ELSEVIER

Food Chemistry 80 (2003) 249–254

Food Chemistry

www.elsevier.com/locate/foodchem

# Protein contents and physicochemical properties in honey samples of *Apis mellifera* of different floral origins

L. da C. Azeredo*, M.A.A. Azeredo, S.R. de Souza, V.M.L. Dutra

*UFRRJ—Instituto de Ciências Exatas, Depto de Química, BR-465, km 07, CEP: 23890-000, Seropédica, RJ, Brazil*

Received 22 March 2002; accepted 31 May 2002

**Abstract**

The protein contents in honey samples of different floral origins, commercialized in several states of Brazil, were determined using the method of Bradford. The spectra of pollen of the honeys collected in those areas were studied, in order to establish the correlation between the different botanical species and the protein contents. The physicochemical properties of the honeys (colour, moisture, pH and acidity, lund test, lugol test, diastase index, reducing and non-reducing sugars and hydroxymethylfurfural contents) were also determined. The colorimetric determination of the protein content of honey samples, using the method of Bradford, was shown to be efficient and it allowed the detection of elevated protein in honey samples of *Borreria verticillata*, known in Brazil as ''vassourinha'', from Piauí State.



*Keywords:* Honey; Protein; *Apis mellifera*; Floral origin

## 1. Introduction

Honey has been used as an alimentary supplement, in medical therapies and a natural food, without the addition of any substance in its elaboration. The physicochemical characteristics of honey, such as high viscosity and density, consistency and sweetness, are due to the fact that it is actually a solution with a high concentration of sugars. Honey is variable in its composition, due to contribution of the plant, climate, environmental conditions and the ability of the beekeeper (White, 1978). The diversity of the physical and chemical properties of honey depends on the nectar and pollen of the original plant, colour, flavour, moisture and contents of proteins and sugars (Barth, 1989; White, 1978; White & Maher, 1980).

The method of Lowry, Rosebrough, Farr, and Randall (1951) is the procedure most used for the quantitative determination of proteins. Lowry and co-workers combined the use of copper, as suggested by Herriott (1941), with the Folin-phenol method, which originated from the work of Wu (1920), to produce a more reliable and sensitive protein assay. However, the main disadvantage of this method is its lack of specifity. Many substances are known that interfere with this method, such as EDTA (Neurath, 1966), tris (Kuno & Kihara, 1967), thiol reagents (Vallejo & Lagunas, 1970) and carbohydrate (Lo & Stelson, 1972). Potassium and magnesium also interfere with this method. Other disadvantages of the method include relatively slow reaction rates, instability of some reagents and non linearity of the standard curve (Peterson, 1979). Several authors report advantages (Bensadoun & Weinstein, 1976; Dulley & Grieve, 1975; Horikawa & Ogawara, 1979; Makkar, Sharma, & Negi, 1980; Markwell, Hass, Bieber, & Tolbert, 1978; Mather & Tamplin, 1979; Ross & Schatz, 1973; Wessel & Flügge, 1984) of proposed alternative methods but they present more complication and involve more time of analysis.

The procedure described by Bradford (1976) eliminates most of the problems involved in the procedure of Lowry and in the modified methodologies and it is easily adapted to the determination of a great number of samples being therefore, suitable for automation. It is based on the observation that the Coomassie Brilliant Blue G-250 reagent exists in two coloured forms, red and blue (Reisner, Nemes, & Bucholtz, 1975). The red

* Corresponding author. Tel.: +55-21-2682-2807.
*E-mail address:* azeredo@ufrrj.br (L. da C. Azeredo).

0308-8146/02/$ - see front matter 
PII: S0308-8146(02)00261-3

form is transformed into the blue form by protein-dye binding. The protein-dye complex has a high extinction coefficient, causing a larger sensitivity in the measure of the protein. Protein-dye binding is a very fast process (approximately 2 min) and the protein-dye complex stays dispersed in solution for a relatively long time (approximately 1 h), making this a very fast procedure.

The first pollen analysis in Brazilian honeys, was done on species whose pollen grains were found in a series of samples removed over successive months from beehives located in Piracicaba county (SP) (Santos, 1961) and in five honey samples of native bees examined by Maurizio (1964), as well as by Absy, Camargo, Kerr, and Miranda (1984) and Carreira, Jardim, Moura, Pontes, and Marques (1986) in the north area of the country. Generally, the determination of the plant families, by the pollen in the honey does not constitute a great obstacle. However, genus is not always differentiable by the pollen morphology and nor is the species, so that it is necessary to limit the pollen type (morphologic type) in order not to introduce error in the analysis (Barth, 1989).

## 2. Materials and methods

### 2.1. Pollen analysis

The preparation of honey samples was performed according to the method described previously (Louveaux, Maurizio, & Varwohl, 1978). Ten grammes of honey were dissolved in 20 ml of distilled water. This mixture was divided into two centrifuge tubes of 15 ml, and centrifuged for about 5 min, at low speed. Distilled water was again added to the sediment, repeating the previous operation. Approximately 5 ml of glycerine–water 1:1 were added to the sediment, and it was left to rest for 30 min. After this time, the sample was centrifuged. The sediment was removed with aid of a stylet, embedded in glycerine jelley and deposited on a microscopic slide, sealing with paraffin wax.

### 2.2. Physicochemical analysis

In order to guarantee the quality of the analyzed honey samples, the following qualitative and quantitative analyses were carried out: moisture, Lund, Lugol and diastase qualitative index (Instituto Adolfo Lutz, 1985), diastase quantitative index, reducing and non-reducing sugars and quantitative hydroxymethylfurfural (AOAC, 1984) and Fiehe, colour, pH and acidity (Lanara, 1981).

### 2.3. Determination of proteins

The protein content was determined by the method of Bradford (1976). To 0.1 ml solution of protein extract (honey sample 50% w/v), were added 5 ml of Coomassie Brilliant Blue (200 mg of Coomassie Brilliant Blue G-250 dissolved in 100 ml 95% ethanol and, finally, 200 ml 85% $H_3PO_4$ added. The resulting solution was diluted to a final volume of 2 l). The Coomassie Brilliant Blue forms a blue complex with the proteins. After 2 min of incubation, the absorbance was measured at 595 nm, against an albumin standard solution of bovine serum (10–100 μg/0.1 ml) in 0.15 M NaCl.

Table 1
Characterization of the analyzed honey samples

| Sample | Type of honey | Predominant plant |
|---|---|---|
| M01 | Monofloral | *Eucalyptus* sp |
| M02 | Monofloral | *Myrtaceae* |
| M03 | Monofloral | *Citrus* |
| M04 | Heterofloral | *Citrus; Eucalyptus* |
| M05 | Monofloral | *Vernonia* sp |
| M06 | Heterofloral | *Citrus, Eucalyptus, Anadenanthera* |
| M07 | Monofloral | *Borreria verticillata* |
| M08 | Heterofloral | *Borreria verticillata; Mimosa verrucosa* |
| M09 | Monofloral | *Sapindaceae* |
| M10 | Monofloral | *Eucalyptus* sp |
| M11 | Heterofloral | *Cassia; Eupatorium* sp |
| M12 | Heterofloral | *Eucalyptus; Mimosa scabrella* |

## 3. Results and discussion

### 3.1. Types of honey

Twelve different honey samples were properly characterized, according to floral origin, and the data are presented in Table 1.

The main apicultural plants found after pollen analysis and the frequency of pollen types of the honey samples studied in this work are presented in Tables 2 and 3, respectively.

### 3.2. Physicochemical analysis

#### 3.2.1. Moisture

Table 4 shows the results for moisture in the different analyzed samples.

All the values obtained were below 20%, the maximum value allowed by the Brazilian legislation (Lanara, 1981).

#### 3.2.2. Lund test

Table 5 shows the results for the Lund test, in the analyzed samples.

The values found for the deposits of proteins after Lund test application were within the range established by the official methods applied in Brazil (Instituto Adolfo Lutz, 1985).

Table 2
Main apicultural plants by pollen analysis

| Famíly | Scientific name | Common name | Property |
|---|---|---|---|
| Compositae | *Eupatorium* sp | "erva de Santa Cruz" | – |
| | *Vernonia* sp | "assa-peixe" | Nectariferous |
| Leguminosae | | | |
| Caesalpiniaefolia | *Cassia* sp | "acácia" | Polliniferous |
| Leguminosae | *Mimosa scabrella* | "sensitiva" | Pollinífera |
| Mimosoideae | *Mimosa verrucosa* | "espinheiro" | Nectariferous and polliniferous |
| Myrtaceae | *Eucalyptus* sp | "eucalipto" | Nectariferous and polliniferous |
| Rubiaceae | *Borreria verticillata* | "vassourinha" | Nectariferous and polliniferous |
| Rutaceae | *Citrus* sp | "laranjeira" | Nectariferous |

Table 3
Most frequent pollen types in the analyzed honey samples

| Pollen types | M01 | M02 | M03 | M04 | M05 | M06 | M07 | M08 | M09 | M10 | M11 | M12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Amaranthaceae** | | | | | | | | | | | | |
| *Alternanthera* | | | | | | PO | | | | | | |
| **Anacardiaceae** | | | | | | | | | | | | |
| *Schinus* | | | | | | | | | PO | | | |
| **Combretaceae** | | | | | | | | | PO | | | |
| **Compositae** | | | | | | | | | | | | |
| *Baccharis* | PI | | | | | | | | | PI | | |
| *Elephantopus* | | | | | | | PO | PO | | | | |
| *Eupatorium* | PI | PA | | | | PO | | | | PI | | |
| *Montanoa* | | | | | | PO | | | | PI | | |
| *Vernonia* | | | | | PD | | PI | PI | | | | PO |
| **Convolvulaceae** | | | | | | | | | | | | |
| *Merremia* sp | | | PO | | | | | | | | | |
| **Cruciferae** | | | | PO | | | | | | | | |
| **Euphorbiaceae** | | | | | | | | | | | | |
| *Croton* | | | PO | | | | | | | | | |
| *Ricinus* | | | PO | | | | | PO | | | | |
| **Gramineae** | | | PO | PO | | | | PO | | PO | | PO |
| *Zea* | | | | | | | | | | PO | PO | PO |
| **Labiateae** | | | | | | | | | | | | |
| *Hyptis* | | | | | | | PO | PO | | | PO | PO |
| **Leg. Caes.** | | | | | | | | | | | | |
| *Cassia* | | | | | | | | | | | PD | |
| **Leg. Mimosaceae** | | | | | | | | | | | | |
| *Anadenanthera* | | PI | PO | | | PA | | | | | PO | |
| *Mim. Scabrella* | | | | | | | | | | | PO | PD |
| *Mim. Verrucosa* | | | | | | | PO | PA | | | | |
| *Schrankia* | | | | PO | | | | | | | | |
| **Leg. Pap.** | | | | | | | | | | | | PI |
| **Loranthaceae** | | | | | | | | | PO | | | |
| **Malvaceae** | | | | | | PO | | | | PO | | |
| **Moraceae** | | | | | | | | | | | | |
| *Cecropia* | PI | | | PD | | PA | | | | PO | PO | |
| **Myrtaceae** | | | | | | | | | | | | |
| *Eucalyptus* | PD | PD | PA | PA | | PO | | PO | | PD | PO | PA |
| **Onagraceae** | | | | | | | | | | | | PO |
| **Palmae** | | PI | | PI | | | | | | PI | | |
| **Polygonaceae** | | | | | | | | | PO | | | |
| **Rubiaceae** | | | | | | | | | | | | |
| *Borreria verticillata* | | | | | | | PD | PA | PO | | | |
| **Rutaceae** | | | | | | | | | | | | |
| *Citrus* | | | PD | PA | | PA | | | | | | |
| **Sapindaceae** | | | | | | | | | | | | |
| *Serjania* | | | | PO | | | | | PD | | | |
| **Solanaceae** | | | | | | | | | | | PO | |
| **Sterculiaceae** | | | | | | | | | | | | |
| *Dombeya* | | | | | | | | | | | PO | PO |

M01–M12: honey samples. PD = dominant (>45%); PA = accessory (15–45%); PI = isolated (3–15%); PO = occasionally (<3%).

Table 4
Moisture contents, in g/100 g, in the honey samples of different floral origins

| Sample | Content[a] | Sample | Content[a] |
|---|---|---|---|
| M01 | 18.98 | M07 | 19.00 |
| M02 | 18.96 | M08 | 19.40 |
| M03 | 19.32 | M09 | 19.58 |
| M04 | 18.59 | M10 | 19.52 |
| M05 | 19.36 | M11 | 19.25 |
| M06 | 19.10 | M12 | 19.20 |

[a] Mean values obtained after five repetitions of each sample.

Table 5
Volume, in ml, of the precipitate obtained after application of Lund test to the different samples

| Sample | Volume[a] | Sample | Volume[a] |
|---|---|---|---|
| M01 | 1.89 | M07 | 1.60 |
| M02 | 1.52 | M08 | 1.54 |
| M03 | 1.58 | M09 | 1.84 |
| M04 | 1.54 | M10 | 1.46 |
| M05 | 1.28 | M11 | 1.50 |
| M06 | 2.00 | M12 | 1.92 |

[a] Mean values obtained after five repetitions of each sample.

### 3.2.3. Lugol test

All the analyzed samples were submitted to the qualitative test, which detects the presence of dextrins, immediately after receipt of samples, which showed that these samples were authentic.

### 3.2.4. Diastase index

*3.2.4.1. Qualitative test*. The olive-green colour developed by the diastase qualitative test immediately after the receipt of the samples, showed that these were authentic honey samples.

*3.2.4.2. Quantitative test*. Table 6 shows the diastase indices in the different analyzed samples.

The minimum standard value for diastase index is 8, according to the rules, dating from 1980, of the Division for the Inspection of Milk and Derivatives, of the Office for the Inspection of Animal Products (BRASIL, 1980). Based on these criteria, the results obtained for the samples suggest that these are good quality honeys.

### 3.2.5. Reducing and non-reducing sugars

The amounts of total reducing sugars, reducing and non-reducing sugars are shown in Table 7.

The method allowed an estimate of the quality of the samples through the determination of sucrose. A high content of this sugar means, most of the time, an early harvest of the honey, i.e. a product in which the sucrose has not been fully transformed into glucose and fructose by the action of invertase. Generally, the sucrose content does not exceed 8% for authentic honey samples.

Table 6
Diastase index (D.I.) for the different analyzed samples

| Sample | D.I[a] | Sample | D.I[a] |
|---|---|---|---|
| M01 | 12.45 | M07 | 17.40 |
| M02 | 13.25 | M08 | 15.24 |
| M03 | 14.20 | M09 | 14.23 |
| M04 | 13.56 | M10 | 12.10 |
| M05 | 11.46 | M11 | 10.24 |
| M06 | 10.80 | M12 | 11.50 |

[a] Mean values obtained after five repetitions of each sample.

Table 7
Sugar content in the analyzed honeys

| Sample | Sugar content (g/100 g)[a] | | |
|---|---|---|---|
| | T.R.S | R.S | N.R.S |
| M01 | 72.4 | 67.0 | 5.1 |
| M02 | 71.9 | 67.5 | 4.2 |
| M03 | 72.8 | 69.1 | 3.5 |
| M04 | 68.4 | 63.9 | 4.3 |
| M05 | 68.0 | 63.9 | 3.9 |
| M06 | 73.5 | 67.8 | 5.4 |
| M07 | 72.5 | 68.8 | 3.5 |
| M08 | 68.8 | 64.2 | 4.4 |
| M09 | 71.8 | 66.6 | 4.9 |
| M10 | 72.0 | 66.8 | 5.2 |
| M11 | 67.6 | 62.6 | 4.8 |
| M12 | 71.9 | 66.6 | 5.0 |

T.R.S. = total reducing sugar; R.S = reducing sugar; N.R.S. = non-reducing sugar expressed as sucrose.
[a] Mean values obtained after five repetitions.

Table 8
HMF content, mg/100 g[a], for honey samples of different floral origins

| Sample | HMF | Sample | HMF |
|---|---|---|---|
| M01 | 3.76 | M07 | 3.76 |
| M02 | 3.24 | M08 | 3.84 |
| M03 | 3.45 | M09 | 3.42 |
| M04 | 3.28 | M10 | 2.15 |
| M05 | 3.90 | M11 | 4.12 |
| M06 | 3.85 | M12 | 4.06 |

[a] Mean values obtained after five repetitions of each sample.

### 3.2.6. Hydroxymethylfurfural (HMF)

*3.2.6.1. Qualitative test (Fiehe)*. The results from the HMF qualitative analyses, done immediately after receipt of samples, did not show any coloration indicative of high levels of HMF, meaning that no samples were adulterated with commercial sugar or had been submitted to high temperatures.

*3.2.6.2. Quantitative test*. Table 8 shows the contents of HMF found in the honey samples of different floral origins.

Table 9
Classification of the honey samples according to Pfund scale

| Sample | Colour | Sample | Colour |
|---|---|---|---|
| M01 | Amber | M07 | Dark amber |
| M02 | Amber | M08 | Amber |
| M03 | Dark amber | M09 | Amber |
| M04 | Dark amber | M10 | Amber |
| M05 | Amber | M11 | Dark amber |
| M06 | Amber | M12 | Amber |

Table 10
pH[a] and acidity[a] of the analyzed honey samples

| Sample | pH | Acidity | Sample | pH | Acidity |
|---|---|---|---|---|---|
| M01 | 3.56 | 38.5 | M07 | 4.05 | 39.5 |
| M02 | 3.84 | 38.2 | M08 | 3.84 | 32.2 |
| M03 | 4.00 | 28.5 | M09 | 3.54 | 36.3 |
| M04 | 3.75 | 39.0 | M10 | 3.10 | 28.2 |
| M05 | 3.65 | 36.4 | M11 | 3.46 | 28.2 |
| M06 | 3.82 | 35.0 | M12 | 3.20 | 32.1 |

[a] Mean values after five repetitions.

Table 11
Protein content, in μg $g^{-1}$, of honey samples of different floral origins

| Honey samples | Protein content[a] | Proteic classification | Floral origin |
|---|---|---|---|
| M07 | 2236 a | High content | *Borreria verticillata* |
| M08 | 2212 a | High content | *Borreria verticillata*, *Mimosa verrucosa*) |
| M09 | 1203 b | High content | *Sapindaceae* |
| M10 | 734.5 c | Medium content | *Eucalyptus* sp |
| M01 | 670 cd | Medium content | *Eucalyptus* sp |
| M03 | 628 cde | Medium content | *Citrus* |
| M06 | 577 def | Medium content | *Citrus*, *Eucalyptus*, *Anadenanthera* |
| M04 | 552 def | Medium content | *Citrus*; *Eucalyptus* |
| M12 | 512 ef | Medium content | *Eucalyptus*, *Mimosa scabrella* |
| M02 | 500 f | Medium content | *Myrtaceae* |
| M11 | 296 g | Low content | *Cassia; Eupatorium* sp |
| M05 | 199 g | Low content | *Vernonia* sp |

[a] The same letters do not differ significantly from each other (test of Tukey 5%).

The results obtained showed that, in just two cases, the contents were higher than the maximum allowed, which is 4.0 mg/100 g) (AOAC, 1984). As these two samples were harvested after a relatively long time, the results suggest that the amount of HMF tends to increase gradually with time, a fact that accords with the literature.

### 3.2.7. Colour

The classification of honeys by their colour was carried out immediately after receipt of samples, using the Pfund scale (Lanara, 1981). Table 9 shows the distribution of the honeys with the respective colours.

### 3.2.8. pH and acidity

Table 10 shows the results for pH and acidity of the different analyzed samples. The mean values were in the range of 3.65 and 34.3 meq/kg, respectively, and were within the standards of the Ministry of Agriculture (Lanara, 1981).

### 3.2.9. Quantitative determination of proteins

Table 11 shows the protein contents, in μg/g, of different analyzed honey samples.

The higher protein contents of the samples M07, M08 and M09, shown in Table 11, were not detected by the qualitative test of Lund. For example, the samples M11 and M5, that present about 10% of the protein content of the samples M07 and M08, when evaluated by the test of Lund showed indefinite values (Table 5). Therefore, according to the results observed in these assays, the test of Bradford should be used for the evaluation of the protein content of honeys. In this work, high protein contents were considered to be those higher than 1000 μg $g^{-1}$ (test of Tukey 5%).

The samples M07 and M08, that had the highest protein contents (greater than 2000 μg $g^{-1}$), had pollen predominance of the same floral origin (*Borreria verticillata*; Tables 3 and 11).

These results indicate that the colorimetric determination of the protein content of honey samples using the method of Bradford, was efficient and it allowed the detection of high values in the samples M07 and M08, compared to the mean of the other analyzed honeys. They are honeys of *B. verticillata*, known in Brazil as "vassourinha", coming from Piauí State. These honeys are therefore recommended as alimentary complements for the population of the northeast area of the country.

## References


Absy, M. L., Camargo, J. M. F., Kerr, W. E., & Miranda, I. P.de A. (1984). Espécies de plantas visitadas por Meliponinae (Hymenoptera; Apoidea), para coleta de pólen na região do Médio Amazonas. *Ver. Brasil. Biol.*, *44*(2), 227–237.

AOAC. (1984). *Official methods of analysis* (14th ed). Washington DC: Association of Official Analytical Chemists.

Barth, O. M. (1989). *O pólen no mel brasileiro* (pp. 1–180). Gráfica Luxor.

Bensadoun, A., & Weinstein, D. (1976). Assay of proteins in presence of interfering materials. *Analytical Biochemistry*, *70*, 241–250.

Bradford, M. M. (1976). Rapid and sensitive method for quantification of microgram quanties of protein utilizing the principle dye binding. *Analytical Biochemistry*, *72*, 248–254.

BRASIL (1980). Ministério da Agricultura. Secretaria Nacional de Inspeção de Produtos de Origem Animal, Diário Oficial. Brasília, DF, 28 de março de 1980. Seção I, p. 5570.

Carreira, L. M. M., Jardim, M. A. G., Moura, C., de, O., Pontes, M. A., de, O., & Marques, R. V. (1986). Análise polínica nos méis de alguns municípios do estado do Pará, I. *Anais do 1º Simpósio do Trópico Úmido, Belém, Pará*, *2*, 79–84.

Dulley, J. R., & Grieve, P. A. (1975). Simple technique for eliminating

interference by detergents in Lowry method of protein determination. *Analytical Biochemistry*, *64*, 136–141.

Herriott, R. M. (1941). Reaction of Folin's reagent with proteins and biuret compounds in presence of cupric ion. *Proceedings of the Society for Experimental Biology & Medicine*, *46*, 642–644.

Horikawa, S., & Ogawara, H. (1979). A simple and rapid procedure for removal of Triton X-100 from protein solution. *Analytical Biochemistry*, *97*, 116–119.

Instituto Adolfo Lutz. (1985). *Normas Analíticas do Instituto Adolfo Lutz, v. 1* (3a ed).

Kuno, H., & Kihara, H. K. (1967). Simple microassay of protein with membrane filter. *Nature*, *215*, 974–975.

Lanara. (1981). Laboratório Nacional de Referência Animal. Métodos analíticos oficiais para controle de produtos de origem animal e seus ingredientes. II—Métodos físicos e químicos. *Mel. Ministério da Agricultura. Brasília*, *2*(25), 1–15.

Lo, C., & Stelson, H. (1972). Interference by polysucrose in protein determination by the Lowry method. *Analytical Biochemistry*, *45*, 331–336.

Louveaux, J., Maurízio, A., & Vorwohl, G. (1978). Methods of Melissopalynology. *Bee World*, *59*, 139–157.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*, 265–275.

Makkar, H. P. S., Sharma, O. P., & Negi, S. S. (1980). Assay of proteins by Lowry method in the presence of high cof beta-mercaptoethanol. *Analytical Biochemistry*, *104*, 124–126.

Markwell, M. A. K., Hass, S. M., Bieber, L. L., & Tolbert, N. E. (1978). Modification of Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Analytical Biochemistry*, *87*, 206–210.

Mather, I. H., & Tamplin, C. B. (1979). Method for the determination of protein in the presence of Trition-X-100. *Analytical Biochemistry*, *93*, 139–142.

Maurízio, A. (1964). Mikroskopische und papierchromatographische untersuchungen in the honig von hummeln, meliponinen und anderen zuckerhaltige saefte sammelnde. *Insekten.z.f.Bienenf.*, *7*(4), 98–110.

Neurath, A. R. (1966). Interference of sodium ethylenediaminetetraacetate in the determination of proteins and its elimination. *Experientia*, *22*, 290.

Peterson, G. L. (1979). Review of the Folin phenol protein quantitation method of Lowry, Rosebrough, Farr and Randall. *Analytical Biochemistry*, *100*, 201–220.

Reisner, A. H., Nemes, P., & Bucholtz, C. (1975). Use of Coomassie Brilliant Blue G250 perchloric acid solution for staining in electrophoresis and isoelectric focusing on polyacrylamide gels. *Analytical Biochemistry*, *64*, 509–516.

Ross, E., & Schatz, G. (1973). Assay of protein in the presence og high concentrations of sulfhydryl compounds. *Analytical Biochemistry*, *54*, 304–306.

Santos, C. F. de O. (1961). *Morfologia e valor taxonômico de pólens das principais plantas apícolas*. Thesis, Esc. Sup. Agric. Luiz de Queiroz, Univ. São Paulo, Piracicaba, SP.

Vallejo, C. G., & Lagunas, R. (1970). Interference by sulfhydryl disulfide reagents and potassium ions on protein determination by Lowry's method. *Analytical Biochemistry*, *36*, 207–212.

Wessel, D., & Flügge, U. I. (1984). A method for the quantitative recovery of protein in dilute-solution in the presence of detergents and lipids. *Analytical Biochemistry*, *138*, 141–143.

White, J. W. (1978). Honey. *Advances in Food Research*, *24*, 287–374.

White, J. W., & Maher, J. (1980). Hidroxymethylfurfural content of honey as an indicator of its adulteration with invert sugars. *Bee World*, *61*, 29–37.

Wu, H. (1920). Contribution to the chemistry of phosphomolybdic acids, phosphotungstic acids and allied substances. *Journal of Biological Chemistry*, *43*, 189–220.