

DETERMINATION OF TOTAL PROTEIN CONTENT IN ROYAL JELLY: A COMPARISON OF THE KJELDAHL, THE BRADFORD AND THE LOWRY METHODS

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Abstract

The bee products gained lots of popularity as healthy food nowadays. Protein content is an recognized parameter used for the quality evaluation of royal jelly. A comparative study of the Kjeldahl and two spectrophotometric methods (Bradford and Lowry) was carried out in order to find the most relevant assay for total protein determination on pure royal jelly samples. The protein values obtained by Kjeldahl and Lowry methods were significant different. The Kjeldahl method showed higher protein values and lowest variation of protein content in royal jelly than Bradford and Lowry methods. Both the Bradford and Lowry methods had a higher sensitivity than Kjeldahl method and equivalent variability (standard deviation about the means). The results showed that all three assays can be used for protein analyze in royal jelly, but Bradford method provide more reliable protein values than Kjeldahl method and even than Lowry method due to a higher sensitivity and precision. In a context of lack of standard methods for evaluating royal jelly composition, this study is very useful for the control laboratories food and bee-products quality.

Key words: Protein analysis, Kjeldahl method, Bradford method, Lowry method, Royal jelly

INTRODUCTION

The bee products gained lots of popularity as healthy food nowadays. Royal jelly (RJ) is a thick and milky secretion from the hypopharyngeal and mandibular glands of young worker bees (*Apis mellifera* L.) and is used to feed the larvae [12]. Due to its exceptional biological activities RJ is one of the most attractive functional foods especially used in pharmaceutical, food industries, cosmetic and manufacturing sectors.

The composition of RJ is essential for its quality evaluation. Chemically, fresh RJ comprises water (60–70%), proteins (9–18%), carbohydrates (7–18%), lipids (3–8%), mineral salts (ca. 1.5%) and small amounts of polyphenols and vitamins [11]. Up to now, there are no international standards for analysis methods of RJ, even if some countries like Brazil, Bulgaria, Japan and Switzerland had established national standards.

Determination of protein concentration is an important tool that is used as a routine

procedure in many research or food control laboratories, being essential to ensure quality and authenticity of food. Total protein content is determined by nitrogen quantification and spectrophotometric methods. Unfortunately, no protein assay method exists that is either perfectly specific to proteins (not affected by any nonprotein components) or uniformly sensitive to all protein types (not affected by differences in protein composition).

The aims of this study are to compare protein content determined by Kjeldahl, Lowry and Bradford methods for RJ samples and to investigate which method gives the most accurate and precise results, being known that food matrix components can influence these both parameters. These methods were chosen because they are easy to carry out and they are based on different reactions.

The Kjeldahl method [7] is the international reference method used on food and feeds, being the most commonly used assay on RJ. The method has lack analytical selectivity for protein because it measure protein on the basis of total nitrogen content and does not distinguish protein-based nitrogen

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from non-protein nitrogen. The basis of the Kjeldahl method is digestion of the sample with sulfuric acid in the presence of catalysts. Organic nitrogen is reduced to ammonium sulfate, which is distilled in the presence of sodium hydroxide, liberating ammonia gas. The distillate is collected into boric acid solution, and the borate anions formed are titrated with standardized hydrochloric acid solution. The milliequivalents of acid required for titration are used to calculate the nitrogen content in the sample [2]. This more than 100-yr-old method has been modified over the years to be more convenient and is used in analytical laboratories as a routine method. Despite its lack of selectivity for protein, total nitrogen protein quantification method has minimal matrix effects due to the fact that as the nitrogen is analyzed, and the food matrix is almost completely oxidized during analysis. However, this method has the disadvantages of using corrosive and/or toxic chemicals with consequent waste production and risk to human health, long analysis time and multiple steps providing many opportunities for error [6].

The Lowry method [8] for total protein determination is one of the most common colorimetric assays performed by biochemists. This procedure lies in the reactivity of the peptide bonds with the Cu^{2+} under alkaline conditions and the subsequent reduction of the Folin-Ciocaltey phosphomolybdicphosphotungstic acid to heteropolymolybdenum blue by the copper-catalyzed oxidation of aromatic acids [3]. The Lowry method is sensitive to low concentrations of protein. The major disadvantage of the Lowry method is the narrow pH range within which it is accurate. However, in this experiment very small volumes of sample are used which have little or no effect on pH of the reaction mixture. A variety of compounds (some amino acid derivatives, zwitterionic and nonionic buffers, drugs, lipids, sugars, salts, nucleic acids, sulphhydryl reagents, ammonium ions and thiol compounds) can interfere with the Lowry procedure [3,9].

The Bradford assay [1] is another colorimetric method commonly used for the assay of protein. It is based on a shift in the

spectrum of a dye upon binding to various concentrations of protein. The absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when noncovalent electrostatic and van der Waals interactions with proteins occur. Since the dye is anionic, it is more sensitive to proteins with high arginine, lysine, and histamine content. This method has gained popularity in research and other applications since it is a simple, quick, one-step procedure that forms a relatively stable colored complex and is free of many of the interferences that limit the application of other methods. Among the assays of this study, Bradford method is the only one that measures proteins.

MATERIAL AND METHOD

Samples of crude Royal jelly (RJ) ($n=9$) were acquired in the year 2011 from all different companies/distributors in Romania. Samples were kept in the dark at 4°C until further analyses in the same year.

The Kjeldahl method was used as described by AOAC [5] with distillation parameters optimization (Digester K-424, Distiller KjelFlex K-360 and titrator Schott-Titro Line).

The Lowry method [8] was carried out using 0.05 g RJ dissolved in 10 ml bidistilled water then centrifuged (25 min, 2300 g). Folin-Ciocalteu reagent was added to the supernatant and the absorbance measured at 750 nm.

The Bradford method [1] used 0.05 g RJ mixed with 5 ml sodium phosphate buffer (pH 6.1), homogenized and centrifuged (20 min, 2300 g). To the supernatant Bradford reagent was added and the absorbance measured at 595 nm.

For the last two assays a standard curve where constructed using BSA (bovine serum albumin, concentration of 0.1 - 1%) and the absorbance was measured on a spectrophotometer (UV-VIS 1700, Shimadzu).

Statistical analysis was performed using STATISTICA 8.0, *t*-test at a significance level of $P < 0.05$.

RESULTS AND DISCUSSIONS

Table 1 presents the means ± standard deviation (each determination performed in triplicate) and the range of protein content. The comparison between Kjeldahl and Lowry methods, Kjeldahl and Bradford methods and Lowry and Bradford methods revealed significant difference between the protein values obtained by Kjeldahl and Lowry methods ($P = 0.006$). The Kjeldahl method showed the lowest variation of total protein content among the samples ($SD = 1.12$). All three methods showed normal values of protein within RJ samples [11].

The majority of samples had highest protein values using Kjeldahl method and Lowry methods shows higher values than Bradford method (Fig. 1). These higher protein values obtained by the Kjeldahl methods can be explained by the lack of selectivity for protein due indirect determination of proteins via nitrogen – all proteic and non-proteic. The difference between the Lowry and Bradford values is caused by the Folin-Ciocalteu

reagent, within the Lowry assay, that does not react specific with Cu^{+} , but with any reducing substance including polyphenols and carbohydrates that are contained in RJ. The Coomassie Brilliant Blue G-250, used in the Bradford assay, interferes less with other molecules. Whiffen *et al.*, 2007 [14] found that the polyphenol tannic acid react with the dye, but it occurs mainly in stem tissues and storage sites of plants and up to now was never been found in RJ.

Table 1 Total protein content in royal jelly measured by three different methods

Method	Protein (%)
Kjeldahl	13.79 ± 1.12 (11.81 – 14.98)
Lowry	12.98 ± 1.75 (9.93 - 15.70)
Bradford	11.71 ± 2.29 (7.07 - 15.56)

Values are given as means with standard deviation including the range in brackets with each determination performed in triplicate

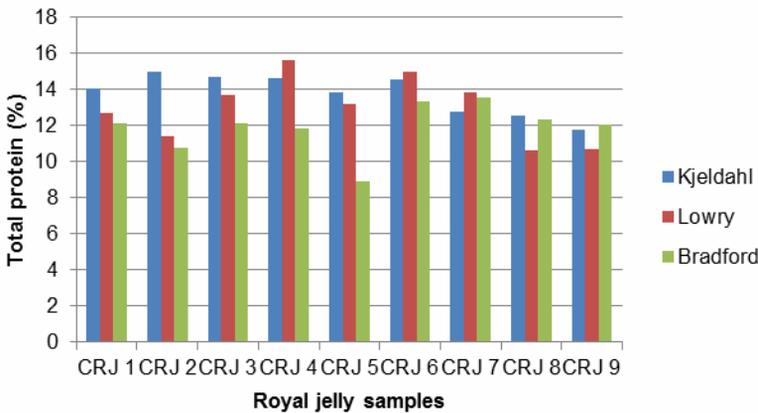


Fig. 1 Total protein content in each royal jelly sample measured by three different methods

Comparing the three methods used for the protein content determination from another point of view, the accuracy and the precision of the assays were used. Accuracy is defined as the concordance between it [a determination] and the true or most probable value, while precision as the concordance of a series of measurements of the same quantity [13]. As far as we know, no studies on

comparing the protein assays on RJ were made before. The results obtained during the Kjeldahl method (Table 2) showed widespread values, but the average of the results was within the normal range of proteins contained in RJ. These results are shown to be accurate, but not precise. The Lowry (Table 2) and Bradford (Table 3) methods showed different results that are

both precise and accurate, the absorbance values being more condensed.

Therefore, even the Kjeldahl method have minimal matrix effects, Lowry and Bradford methods showed more accurate and precise results and from the latest two assays the Bradford method would be preferred due to fewer interferences that can occur in this reaction. The Bradford method showed the highest sensitivity for proteins (range of protein concentration varied between 7.07 and 15.56% and the Kjeldahl method showed the lowest sensitivity for proteins (range of protein concentration varied between 11.81 and 14.98%).

Table 2 Results obtained in a royal jelly sample using Kjeldahl method - protein content

Concentration of the titration acid (mol/L sulfuric acid)	Protein (%)
0.01	5.59
0.05	14.22
0.1	22.43
Average protein (%)	14.08 ± 8.42

Table 3 Results obtained in using Lowry and Bradford methods - absorbance

Absorbance	Lowry	Bradford
1 st Absorbance	0.675	0.896
2 nd Absorbance	0.783	0.918
3 rd Absorbance	0.796	0.945
Average absorbance	0.751 ± 0.7	0.920 ± 0.02

CONCLUSIONS

The Kjeldahl, Lowry and Bradford methods used for total protein determination on royal jelly offer values within the normal ranges for proteins in this bee-product.

The Bradford method offers more reliable protein values than the Kjeldahl method and even the Lowry method because of its higher sensitivity and precision and furthermore is a simple and quick assay. In a context of lack of standard methods for evaluating royal jelly composition, this study is very useful for the control laboratories of bee-products quality.

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REFERENCES

- [1] Bradford M.M., 1976: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, *Analytical Biochemistry*, 72: p 248-54.
- [2] Chang S.K.C., 1998: *Protein Analysis*, in Food Analysis, S. Nielsen Eds., Aspen Pub, Gaithersburg, MD, p: 237–250.
- [3] Dunn M. J., 1992: Protein determination of total protein concentration, In Harris, E.L.V., Angal S. Eds, *Protein Purification Methods*, Oxford: IRL Press.
- [4] Moore C.J., DeVries J.W., Lipp M., Griffiths J.C., Abernethy D.R., 2010: Total Protein Methods and Their Potential Utility to Reduce the Risk of Food Protein Adulteration, *Compr. Rev. Food Sci. F*, 9: p 330-357.
- [5] Helrich K., 1990: Official methods of analysis of the association of official analytical chemists Arlington: Association of Official Analytical Chemists Inc., p 807.
- [6] Jung S., Rickert D.A., Deak N.A., Aldin E.D., Recknor J., Johnson L.A., Murphy P.A., 2003: Comparison of kjeldahl and dumas methods for determining protein contents of soybean products, *Journal of the American Oil Chemists' Society*, nr 12(80): p 1169-1173.
- [7] Kjeldahl, J., 1883: Neue Methoden zur Bestimmung des Stickstoffs in Organischen Korpern, *Z. Anal. Chem.*, 22: p 366–382.
- [8] Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J., 1951: Protein measurement with the Folin phenol reagent, *Journal of Biological Chemistry*, nr 193(1): p 265-75.
- [9] Price, N. C., 1996: *Proteins*, Labfax, Oxford, Academic Press.
- [10] Ramadan M.F., Al-Ghamdi A., 2012: Bioactive compounds and health-promoting properties of royal jelly: a review, *Journal of Functional Foods* nr 4: p 39-52.
- [11] Sabatini, A.G., G.L. Marcazzan, M.F. Caboni, S. Bogdanov and L. Biculo de Almeida- Muradian (2009). Quality and standardisation of Royal Jelly. *J. ApiProd. ApiMed. Sci.*, nr 1(1): p 1-6.
- [12] Townsend G.F., Lucas C.C., 194: The chemical nature of royal jelly. *Biochem. J.*, nr 34(8-9): p 1155-62.
- [13] Vogel A.I., 1961: *A Textbook of Quantitative Inorganic Analysis Including Elementary Instrumental Analysis*. Longmans.
- [14] Whiffen L.K., Midgley D.J., McGee P.A., 2007: Polyphenolic compounds interfere with quantification of protein in soil extracts using the Bradford method. *Soil Biology and Biochemistry*, nr 39(2): p 691-94.