

## PROTEINS IN HONEY

### BAL PROTEİNLERİ

Hayrullah YILMAZ<sup>1</sup>, Ö. İrfan KÜFREVLİOĞLU<sup>2</sup>

<sup>1</sup>University of Dicle, Faculty of Education, Department of Chemistry, Diyarbakır

<sup>2</sup>University of Atatürk, Faculty of Art and Science, Department of Chemistry, Erzurum

**ÖZET:** The proteins of honey from south-eastern Anatolia were identified by sodium dodecyl sulfate-polyacrylamid gel electrophoresis (SDS-PAGE). It was found 12 protein bands in various honey samples. The molecular weights of these proteins were determined approximately between 22,000 and 75,000. Also, determined in the honey samples were hydroxymethylfurfural, diastase number, invert sugar, sucrose, proline, moisture, free acid, pH, lactone and ash.

**ABSTRACT:** Güneydoğu Anadolu bölgesinde toplanılan bal örneklerindeki proteinler sodyum dodesil sülfat poliakrilamid jel elektroforez (SDS-PAGE) metodu ile belirlendi. Bal örneklerinde molekül ağırlığı yaklaşık 22.000 - 75.000 arasında olan 12 farklı protein bandı görüldü. Ayrıca bu bal örneklerin hidroksimetilfurfural, diastaz sayısı, invert şeker, sakaroz, prolin, nem, kül, serbest asit, pH ve lakton değerleri de tayin edildi.

#### INTRODUCTION

Honey is a complex natural product, made mainly of carbohydrates and water, but containing a large number of minor components of which only a fraction is known. The composition of a particular honey sample greatly depends on the composition of nectar whence it originates. Honey contains about 80 different components and 95 to 99 per cent of the total solids are sugars (GUPTA et al., 1992). Besides glucose and fructose, honey contains protein, amino acids, enzymes, organic acids, mineral substances, pollen and other substances (CRANE, 1975). The studies on physical and chemical properties of honey produced in different countries have been reported by many scientist (DONER, 1977; THRASYVOULOU, 1986; SINGH et al., 1988; SANCHO et al., 1992; RODRIGUEZ-OTERO et al., 1994).

It has been known for many years that honeys contain a small and variable amount of protein. The protein content could prove useful of the adulteration and misrepresentation of honey (CROFT et al., 1986). It contains approximately 0.2% protein (WHITE et al., 1978) of bee and plant origin (LEE et al., 1985). Other a scientist has reported the protein content range 50-200-mg/100g in honeys (BOGDANOV, 1981). Also, 19 protein bands were detected in native honey by SDS-PAGE (MARSHALL et al., 1987).

In south-eastern Anatolia the honey is all blossom (floral source), and honey production in this region is about 1,000 ton per year (ANONYMOUS, 1997). Honey in south-eastern Anatolia has been studied previously (BOZKURT and AYDOĞAN, 1986). Proteins in honey, however, has not been investigated until now; hence it seemed of interest to determined this parameter.

This study detected the proteins bands in the south-eastern Anatolia honeys by SDS-PAGE and Coomassie Brilliant Blue staining. Also, determined in honey samples were invert sugar, sucrose, hydroxymethylfurfural (HMF), diastase number, free acid, lactone, pH, ash, proline and moisture.

#### MATERIAL AND METHODS

##### Samples

Seven different honey samples (floral source) were collected from beekeepers in south-eastern Anatolia. The honey samples were prepared by straining the comp honey at room temperature.

### Sample preparation

10 g sample was diluted 10 ml distilled water, thoroughly mixed. This mixture was dialysed and lyophilized respectively. Then, sample-denaturing solution (0.2 M  $\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^-$ , pH=7.5 containing 2% (w/v) SDS, 5% (v/v)  $\beta$ -mercaptoethanol and 20% (v/v) glycerol) was mixed sample with an equal volume, heated at 90 °C for 10 minute.

It used that proteins as standard; bovine albumine(66,000), egg albumine(48,000), glyceraldehyde-3-phosphate dehydrogenase(36,000), carbonic anhydrase(29,000), trypsinogen(24,000), trypsin inhibitor (20,000) and  $\alpha$ -lactalbumin(14,000). The standard proteins have been provided from Sigma Chemical Comp.

### SDS-PAGE

The samples were loaded in gel (polyacrylamide, bis-acrylamide) and electrophored at 8 mA/gel for 5 hour in 0.1 M  $\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^-$  containing 0.1% (w/v) SDS. The electrophoresis gels were soaked for 2 hour in 0.025% Coomassie Brilliant Blue, 50% methanol, 10% acetic acid, and the proteins were detected using the destain strategy of acetic acid-methanol-water (1:0.5:8.5)(LAEMMLI, 1970)

### Chemical analysis

Invert sugar, sucrose, HMF, diastase number, free acid, lactone, pH, ash, proline and moisture were determined by standard methods of the Association of Official Analytical Chemists (ANONYMOUS, 1990).

## RESULTS AND DISCUSSION

Coomassie Brilliant Blue stained SDS-PAGE proteins bands of the honey are shown in Figure 1. In samples analyzed 12 clearly distinctive bands were reproducibly detected following SDS-PAGE (Figure 1). The molecular weights of proteins were determined to be approximately between 22,000 and 75,000. The maximum number of protein bands was found to be 7 in the third sample, and the minimum number of protein bands was seen to be 1 in the second sample.

In literature, various protein bands have been detected in honey. In previous studies the number of protein bands is seen to vary; for instance, 6 protein bands were found by DOLINSEK (1981); 11 protein bands by CROFT et al. (1986); and 19 protein bands by MARSHALL et al. (1987).

We found out in our research that the number of protein bands, which we obtained from the honeys of the south-eastern Anatolia region, was quite close to those which were calculated in the previous researches.

Protein bands were detected in all honey samples analyzed, but it was found out that the number of protein bands was different in each sample. As it is possible to obtain protein from bees and plants(LEE et al., 1985), different protein bands may be detected in each honey sample.

Chemical composition together with the corresponding standard deviations and coefficients of variation of honey samples that the protein bands were determined are shown in Table 1. The most analytical values determined in the honeys showed very high coefficient of variation (Table 1). The chemical composition fit the standards(ANONYMOUS, 1990)

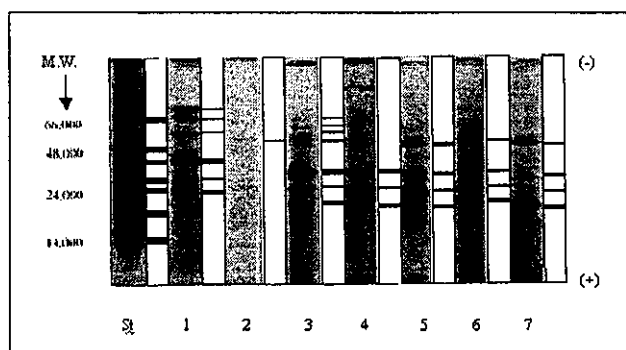


Figure 1. Coomassie brilliant blue-stained SDS-PAGE protein bands of seven honey samples(lanes 1-7).Molecular weight of standards are given on the left side of gel (St : Standard protein).

**Table 1. Statistical Analysis and Chemical Compositions of the Honey Samples**

Analytical values	Mean values	Minimum values	Maximum values	Standard deviation	Coefficient of variation
Moisture(%)	15.4	14.6	16.6	0.8	0.05
Invert sugar(%)	69.8	67.6	73.5	2.3	0.03
Sucrose(%)	2.5	0.4	4.4	1.4	0.56
Ash (%)	0.09	0.02	0.16	0.05	0.55
Proline (mg /100g)	58	30	84	17	0.29
Diastase number	14.7	8.7	24.5	4.9	0.33
HMF (mg /kg)	3.2	0.0	4.8	1.6	0.50
Free acid (meq/ kg)	21.7	12.0	32.0	7.0	0.32
Lactone (meq/ kg)	7.6	5.2	10.5	1.6	0.21
pH Valve	3.8	3.2	4.2	0.3	0.08

**REFERENCES**

- ANONYMOUS , (TSE 3036), 1990. Türk Standartları Enstitüsü, Ankara, Turkey.
- ANONYMOUS , 1997. Devlet İstatistik Enstitüsü(DİE), Ankara, Turkey.
- ANONYMOUS , AOAC. 1990. Methods of Analysis (15th ed.).Association of Official Analytical Chemists, Washington, DC, USA.
- BOGDANOV, S. 1981. Determination of honey protein with coomassie brilliant blue G 250. Mitt. Geb. Lebensm. u. Hgy. 72,411-417.
- BOZKURT, M. and AYDOĞAN, A. 1986. Research on the chemical composition of honey at different regions of Turkey. Turkish Bull. of Hyg. and Experimen. Biol. 43(1) , 1 – 22.
- CRANE, E.1975. Honey. A Comprehensive survey. International Bee Research Association, Helnemann,London. UK.
- CROFT, L.R.; MISTRY, R. P. and WASHINGTON, R.J.1986. In electrophoresis'86 pp. 338 - 389. VCH publishers, Deerfield beach.F1.
- DOLINSEK, B. 1981. Electrophoretic study of proteins in honey and products with pollen. Zborn. Bioteh. Fakul. Univ. v Ljubljani 36, 303-308.
- DONER, L.W.1977.The sugars of honey: a review. J.Sci.Food Agric. 28, 443-456
- GUPTA, J.K. ; KAUSHIK, R. and JOSHI, V. K. 1992. Influence of different treatments, storage temperature and period on some physico-chemical characteristics and sensory qualities of Indian honey. J.Fd.Sci.Technol. 29(2), 84-87.
- LAEMMLI, U.K.,1970 Cleage of structural proteins during in assembly of the head of Bacteriophage T4. Nature 227,680-685.
- LEE, C.Y.; SMITH, N. L.; KIME, R. W. and MORSE, R. A. 1985. Source of honey protein responsible for apple juice clarification. J. Apic. Res. 24(3),190-194
- MARSHALL, T. and WILLIAMS, K. M. 1987. Electrophoresis of honey: Characterization of trace proteins from a complex biological matrix by silver staining. Anal. Biochem. 167, 301-303.
- RODRIGUEZ-OTERO, J.L.; PASEIRO, P.; SIMAL, J. and CEPEDA, A.1994. Mineral content of the honeys produced in Galicia. Food Chem. 9, 169-171
- SANCHO, M.T. ; MUNIATEGUI, S. ; HUIDOBRO, J.F. and SIMAL, J. 1992. Aging of honey. J. Agric. Food Chem. 40, 134-138.
- SINGH, N. ; SINGH, S. ; BAWA, A.S. and SEKHON, K. S. 1988. Honey-its food uses. Indian Food Packer. 42, 15-25.
- WHITE, J. W. Jr; RUDYJ, O. N. 1978. The protein content of honey. J. Apic. Res. 17(4), 234-238.