PHYSICOCHEMICAL ANALYSES INDICATED TO THE QUALITY CONTROL OF ROYAL JELLY WITH HONEY[1]

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SUMMARY

Royal jelly (RJ) is used as a revitalizing tonic. In order to avoid rejection to its acid taste, it is added to honey. There are regulations for honey and for royal jelly separately but not for the mixture. The objective of this work is, therefore, to verify if the same methods used for pure honey quality control can be used for honey mixed with royal jelly and also the presence of RJ through 10-HDA determination. The methods used were: moisture, reducing sugars, apparent sucrose, ash, hydroxymethylfurfural, insoluble solids, diastase activity, acidity and 10-HDA. Samples were prepared by adding 0-100% of RJ in honey. The results showed that the ash method was the only suitable one to all the samples. The acidity analysis (direct titration) was suitable to 0-30%RJ samples; the reducing sugar analysis was suitable to 0-20% RJ samples. Concerning moisture analysis the refractometric method is suitable to 0-10% RJ and the Infra Red method is suggested to be used for samples with more than 10% RJ. The methods for diastase activity, HMF, apparent sucrose and insoluble solids were inadequate for all samples with RJ. The presence of RJ in the samples was confirmed by the 10-HDA analyses.
Keywords: honey; royal jelly; analysis; bee products; honey with royal jelly; quality control.

RESUMO

ANÁLISES FÍSICO-QUÍMICAS INDICADAS PARA O CONTROLE DE QUALIDADE DE MEL COM GELÉIA REAL. A geléia real (GR) é utilizada como produto revitalizante com sabor ácido e adstringente. Para evitar rejeição a este sabor, existem misturas de mel com GR. Existe legislação específica para o mel e para a GR separadamente, mas não para a mistura. Os objetivos do trabalho são: verificar se os métodos usados para o controle de qualidade do mel puro podem ser utilizados no controle do mel com GR e verificar a presença de GR pela determinação do 10-HDA. As análises incluíram: umidade, açúcares redutores, sacarose aparente, cinzas, hydroxymethylfurfural, sólidos insolúveis, atividade diastásica, acidez e 10-HDA. As amostras foram preparadas com 0-100% de GR no mel. Os resultados obtidos sugerem que dos métodos citados na legislação do mel somente o de cinzas foi adequado para todas as amostras. A análise de acidez (titulação direta) foi adequada de 0–30%GR; açúcares redutores 0-20%GR; umidade (método refratométrico) 0–10%GR e sugere-se o método de secagem por IV para as demais amostras. Os métodos para as análises da atividade diastásica, HMF, sacarose aparente e sólidos insolúveis foram inadequados para as amostras com GR. A presença de GR nas amostras foi comprovada pela análise do10-HDA.
Palavras-chave: mel; geléia real; análise; produtos apícolas; mel composto; controle de qualidade.

1 – INTRODUCTION

Honey mixed with other bee products is usually found in retail markets, being honey with propolis and royal jelly the most common. All of them are sold by the authorization of the Ministry of the Agriculture, and although there are no technical regulations for identity and quality to determine the necessary parameters to guarantee the authenticity or adulteration to these products they are frequently available for consumption.

Basically honey is a complex mixture of highly concentrated sugars whose chemical composition has been the subject of bibliographic revisions such as those made by CAMPOS [9] and SERRANO [22]. Besides the soluble sugars honey also contains organic acids, enzymes, vitamins, acetilcholine flavonoids, minerals and other organic compounds that provide its color, smell and taste [1].

Honey from other countries has had its composition studied by several authors such as WHITE [25], HAYDAK [14] and SERRANO [22].

Royal jelly is secreted by the young bees and it is an important product for the bee hive serving as food for developing larva, for worker bees and for the drones (until 3rd day of larval stage) and for the queen bee during its whole life [3, 8, 23].

The vitality provided to the queen bee by the diet rich in royal jelly has attracted many consumers who search for a long healthy life. Some of its effects on human beings are: memory, physical performance and skin improvement. Furthermore, royal jelly also has antibiotic and antiviral action [17, 21].

Royal jelly has a complex composition of proteins, amino acids, organic acids, sterols, phenols, sugars, minerals and other components [26]. The main component of the lipidic fraction [4] is 10-hydroxy-2-decenoic acid (10-HDA), considered the most important active principle [11, 13, 16, 18, 20] in the royal jelly. The concentration of 10-HDA can be considered as an index of freshness and quality of the products that contain royal jelly serving as a parameter for its quality control [2,11,16,19].

Royal jelly has been mixed with honey for a better acceptance to the consumers because of its acid taste.

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Also the presence of honey can contribute to the conservation of the royal jelly at room temperature.

In Brazil, there is a regulation for honey [6] and for royal jelly [5] separately but not for their combination. A provisional regulation was published for honey mixed with other bee products in 2001 [7] but the final regulation has not been published yet. However, neither the methods of analysis nor the standards of identity and quality to be used in these products quality control were mentioned

The objectives of this paper are: to verify if the same methods used in honey quality control regulation (Instrução Normativa n. 11 de Outubro de 2000) [6] can be applied for royal jelly mixed with honey and to confirm the presence of royal jelly trough 10-HDA analysis.

2 – MATERIAL AND METHODS

2.1 – MATERIAL

Samples of pure royal jelly and pure honey were obtained directly from beekeepers and marts under the federal authorization and supervision. All the products have a seal labeled on them – SIF (Serviço de Inspeção Federal). The mixture of royal jelly with honey was made in laboratory in the proportions described in *Table 1.*

TABLE 1. Proportions of royal jelly mixed with honey.

Samples	Royal Jelly (%)
1 (Pure Honey)	0
2	10
3	20
4	30
5	40
6	50
7	60
8	70
9	80
10	90
11 (Pure royal jelly)	100

2.2 – METHODS

2.2.1 – Methods used for pure honey and royal jelly mixed with honey

Physicochemical methods, cited in the Brazilian honey regulation (Instrução Normativa nº 11 de 20 de Outubro de 2000) [6] were used as follows: free acidity, reducing sugars, diastase activity, ash, hydroxymethylfurfural (HMF), apparent sucrose and insoluble solids.

The method used for 10-HDA was based in the method cited in the Brazilian regulation of royal jelly [5] substituting the Shimadzu® ODS-H column (C18-H reverse phase, dimension 150 x 4,0mm) for Vydac® ODS column (C18 reverse phase, dimension 250 x 4,6mm).

The method for moisture analysis used was the infrared moisture analyzer, according to GARCIA-AMOEDO & ALMEIDA-MURADIAN [12].

2.2.2 – Methods used for pure royal jelly

Physicochemical analyses, cited in the Brazilian royal jelly regulation [5] were used as follows: Fehling method for reducing sugars and apparent sucrose [15], gravimetric method for ash content [16] titrimetric method for free acidity [15] and HPLC method for 10-HDA [5, 11].

Infrared moisture analyzer was used for moisture content analysis according to GARCIA-AMOEDO & ALMEIDA-MURADIAN [12].

2.2.3 – Statistical analysis

Statistical analysis was made using the software STATISTICA for Windows (Statsoft. Inc,1995, Tulsa, USA), as described by COSTA NETO [10].

3 – RESULTS AND DISCUSSION

For the evaluation of the pure honey and pure royal jelly, physicochemical analyses were made as follows:

3.1 – Pure royal jelly

The analytical results obtained and the values of the Brazilian royal jelly regulation are presented in *Table 2.*

TABLE 2. Mean values of the physicochemical analyses of pure royal jelly and the values of the Brazilian royal jelly regulation.

Parameters	Results*	Brazilian regulation
Reducing sugars (g/100g)	14.9 ± 0.2	Minimum of 10
Ash (g/100g)	0.89 ± 0.02	Maximum of 1.5
Free acidity (mg KOH/g)	15.4 ± 0,1	23 to 53
Apparent Sucrose (g/100g)	0.0 ± 0.0	Maximum of 5.0
Moisture (g/100g)	64.5 ± 0.1	60 to 70
10-HDA (μg/100g)	4.98 ± 0.12	Minimum of 2 (dry basis)

*Mean ± standard deviation, n=3.

Comparing the values obtained for moisture, reducing sugars, ash, apparent sucrose and 10-HDA with the Brazilian regulation values it can be verified that all the results are in accordance with this regulation (Instrução Normativa n. 3/2001) [5], except from the values obtained for the free acidity that are lower than the limits recommended. Similar results were obtained by GARCIA-AMOEDO [11] who analyzed royal jelly samples from São Paulo city, Brazil in 1999.

3.2 – Pure honey

The analytical results obtained and the values of the Brazilian pure honey regulation are presented in *Table 3.*

TABLE 3. Results from the physicochemical analyses of pure honey and the Brazilian regulation parameters.

Parameters	Results*	Brazilian regulation
Free acidity (mEq/Kg)	32.4 ± 0.2	Maximum of 50
Reducing sugars (g/100g)	71.8 ± 0.8	Minimum of 65
Diastase activity	9.64	Minimum of 8
Ash (g/100g)	0.19 ± 0.02	Minimum of 0.6
HMF (mg/kg)	22 ± 2	Maximum of 60
Apparent sucrose (g/100g)	1.44 ± 0.02	Maximum of 6
Insoluble solids (g/100g)	0.008 ± 0.002	Maximum of 0.1
Moisture (g/100g)	16.5 ±0.1	Maximum of 20

*Mean ± standard deviation, n=3.

All the results (*Table 3*) are in accordance with the limits proposed by the Brazilian regulation [6], being similar to those obtained by VILHENA & ALMEIDA-MURADIAN [24].

3.3 – Honey mixed with royal jelly

The results for physicochemical analyses of honey mixed with royal jelly are presented in *Table 4.*

TABLE 4. Results from physicochemical analyses of honey mixed with royal jelly.

Sample	DT	IT	RS	Ash	IS	10-HDA	Moisture
Honey	1.31±0.01	--	71.8±0.8	0.19±0.02	0.008±0.002	0.00	16.5±0.1
Honey with % RJ							
10	3.4±0.1	4.3±0.2	61.9±0.8	0.25±0.03	0.34±0.01	0.13±0.01	22.60±0.30
20	4.2±0.0	5.2±0.1	58.8±0.8	0.36±0.03	0.38±0.02	0.34±0.04	24.25±1.05
30	5.8±0.1	6.8±0.2	38.2±0.6	0.41±0.03	0.26±0.02	0.51±0.03	26.85±0.85
40	6.6±0.2	6.9±0.1	22.6±1.1	0.49±0.04	0.40±0.02	0.67±0.03	33.85±0.85
50	8.0±0.1	8.7±0.1	28.5±2.2	0.58±0.04	0.44±0.02	0.85±0.01	39.85±0.15
60	10.1±0.5	10.7±0.4	19.3±1.0	0.66±0.02	0.76±0.05	1.08±0.11	46.10±0.20
70	10.6±0.2	11.4±0.2	-	0.73±0.05	0.52±0.05	1.57±0.03	50.45±0.95
80	12.2±0.1	12.8±0.0	-	0.80±0.05	1.05±0.06	1.75±0.03	55.9 ±0.14
90	14.5±0.6	15.1±0.6	-	0.87±0.05	0.84±0.04	1.91±0.05	59.1±0.10
RJ	11.0±0.1	11.4±0.0	14.9±0.2	0.89±0.02	--	1.77±0.04	64.5±0.1

DT– free acidity by direct titration (mg NaOH/g); IT – free acidity by indirect titration (mg NaOH/g);
RS – reducing sugars (g/100g); Ash (g/100g); IS – insoluble solids (g/100g); RJ – royal jelly.

3.3.1 – Free acidity

The obtained values concerning free acidity (direct titration in *Table 4*) showed that the results increased as long as the percentage of royal jelly increased in the samples. However, as problems at the endpoint visualization occurred from 30% RJ to 100% RJ samples, the indirect titration was used. Comparing these two methods and using statistical analysis (Pearson's correlation r = 0.998) it could be verified that direct titration results are significantly lower in acidity values than those obtained by indirect titration. Once that both the regular Brazilian legislation to pure honey and to pure royal jelly do not specify which titration method should be used, the suggested one for standardizing is the indirect titration for mixed samples, as recommended by GARCIA–AMOEDO [11].

3.3.2 – Reducing sugars

According to the official method for pure honey adopted by the Brazilian regulation [6], a constant volume of 5mL of titration solution during Fehling's reaction is usually used. However, samples from 30% RJ requested higher volumes than those recommended by the method and from 70% RJ samples the endpoint did not occur even if 100 mL of titration solution was added. Thus, this method is suggested only for 0 – 30% RJ samples.

3.3.3 – Diastasic activity

Only the samples of pure honey (9.64) and 10% RJ (8.75) could be analysed using the official method [6]. Samples from 20% of RJ presented precipitation in the solution, harming the absorption measurement.

3.3.4 – Ash

The official gravimetric method [6] for ash analysis was suitable for all the samples being the ash and royal jelly values increased proportionally (*Table 4*).

3.3.5 – Hydroxymethylfurfural (HMF)

The official hydroxymethylfurfural method [6] was suitable only for pure honey. For samples with RJ the method was not suitable because the results were sometimes negative and not constant.

3.3.6 – Apparent sucrose

The official method for apparent sucrose [6] was suitable only for pure honey. For samples with RJ the method was suitable only for pure honey. For samples with RJ the method was not suitable because the results sometimes were negative and not constant.

3.3.7 – Insoluble solids

The official method for insoluble solids analysis [6] had to be modified concerning the weight of samples (from 20g to 1g), as royal jelly presents much higher amount of insoluble solids than pure honey what causes filtration blockage. In *Table 4* it can be seen that insoluble solids results increased with the presence of royal jelly in honey, but it was not proportional to the percentage of royal jelly added. Thus, this method seems to be inadequate to analyze samples with royal jelly.

3.3.8 – Moisture

The Brazilian regulation for pure honey [6] recommends the refractometric method for moisture analysis which proved to be suitable only for 0 – 10%RJ samples. However, as the samples ranking to 20 – 100% RJ did not match suitable values in the Chataway table, it was necessary to use the infrared gravimetric method instead.

As commercial samples usually contain around 2% royal jelly, the refractometric method can be applied.

3.3.9 – 10-HDA

Table 4 shows that the enhancement of the royal jelly percentage results in a proportional increase of the 10-HDA values but the values obtained for RJ (1.77), 80%RJ (1.75) and 90% (1.91) were not significantly different (t-Student statistical analysis).

4 – CONCLUSIONS

Among all the official methods, the gravimetric method for ash analyses was the only suitable one for all the samples.

The acidity analysis by direct titration could be used for samples ranking 0 -30% RJ and the indirect titration was suitable for all the samples with RJ.

The reducing sugars determination was suitable for samples ranking 0 -20% RJ.

The refractometric method for moisture determination was suitable for samples ranking from 0-10% RJ and the Infrared gravimetric method was suitable for all samples with RJ.

The methods for diastasic activity, HMF, apparent sucrose and insoluble solids were inadequate for samples with royal jelly.

Regarding commercial samples, which usually have about 2% of RJ, the following methods of the regulation can be used: acidity, reducing sugars, moisture and ash.

The presence of royal jelly in samples was confirmed through chromatographic analysis of 10-HDA.

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