Chemical Composition and Antimicrobial Activity of Royal Jelly - Review

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Abstract
The present paper presents the literature data regarding the chemical composition and antimicrobial activity of Royal Jelly. Royal Jelly is a secretion from the hypofaringeal glands of worker bees which serves as a food for queen bee and to the growing up larvae. Having biological properties already proven, Royal Jelly has considerable commercial appeal and is today used in many sectors (pharmaceutical, food industries and cosmetic products). The physicochemical composition of pure royal jelly are analyzed by determining moisture, ash, lipids, proteins,vitamins, aminoacids, carbohydrates, 10-HDA; RJ is the key substance in the antimicrobial function of the system Apis mellifera. The intact Royal Jelly exhibited the highest antibacterial activity.

Keywords: antimicrobial activity, chemical composition, royal jelly.

1. Introduction

Royal Jelly, or bee's milk, is a creamy product secreted by the hypo pharyngeal glands in the head of the young nurse worker bees primarily for developing and maintaining the queen bee. [1]
The Royal Jelly (RJ) is a yellowish-white, acidic secretion, with a slightly pungent odor and taste produced by the honeybees. It is the principal food of the queen honeybees and is produced by the hypopharingeal and mandibular glands of worker bees. RJ is a nutritive secretion produced by the worker bees, rich in proteins, carbohydrates, vitamins and minerals which is stored in the nest, under field conditions. RJ appears as a substance with a gelatinous consistency, often not homogenous due to the presence of undissolved granules of varying size. It has a distinctively sharp odor and taste. [2] It is partially soluble in water and highly acidic (pH 3.4–4.5) with a density of 1.1 g/mL [3].

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After harvesting, in contact with the air at a temperature of 15°C royal jelly turn yellow, it is deteriorate because the albumin present in composition is due to drying. It contains remarkable amounts of proteins, lipids, sugars, vitamins, hormones, enzymes, mineral substances, and specific vital factors that act as biocatalysts in cell regeneration processes within the human body.
RJ contains pollen grains derived from the foraging activity of honeybees that reflect the environmental location of the beehives [4]. RJ is of particular interest because, as a natural material, it offers probably the greatest potential to find new substances with pharmaceutical potential. The physiological effects of RJ in humans are still not fully understood. However, RJ has been recognized in the world market as a dietary supplement and is used in cosmetics for its alleged tonic and bio-stimulating effects. Biological activities of RJ are variable and have been correlated to their content of trace elements [5]. The chemical composition of the RJ remains relatively constant when comparing between

different colonies, bee breeds and variations of temperature.
Quality and freshness evaluation indexes, such as furosine, superoxide dismutase, glucose oxidase, (E)-10-hydroxydec-2-enoic acid are usually used for RJ. For centuries, fresh RJ has been used as all natural energy boost and alternative medicine. As far as nutrition goes, it is packed full of a wide spectrum of vitamins, mineral and amino acids that have been shown to increase energy, reduce stress and boost the immune system.
A lot of studies done before show a wide range of medical activities in RJ. Some of these effects are as follows: anti-microbial effects [6], suppression of allergic reactions, lowering the amount of blood cholesterol [7], preventing cell damage in cancer and HIV patients, as well as wound healing and growth acceleration.
The aim of this paper is to review the literature data regarding the chemical composition and antimicrobial activity of royal jelly.

2. Chemical composition

The chemical composition is complex, consisting of proteins, amino acids, sugars, lipids and vitamins. Parameters that are usually determined for the general chemical characterization are the following [1]:
Water content - determined by freeze-drying [8], Karl Fischer [9], vacuum oven, dessication [10].
Total protein - nitrogen determined with the Kjeldahl method [11-10].
Free amino acids - determined by ion chromatography [12].
Carbohydrates - determined by gas [11] or liquid chromatography [13].
Lipids - determined as free and total organic acids by gas chromatography [11] or as total lipids, by solvent extraction [10].
10-HDA - determined by HPLC [10, 14-16]
Minerals - determined by atomic absorption [17]
Acidity - titration method [18]
Sediment analysis - microscopical analysis [19]
Furosine, [20]

Water

RJ has the largest amount of water between all bee products. Water content shows to be fairly uniform, greater than 60%, and 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 efforts to maintain a level of ambient moisture; moreover the non solubility of some compounds can explain the variations in water content.

Minerals

RJ is also rich in minerals, especially in potassium, magnesium, calcium, iron, phosphorus, sulfur, manganese, silicon, lead and others.
Ash content represents 0.8-3% of RJ (fresh matter) [21, 10]. 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).

Proteins

A significant amount of RJ is made up of proteins that make up about 50% of the dry mass [22]. Important proteins form 90% of the total amount of proteins with molecular mass of 49-87 kDa proteins and attributed to a gene family. Minor proteins contained in RJ are made from proteins and peptides with different functions, including antimicrobial and antifungal properties [23].
Protein fraction of RJ contains many valuable components and biologically active substances. Besides the major royal jelly proteins, it also contains small amounts of minor proteins, including peptide antibiotics. These bioactive peptides present in protein amino acid sequence shows a keen interest in food. Once released into the body they can act as regulatory components with activity similar to that of hormones. The recent discovery that proteins of royal jelly may have physiological immunoregulatory functions, suppression of allergic reactions, but their antihypertensive properties and stimulate the proliferation opened a new era in the use of royal jelly and honey [22].

Lipids

The lipid fraction in royal jelly is 3-6%, but is has also been have also been identified as responsible for important biological activities tied to the development strategies of the colony. Lipids determination provides useful information on quality based on the presence of natural lipids in royal jelly. Exogenous lipids, due to harvest process or fraudulently introduced in the product, can also be easily identified by gas

chromatographic analysis using appropriate standards [23].

The lipid content of RJ is interesting because the structure is uncommon, consisting of primarily short-chain fatty acids. These lipids are credited with most of the biological properties associated with royal jelly, particularly the lowering of cholesterol levels and stimulation of glial cells, which are non-neuronal cells that produce the myelin insulation that coats healthy neurons.

Carbohydrates

On average this portion accounts for 30% of the dry matter of RJ. 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. These constituents, even if present only in small quantities, are characteristic and thus play a useful role in checking the authenticity of the product [3].

Amino acids

RJ is one of the richest natural products in amino acids. Amino acids are very important for humans and animals. RJ contains at least 17 amino acids, including the 8 essential ones, plus 5 non-identified related compounds. The main RJ acid, 10-hydroxy-2-decenoic acid is known to have various pharmacological effects, including antibiotic, antitumoral.

Vitamins

The composition of RJ consists of vitamins, aminoacids and also unidentified compounds [12]. Only trace amounts of vitamin C that may be present in the composition of RJ. Royal jelly contains the vitamins B1, B2, B6, B5 (large amounts), B8, B9, C and PP.

Table 3 shows the vitamins in royal jelly.

3. Antimicrobial activity

Protein and peptides from RJ can participate in defense mechanism of honeybee against microbial pathogens by means of direct inactivation of microorganism occurring in honeybee products, as well as trough induction of cytokines participating in regulation of transcription of defensive proteins and peptides.

Table1. Free amino acids in royal jelly [24]

Free amino acids	%
Alanine	1.7
Valine	1.7
Glycine	2.1
Isoleucine	1.3
Leucine	13.3
Proline	139.8
Threonine	1.0
Serine	3.5
Amino butyric acid	3.5

Table 2. Amino acid composition of royal jelly proteins [24]

Amino acids	%
Valine	1.6
Glycine	3.0
Isoleucine	1.6
Leucine	3.0
Proline	3.9
Threonine	2.0
Serine	2.9
Methionine	3.7
Phenylalanine	0.5
Aspartic acid	2.8
Glutamic acid	8.3
Tyrosine	4.9
Lysine	2.9
Arginine	3.3
Tryptophan	3.4

Table 3. Vitamins in royal jelly.[24]

Vitamins	mg/100g
Vitamin A	1.10
Vitamin D	0.2
Vitamin E	5.00
Vitamina B1	2.06
Vitamina B2	2.77
Vitamina B6	11.90
Vitamina B12	0.15
Vitamina B5 (acid Panthotenic)	52.80
Niacină (PP)	42.42
Vitamina C (acid ascorbic)	2.00
Vitamina B9 (acid folic)	0.40

Protein and peptides presenting simple structures, no complex modifying moieties or rare amino acids are expressed and frequently secreted to the hemolymph, providing a broad – spectrum antibiosis against bacteria and fungi [25]. Major proteins accounting for 90% of total RJ proteins. The physico – chemical properties of these proteins are similar to typical albumin proteins

(such as ovalbumin, serum albumin) and therefore was proposed the name major royal jelly proteins as albumins. The most abundant protein of RJ is albumin1 representing 50% of proteinous content of RJ. The peptide RJ showed total inhibition of bacterial growth for *S. aureus* , *L. monocytogenes* and *S. typhimurium* only at very high concentrations (≥200 μg/ml) [26]. In the chemical composition of royal jelly have been identified a number of antimicrobial peptides including royalisin, apisimin, jelleines I, II, III,IV,10-HDA, apalbuminaα.

Royalisin

Is one protein was found in RJ of the honeybee *Apis mellifera L*. and purified to homogeneity for the first time by acid extraction, gel filtration, and reverse-phase high pressure liquid chromatography. The primary structure of royalisin was determined to consist of 61 residues, with three intramolecular disulfide linkages, having a calculated molecular mass of 5523 Da and 51 aa. Royalisin is an amphipathic protein, with the C-terminal half of the molecule being rich in charged amino acids; and it showed extensive sequence homology to two other antibacterial proteins, sapecin from embryonic *Sarcophaga peregrina* cells and phormicins from *Phormia terranovae* larvae.

Royalisin was found to have potent antibacterial activity against Gram-positive bacteria at low concentrations, but not against Gram-negative bacteria. Royalisin may be involved in a defense system active against bacterial invasion of the honeybee [27]. Royalisin, an antibiotic polypeptide was previously isolated from the RJ of *Apis mellifera*, providing protection against infection of RJ by Gram-positive bacteria. Bilikova et al. [28] described that royalisin fractions in the concentration 180 μg/ml showed a clearly weaker inhibition against *Bacillus subtilis* as tetracyclin in the concentration 50 μg/ml. Additionally antifungal activity of a royalisin-fragment isolated with dialysis membranes against the fungus *Botrytis cinerea* was described [28]. With agar diffusion tests the antibacterial activity also for this royalisin fragment against the gram-positive bacteria like *Bacillus subilis*, *Sarcina lutea* and 2 *Paenibacillus larvae* strains were described. The tested gram-negative strains *Escherichia coli* and *Serratia marcescens* were not inhibited.

Royalisin is stable at low pH and high temperature probably because of the three disulfide bonds. Moreover, the inhibitory effect of royalisin toward the growth of Gram-positive bacteria and fungi has been demonstrated. It shows the antibacterial activity particularly toward honeybee pathogen, *Paenibacillus larvae* that would cause American foulbrood, a serious disease found in honeybee larvae.

Apisimin

Apisimin was found in honeybee RJ. Is a peptide composed of 54aa, 5540Da which stimulates the proliferation of human monocites. It is rich in valine (18.5%), and serine (16.7%), no cysteine, only two basic amino acids, and contains only one aromatic aminoacid, phenylalanine. The 54 amino acids of apisimin do not include Cys, Met, Pro, Arg, His, Tyr, and Trp residues [29].

Jelleines

The Jelleines are the peptides with antimicrobial activity of RJ. Four antimicrobial peptides were purified from RJ of honeybees, by using reverse phase-HPLC and sequenced by using MS: (Jelleine-I), (Jelleine-II), (Jelleine-III), and (Jelleine-IV). The peptides were synthesized on-solid phase, purified and submitted to different biological assays: antimicrobial activity, mast cell degranulating activity and hemolysis. The Jelleines-I–III presented exclusively antimicrobial activities against yeast, Gram+ and Gram− bacteria; meanwhile, Jelleine-IV was not active in none of the assays. These peptides do not present any similarity with the other antimicrobial peptides from the honeybees; they are produced constitutively by the workers and secreted into RJ [30]. The Jelleines are very short peptides, presenting hydrophobic sequences; these peptides do not present any similarity with any other known antimicrobial peptides.

Apalbumina

Apalbumin is the major protein in (RJ) having various biological properties [31]. Apalbumina formed α-subunit structure [22]. Basic subunit is approximately 420 kDa and was composed of 55 kDa basic monomer. Microscopic observations show that apalbumina-α in aqueous form structures similar to those which occur in royal jelly. Depending apalbuminei-α concentration, different structures were generated with a regular

repetition. It is a autoasamblatoare structure of the protein, the result of oligomerization of these subunits. It is interesting that other royal jelly protein oligomerization has ability, but has a high degree of sequence similarity with apalbumina-α.

10- HDA

10-Hydroxy-2-Decenoic Acid is a kind of special active substance which exists only in royal jelly in the nature. It is also called 10-HDA or royal jelly acid. The content of 10-HDA in RJ is usually around 1.5% - 2,0%. Since it has the function of anticancer and antibacterial effect, it can be added to health food or cosmetics as an active ingredient. 10-HDA represents the main criterion in quality control for the determination of RJ authenticity [14]. The content of 10-HDA in RJ is the international standard of the quality of royal jelly and it directly determines the price of RJ on the international market. Recent studies have shown that 10-HDA promotes the growth of T-lymphocyte subsets and interleukin-2, which might suggest that this fatty acid possesses immunoregulation and anticancer activities [31]. The antimicrobial action of 10-HDA that extracted from RJ against a series of bacterium was studied. The studies showed that the antimicrobial effect of 10-HDA varied with the pathogenetic microbes. The antimicrobial minimum dosage of 10-HDA were as follows: (*Escherichia coli* 0.625 mg·mL-1,*Bacillus subtilis* 1.25 mg·mL-1, *Staphylococcus aureus* 2.5 mg·mL-1. The antimicrobial effection of 10-HDA were as follows*: Escherichia coli, Bacillus subtilis Staphylococcus aureus*. The study showed that the 10-HDA could effectively inhibit the growth of bacterium.

4. Conclusions

Royal jelly with its manifold inhibitory properties offers a great potential of applications. It is the bee product with the highest percentage of water and a rich chemical composition. It contains many proteins, aminoacids (at least 17 - including the 8 essential), minerals, sugars, trace elements and other constituents.

The Jelleines and royalisin are produced constitutively by the workers and secreted into RJ, to provide a broad-spectrum protection of this bee hive product against microbial infections. The major antibacterial effect of RJ is attributed to a particular 10-carbon molecule of fatty acid (10-hydroxy-decenoic acid). 10-HDA is the most important active ingredients in royal jelly, and the HDA content can be considered as an index for estimation of quality.

In conclusion, the literature data reviewed so far regarding the chemical composition of RJ proved inconclusive regarding the quantitative and qualitative methods applied. The results published so far are mainly focused on determining the main classes of RJ compounds and do not cover in detail the chemical compounds of each class.

The methods applied for antimicrobial activity of RJ are very different and therefore not comparable: starting with the raw material preparation, the solvents' choice and the method parameters – strains of bacteria, fungi.

There is a real need of a standardized method for quality evaluation of royal jelly: qualitative, quantitative and biological activity.

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