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RESEARCH ARTICLE

The Investigation of Protein Prophile of Different Botanic Origin Honey and Density Saccharose- Adulterated Honey by SDS-PAGE Method [1]

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Summary

Protein content and quality of honeys are varied according to the plant source as well as production and handling conditions. Lots of methods were developed to determine the botanical origins and qualities of honeys. The aim of this study was to comparatively evaluate the protein profiles of the honeys produced from bee colonies exposed to different plant sources (rhododendron, chestnut, blossom, pine) and feeding (Saccharose nutrition) conditions by using SDS-PAGE method. In this study, the protein profiles, but not protein densities, were found similar between pure honeys produced from rhododendron, chestnut, blossom, pine and high sugared (saccharose) honeys. Three main protein bands (94 kDa, 87 kDa and 84 kDa) were determined in the electrophoretic analysis of the honeys. Protein ratios (%) were determined as 1.31±0.07, 1.02±0.04, 0.90±0.03, 1.16±0.09 and 0.23±0.01, respectively. Protein band densities were found higher in rhododendron, chestnut and pine honeys than those in honeys produced by using excessive saccharose syrup. It was concluded that protein profile can be used in differentiation between pure and adulterated honeys.

Keywords: *Honey, Protein electrophoresis, Saccharose syrup honey*

Farklı Bitki Türlerinden Üretilmiş Saf ve Şekerli Balların Protein Profilinin SDS-PAGE Yöntemi ile İncelenmesi

Özet

Balın protein içeriği ve kalitesi bitki kaynağı, balın üretim ve işleme şartlarına bağlı olarak değişir. Balların botanik kaynağını ve kalitesini belirlemek üzere birçok yöntem geliştirilmiştir. Bu çalışmanın amacını farklı bitki kaynakları (ormangülü, kestane, çiçek, çam) ve farklı besleme koşullarına (glukozlu) tabi tutulan arı kolonilerinden üretilen balların protein profilinin SDS-PAGE yöntemi ile karşılaştırmalı olarak değerlendirilmesi oluşturmaktadır. Çalışmada ormangülü, kestane, glukozlu, çiçek ve çam bitki kaynaklarından üretilen saf ballar ile yoğun şekerli (glukozlu) balın protein profilinin aynı olduğu, ancak protein yoğunluğunun farklılık gösterdiği belirlendi. Balların elektroforez analizi sonucunda 94 kDa, 87 kDa ve 84 kDa olmak üzere başlıca 3 protein bantı belirlenmiştir. Protein (%) oranları ise sırasıyla 1.1±0.07, 1.02±0.04, 0.90±0.03, 1.16±0.09 ve 0.23±0.01 olarak bulunmuştur. Ormangülü, Kestane, çiçek ve çam ballarının protein bant yoğunluklarının aşırı glukoz şerbetiyle yapılan besleme sonucu üretilen baldan yaklaşık 4-7 kat daha fazla olduğu saptandı. Bu çalışma ile saf ve hileli balların ayrımında protein profilinin değerlendirilmesinin önemli kaynak teşkil edeceği ve bu hususta yapılacak olan analizlere de destek olacağı kanısındayız.

Anahtar sözcükler: *Bal, Potein elektroforezi, Şekerli bal*

INTRODUCTION

Most of the nutrients are found in honeys in their original forms obtained from plant. Part of the remaining nutrients is produced during the handling (invert sugar) and the other part is added by honeybees during handling (enzymes and some proteins) [1,2] Consequently, sugars, proteins, minerals and vitamins are among the most important nutrients in honey [1-4]. The nutrient content of honey varies mainly according to the botanical source from which honeybees collect nectar. Plant nectar content varies according to the geography, soil productivity, precipitation, light, altitude and the other environmental factors [5-7]. Pollen is the most important source of free amino acids and proteins which are used by honeybees. Protein content of strained honey is both low and variable as the pollen remained in honeycomb during extraction process.

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Amino acids in honey are sourced from worker bees, nectars and insects in addition to pollens. The main producer of proteins in pine honey, which is a prominent secretion honey, are the microorganisms found in ventriculus of Marchellina hellenica [2,8]. Although the protein content of honey varies due to these factors, it is an important criterion for naturalness of honey [5].

Honey is one of the animal origin foods which are most exposed to adulteration due to its liquid form and its production and handling methods. Some beekeepers tend to meet protein and carbohydrate requirements of colonies from other sources in order to obtain high yields from their bee colonies [9,10]. In recent years, some commercial sugars and sugar derivatives are given to colonies in syrup form in excessive amounts due to its lower prices and liquid forms. This practice was determined to have negative influence on sugar, protein, mineral and prolin contents of honey and health of honeybees [5,9-11]. This negativeness not only causes a risk in terms of public health but also it can be harmful to real honey producer. Although it can be possible to obtain some knowledges on adulterated honeys by using routine biochemical analyses such as enzyme activity, hydroxi-metylfurfurol, ash, acid, pH, electrical conductivity, carbon count, evaluation of these parameters separately could not be enough to get favorable results [6,9]. Taken together, all these factors indicate that new methods are required to be developed [5,8,12,13]. Consequently, protein purification has become most common method in drug and food industry in recent years. But, the studies related to discrimination of adulterated honeys produced by heavy feeding are scarce.

In this study, it was aimed to develop a practical and efficient method which can be used in differentiation of pure and adulterated honeys and also in diagnosis of adulterated honeys by protein molecule density by using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE).

MATERIAL and METHODS

Honey samples: Rhododendron, chestnut, blossom and pine (honeydew) honeys standard rearing methods has been applied to colonies [14,15]. In April, 7-8 kg syrup was given to each colony to ensure adequate nutrition. Neither cake and syrup nor chemical for honey bee diseases were given to colonies in main nectar flow period. Main honeycomb and supper were given to colonies in case they need. While chestnut *(Castanea sativa)* and rhododendron *(Rhododendron luteum)* honeys were produced in Turkeli district of Sinop in which these plants were common, blossom honey was produced in Kelkit district of Gumushane where is rich in prairie blossoms. Pine (honeydew, Marchellina hellenica) honey was obtained from Aegean region of Turkey. Honeys were produced in August-September months. Honeys were extracted and were wrapped up following filtration with 0.2 mm filter.

Saccharose nutrition honey: Saccharose honey, which is considered as adulterated honey, was produced by shook swarm method. In spring (April and May), 8-10 kg sucrose syrup was given to each colony to ensure adequate nutrition. Developed bee colonies were shook into empty hive together with queen bee and worker bees in the end of May [12]. Following this process, a saccharose solution (80 kg/colony) was given in syrup form (1 L water +3 L saccharose) up to harvest period. In this group, a total of 110 L syrup was given to each colony. Syrup was prepared every 2 days, rinsed with short intervals and finally was given to colonies following a day rest period. In this group, neither cake and foundation comb nor chemical were given to the colonies after transfer process. Honey was produced in Sutveren village of Kelkit, Turkey.

SDS-PAGE: Serum protein electrophoresis was performed by Laemmli method [16]. In this study, 5 different honey samples (rhododendron, chestnut, blossom, pine, honeydew) honeys and the honeys produced by heavy saccharose feeding were studied. The protein profiles of honey samples were determined by 7.5% polyacrylamide gel electrophoresis. Honey samples were weighed in equal amounts and were diluted with physiological saline at ¼ ratio. Protein denaturation was ensured by boiling honey samples in boiling water at 95ºC for 5 minutes after rinsing them with sample buffer (SDS 2%, βmerkaptoetanol 5%, 10% glycerol and 0.001% bromphenol blue, pH 6.8). To achieve comparison equal amounts of honey samples Honey samples (30 µl) were taken from five colonies, which were chosen randomly from among all available groups, and total of 25 samples (5 x 5 = 25) were put into wells and proteins of honey samples were decomposed at denature conditions (20 mAmp/jel). Protein standard with 205-6. kDa molecule weight was used in SDS-PAGE method. Protein bands were visualized by staining with Blue Silver [17]. The molecule weights of bands observed at the end of SDS-PAGE were computed using Kodak Molecular Image Analysis Software.

Determination of the protein content of the honey samples: Protein contents $(100g)^{-1}$ of honey

samples were determined according to the Kjeldahl method [18,19].

Statistical analysis was performed with the use of the SPSS program. Results are presented as mean ± SD of each group [20].

RESULTS

Electrophoretic band appearance of the honey samples was presented in *Fig 1* Three different protein bands (94 kDa, 87 kDa ve 84 kDa) were determined in comparison of protein profiles of honey samples with SDS-PAGE method. Protein band intensity in honeys produced by heavy saccharose feeding was found lower compared to that in chestnut, rhododendron, blossom and pine (honeydew) honeys. Protein contents of rhododendron, chestnut, blossom, pine (honeydew) and saccharose honeys were determined as 1.31±0.07, 1.02±0.04, 0.90±0.03, 1.16±0.09 and 0.23±0.01 $(100g)^{-1}$ respectively. The highest and the lowest protein contents were determined in rhododendron and saccharose honeys, respectively *Fig 2*.

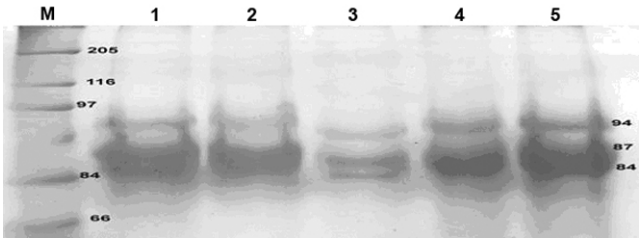

Fig 1. Protein prophiles of the honey samples
M: Marker, 1: Rhododendron honey, 2: Chestnut honey,
3: Saccharose-syrup-honey, 4: Blossom honey, 5: Pine honey

Şekil 1. Bal örneklerinin protein profilleri
M: Standart, 1: Ormangülü balı, 2: Kestane balı, 3: Şekerli bal,
4: Çiçek balı, 5: Çam balı

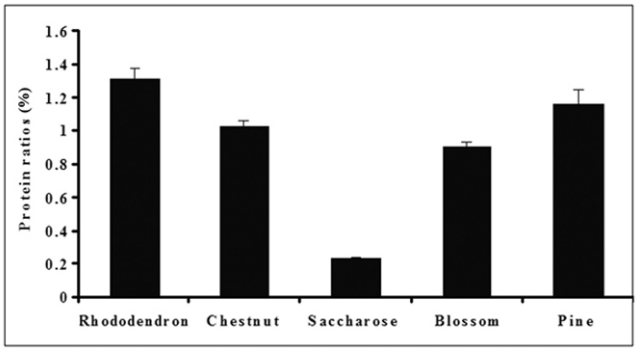

Fig 2. Protein ratios (%) of the rhododendron, chestnut, saccharose-syrup, blossom and pine honeys

Şekil 2. Ormangülü, kestane, sakkarozlu, çiçek, çam balı örneklerindeki protein oranları (%)

DISCUSSION

Both protein band densities and protein contents were found different between pure honeys produced from different botanic sources and the honeys produced by heavy feeding. In electrophoretic analysis the protein weights (94 kDa, 87 kDa, and 84 kDa) were found similar for each honey sample. This indicated that there was no protein specifity in honeys produced from different botanical sources (rhododendron, chestnut, blossom, pine etc). Thus, arginine, tryptophan and cystine contents were found similar for honeys produced from different botanical sources and this finding indicated that an amino acid or an amino acid group was not enough for discrimination of specific honeys [5,21,22]. Similarly, it was reported that there was no protein specifity in honeys produced from different botanical sources and thus amino acid profile can not be used for determining botanical origin. This method was reported to be more efficient in discrimination of adulterated honeys from pure honeys [23,24] .

Protein contents were found as 1.31±0.07, 1.02±0.04, 0.90±0.03, 1.16±0.09 and 0.23±0.01 $(100g)^{-1}$ in rhododendron, chestnut, blossom, pine (honeydew) and saccharose honeys, respectively. The protein contents were found different between honey samples, but main difference was found between adulterated (saccharose nutrient) honey and the other honeys. Protein content of adulterated honey was found 6 times, 5 times, 4.5 times and 5.5 times lover than that of rhododendron, chestnut, blossom and pine honeys, respectively. This finding indicates that protein density has more importance in discrimination of pure honeys and adulterated honeys compared to the discrimination of honeys produced from different botanical sources. This finding is in consistence with some previous studies. Hermosin et al.[21] reported that comparing protein density in addition to amino acid composition was more efficient in discrimination of adulterated honeys.

The difference in protein density was found in parallel with protein band intensity in electrophoresis method *Fig1.*The findings indicates that protein content of honey produced by heavy saccharose feeding is lower than the natural honeys of different botanical origin. According to us, the lower protein content in adulterated honeys is caused due to the fact that worker bees prefer using ready-carbohydrate source in hives instead of collecting nectar from plants. A similar finding was obtained in an our previous

study [9]. But, in that study heavy sucrose feeding was applied in place of heavy saccharose feeding and proline was evaluated as protein source in honey samples. Proline contents were determined as 40 and 63 mg $(100g)^{-1}$ in honeys produced by heavy sucrose feeding and pure honeys, respectively. It is suggested that heavy (saccharose) feeding lowers the bees capacity of utilizing the natural sources out of hives. Protein content and pollen diversity in honeys increases if the bee uses the natural sources. Rodriguez et al.[25] determined 12 different protein fractions in honeys obtained from different botanic origin plants in their study in which electrophoresis method was used. However, the researchers reported that not characteristic these bands. Paramas et al.[26] reported that protein content could be both low and variable in honeys. Although the protein bands are not enough in discrimination of adulterated honeys in this study, the extremely high concentrations in natural and pure honeys can be a distinctive factor.

In result it was determined that protein profiles of pure honeys of different botanical origin and adulterated honeys produced by heavy feeding (saccharose syrup) are similar, but protein content (nutrition value) of adulterated honeys is low. For this reason, this method can be used in studies which are conducted with the aim of determining the protein contents of pure honeys and adulterated honeys.

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