

PHYSICOCHEMICAL COMPOSITION OF PURE AND ADULTERATED ROYAL JELLY

Luis Henrique Garcia-Amoedo e Ligia Bicudo de Almeida-Muradian*

Departamento de Alimentos e Nutrição Experimental, Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, Av. Prof. Lineu Prestes 580, Bloco 14, 05508-900 São Paulo - SP, Brasil

Recebido em 14/3/05; aceito em 5/5/06; publicado na web em 31/10/06

The physicochemical composition of pure royal jelly as well as of some adulterated samples was analyzed by determining moisture, ash, lipids, nitrogen/proteins, carbohydrates, starch and 10- HDA (10-hydroxy-2-decenoic acid). The solubility in alkaline medium was used to detect the main frauds for adulterating royal jelly which comprise addition of yogurt, water, egg white, sweet condensed milk mixed with propolis, unripe banana and corn starch slurry.

Keywords: adulteration; royal jelly; beehive products.

INTRODUCTION

Royal jelly (RJ) is a secretion from the hypopharyngeal glands of worker bees which serves as a food for the queen bee and to the growing up larvae (from the first to the third day). RJ has a complex composition of proteins, amino acids, sterols, phenols, sugars, minerals and other components and it is hard to obtain it¹.

In the market, this product has been sold as a food supplement, popularly known to have many nutrients such as carbohydrates, vitamins, minerals and lipids including its characteristic acid (10-HDA or 10-hydroxy- 2-decenoic acid). Because of the high price of the product, some people can adulterate RJ by adding other products, less expensive, which can not be detected organoleptically like starch corn slurry, yogurt, white of the egg, condensed milk mixed with propolis, unripe banana and water.

Howe *et al.*² analyzed gross composition of fresh samples of RJ and others from commerce using water, protein, lipid, amino acid and fatty acid determination. Commercial samples were compared with fresh samples which were adopted as standards being classified either as authentic or adulterated.

Considering the fact that in 1992 there was not a Brazilian regulation for RJ quality control, Palma³, started studying the chemical composition of Brazilian samples of RJ analyzing their moisture, proteins, ash, lipids, carbohydrates and acidity. He concluded that the composition of Brazilian RJ agreed with other values described in literature. However, some differences could be observed, especially in water content that was higher in Brazilian samples.

Bloodworth *et al.*⁴, knowing the fact that 10-HDA is the active substance of RJ, and admitting its importance to the quality of RJ, proposed a method for its determination by HPLC. As RJ is the only product that contains 10-HDA naturally, it could be used as an indicator of its authenticity and the presence of RJ in products in which it was added.

Chen and Chen⁵ studied some physicochemical changes that occurred in the RJ when it was stored at -20 °C, at 4 °C and at room temperature exposed and protected from the light by seven months. The results showed that the viscosity, colour, soluble protein fraction, and the carbohydrates changed significantly when RJ was stored at room temperature; the same did not occur when storage was at -20°C. The main changes verified were an increment of viscosity,

browning of the product, and the diminution of carbohydrate content and soluble protein content.

The main purpose of this work is to obtain the physicochemical profile of the RJ adulterated in different proportions of the following adulterants: natural yogurt, pure water, white of the egg, starch corn slurry, a mixture of sweet condensed milk with propolis and unripe banana. This could be useful to detect frauds.

EXPERIMENTAL

Material

Seven samples of pure royal jelly obtained directly from beekeepers and from commerce in São Paulo State, Brazil were used. Each sample received a code from A to G being samples A, B, D and G acquired from the market, samples C and F were acquired directly from beekeepers and sample E was declared as Chinese. After the analysis, Sample B was chosen to be adulterated.

All samples were kept frozen (-18 °C) and protected from light, from the receiving time until the analysis procedure.

Preparation of the adulterated samples

Natural yogurt, pure water, white of the egg, starch corn slurry, a mixture of sweet condensed milk with propolis and unripe banana were added to Sample B in a proportion of 10, 25 and 50% (m/m). The adulteration was prepared in the laboratory, being all the samples kept in the freezer at -18 °C and protected from light since the adulteration step until the moment of the analysis.

Standards and solvents

For the 10-HDA analysis, a reference standard of *trans*-10-hydroxy-2-decenoic acid (acquired from Nippon Shoji Kaisha Ltd. - Chuo-Ku, Osaka, Japan), was used. All solvents used were HPLC grade.

Methods

Moisture content

Moisture content was determined by gravimetric analysis using desiccator with sulfuric acid⁶.

*e-mail: ligiabi@usp.br

Ash content

Ash content was determined by gravimetry using oven at 550 °C⁷.

Lipid content

Lipid content was determined by Soxhlet procedure using diethyl ether as solvent⁷.

Total nitrogen and protein content

The total nitrogen content was obtained using the Micro-Kjeldahl method using the factor of 6.25 for conversion to protein content⁸.

Total carbohydrate

Total carbohydrate was obtained by difference⁹ using the formula:

Total carbohydrate = (100 g – g of moisture, protein, lipids and ash).

Starch/ iodine reaction

To identify the presence or absence of starch in the samples, the iodine reaction¹⁰ was used. 1.5 g of the sample was weighed, diluted in 20 mL of distilled water and the solution was heated till boil and then lowered the temperature to cold. Three drops of the iodine solution were added. A standard with pure starch was prepared for a positive control for comparison data.

Alkaline solubility

The water solubility of the samples was tested using 1.0 g of the sample, diluted to 20 mL with distilled water resulting in a characteristic turbidity. NaOH solution (20%) was added, drop to drop, from the pipette till complete solubilization of the sample¹⁰.

HPLC determination of trans-10-hydroxy- 2-decenoic acid (10-HDA)

The 10-HDA determination was similar to that used by Garcia-Amoedo and Almeida-Muradian¹¹, Koshio and Almeida-Muradian¹² and Pamplona *et al.*¹³. High Performance Liquid Chromatography (HPLC) was used under the following conditions: an isocratic system with a Shimadzu® LC9A pump, an auto sampler and a diode array detector (Shimadzu® SPD-MXA). The column used was a reversed phase column C₁₈-H (Shimadzu®, 150 x 4.0 mm x 5 µm). The mobile phase was composed by methanol/water (45:55); the pH was adjusted to 2.5 with phosphoric acid, filtered through 0.45 µm membrane (Millipore®) and degassed during 5 min with helium gas. The flow rate was 0.5 mL/minute. The detector was adjusted to 225 nm. The running time for analysis was 30 min. It was used α-naphthol as internal standard. Data were obtained using the software SPD-MXA (Shimadzu®) which is part of the chromatographic system.

A standard curve was constructed with 5 points of concentrations: 2.73, 5.46, 10.92, 16.38 and 21.84 µg/mL of 10-HDA, adding α-naphthol solution to obtain a final concentration of 30 µg/mL in mobile phase, filtered through 0.22 µm membrane (Millipore®), and 5 µL were injected in the chromatographic system.

30 mg of RJ was weighed in a volumetric flask of 10 mL; 1 mL of α-naphthol solution (with concentration of 30 µg/mL) was added, and the volume was completed with mobile phase. The solution was filtered through a 0.22 µm membrane, and 5 µL were injected into the chromatographic system.

RESULTS AND DISCUSSION

The results for pure royal jelly samples are presented on Table 1 for the proximate analysis and on Table 2 for 10-HDA content.

The results of comparison of all physicochemical analysis of

Table 1. Proximate analysis of seven samples of pure royal jelly

Samples	Determinations (%)*				Obtained from calculation(%)
	Moisture	Ash	Lipids	Proteins	Carbohydrates
A	61.45	1.17	2.17	14.01	21.20
B	67.58	0.93	4.20	11.99	15.30
C	63.44	0.97	4.22	12.42	18.95
D	61.50	1.11	3.25	13.63	20.51
E	63.76	1.06	2.86	13.28	19.04
F	62.33	1.11	3.90	13.26	19.40
G	62.14	1.08	2.38	13.25	21.15
Means	63.17	1.06	3.28	13.12	19.36
RSD(%)	1.98	0.08	0.78	0.64	1.88

* means of three replications; RSD = relative standard deviations

Table 2. 10-HDA content of the seven samples of pure royal jelly

Samples	10-HDA*(means)	RSD (%)
A	1.58	0.10
B	3.17	0.23
C	3.39	0.37
D	2.70	0.30
E	1.98	0.12
F	3.10	0.14
G	1.82	0.28

*means of three replications; (HPLC analysis using internal standard and calibration curves)

Table 3. Proximate analysis and 10-HDA data from regular and adulterated royal jelly samples (in percentage)

Samples	Determinations (%)*					
	Moisture	Ash	Lipids	Proteins	Carbo- hydrate	10- HDA
Pure Royal Jelly	67.58	0.93	4.20	11.99	15.30	3.17
(sample B)						
10% CM	59.96	0.90	4.67	12.21	22.26	2.88
25% CM	52.96	1.01	5.25	11.08	29.7	2.44
50% CM	40.70	1.89	6.31	9.83	41.27	1.63
10% Yogurt	67.87	0.85	3.92	11.16	16.20	2.75
25% Yogurt	70.07	0.74	3.43	9.98	15.15	2.51
50% Yogurt	75.11	0.67	2.70	7.65	13.87	0.96
10% WE	66.74	0.84	3.65	12.12	16.65	2.80
25% WE	70.21	0.74	3.12	11.76	14.17	2.48
50% WE	76.16	0.66	2.21	11.45	9.52	1.47
10% Water	69.01	0.81	3.70	11.24	15.24	2.83
25% Water	73.99	0.66	2.98	9.21	13.16	2.47
50% Water	81.95	0.48	2.25	6.30	9.02	1.42
10% Starch	67.43	0.85	3.73	11.44	16.55	2.81
25% Starch	72.69	0.61	3.09	9.22	14.39	2.48
50% Starch	79.57	0.42	2.18	6.52	11.31	1.41

* means of two replications, CM = condensed milk, WE = white of the egg

pure and adulterated samples are presented on Table 3.

As the adulteration of RJ was not possible with unripe banana because of changes in some organoleptic characteristics like flavor (typical from banana) and colour (dark), this kind of adulteration was not used for the physicochemical analysis.

Solubility test showed that all the pure samples and the one adulterated with water became soluble in alkaline medium. All the other adulterated samples showed some degree of turbidity in alkaline medium, depending on the kind and the percentage of each adulterant. Samples adulterated with yogurt and white of the egg showed higher turbidity compared with the samples adulterated with starch. This analysis can be useful for a previous trial for adulteration check.

10-HDA content decreased when adulterants were added, having the minimum of 0.96 when 50% of yogurt was added.

CONCLUSIONS

Adulterations in RJ can be detected by interaction of the solubility test in alkaline medium with determinations of proximate analysis, 10-HDA content, and iodine-starch reaction. The main changes in physicochemical properties of adulterated samples were: samples adulterated with yogurt, white of the egg, water and starch corn slurry, in concentrations higher than 25%, can be detected by the enhancement of moisture, diminishing in lipid, protein and 10-HDA content, and insolubility in alkaline medium; samples adulterated with yogurt, white of the egg, water and starch corn slurry, in concentrations near 10% can be detected by a little enhancement in moisture, and a diminishing of lipid, protein and 10-HDA content and insolubility in alkaline medium; samples

adulterated with condensed milk in concentrations higher than 10% can be detected by an enhancement of lipid content and a diminishing of moisture, protein and 10-HDA; the absence of 10-HDA showed a total substitution of royal RJ.

REFERENCES

1. Krell, R.; *FAO Agricultural Services Bulletin*, No. 124, Rome, 1996.
2. Howe, S. R.; Dimick, P. S.; Benton, A. W.; *J. Apic. Res.* **1985**, 24, 52.
3. Palma, M. S.; *J. Apic. Res.* **1992**, 31, 42.
4. Bloodworth, B. C.; Harn, C. S.; Hock, C. T.; Boon, Y. O.; *J. AOAC Int.* **1995**, 78, 1019.
5. Chen, C.; Chen, S. Y.; *Food Chem.* **1995**, 54, 195.
6. Garcia-Amoedo, L. H.; Almeida-Muradian, L. B.; *Quim. Nova* **2002**, 25, 676.
7. Instituto Adolfo Lutz; *Normas analíticas do Instituto Adolfo Lutz*, 3ª ed., Instituto Adolfo Lutz: São Paulo, 1985.
8. Association of Official Analytical Chemists; *Official methods of analysis*, 14th ed., AOAC: Arlington, 1984.
9. <http://www.fcf.usp.br/tabela/>, acessada em Maio 2006.
10. Garcia-Amoedo, L. H.; *Dissertação de Mestrado*, Universidade de São Paulo, Brasil, 1999.
11. Garcia-Amoedo, L. H.; Almeida-Muradian, L. B.; *Ciência e Tecnologia de Alimentos* **2003**, 23, 62.
12. Koshio, S.; Almeida-Muradian, L. B.; *Quim. Nova* **2003**, 26, 670.
13. Pamplona, L. C.; Azedo, R. A. B.; Oliveira, K.; Garcia-Amoedo, L. H.; Almeida-Muradian, L. B.; *Ciência e Tecnologia de Alimentos* **2004**, 24, 608.