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REVIEW ARTICLE

# Quality and standardisation of Royal Jelly

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## Introduction

Given the exceptional biological properties attributed to it, royal jelly (RJ) has considerable commercial appeal and is today utilised in many sectors, ranging from the pharmaceutical and food industries to the cosmetic and manufacturing sectors. This has resulted, among other things, in large-scale importation in countries where production is insufficient to meet domestic demand. Research capabilities thus need to be reinforced to permit both a reliable qualitative and quantitative evaluation of the different components and the implementation of analytical tests on commercially available products – RJ on its own or as an additive to new or traditional products – also for the purpose of identifying possible adulteration.

No official data exist about the RJ market (Grillenzoni, 2002), but China is unanimously acknowledged as being the leading world producer and exporter of RJ, which it sells at highly competitive prices. Chinese production of RJ is estimated as 2 000 t/year (a quantity that represents over 60% of production worldwide), almost all of which is exported to Japan, the United States and Europe. Other countries like Korea, Taiwan and Japan are important producers and also exporters. Elsewhere in the world, RJ is produced mainly in Eastern Europe and to a lesser extent in Western Europe and in America: Mexico, in particular, is quite a big producer.

Numerous studies have been dedicated to RJ since as far back as the late 19$^{th}$ century (Planta, 1888; Lercker, 2003). However, it is difficult to bring together the data collected by different authors into an organic whole, as the data themselves are not always comparable due to the lack of homogeneity among the materials used, the different sampling procedures and production conditions. Additional complicating factors are the multiplicity of experimental conditions, as well as the diversity of the analytical methods used and their continual evolution.

Knowledge of the composition of recently produced RJ is essential in order to define a standard composition, evaluate the quality of commercial products and detect the presence of RJ in other products which containing it.

At present some countries, like Switzerland (Bogdanov *et al.*, 2004), Bulgaria, Brazil (Brasil Leis e decretos, 2001) and Uruguay have defined national standards for this product. A group of the International Honey Commission is dealing presently with royal jelly standardisation.

## Studies on royal jelly quality

In the 1980s a workgroup was formed in Italy which has devoted much effort to the study of RJ (Lercker *et al.*, 1981; Lercker *et al.*, 1982; Lercker *et al.*, 1984a; Lercker *et al.*, 1984b; Lercker *et al.*, 1985; Lercker *et al.*, 1986; Vecchi *et al.*, 1988; Lercker *et al.*, 1993; Antinelli *et al.*, 2003; Boselli *et al.*, 2003; Lercker, 2003). The data presented in this article refer to the results obtained by the Italian group cited, completed by findings of researchers from other countries.

Samples of recently produced, commercial grade RJ directly gathered from specialised beekeeping facilities located in different Italian regions were used both for the purpose of developing methods and conducting the analyses. The same samples were used to assess the changes occurring in RJ during storage.

Apart from this project, other studies were also carried out. Most of them were concerned with RJ authenticity. RJ adulteration is the most important quality problem. Adulteration by the nursing jellies for worker and drones is improbable because of the very little amounts available for harvest. Adulteration with honey is more probable, causing an increase of the sugar values, the other values being lowered (Serra Bonvehi, 1991). The most important quality criteria for RJ adulteration is 10-Hydroxy-2-Decenoic Acid (HDA). However, the composition limits, reported in the literature are very broad. 10-HDA content decreases with storage of RJ (Antinelli *et al.*, 2003). This decrease is higher in honey containing RJ (Matsui, 1988). Thus, the determination of all fatty acids, as carried out in the Italian studies (Lercker *et al.*, 1981; Lercker *et al.*, 1993), might be the better approach that the determination of 10-HDA only.

It was recently reported that authenticity of RJ production can be determined by measuring of the ratios stable isotopes of the elements C and N (Stocker, 2003). The authenticity of production can be measured by determining the fatty acid composition of RJ (Howe *et al.*, 1985; Lercker *et al.*, 1993).

The geographical authenticity can be determined also by pollen analysis (Ricciardelli d'Albore *et al.*, 1978; Ricciardelli d'Albore, 1986). The $^{87}Sr/^{86}Sr$ ratios indicate also the geographic regions of the samples (Stocker, 2003).

The amount of pollen, as well as visible wax and larvae particles should be minimal. RJ has relatively low concentration of bacteria (Serra Bonvehi and Escola Jorda, 1991).

The parameters investigated concerned in the above mentioned studies concern the organoleptic characteristics and physicochemical properties as well as the following composition factors:

| | |
|---|---|
| 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; Garcia-Amoedo and Almeida-Muradian, 2007). Free amino acids determined by ion chromatography (Boselli *et al.*, 2003) |
| Carbohydrates | Determined by gas (Lercker *et al.*, 1993) 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.*, 1986; Garcia-Amoedo and Almeida-Muradian, 2007) |
| 10-HDA | Determined by HPLC (Bloodworth *et al.*, 1995; Genc and Aslan, 1999; Koshio and Almeida-Muradian, 2003; Garcia-Amoedo and Almeida-Muradian, 2003, 2007; Pamplona *et al.*, 2004) |
| 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) |

**Contamination**

There are very few studies concerning the possible contamination of RJ. The content of RJ contaminants, compared to other bee products, is relatively low (Fleche *et al.*, 1997). Recently, the problem of honey and RJ contamination by antibiotics has arisen. Although most studies concern residues in honey, antibiotic use in the colony can contaminate also royal jelly (Matsuka and Nakamura, 1990). On the other hand, experience has shown that RJ residue analysis is difficult and that old analysis methods are questionable. There are very few publications on antibiotic residues in RJ, mainly on chloramphenicol (Dharmananda, 2003; Reybroeck, 2003; Calvarese *et al.*, 2006). The first two papers do not report details, only in the last publication details on the methods and the contamination levels are given. The presence of chloramphenicol (CA) was detected in 29 out of 35 tested samples imported in Italy, the concentrations ranging from 0.6 µg/kg to 28 µg /kg, with an average content of 6.1 µg/kg. As antibiotics are not allowed for use in beekeeping, there is no MRL for honey or other bee products in the European Union. For CA in honey the EU has established an MPRL of 0.3 µg/kg. By using method developed by Calvarese and coworkers (Calvarese *et al.*, 2006) this MPRL can also be used for royal jelly.

## Composition and quality criteria for royal jelly

**Organoleptic description and physical characteristics**

RJ appears as a whitish substance with a gelatinous consistency, often not homogenous due to the presence of undissolved granules of varying size. It has a distinctively sharp odour and taste.

It is partially soluble in water and highly acidic (pH 3.4-4.5) with a density of 1.1 g/mL (Lercker, 2003).

**Main components**

The composition of the main constituents of RJ, proteins, carbohydrates and lipids is reported in the literature (Takenaka and Echigo, 1980; Bonomi *et al.*, 1986; Pourtallier *et al.*, 1990; Lercker, 2003, Garcia-Amoedo and Almeida-Muradian, 2007).

The values obtained by the various authors are fairly in agreement, notwithstanding the high variability displayed by some parameters (sugars and lipids). It should be kept in mind that the reported findings refer to different number of samples taken in different places and at different times of production and that different methods of sampling and analysis were used. Moreover, RJ is naturally inhomogeneous.

Our own analyses of RJ samples of different geographical origins showed no differences in composition such as to distinguish one product from another.

It may similarly be affirmed that environmental conditions do not significantly influence the main components.

**Water**

Water content shows to be fairly uniform, greater than 60%, and with an activity ($a_w$) above 0.92, in spite of which RJ displays considerable microbial stability. The constancy of the moisture content is basically assured, inside the hive, by the continuous provision of fresh supplies of this substance by nurse bees, by the natural hygroscopicity of RJ and the entire colony's efforts to maintain a level of ambient moisture; moreover the non solubility of some compounds can explain the variations in water content.

**Proteins**

From a quantitative viewpoint, proteins (27-41%) represent the most important portion of the dry matter of RJ.

The amino acids present in the highest percentages were proline, lysine, glutamic acid, β-alanine, phenylalanine, aspartate and serine (Boselli *et al.*, 2003). The concentration of series D amino acids was below the detection limit of the method (0.1mg/g of RJ) in all samples.

The study aimed to assess how this parameter evolved during storage of the product. No significant changes were observed in the overall concentration of free amino acids in RJ stored at 4°C for 10 months. However, in the same samples stored at room temperature, the proline and lysine content showed an increase in the first three months and after 6-10 months decreased to levels slightly lower than those in the control samples. This suggests that, in favourable temperature conditions, a proteolytic enzymatic activity continues to occur over time.

**Carbohydrates**

On average this portion accounts for 30% of the dry matter of RJ. However, while the components are highly constant in qualitative terms, considerable variability may be observed from a quantitative standpoint.

As in honey, the monosaccharides fructose and glucose are the main sugars. They often account for over 90% of the total sugars and, of the two, fructose is prevalent. Sucrose is always present but often in highly variable concentrations. It is also possible to find oligosaccharides such as trehalose, maltose, gentiobiose, isomaltose, raffinose, erlose, melezitose; though present in very small concentrations they are useful for identifying a characteristic pattern, which is comparable to that of honey and in some cases indicative of the genuineness of the product.

**Lipids and 10-Hydroxy-2-decenoic acid (10-HDA)**

This fraction is likewise present in fairly modest, variable concentrations (8-19% of dry matter), but no doubt represents the most important of RJ components.

The lipid portion in fact consists primarily of organic acids (80 -90%), most of which free, with a rather unusual structure rarely encountered in nature: they are in fact mono- and dihydroxy acids and dicarboxylic acids with 8 and 10 carbon atoms, which show a characteristic arrangement (Lercker *et al.*, 1992-93).

Hydroxy acids with 10 carbon atoms (10-hydroxydecenoic and 10-hydroxy-2-decenoic acid) above all can be found in high concentrations. Not only may they be ascribed a role as a marker component, but they have also been identified as responsible for important biological activities tied to the development strategies of the colony (Wu *et al. 1991)*

The identification of this fraction – in particular as regards the pattern and quantitative analysis of free organic acids – is believed to represent the criteria of choice for defining the genuineness of RJ and the presence of RJ in other products, be they foods or cosmetics (Caboni *et al.*, 1994). The analyses we performed showed that the composition remained stable for as long as 2 years, regardless of whether the samples were stored at 4°C or at room temperature.

A recent study (Antinelli *et al.*, 2003) showed a 0.4 and 0.6% reduction in 10-hydroxy-2-decenoic acid in two RJ samples stored at room temperature for 12 months. It is difficult to evaluate such a reduction in a sample in the control phase. Moreover it is difficult to use 10-hydroxy-2-decenoic-acid decrease as a freshness marker because their variable amount on fresh RJ. Both HPLC and electrophoretic analysis of 10-HDA showed that samples of RJ from extra-european origin contain smaller amount of this compound; this evidence was confirmed measuring total lipids after organic extraction (Ferioli *et al.*, 2007).

**Minerals**

Ash content represents 0.8-3% of RJ (fresh matter) (Messia *et al.*, 2003; Garcia-Amoedo and Almeida-Muradian, 2007). The major elements are, in descending order: K, Ca, Na, Mg, Zn, Fe, Cu and Mn (Nation and Robinson, 1971; Ivanov and Chervenakova, 1985; Benfenati *et al.*, 1986), present in specific ratios such as K/Na and Ca/Mg.

The hypotheses regarding the quantitative presence of these metals have focused on factors outside the colony (environment,

procurement of food, production period) and to some extent internal factors (biological factors tied to the bees).

**Authenticity**

The main quality factors of RJ have been described and studies have revealed the importance of the lipid fraction as a marker and hence a criterion by which to determine the product's genuineness. Presently, 10-HDA is mostly used for routine testing of RJ authenticity. However, the concentration of this acid varies in wide limits. Further studies are necessary to determine whether the determination of the stable isotopes of the elements C and N (Stocker, 2003) is a promising approach for the determination of the authenticity of production. Adulteration by honey results in a general diminution of proteins and lipids and a relative increase of sugars (Serra-Bonvehi, 1991).

Adulteration with more than 25% of yoghurt, egg white, water and corn starch slurry can be detected by the enhancement of moisture, diminishing in lipid, protein and 10-HDA content as well as the insolubility in alkaline medium. (Garcia-Amoedo and Almeida-Muradian, 2007).

Furthermore, microscopic analyses of RJ sediment, applied according to the basic principles of melissopalynology (Louveaux *et al.*, 1978; Ricciardelli, 1986) and in particular the identification of the pollens it contains, make it possible to define the geographical origins of the product and detect mixtures where they occur. Pollen identification is made easier by the fact that only a few countries actually produce RJ and specialists are capable of formulating their respective characteristic pollen associations.

Another promising parameter for the evaluation of RJ authenticity is the presence of apalbumin (Simuth *et al.*, 2004). This marker, if confirmed by further research, could gain high importance.

**Freshness definition**

Another fundamental aspect lies in the possibility of defining a parameter of RJ freshness.

It has been noted that the macroscopic composition of RJ is fairly stable on the whole but also variable, above all as far as certain components are concerned. Thus it is not a suitable parameter for defining product freshness.

For the latter purpose, experiments were conducted on RJ samples stored at 4 and 20°C over a period of 24 months to assess changes in the content of the enzyme glucose oxidase. The results obtained showed that the enzyme contained in RJ is influenced both by storage temperature and time. At 20°C it had decreased significantly after one month and degraded completely after one year. Even at 4°C there was an evident, albeit modest, reduction in the enzyme.

The determination of glucose oxidase is analytically very simple and thus within the capabilities of all laboratories. This method could be used to evaluate the product's freshness; however, further investigation must first be conducted into the natural variability of this component in the fresh product (Boselli *et al.*, 2002). Marconi *et al.* (2002) quoted several experiments were performed to evaluate the possibility of using furosine content as a marker for RJ freshness.

The value of furosine, a product of Maillard's reaction, proved very low (from 0 to 10 mg/100g of protein) in freshly produced RJ samples (Messia *et al.*, 2003) but increased over time and in relation to temperature. Specifically, the content rose to as high as 500 mg/100g of protein after 18 months' storage at room temperature and 50 mg/100g at 4°C. Samples taken from store shelves showed values ranging from 40 to 100 mg/100g protein. By contrast, freeze-dried RJ showed strong tendency to form furosine during storage (Messia *et al.*, 2005).

**Table 1:** Royal Jelly Composition

| | **Fresh** | **lyophilized** |
|---|---|---|
| Water % | 60 – 70 | < 5 |
| Lipids % | 3 – 8 | 8 – 19 |
| 10-Hydroxy-2-decenoic acid (10-HDA) % | > 1,4 | > 3,5 |
| Protein % | 9 – 18 | 27 – 41 |
| Fructose + glucose+ sucrose % | 7 – 18 | - |
| Fructose % | 3 – 13 | - |
| Glucose % | 4 – 8 | - |
| Sucrose % | 0,5 – 2,0 | - |
| Ash % | 0,8 - 3,0 | 2 – 5 |
| *pH* | 3,4 - 4,5 | 3,4 – 4,5 |
| Acidity (ml 0.1N NaOH/g) | 3,0 - 6,0 | - |
| Furosine (mg/100g protein) | < 50* | - |

Table 1 shows some data from literature that could be used as a preliminary proposal for fresh and lyophilized royal jelly standards. Data come from many countries investigations and regulations but for the establishment of a paper general standard, further investigation is needed.

Given the product's high water content, the composition values are also proposed for the freeze-dried sample. This enables a more direct comparison of data; plus RJ is also marketed in this form.
Although the overall analytic data confirm that exposure to a temperature of 4°C causes no alterations in RJ composition, recently it was also shown that only storage of RJ in frozen state prevents decomposition of biologically active RJ proteins and thus RJ should be frozen as soon as it is harvested (Li *et al.,* 2007).

The next steps should be: 1. Validate the respective methods of analysis 2. Use the method and create a royal jelly standard, based measurements on royal jelly samples produced in different countries. To this end, the UNI (Italian certification body) is presently drawing up standards for these methods based on the available know-how.

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# 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 Chemistry* 80: 85-89.

BENFENATI, L; SABATINI, A G; NANETTI, A (1986) Composizione in sali minerali della gelatina reale. *Apicoltura* 2: 129-143.

BLOODWORTH, B C; HARN, C S; HOCK, C T; BOON, Y O (1995) Liquid chromatographic determination of trans-10-hydroxy -2-decenoic acid content of commercial products containing royal jelly. *Journal of AOAC International* 78 (4): 1019-1023.

BOGDANOV, S; BIERI, K; GREMAUD, G; IFF, D; KÄNZIG, A; SEILER, K; STÖCKLI, H; ZÜRCHER, K (2004) *Swiss Food Manual: Gelée Royale*. Bienenprodukte, BAG (Swiss Federal Office for Public Health); Berne.

BONOMI, A; MARLETTO, F; LUCCELLI, L; ANGHINETTI, A; BONOMI, A; SABBIONI, A (1986) Composizione chimico-bromatologica della gelatina reale in rapporto alla flora nettarifera e pollinifera. *La Rivista della Società Italiana di Scienza dell'Alimentazione* 15(1-2): 53-62.

BOSELLI, E; CABONI, M F; LERCKER, G; MARCAZZAN, G L; SABATINI, A G; BAGGIO, A; PRANDIN, L (2002) Valutazione di produzioni apistiche: gelatina reale e cera. *In* Atti del convegno finale del Progetto Finalizzato AMA "*Il ruolo della ricerca in apicoltura*". Istituto Nazionale di Apicoltura; Bologna; pp. 321-329.

BOSELLI, E; CABONI, M F; SABATINI, A G; MARCAZZAN, G L; LERCKER, G (2003) Determination and changes of free amino acids in royal jelly during storage. *Apidologie* 34: 1-7.

BRASIL, LEIS E DECRETOS (2001) Instrução Normativa nº 3, de 19 de janeiro de 2001. Regulamentos técnicos de identidade e qualidade de apitoxina, cera de abelha, geléia real, geléia real liofilizada, pólen apícola, própolis e extrato de própolis. http://extranet.agricultura.gov.br/sislegis-consulta/consultarLegislacao.do?operacao=visualizar&id=1798

CABONI, M F; SOLAZZO, P; LERCKER, G (1994) Proposta di un metodo per la valutazione quali-quantitativa della gelatina reale nei prodotti alimentari. *Bollettino dei Chimici Igienisti* 45: 195-202.

CALVARESE, S; FORTI, A F; SCORTICHINI, G; DILETTI, G (2006) Chloramphenicol in royal jelly: analytical aspects and occurrence in Italian imports. *Apidologie* 37: 673-678.

DHARMANANDA, S (2003) Traces of Chloramphenicol in Chinese Bee Products: Origin, Development and Resolution, 1-5. www.itmonline.org/arts/bees.htm

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. *Journal of separation Science*: 30, 1061 – 1069.

FLECHE, C; CLÉMENT, M C; ZEGGANE, S; FAUCON, J P (1997) Contamination of bee products and risks for human health: the situation in France. *Revue Scientifique et Technique de l'Office International des Epizooties* 16: 609-619.

GARCIA-AMOEDO, L H; ALMEIDA-MURADIAN, L B (2002) Comparação de metodologias para a determinação de umidade em geléia real. *Química Nova* 25: 676-679.

GARCIA-AMOEDO, L H; ALMEIDA-MURADIAN, L B (2003) Determination of trans 10-hydroxy-2-decenoic acid (10-HDA) in Brazilian royal jelly. *Ciencia y tecnología de alimentos* 23, Supl.: 62–65.

GARCIA-AMOEDO, L H; ALMEIDA-MURADIAN, L B (2007) Physicochemical composition of pure and adulterated royal jelly. *Química Nova* 30(2): 257-259.

GENC, 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. *Journal of Chromatography A* 839(1-2): 265-268.

GRILLENZONI, F G (2002) Prodotti dell'alveare: aspetti economici e di mercato. *In* Sabatini, A G; Carpana, E (a cura di) *I prodotti dell'apicoltura*. LEADER II (Galizia, Portogallo, Italia); Portodemouros, Spagna; pp. 99-105.

HOWE, S R; DIMICK, P S; BENTON, A W (1985) Composition of freshly harvested and commercial royal jelly. *Journal of Apicultural Research* 24: 52-61.

IVANOV, T S; CHERVENAKOVA, I (1985) La quantité de macro et micro éléments contenus par le miel, la gelée royale et le pollen. *In XXX Congres International d'Apiculture* Nagoya, Apimondia, Bucarest

KOSHIO, S; ALMEIDA-MURADIAN, L B (2003) Aplicação da CLAE para determinação do ácido 10-hidróxi-2-decenóico (10-HDA) em geléia real pura e adicionada a mel brasileiro. *Química Nova* 26(5): 670-673.

KARAALI, A; MEYDANOGLU, F; EKE, D (1988) Studies on composition, freeze-drying and storage of Turkish royal jelly. *Journal of Apicultural Research* 27(3): 182-185.

LERCKER, G (2003) La gelatina reale: composizione, autenticità ed adulterazione. *In* Atti del Convegno "*Strategie per la valorizzazione dei prodotti dell'alveare*". Università degli Studi del Molise; Campobasso; pp. 67-81.

LERCKER, G; CAPELLA, P; CONTE, L S; RUINI, F; GIORDANI, G (1981) Components of royal jelly: I. Identification of the organic acids. *Lipids* 16: 912-919.

LERCKER, G; CAPELLA, P; CONTE, L S; RUINI, F; GIORDANI, G (1982) Components of royal jelly: II. The lipid fraction, hydrocarbons and sterols. *Journal of Apicultural Research* 21(3): 178-184.

LERCKER, G; VECCHI, M A; PIANA, L; NANETTI, A; SABATINI, A G (1984a) Composition de la fraction lipidique de la gelée royale de larves d'abeilles reines et ouvrières (*Apis mellifera ligustica*, Spinola) en fonction de l'âge des larves. *Apidologie* 15(3): 303-314.

LERCKER, G; VECCHI, M A; SABATINI, A G; NANETTI, A (1984b) Controllo chimico analitico della gelatina reale. *Rivista di Merceologia* 23(1): 83-94.

LERCKER, G; CABONI, M F; VECCHI, M A; SABATINI, A G; NANETTI, A; PIANA, L (1985) Composizione della frazione glucidica della gelatina reale e della gelatina delle api operaie in relazione all'età larvale. *Apicoltura* 1: 123-139.

LERCKER, G; SAVIOLI, S; VECCHI, M A; SABATINI, A G; NANETTI, A; PIANA, L (1986) Carbohydrate determination of royal jelly by high resolution gas chromatography (HRGC). *Food Chemistry* 19: 255-264.

LERCKER, G; CABONI, M F; VECCHI, M A; SABATINI, A G; NANETTI, A (1993) Caratterizzazione dei principali costituenti della gelatina reale. *Apicoltura* 8: 27-37.

LI, J K; WANG, T; PENG, W J (2007) Comparative analysis of the effects of different storage conditions on major royal jelly proteins. *Journal of Apicultural Research* 46 (2): 73-80. DOI: 10.3896/IBRA.1.46.2.02

LOUVEAUX, J; MAURIZIO, A; VORWOHL, G (1978) Methods of melissopalynology. *Bee World* 59(4): 139-157.

MARCONI, E; CABONI, M F; MESSIA, M C; PANFILI, G (2002) Furosine: a suitable marker for assessing the freshness of Royal Jelly. *Journal of Agricultural and Food Chemistry* 50: 2825-2829.

MATSUI, M (1988) Decreasing effect of honey on hydroxy acids in royal jelly products. *Shokuhin Eiseigaku Zasshi* 29(5): 297-300. (in Japanese)

MATSUKA, M (1980) Royal jelly. *Honeybee Science* 1(1): 31-41.

MATSUKA, M; NAKAMURA, J (1990) Oxytetracycline residues in honey and royal jelly. *Journal of Apicultural Research* 29(2): 112-117.

MESSIA, M C; CABONI, M F; MARCONI, E (2003) Valutazione della freschezza della gelatina reale. *In* Atti del Convegno "*Strategie per la valorizzazione dei prodotti dell'alveare*". Università degli Studi del Molise; Campobasso; pp. 83-92.

MESSIA, M C; CABONI, M F; MARCONI, E (2005) Storage stability assesment of freeze dried RJ by furosine determination. *Journal of Agriculture and Food Chemistry* 53: 4440-4443.

NATION, J L; ROBINSON, F A (1971) Concentration of some major and trace elements in honeybees, royal jelly and pollen, determined by atomic absorption spectrophotometry. *Journal of Apicultural Research* 10(1): 35-43.

PAMPLONA, L C; AZEDO, R A B; OLIVEIRA, K; GARCIA-AMOEDO, L H; ALMEIDA-MURADIAN, L B (2004) Physicochemical analyses indicated to the quality control of royal jelly with honey. *Ciencia y tecnología de alimentos* 24: 608-612.

PLANTA, VON A (1888) Über den Futtersaft der Bienen. Hoppe-Seylers *Zeitschrift fur Physiologische Chemie* 12: 327-354.

POURTALLIER, J; DAVICO, R; ROGNONE, M C (1990) Les analyses dans le contrôle de pureté de la gelée royale. *Abeille de France et l'Apiculteur* 753: 405-407.

REYBROECK, W (2003) Residues of antibiotics and sulphonamides in honey on the Belgian market. *Apiacta* 38: 23-30. www.apimondia.org/apiacta/articles/2003/reybroek.pdf

RICCIARDELLI D'ALBORE, G (1986) Analisi microscopica del miele e della gelatina reale: possibilità di applicazione e limiti. *Atti della Accademia gioenia di Scienze Naturali in Catania* 19: 45-60.

RICCIARDELLI D'ALBORE, G; BATTAGLINI, M; BERNARDINI, M (1978) Origine géographique de la Gelée royale. *Apidologie* 9: 1-17.

SERRA BONVEHI, J (1990) Estudio de la proteina y aminoacidos libres de la jalea real. *Anales de Bromatologia* 42(2): 353-365.

SERRA BONVEHI, J (1991) Study of adulteration of royal jelly with other honey bee products and water. *Prod. Sanidad Anim.* 6(2): 99-111.

SERRA BONVEHI, J (1992) Azucares, acidez y pH de la jalea real. *Anales de Bromatologia* 44(1): 65-69.

SERRA BONVEHI, J; ESCOLA JORDA, R (1991) Studie über die mikrobiologische Qualität und bakteriostatische Aktivität des Weiselfuttersaftes (Gelée Royale): Beeinflussung durch organische Säuren. *Deutsche Lebensmittel-Rundschau* 87: 256-260.

SESTA, G (2006) Determination of sugars in royal jelly by HPLC. *Apidologie* 37: 84-90.

SIMUTH, J; BILIKOVA, K; KOVACOVA, E; KUZMOVA, Z; SCHRODER, W (2004) Immunochemical approach to detection of adulteration in honey: Physiologically active royal jelly protein stimulating TNF- alpha release is a regular component of honey. *Journal of agricultural and food chemistry* 52 (8): 2154-2158

STOCKER, A (2003) *Isolation and characterisation of substances from Royal Jelly.* PhD Thesis; Université d'Orléans; Orléans (France).

TAKENAKA, T; ECHIGO, T (1980) General chemical composition of the royal jelly. *Bulletin of the Faculty of Agriculture*, Tamagawa University 20: 71-78.

VECCHI, M A; SABATINI, A G; GRAZIA, L; TINI, V; ZAMBONELLI, C (1988) Il contenuto in vitamine come possibile elemento di caratterizzazione della gelatina reale. *Apicoltura* 4: 139-146.

WU, G; LI, Y; LIU, G (1991) The immunoregulative effect of royal jellyacid, 778. *Zhongguo Yaoke Daxue Xuebao* 22 (2): 117-118.