Seasonal Variation of Chemical Composition of Royal Jelly Produced in Thailand

Vichai Wongchai
Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand, 50002

Treetip Ratanavalachai
Division of Biochemistry, Department of Preclinical Sciences, Faculty of Medicine, Thammasat University, Pathumthani, Thailand, 12121

Correspondence : Treetip Ratanavalachai, Division of Biochemistry, Department of Preclinical Sciences, Faculty of Medicine, Thammasat University, A. Klongluang, Pathumthani, Thailand, 12121 ; email address: treetip2000@hotmail.com

Abstract

A study was conducted on the chemical composition of royal jelly produced by honeybees (*Apis mellifera*) in Chiang Mai, Thailand. It was shown that seasonal variations had a moderating influence on the chemical composition of royal jelly, especially on carbohydrate and lipid contents, causing slight changes in protein and moisture contents but no alteration in ash content and pH value. The lipid mostly contained acidic polar compounds. The protein mostly contained a water-soluble fraction makeing up to 70% of the total protein, mainly having rather low molecular weights. As compared with other studies, royal jelly from various sources demonstrated similar chemical compositions.

Keywords: *Apis mellifera*, royal jelly, chemical composition, seasonal variation, Chiang Mai, Thailand

1. Introduction

Royal jelly, or bee's milk, is a creamy product secreted by the hypopharyngeal glands in the head of the young nurse worker bees primarily for developing and maintaining the queen bee. It is a special food serving only the queen bee throughout her life, while other sexually immature females are served royal jelly for only the first 2-3 days. The queen bee is 50% larger than worker bees and also lives longer, about 4-5 years, compared to worker bees that live only through one season. The nutritional significance of royal jelly has been proven since any larvae from a fertile egg, if given royal jelly throughout its larvae period, develops sexually so that it becomes a perfect queen, otherwise, the larvae develops into a sexually immature worker [1]. Now, beekeeping has become popular in Thailand especially in the northern part such as Chiang Mai. It has many advantages such as the farmers and gardeners can get more crops in their cultivation because honeybees are good native pollinators. In addition, beekeepers can get bee's products such as honey, wax, and royal jelly, which are of monetary importance since they are used as traditional medicine. In Thailand, honey has been used for clinical treatments and as therapeutic agents, but scientific literature about royal jelly has been scarcely reported. In other countries, such as U.S.A., France, West Germany, Russia, China, Taiwan, and Japan there is various scientific literature reporting on royal jelly produced in their countries [2-6]. Therefore, our research study was conducted to investigate the chemical composition of royal jelly produced by honeybees (*Apis mellifera*) in Chiang Mai,

Thailand in order to perceive how it is different from foreign royal jelly and how the seasonal variations affected the chemical composition of royal jelly. Furthermore, we analyzed the biochemical composition of the constituents in royal jelly in detail in order to postulate their role in biological activities as a healthy food.

2. Materials and Methods

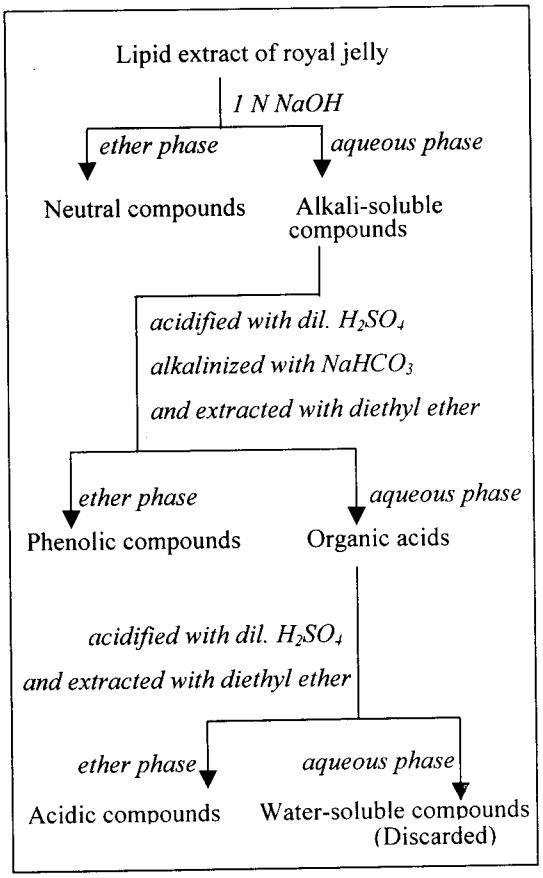
Soxhlet apparatus (Kra□nich, Germany), Kjeldahl apparatus (B□chi 430, Germany), Furnace (Thermolyne 1400, U.S.A.), Disc electrophoresis (Analco, U.S.A.), UV-spectrophotometer (Beckman, U.S.A.), Bio Gel (Bio-Rad Laboratories, Germany), anthrone (AR grade, Fischer Scientific company, U.S.A.), Glucose (AR grade, Sigma chemical company, U.S.A.), Standard protein calibration kit (Pharmacia Fine Chemicals, Sweden). All other chemicals were laboratory grade.

Royal jelly samples purchased from an apiary farm in Chiang Mai, Thailand were freshly collected from an Italian strain of honeybee: *Apis mellifera* after 72 hours grafted larvae into the artificial queen cells. The samples were obtained in each consecutive month for 9 months (November-August except March, September, and October because of the lack of royal jelly production by beekeepers). The chemical composition of intact royal jelly samples was determined. The intact royal jelly was fractionated into the lipid extract and the defatted extract. All the fractions were fractionated further and their biochemical compositions were analyzed in detail.

The carbohydrate content was determined by the anthrone method. The lipid fraction was extracted using Soxhlet apparatus and the lipid content was determined. The protein content was determined by Kjeldahl method. Ash and moisture contents were determined followed AOAC method (1960).

2.1 Analysis of lipid mixture of royal jelly by thin-layer chromatography (TLC)

Intact royal jelly samples were extracted by diethyl ether for 24 hrs using Soxhlet apparatus resulting in the lipid fraction and the defatted fraction. The lipid fraction was extracted further to obtain the acidic fraction, phenolic fraction, and the neutral fraction according to the protocol of Townsend and Lucas [1] as shown in scheme 1. All lipid samples were redissolved in diethyl ether and applied on silica gel 60G thin-layered chromatograms. The plates were developed at room temperature when pure chloroform or 29%

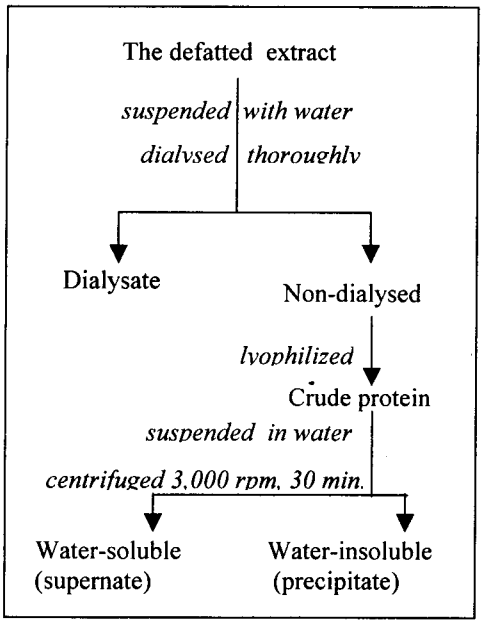


Scheme 1 Fractionation of the lipid extract [1]

methanol in chloroform or pure methanol were used as solvents. Whereas, the plates were developed at refrigerated temperature when hexane–diethyl ether 1.5:1 (V/V) was used as a solvent. After developing, the plates were dried and the spots were located under UV-light and iodine vapor. The R_f value of each spot was determined. In addition, all the lipid extracts (acidic fraction, phenolic fraction, and neutral fraction) were also analyzed by scanning ultraviolet spectrophotometer.

2.2 Analysis of proteins in royal jelly by gel filtration

The water-soluble fraction of royal jelly and water-soluble crude protein fraction were both analyzed by gel filtration. The water-soluble fraction of royal jelly obtained from suspended royal jelly in distilled water and centrifuged at 3,000 rpm for 30 min. The supernatant was water-soluble royal jelly. The water-soluble crude protein fraction was obtained from dialysis and lyophilization of the defatted extract as shown in scheme 2. The crude protein fraction was suspended in distilled water and centrifuged at 3,000 rpm for 30 min. The supernatant was water-soluble crude protein fraction.



Scheme 2 Fractionation of the defatted extract

The protein samples were loaded onto three types of gel filtration columns: Bio-Gel P-10, Bio-Gel P-60, and Bio-Gel P-150 equilibrated with 0.01 M sodium phosphate buffer pH 7.0. The protein concentration in each eluted fraction was measured at the wavelength of 280 nm and the molecular weight (MW) of solute samples were estimated by comparing to the three known molecular weight compounds (bovine serum albumin, 67K; ovalbumin, 43K; and chymotrypsinogen, 25K)

2.3 Analysis of proteins in royal jelly by disc-polyacrylamide gel electrophoresis

The water-soluble fraction of royal jelly was used to study in the native-disc polyacrylamide gel electrophoresis system (native-PAGE) and Sodium dodecyl sulfate - polyacrylamide gel electrophoresis using Laemmli discontinuous buffer system (SDS-PAGE). The separating gel, using a 7.5% acrylamide concentration with a 2.5% cross linker concentration and the stacking gel solution using a 3.5% cross linker concentration were prepared. Pooled normal human serum dissolved in 40% sucrose solution was used as a standard control for native-PAGE. The three known MW proteins (Bovine serum albumin, ovalbumin, and chymotrypsinogen A) were used as standard controls for SDS-PAGE. The power supply was energized and operated at 3 mA constant current. Coomasie blue solution was used as a staining solution.

3. Results and Discussion

3.1 Analysis of general chemical basis of royal jelly

Royal jelly samples collected from Northern Thailand were thick, milky in appearance, slightly yellow with a pungent odor and a bitter taste. The study of the chemical composition of royal jelly collected over a year demonstrated the quantitative differences among the constituents in royal jelly as shown in Table 1 and figure 1. Carbohydrate content markedly increased in hot season (April-June) and maximally increased in rainy season (July-Aug) while it decreased to constant level during the cool season (Nov-Feb). The lipid content abruptly increased during the transition from cool season to hot season. The Lipid value notably increased to about 6% while it slowly decreased to a constant level from rainy season to cool season. The protein content changed only slightly. Its value remained constant during the cool season and then slowly decreased during verging into the hot season finally it increased to the former level again in the rainy season The moisture content slowly decreased in hot season and increased in rainy season. However, ash content and pH value of royal jelly were constant throughout the year. Therefore, this suggests that seasonal variations have an influence in the chemical composition

Table 1. General biochemical composition of royal jelly produced in Chiang Mai, Thailand

I. % of fresh matter basis (V/V)

Collection of royal jelly (month)	pH	% of fresh matter basis (V/V)					
		Moisture	Dry matter	Carbohydrate	Lipid	Protein	Ash
A. Cool Season							
November	3.45	66.92±0.06	33.08	11.34±1.83	4.04±0.22	13.46±0.50	1.06±0.07
December	3.60	68.99±0.39	31.01	8.95±1.07	2.93±0.20	12.97±0.43	1.10±0.09
January	3.45	68.79±0.10	31.21	8.15±1.00	3.01±0.25	13.60±0.85	0.98±0.10
February	3.45	68.58±0.18	31.42	10.03±1.05	5.91±0.20	12.52±0.83	1.10±0.06
B. Hot season							
April	3.40	65.61±0.19	34.39	12.78±1.10	5.16±0.21	12.05±0.55	1.22±0.09
May	3.50	64.38±0.09	35.62	12.62±0.31	5.16±0.21	12.05±0.56	1.24±0.25
June	3.60	65.02±0.20	34.98	15.19±1.10	2.74±0.22	13.76±0.33	1.24±0.12
C. Rainy Season							
July	3.60	68.92±0.10	31.08	14.30±0.85	3.96±0.21	12.24±0.28	1.17±0.06
August	3.50	69.70±0.27	30.30	15.94±0.74	3.15±0.23	13.18±0.02	1.12±0.09
Mean	3.50	67.43	32.56	12.14	4.01	12.87	1.14
±S.D.	±0.08	±1.99	±1.99	±2.74	±1.16	±0.67	±0.09

of royal jelly, especially on carbohydrate and lipid content. There were also slight variations in protein and moisture content. This result agreed with some of the preliminary reports that there was a marked increase in free fatty acid in summer [6]. Moreover, the mean values of the general chemical composition of royal jelly collected in this study were somewhat comparable with those of royal jelly samples produced in other countries such as Germany, U.S.A., Bulgaria, Japan, and Italy [1,2,4,6].

Some slight differences between these values may be on account of various conditions such as collection procedure, analytical procedures, and localities of royal jelly production, which depend on soil fertility, climate and floral sources. Especially, the locality may influence the composition of the incoming pollen and nectar, the food of nurse bees that secrete royal jelly.

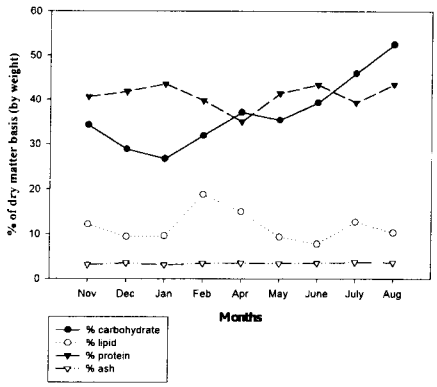


Figure 1 General composition of royal jelly (% of dry matter basis)

3.2 Analysis of lipid extract of royal jelly by thin-layer chromatography (TLC)

The TLC analysis of total lipid extract from royal jelly indicated that most of the lipid components contain polar compounds that may be unsaturated compounds. Because it can be seen that when more polar solvent systems were used, higher R_f values were obtained. For example, using pure chloroform, 29% methanol in chloroform, and pure methanol as the solvent systems, developed at room temperature and revealed with long wavelength UV-light and iodine vapor, there was only one spot at the R_f values of 0.30, 0.67, and 0.80 for each chromatogram respectively. Furthermore, those spots became visible under UV light. However, the most effective solvent system to separate total lipid extract from royal jelly into at most 7 fractions (with the R_f of 0.12, 0.16, 0.19, 0.22, 0.44, 0.53, 0.79, and a band at solvent frontline) was hexane:diethyl ether 1.5:1 (V/V) developed at refrigerated temperature. By using the latter solvent system, the acidic fraction was illustrated two spots with R_f values of 0.09 and 0.15. For the phenolic fraction, there were five spots with R_f values of 0.22, 0.33, 0.56, 0.83, and 0.99 including a band at the solvent front line. The major component (R_f = 0.83) was fluorescent under UV-light. At last, the neutral fraction had only one band at the solvent front line. These lipid fractions were also analyzed by scanning with the UV-spectrophotometer from 200 nm to 360 nm. The neutral fraction showed a small peak at 275 nm. The phenolic fraction showed a medium peak at 256 nm. Whereas the acidic fraction showed a strong absorption peak at 255 nm and a medium peak at 256 nm. The latter, medium peak may be contaminated with chromophoric compounds from the phenolic fraction because they also showed the absorption at 256 nm. Additionally, the lipid extract of royal jelly rapidly absorbed iodine by testing with H□bl's iodine solution. Therefore, it may be concluded that the major components of lipid extract should contain acidic, polar and unsaturated compounds. Several preliminary studies of the lipid of royal jelly support this finding that the major portions of ether-soluble fraction of royal jelly are free and esterified fatty acids [7-10].

3.3 Analysis of water-soluble proteins in royal jelly by gel filtration

The analysis of proteins in royal jelly showed that water-soluble protein fraction was the major fraction making up to 70% of the total protein in royal jelly. The elution profile from Bio-Gel P-10 as shown in figure 2 illustrated two peaks with the corresponding estimated MW of more than 20,000 and close to 12,000-13,000. Their relative concentrations seemed rather equal. On Bio-Gel P-60 (as shown in figure 3), three peaks of the elution profile were shown. The estimated MW of the

were more than 60,000; close to 25,000; and less than 25,000 respectively. On Bio-Gel P-150 (as shown in figure 4), the elution profile had three peaks. The estimated MW of these proteins were more than 150,000; close to 62,000; and close to 22,000 respectively. The concentration of the third peak (22,000) was much greater than the others. These results indicated that there were at least four groups of proteins in water-soluble fraction of royal jelly, group 1 should have MW over 150,000; group 2, MW close to 62,000; group 3, MW close to 25,000 and group 4, MW less than 20,000. The low MW proteins and peptides of group 3 and group 4 were the predominated ones.

Concerning the water-soluble fraction of crude protein, the elution profile on Bio-Gel P-10 had two peaks. The relative concentrations of the second peak were a half of the first peak. The estimated MW of first peak was more than 20,000 and the second was around 12,000-13,000. The elution profile on Bio-Gel P-60 had two peaks, one estimated MW of 60,000 was the major fraction and the other, with an estimated

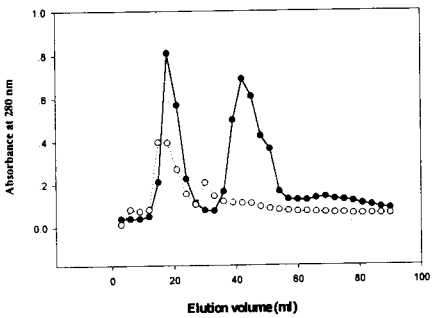


Figure 2 Elution profiles of water-soluble royal jelly and water-soluble crude extract of royal jelly on Bio-Gel P-10

MW of less than 25,000 was the minor. Finally, the elution profile on Bio-Gel P-150 had four peaks. The first peak wither estimated MW of more than 150,000, and the second peak, estimated MW of close to 54,000 were the major fractions. The third and the fourth peaks, with an estimated MW of close to 31,000 and 20,000 were the minor fractions. The results of the analysis of the proteins in water-soluble fraction of royal jelly were somewhat different from the analysis of the proteins in water-soluble fraction of crude protein. It showed that the high MW proteins (group 1 and group 2) were predominated in the water-soluble crude protein fraction, whereas the low MW proteins and peptides (group 3 and group 4) were predominant in the water-soluble fraction of royal jelly.

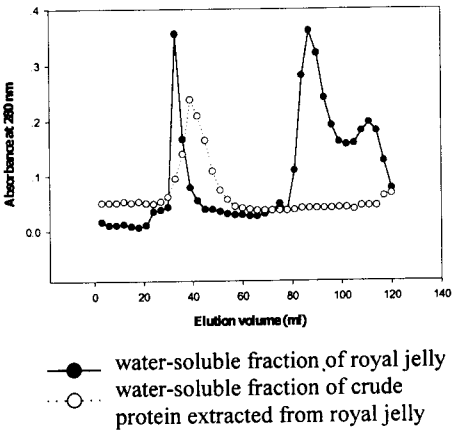


Figure 3 Elution profiles of water-soluble royal jelly and water-soluble crude extract of royal jelly on Bio-Gel P-60

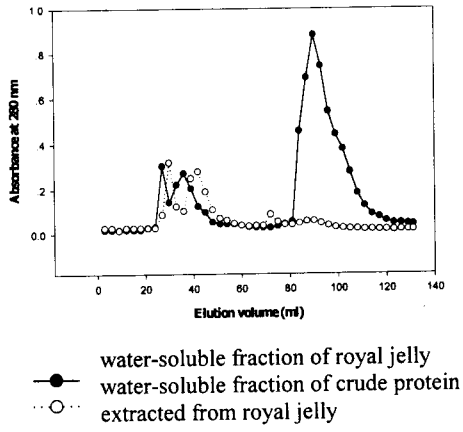


Figure 4 Elution profiles of water-soluble royal jelly and water-soluble crude extract of royal jelly on Bio-Gel P-150

The discrepancy may be on account of the dialysis of protein procedure, which may allow the passage of low MW proteins and peptides through the dialysing membranes and remained only high MW in the bag. This result is similar to that reported by Tomoda, 1977 [9]. They found 2 major protein fractions in the when analyzed by gel filtration. water-soluble part of royal jelly, which had been previously defatted, and dialysed. Their proteins had rather low MW of about 14,000 and 33,000 when analyzed by gel filtration.

3.4 Analysis of water-soluble proteins in royal jelly by disc-polyacrylamide gel electrophoresis

The native protein patterns of water-soluble proteins in royal jelly collected in different seasons were demonstrated by three major bands and three minor bands. However, they were somewhat different in minor bands between these protein samples. Compared with the mobility of pooled human serum proteins, two major protein bands: band 1 and band 2, had equal mobility to β-globulin and α_2-globulin respectively while the major protein band 6 had equal mobility to albumin serum protein. The amount of protein No. 2 was the highest. The water-soluble proteins isolated from royal jelly samples from Sigma Chemical (U.S.A.) were also analyzed. There was a slight variation from the royal jelly sample collected in Thailand. There were also three bands in major and three bands in minor, but the amount of protein No. 1 was the largest, whereas the amount of protein No.2 was the largest in the latter group. Regarding the water-soluble fraction from defatted royal jelly, there were three major bands only.

Concerning the SDS-PAGE, the protein pattern of the water-soluble royal jelly collected in different seasons was generally the same but was different from the protein pattern of the defatted fraction of royal jelly. The former groups had five major bands and two minor bands whereas the latter had only three bands. The estimated MW of the major protein bands were 94,000±8,000; 76,000±4,000; 66,000±6,000; 54,000±4,000 and 46,000±4,000 respectively The amount of protein MW equal to 54,000 was the highest. While the estimated MW of the minor protein bands were 34,000 and 26,000. For the minor bands, proteins were somewhat changed due to seasonal variations and there were some variation among major bands and minor bands. Some showed a usual minor protein band MW of 34,000 as a major protein band. For water-soluble of defatted extract, it was shown that they had less protein components than water-soluble fraction of royal jelly. Only the low MW proteins of 64,000; 54,000; and 46,000 were seen which might result from the process of extraction. A previous study showed 4 bands in water-soluble proteins using paper electrophoresis [10]. Therefore, this study has shown that the water-soluble proteins in royal jelly collected in Thailand vary somewhat in protein components and there is some small variation among different countries. In addition, due to different locality, it may due to different royal jelly samples collected from queen larvae of different ages [2] and to various form of royal jelly.

4. Conclusion

This chemical analysis of royal jelly produced in Chiang Mai, Thailand demonstrated that it is composed of carbohydrate content 12.14±2.74 % (V/V), lipid content 4.01±1.16%(V/V), protein content 12.87±0.67% (V/V), ash content 1.14±0.09% (V/V), moisture content 67.43±1.99 % (V/V), and pH value was 3.5±0.08. Seasonal variations also affected the chemical composition of royal jelly. Carbohydrate and moisture contents were the highest in the rainy season while the lipid content was the highest in the hot season. Protein content was slightly altered whereas ash content and pH value were constant throughout the year. Characterization of the lipid fraction indicated that its major components contained acidic, polar, unsaturated compounds which maximally absorbed UV wavelength at 255 nm. The protein fraction mainly contained water-soluble components having small MW lower than 66 KD.

5. Acknowledgements

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6. References

[1]. Townsend, G.F., and Lucas, C.C., Chemical Examination of Lipid Fraction of Royal Jelly, Science, Vol. 92, p. 43, 1940.

[2]. Haydak, M.H., Larvae Food and Development of Castes in the Honeybee, J.Econ.Entomol., Vol. 36, pp.778-792, 1943.

[3]. Johansson, T.S.K., Royal Jelly, Bee World, Vol. 36, No. 2, pp. 1-13, 21-32, 1965.

[4]. Dayan, A.D., A Note on Royal Jelly a Critical Evaluation, J.Pharm.Pharmacol., Vol. 12, pp. 377-383, 1960.

[5]. Lerckner, G., Capella, P., Conte, L.S., and Runni, F., Component of Royal Jelly I. Identification of the Organic Acids, Lipid, Vol. 16, No. 12, pp. 912-919, 1981.

[6]. Brown, W.H., and Freure, R.J., Some Carboxylic Acids Present in Royal Jelly, Can.J.Chem., Vol. 37, p. 2042, 1959.

[7]. Matsuyama, J., Saemi, U., and Goro, T., Tamagawa Daigaku Nogakubu KenkynHokaku, Vol. 14, pp. 97-104, 1974, from Chemical Abstract, 84:2590a.

[8]. Takashi, e., Takenaka, T., and Takahashi, K., Chemical Composition of Carboxylic Acids in Royal Jelly, Nogakubu Kenkyn Hokoku, Vol. 22, pp. 67-78, 1982, from Chemical Abstract, 99:50582f.

[9]. Tomoda G., Matsuyama, J., and Matsuka, M., Studies on Protein in Royal Jelly, II. Fractionation of Water-Soluble Protein by DEAE: Cellulose Chromatography, Gel Filtration and Disc Electrophoresis, J. Apis.Res., Vol. 16, No. 3, pp. 125-130, 1977.

[10].Patel, N.G., Haydak, M.H., and Gochnauer, T.A., Electrophoretic Components of the Protein in Honeybee Larval Food, Nature, Vol. 196, pp. 633-634, 1960.