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QUALITY PARAMETERS AND AUTHENTICITY OF ROYAL JELLY

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Review paper

Abstract: Due to specific chemical composition, royal jelly is one of the most valuable bee products used by man since ancient times. Because of the presence of physiologically active compounds that have positive effects on human health, this bee product is primarily used in the pharmaceutical industry, cosmetics and production of healthy food. The present paper aimed to discuss parameters of quality and authenticity of royal jelly, which must satisfy certain national and international standards, in order to be competitive product in the food market. Royal jelly is a creamy yellowish substance produced by the cephalic glands of worker bees (*Apis mellifera* L.) in certain developmental stage, for the purpose of larvae feeding. Considering that this is a highly sought product in the market, whose production is constantly increasing, there is a need for developing new methods of verification of its quality and authenticity. The most important criterion for establishing the quality and authenticity of royal jelly is the determination of 10-hydroxy-2-decenoic acid (10-HDA), whose concentration varies widely, depending on a number of factors. The chemical composition of royal jelly is changed if stored for a long time, or due to the lyophilization. Besides 10-HDA analysis, in recent years, variety of methods for determining the authenticity of royal jelly has been developed, but so far, none of them can be singled out as the most reliable. Improving methods for quality and authenticity assessment, in accordance with established national and international standards, remains a challenge for science.

Key words: royal jelly, quality, geographical authenticity

Introduction

Royal jelly is formed by activity of hypopharingeal glands of worker bees and it represents food of queen bee and larvae, up to three days of age *(Garcia-Amoedo et al., 2007)*. In addition to its use in traditional medicine, many scientific

experiments confirmed positive effect of royal jelly on human health *(Nakaya et al., 2007; Kohno et al., 2004; Morita et al., 2012; Park et al., 2011)*, causing that this product has become very desirable and highly appreciated in the market. Royal jelly is a creamy yellowish substance, which is used fresh or dried (lyophilized), mainly as a dietary supplement while recovering from an illness or during severe stress. The chemical composition of this product is complex, consisting of proteins, amino acids, sugars, lipids and vitamins *(Bărnuţiu et al., 2011)*. In the industry, royal jelly is primarily used for cosmetics, medicines or food products *(Daniele and Casabianca, 2012)*. Due to the high demands in the market, it has reached a high price and became the subject of adulteration. Substances used for adulteration can not be detected by organoleptic methods, and include adding of corn starch, yogurt, egg whites, condensed milk with propolis, unripe bananas and water *(Garcia-Amoedo et al., 2007)*. Today, the largest amount of royal jelly is produced in China, so thousands of tons of this product is placed on the world market per year, at affordable prices *(Daniele and Casabianca, 2012)*. While some countries such as Brazil, Bulgaria, Japan and Switzerland have developed national standards for royal jelly quality, international standards for quality and authenticity of this product do not exist yet *(Ramadan et al., 2012)*. Due to the liberalization of the global food market and increasingly strong competition of producers in the conquest of new markets, it is necessary to develop international standards, which would regulate the issue of royal jelly quality and authenticity, to achieve greater security primarily in terms of food safety and origin.

The Quality of Royal Jelly

According to *Grunert (2005)*, an objective assessment of the quality of products includes physical-chemical characteristics of the product, which are analyzed by experts, and the subjective quality impression about the product, indicated by the average consumer obtained when eating. Scientific research related to the impression of consumers has not been conducted yet but many studies has been devoted to the physical, chemical and sensory properties of this product. Physico-chemical properties of this natural substance, which are most frequently analyzed are as follows: sugar composition, moisture, proteins and 10-HDA *(Daniele and Casabianca, 2012)*. Quality parameters include content and composition of sugars, water and proteins content, as well as sensory properties (smell, taste, color), while the freshness of royal jelly is determined based on the concentration of the 10-HDA, glucose oxidase and values of furosine. More than 80% of sugars are fructose and glucose, and in addition to that, there are 12 more sugars present in small amounts - galactose, mannitol, maltose, maltulose, turanose, trehalose, palatinose, isomaltose, gentiobiose, melezitose, maltotriose,

and erloza *(Daniele and Casabianca, 2012)*. The same authors state that these sugars can serve as markers of geographical authenticity of the product. The water content is quite constant and no greater than 60%. The largest part of dry matter is made of proteins, wherein there are considerable differences in protein content between fresh and lyophilized royal jelly. Namely, according to *Popescu et al. (2009*), the fresh royal jelly has lower protein content (7.7 mg/ml) compared to a lyophilized royal jelly (19.8 mg/ml). *Liming et al. (2009)* showed that the average content of total amino acids amounts to 111.27 mg/g, wherein, the most abundant is the aspartic acid with concentration of approximately 21.04 mg/g. Significantly smaller amount of glutamic acid (12.29 mg/g), lysine (10.05 mg/g) and leucine (9.53 mg/g) has been found. The same survey showed that with the increase of temperature and storage time, the concentration of glutamine, glycine and leucine slightly increased while the concentration of lysine, histidine and methionine decreased. Due to this fact, the same authors suggested that amino acids could be parameters for quality evaluation. Sensory properties, which also indicate quality, include: colour, aroma, taste, odour and viscosity. The color varies from white to pale-yellow (when stored for long period it changes colour to yellow), and the odour and taste are sour *(Popescu et al., 2008)*. Viscosity is an important parameter of quality and varies depending on the water content and the storage conditions. According to *Ramadan et al. (2012)*, the increase in viscosity is related to the increase of nitrogen compounds insoluble in water and dicrease of soluble nitrogen and free fatty acids, which occurs when preserving royal jelly at room temperature. The most analyzed acid is 10-hydroxy-2-decenoic acid (10-HDA) indicating freshness and authenticity of royal jelly if it is contained over 1.8% *(Antinelli et al., 2003)*. By studying qualitative and quantitative properties of this substance, *Antinelli et al. (2003)* concluded that, at room temperature, there is a rapid degradation of the 10-HDA after the third month, which can be detected by sensory observation, whereas in samples stored at very low temperatures this was not the case, or the content of this acid did not significantly decreased during storage. Hence, in practice, royal jelly is considered fresh if not older than 3 months *(Antinelli et al., 2003)*. Degradation process of 10-HDA stops at -18°C, whereas at temperatures above 4°C, 20-50% of its content is lost. However, the same authors stated that the determination of 10-HDA could not be used as a reliable indicator of freshness. According to *Barnutiu et al. (2011)*, the concentration of 10-HDA in royal jelly is about 1.5-2.0%, and this parameter is an internationally recognized standard for determining royal jelly quality, which directly impacts the price in the international market. According to *Sabatini et al. (2009)*, the concentration of the enzyme glucose oxidase can indicate the freshness of royal jelly. Namely, time and storage temperature affect this enzyme content, whereby at the temperature of 20°C after one month, its concentration is significantly reduced, and a year after,

enzyme is completely degraded. Another potential indicator of royal jelly freshness is furosine *(Marconi et al., 2002; Messia et al., 2005)*. In a fresh royal jelly, furosine content is low (0-10 mg/100 g of protein), but it is significantly increased with prolonged storage at higher temperature. Furosin content can be increased up to 500 mg/100g of protein after 18 months of storage at room temperature, or up to 50 mg/100 g of protein after 18 months of storage at 4°C *(Sabatini et al., 2009)*.

Adulteration of royal yelly

Royal jelly is usually adulterated by adding yogurt, egg whites, water and corn starch, which can be detected by changes of the physico-chemical characteristics. Namely, adding more than 25% of these substances in the royal jelly, leads to increase in moisture, followed by lipid, protein, and 10-HAD reduction, as well as insolubility in basic medium. Samples adulterated by mentioned substances in concentrations of about 10% can be detected by a slight increase in moisture, reduce in lipids, proteins and 10-HDA, as well insolubility in basic medium. Royal jelly adulterated by condensed milk in an amount of over 10% can be detected based on the increase of lipids and decrease of humidity, protein and 10-HDA content *(Garcia-Amoedo et al., 2007)*. Adulterated royal jelly is detected by routine analysis of quality and authenticity. To determine geographical authenticity, the easiest and the most accessible method is to ascertain the pollen spectra in this bee product, which requires the engagement of highly skilled people who know not only the pollen morphology, but also the apiflora and vegetation in the area of royal jelly origin. In addition to pollen analysis, other methods for determining the quality and authenticity of royal jelly were developed (Table 1).

Table 1. Some methods for determination of royal jelly quality and authenticity (*Sabatini et al., 2009*)

Parameter	Method
Water content	Determined by freeze-drying (*Messia et al., 2005*), Karl Fischer (*Ferioli et al., 2007*), vacuum oven, dessication (*Garcia-Amoedo and Almeida-Muradian, 2002, 2007*)
Total protein	Nitrogen determined with the Kjeldahl method (*Lercker et al., 1992-93*), free amino acids determined by ion chromatography (*Boselli et al., 2003*)
Carbohydrates	Determined by gas (*Lercker et al., 1992-93*) or liquid chromatographies (*Sesta, 2006*)
Lipids	Determined as free and total organic acids by gas chromatography (*Lercker et al., 1992-93*) or as total lipids, by solvent extraction (*Karaali et al., 1988*)
10-HDA	Determined by HPLC (*Bloodworth i sar., 1995; Genc and Aslan, 1999*)
Minerals	Determined by atomic absorption (*Benfenati et al., 1986*)
Acidity	Titration method (*Serra-Bonvehi, 1992*)
Sediment analysis	Microscopical analysis (*Ricciardelli D'Albore, 1986*)
Furosine	(*Marconi et al., 2002*)

Also, in addition to developing methods, it is necessary to establish international standards for determination of royal jelly quality that comes to market (Table 2).

Table 2. Proposed criteria for royal jelly composition (*Bogdanov and Gallmann, 2008*)

Royal jelly	Fresh(*)	Lyophilized
Water	60-70	<5
Lipids	3-8 (4-8)	8-19
10-HAD	>1,4 (1,4-6)	>3,5
Proteins	9-18	27-41
Fructose-glucose-sucrose	7-18 (11-23)	-
Fructose	3-13	-
Glucose	4-8	-
Sucrose	0,5-2,0	-
Ash	0,8-3,0	2-5
pH	3,4-4,5 (3,5-4,1)	3,4-4,5
Acidity	3,0-6,0	-
Furosine	<50* (-)	-

In its bylaws, the Republic of Serbia did not especially defined royal jelly quality in the market, but in the Regulations on the quality of the honey, products based on honey and other bee products, the term of royal jelly is defined, as well as form of sale (original form, stabilized or lyophilized), way of storage (in the dark, hermetically sealed glass vessels at temperatures ranging from -6 to -4^{o}C) as well as the basic requirements in terms of its physical and chemical characteristics. This

implies that royal jelly contains no more than 70% of water and 30% of dry matter and at least 11% of proteins *(Službeni list SCG br. 448/1, 2003)*.

Royal Jelly authenticity

The authenticity of food products is linked to the area of their origin, and that area is characterized by certain environmental conditions as well as mode of production. Raw materials and food products at the market with known geographic origin are valued more because they are considered of higher quality and healthier. The reason for this is not a small number of adulterated products, for example wine, olive oil and dairy products, which is very detrimental to the economy and reputation of the country of origin *(Anklam, 2001)*. The authenticity of royal jelly production, is routinely estimated by concentration of 10-HDA *(Bogdanov, 2008)*, however, this method can not be used for determining the geographical origin. Methods for determining the geographical authenticity of royal jelly include polen spectra identification and determination of stable isotopes of C and N. The principle of determining the botanical origin of honey, based on the type and percentage of pollen grains, also can be applied in the case of royal jelly. Research of *Dimou et al. (2007)* have shown that the palynological analysis of royal jelly can be used as a reliable indicator of geographic origin, considering that in the samples analyzed, a correlation between the pollen spectra and vegetation in the area of royal jelly origin exists. Since the analysis of stable isotopes of carbon can be used to determine the honey authenticity, in terms of its possible adulteration with sugar of C4 plants, similar principle can be applied in the case of determining the authenticity of royal jelly production. Namely, by analysing the stable isotopes of C and N, *Stocker et al. (2006)*, concluded that on the basis of this ratio, genuine from adulterated jelly can be distinguished, but it is necessary to continue research related to this issue, as in the case of determining honey geographical authenticity. Namely, for the determination of the region of origin, it is necessary also to analyze isotopes of other elements, such as strontium, sulfur, oxygen, and hydrogen.

Conclusion

Due to the its positive impact on human health, royal jelly is a bee product that is increasingly demanded on European and world markets. However, it is necessary to reliably determine the quality and authenticity of royal jelly that is sold to consumers. This could be performed by analyzing the following parameters: the contents of 10-HDA, sugar, water, proteins and fats as well as on the basis of pollen analysis. Methods for accurately determining adulterated royal

jelly and its geographical origin are still developing and there are attempts to establish international standards regarding this issue.

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Parametri kvaliteta i autentičnosti matičnog mleča

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Rezime

Zbog specifičnog hemijskog sastava, matični mleč predstavlja jedan od najdragocenijih pčelinjih proizvoda koje čovek koristi od davnina. Zbog utvrđenih pozitivnih efekata na zdravlje ljudi, usled prisustva fiziološki aktivnih jedinjenja, ova se materija koristi prevashodno u farmaceutskoj industriji, kozmetici i proizvodnji tzv. zdrave hrane. Cilj rada je predstavljanje parametara kvaliteta i autentičnosti matičnog mleča, koji moraju biti u skladu sa nacionalnim i međunarodnim standardima kako bi se mleč plasirao na tržište hrane. Matični mleč je žućkasta kremasta supstanca koja nastaje radom cefaličnih žlezda pčela radilica (*Apis mellifera* L.) u određenom stadijumu njihovog razvića radi ishrane larvi. S obzirom da je u pitanju proizvod koji je veoma tražen na tržištu, i čija se proizvodnja konstantno povećava, javila se neophodnost razvijanja novih i usavršavanja postojećih metoda pomoću kojih je moguće ustanoviti kvalitet mleča i njegovu autentičnost. Najvažniji kriterijum za procenu kvaliteta i autentičnosti ovog pčelinjeg proizvoda je određivanje sadržaja 10-hidroksi-2-decenoične kiseline (10-HDA), čija koncentracija veoma varira u zavisnosti od većeg broja faktora. Naime, hemijski sastav mleča se menja pri dužem čuvanju kao i pri liofilizaciji. Osim određivanja sadržaja 10-HDA, poslednjih godina se unapređuju metode za utvrđivanje autentičnosti mleča ali za sada, nijedna se ne može izdvojiti kao najbolja. Usavršavanje ovih metoda uz uspostavljanje međunarodnih i nacionalnih standarda kvaliteta i autentičnosti, ostaje izazov za nauku.

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