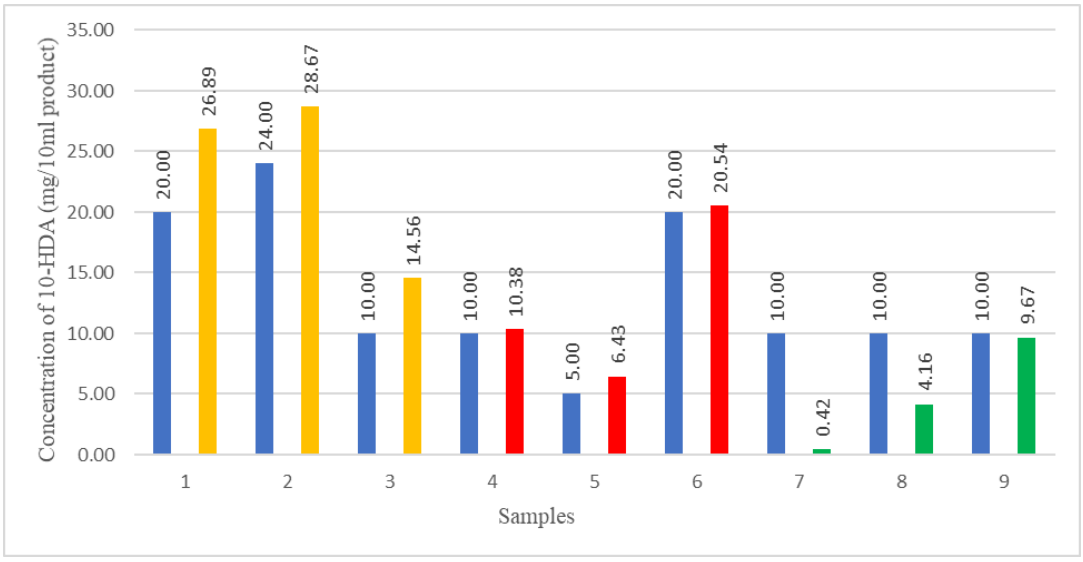

Fig. 4. 10-HDA concentration in commercially available products
(■ declared value, ■ analysis before expiration, ■ analysis after expiration, ■ analysis one year after expiration)

All of the above leads to the question of whether the obtained values of loss of 10-HDA content would be significantly lower if lyophilized royal jelly and finished products containing it were stored in different conditions (in a dark and cool place). Therefore, it would be desirable to continue this pilot study by conducting analyses for the quantitative determination of 10-HDA and to link the results to storage conditions. It would be necessary to store lyophilized royal jelly and finished products in precisely defined conditions of light, temperature (cold chain and room temperature) and humidity, to see if these differences are statistically significant.

Conclusion

The data obtained from the conducted pilot study suggest stability of lyophilized royal jelly under the storage conditions specified by the manufacturer, i.e. at room temperature and protected from a light within the recommended shelf life. The analysis of finished products showed a higher value of 10-HDA than declared in half the shelf life, which is associated with meeting the requirements of the shelf life. According to the legislation, the declared content of 10-HDA must be satisfactory even at the end of the product's shelf life. Therefore, according to the data of reducing the value of 10-HDA in lyophilized royal jelly, it is necessary to increase the share of lyophilized royal jelly as a component of the finished product in order to comply with the regulations, which is (± 10% 10-HDA) at the end of the estimated period.

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