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Comparative analyses of chemical composition of royal jelly and drone brood

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Royal jelly (RJ) is commonly consumed for its nutritional properties and it has been widely used in commercial medical products, health foods and cosmetics in many countries. Because of the high price of this product, sometimes RJ can be adulterated by adding other less expensive products, like drone brood (DB). Proper identification of both important products RJ and DB requires complete analysis and determination of major compounds in order to find precise descriptors for their accurate characterization. To investigate the effect of adulteration with DB, the chemical composition on mixtures of RJ and DB were analyzed. Seven RJ sample and seven DB samples were analyzed for water content, protein, fructose, glucose, sucrose, total sugars, pH, total acidity and electrical conductivity. In addition, these parameters were applied to mixtures of RJ and DB samples. All samples were collected from the experimental apiaries of Institute of Animal Science (IAS) and stored at -20 ºC before analysis.

Key words: Royal jelly, Drone brood, Bee brood, Chemical composition, Cluster analysis.

INTRODUCTION

Royal jelly (RJ) is a secretion from the hypopharyngeal and mandibular glands of worker bees (*Apis mellifera* L.) and it is the exclusive food of the queen honeybee larva. This secretion is a yellowish, creamy and acidic material with a slightly pungent odor and taste [1]. RJ is a very valuable bee product due to its antioxidant, antibacterial, antifungal and therapeutical properties which are related to the content of different bioactive compounds [2–5]. The composition of RJ is quite complex. It comprises water (60–70%), proteins (9–18%), carbohydrates (7–18%), lipids (3–8%), minerals (0.8–3%), vitamins and amino acids [6, 7]. RJ is a valued bee product which is used and sold pure or mixed with other bee products such as honey, propolis, etc.

Drone brood (DB), comprising a mixture of drone larvae, is close to RJ in some physicochemical characteristics and composition but at different rates. Bee brood has nutritional value and it is increasingly used as a health food supplement. This nontraditional product can also offer a readily accessible and cheap source of biologically active substances, but its biological activity is much lower than that of RJ. From chemical point of view, DB contains water (65–80%), proteins (10–20%), carbohydrates (10–15%), fatty acids and lipids (4–8%), minerals K, Na, Ca, Mg (1–1.5%), amino acids and hormones (testosterone and estradiol) [8–11]. Various methods have been identified for adulteration of RJ most frequently by mixing it with DB. Garcia-Amoedo and Almeida-Muradian [12] have analyzed the physicochemical profile of the RJ adulterated in different proportions by the following adulterants: natural yogurt, pure water, starch corn slurry, a mixture of sweet condensed milk with propolis and unripe banana. Proper identification of both important products RJ and DB requires complete analysis and determination of major compounds in order to find precise descriptors for their accurate characterization. In the present paper various RJ and DB samples have been analyzed and characterized for major component content. To the best of our knowledge the composition of RJ adulterated with DB has not been fully studied until now. Statistical analysis of results obtained allows precise identification of both products. Such information and data are essential not only from scientific point of view, but it is also important for practical purposes in order easily and efficiently to assess the authenticity of this both commercial product.

EXPERIMENTAL

Samples. Seven RJ samples, seven DB samples and mixtures of them were analyzed. All samples were collected from experimental apiaries of Institute of Animal Science during the period May – August 2011 and were stored at -20°C until

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analysis.

Preparation of the adulterated RJ samples. 12-14-day-old DB larvae were taken from the DB cells and were immediately smashed. Mixtures of DB larvae were filtrated and added to the RJ samples in a proportion of 1:1, 1:2 and 2:1 (w/w). The samples were shaken to obtain homogeneous solutions. The adulterations with DB were prepared in the laboratory and the samples were kept in the freezer at -20 ºC until the moment of the analysis.

Methods. In brief, the analysis was performed as follows: sugars (fructose, glucose, sucrose) by HPLC as proposed by Sesta [13]; proteins by Folin-Ciocalteu reagent; water content by refractometer; pH values – potentiometricaly; total acidity by titration with 0.1 N NaOH according to ON 2576693-84 Fresh and lyophilized royal jelly [14]; electrical conductivity by conductometer (1% water solution of sample) [15].

Statistical Analysis. Data were statistically processed by Student's t-test for the differences between RJ and DB samples. Means and standards deviations of means were determined with descriptive statistical methods. Differences between means at the 1% ($p<0.001$) level were considered significant.

Cluster analysis is applied as the method for finding different classes and groups within the obtained data. The cluster analysis is a group of multivariate techniques whose primary purpose is to assemble objects based on the characteristics they possess. The obtained results were processed with MS Office 2007 and STATISTICA 7.0.

RESULTS AND DISCUSSION

Results for major compound content in RJ and DB samples are summarized in Table 1 and 2. Additionally, in order to assess possible changes in composition of adulterated RJ, mixtures of RJ and DB (RJ+DB) have been analyzed and the results are shown in Table 3.

Table 1. Main components and physicochemical characteristics of RJ samples (n=7)

Parameters	Mean*±SD**	Range
Water content, %	63.39±1.75	61.00–65.20
Proteins, %	16.73±1.29	14.65–18.33
Fructose, %	4.88±0.37	4.24–5.35
Glucose, %	3.46±0.58	2.70–4.15
Sucrose, %	1.53±0.55	0.59–2.05
Total sugars, %	9.86±0.93	8.47–10.80
pH	3.95±0.09	3.80–4.02
Total acidity, ml 0.1 N NaOH/g	4.07±0.30	3.68–4.42
Electrical conductivity, µS/cm	205.14±8.73	194.00–219.00

*p<0.001; **SD – Standard deviation

Table 2. Main components and physicochemical characteristics of DB samples (n=7)

Parameters	Mean*±SD**	Range
Water content, %	70.97±0.72	70.30–72.30
Proteins, %	9.35±0.63	8.12–10.00
Fructose, %	0.11±0.11	0.00–0.34
Glucose, %	6.74±0.65	5.92–7.88
Sucrose, %	0.05±0.07	0.00–0.18
Total sugars, %	6.92±0.70	6.22–8.22
pH	6.49±0.14	6.23–6.63
Total acidity, ml 0.1 N NaOH/g	0.88±0.15	0.74–1.10
Electrical conductivity, µS/cm	161.43±10.67	144.00–178.00

*p<0.001; **SD – Standard deviation

Table 3. Main components and physicochemical characteristics of RJ samples adulterated with DB (n=7)

	RJ+DB 1:1	RJ+DB 2:1	RJ+DB 1:2
Parameters	Mean±SD**	Mean±SD**	Mean±SD**
Water content, %	67.24±0.59	65.68±0.79	68.13±0.40
Proteins, %	14.90±1.17	15.66±1.12	13.20±0.23
Fructose, %	3.17±0.14	4.24±0.29	2.30±0.13
Glucose, %	6.30±0.48	4.82±0.20	5.64±0.23
Sucrose, %	1.23±0.19	1.02±0.07	0.85±0.12
Total sugars, %	10.70±0.65	10.07±0.42	8.79±0.32
pH	4.25±0.10	4.45±0.08	5.08±0.15
Total acidity, ml 0.1 N NaOH/g	2.72±0.14	3.43±0.11	2.23±0.14
Electrical conductivity, µS/cm	171.14±7.17	188.76±3.33	177.83±4.23

**SD – Standard deviation

The comparison of results from chemical analysis of RJ and DB samples are better visualized in Figure 1.

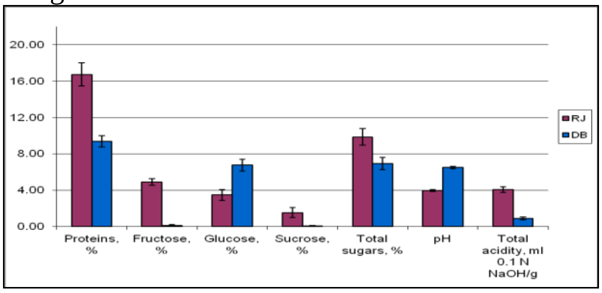

Fig. 1. Average values of proteins, sugars, pH and total acidity of RJ and DB samples (n=7)

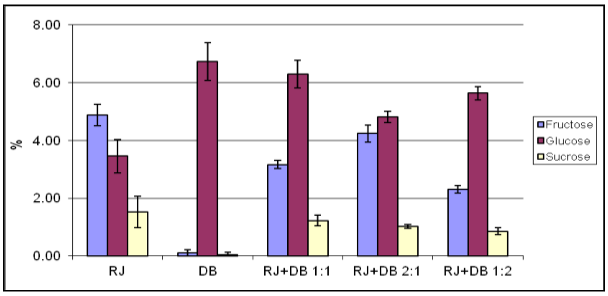

Fig. 2. Average values of sugar profile of RJ, DB samples and their mixtures (n=7)

The results for the average protein content showed relatively narrow range of variation and demonstrated that RJ samples contained almost twice higher protein amount than DB samples. It might be suggested that protein concentration could be a good descriptor for RJ identification. However as can be seen for RJ+DB mixtures protein amount is very close to this of pure RJ (the amount of protein in RJ+DB 1:1 samples is 14.90% and this value is similar to minimal value for pure RJ (14.65%) (see Table 1) which mean that DB proteins quantity should not be the only one descriptor for RJ identification.

Sugars represent second major fraction of compounds in RJ and DB – as a rule the monosaccharides fructose and glucose are the most abundant, while small amounts of disaccharides (mainly maltose and sucrose) are also present; other trisaccharides (erlose), could be found in very small quantities in RJ [13, 16]. As can be seen from results in Table 1 and Table 2 significant differences were observed between average values for total sugar content in RJ and DB samples. Although statistically significant ($p<0.001$) these variances are not very useful for products identification as far as absolute values found are relatively small. Much better descriptor for differentiation between RJ and DB is sugar profile see Figure 2. The most abundant sugar in both products is glucose which is almost twice higher in DB in comparison with RJ. The fructose levels in RJ samples are much higher (average value 4.88%) in comparison with these in DB (average value 0.11%), this compound cold be used as very good descriptor. Sucrose content in RJ samples is relatively higher than this in DB however values are considerably low and not enough informative. Results obtained for DB in this study are close to the results of Burmistrova [11]. They reported that the proportions of the major sugars in DB are glucose (3.16–5.00%), fructose (0.03–0.50%) and sucrose (0.03–0.50%). Higher content of glucose is specific for DB and this parameter is appropriate for identification purposes.

Relatively significant difference was observed between pH values of RJ and DB – for pure products RJ is acidic with average pH 3.95 (value closed to this reported by Isidorov et al. [17]) while DB is almost neutral average pH 6.49 (value close to this reported by Budnikova [10] for DB, between 5.67 and 6.67 in different larvae stages). However due to high buffer capacity of RJ, mixed samples (see Table 3) showed pH very close to this of pure RJ which mean that pH could not be used for precise RJ identification.

Electrical conductivity depends on the chemical composition of the product, mainly from mineral elements, organic acids, amino acids and proteins content. This parameter should be used for identification purposes.

As might be expected taking into account pH values the pure RJ samples showed higher total acidity (average value 4.07 ml 0.1 N NaOH/g), compared to the samples of DB (average value 0.88 ml 0.1 N NaOH per g sample). Samples of RJ adulterated with DB showed total acidity proportional to the RJ content in the mixture. This parameter could be used for identification purposes.

As shown in Table 2, water content in DB samples is significantly higher in comparison to RJ samples. The average amount of water decreased in order: DB (70.97%) > RJ+DB 1:2 (68.13%) > RJ+DB 1:1 (67.24%) > RJ+DB 2:1 (65.68%) > RJ (63.39%) (Table 1–3). Although good parameter for identification purposes water content strongly depend on storage conditions and it is difficult to control experimental conditions during measurement. From this point of view water amount could not be accepted as enough reliable parameter for identification purposes.

Compared to our results, Budnikova [10] established higher values for protein and sugar levels in drone brood. They can vary depending on the age of the larvae during time of collection. Our research matches the data reported in the literature [6]. Sugars, water content and proteins are the most common criteria used to characterize RJ composition.

It is worth mention that there was an effect observed on the organoleptic properties of the RJ samples mixed with DB. Pure RJ is an acidic product with a slightly pungent taste. DB has a stronger flavour than RJ. All adulterated samples (RJ+DB) had strong and untypical flavour.

Result of cluster analysis. All the results have gone through a statistical analysis by cluster analysis. It indicates the linkage between the clustered objects with respect to their similarity (distance measure) and takes into account majority of parameters defined for RJ identification. On Figure 3 the dendrogram for linkage of RJ, DB and their mixtures are presented.

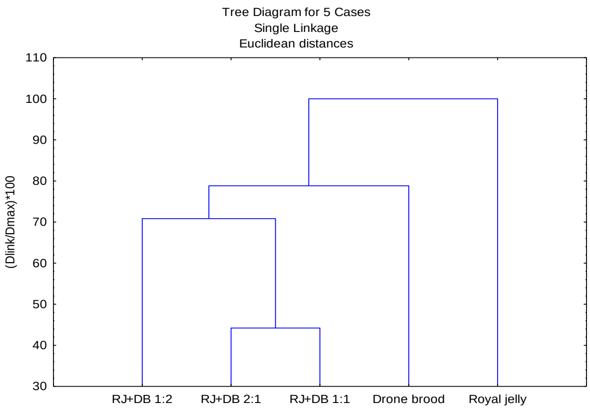

Fig. 3. Dendrogram from cluster analysis of RJ, DB and adulterated samples for all parameters (n=7)

The method of single linkage was chosen as a measure of similarity to classify the RJ, DB and adulterated samples. It could be concluded that Euclidean Distance (the nearest distance method) gives the best result. Two clusters are formed as follows: DB; RJ+DB 1:2, RJ+DB 2:1, RJ+DB 1:1 and RJ as an outlier. It could be concluded from the cluster analysis results that parameters chosen are good descriptors for pure RJ identification as fas as relatively good separation between all samples studied and pure RJ is achieved. As can be seen from dendrogram (Figure 4) parameters such as protein and fructose content, total acidity and electrical conductivity could be used for identification purposes.

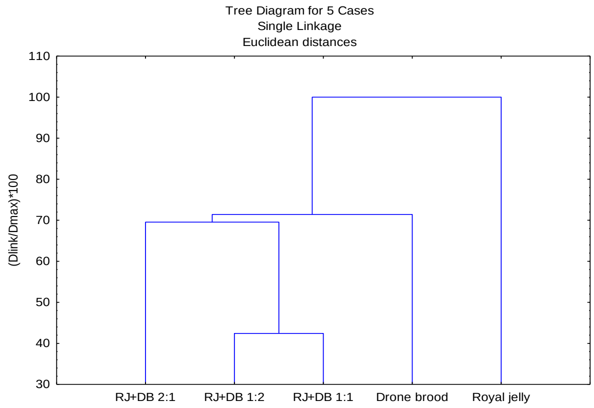

Fig. 4. Dendrogram from cluster analysis of RJ, DB and adulterated samples for parameters protein, fructose, total acidity and electrical conductivity (n=7)

CONCLUSION

The differences in water content, dry mater, proteins, sugars, pH, total acidity, electrical conductivity between RJ and DB samples are significant ($p<0.001$). The cluster analysis turned out to be useful for finding similarity (or dissimilarity) between pure RJ and mixtures with other products. Parameters such as protein and fructose content, total acidity and electrical conductivity are good descriptors for the evaluation of RJ authenticity. Therefore, the addition of DB in RJ would change in a certain direction the composition on RJ. In Bulgaria, there is no regulation for RJ and for DB separately nor for their combination. These results have to be supplemented with additional measurements in order to confirm and to complete these findings.

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СРАВНИТЕЛНИ АНАЛИЗИ НА ХИМИЧНИЯ СЪСТАВ НА ПЧЕЛНО МЛЕЧИЦЕ И ПИЛО ОТ ТЪРТЕИ

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(Резюме)

Пчелното млечице (RJ) обикновено се консумира заради хранителните си свойства и е широко използвано в комерсиалните медицински продукти. Понякога RJ може да бъде смесено с други, по-евтини продукти, подобни на пило от търтеи (DB). Правилната идентификация на двата важни продукта RJ и DB изисква пълен анализ и определяне на основните съединения, за да се намерят точни описания за тяхното охарактеризиране.

За да се изследва ефектът от смесването с DB, е анализиран химичният състав на смеси от RJ и DB. Седем проби от RJ и DB са анализирани за водно съдържание,`протеини, фруктоза, глюкоза, захароза, тотални захари, pH, обща киселинност и електропроводимост. В допълнение, тези параметри са изследвани и за смесени от RJ и DB проби. Всички проби са събирани от експерименталните пчеларници на института по животновъдство и са съхранявани при -20^{o}C преди анализите.