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Royal Jelly and Its SDS-PAGE Electrophoresis Profiles

Arı Sütü ve SDS-PAGE Elektroforez Profili

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Abstract

Royal jelly (RJ) is an important functional food, is used for many purposes. New methods are needed for thepractical characterization of royal jelly. In this preliminary study, SDS-PAGE electrophoretic profiles of royal jelly proteins were in vestigated. Raw royal jelly samples were analyzed by classical SDS-PAGE electrophoresis using 5% and 10% gels. The major protein spot were obtained at the samples collected same regions. Compared the proteins with known marker proteins they were between 5,2 and 116 kDa. Classical SDS-PAGE electrophoresis alone is not sufficient to determine royal jelly proteins, but can be used to basic characterization of royal jelly. Further selectrophoretic techniques are needed for a better separation.

Keywords: Royal Jelly, Electrophoresis, Protein

Özet

Arı sütü birçok amaç için kullanılan önemli bir fonksiyonel üründür. Arı sütünün pratik karakterizasyonu için yeni metodlara ihtiyaç duyulmaktadır. Bu ön çalışmada, SDS-PAGE elektroforezi ile arı sütü proteinlerinin profili araştırılmıştır. Ham arı sütü örnekleri %5 ve %10 oranında jel kullanılarak klasik SDS-PAGE elektroforezi yönteminden yararlanarak analiz edilmiştir. Ana protein noktası aynı bölgelerden toplanan örneklerden elde edilmiştir. Proteinlerin karşılaştırıldığı marker proteinlerin ağırlığı 5,2 ve 116kDa arasında değişmektedir. Klasik SDS-PAGE elektroforezi arı sütü proteinlerini belirlemek için yeterli bir analiz olmamakla birlikte arı sütlerinin temel karakterizasyonunda kullanılabilir. Daha iyi bir ayrım için gelişmiş elektroforetik tekniklere ihtiyaç duyulmaktadır.

Anahtar kelimeler: Arı Sütü, Elektroforez, Protein

Abbreviations:

1. INTRODUCTION

Royal jelly (RJ) is a mixture secreted from young worker bees and constitutes the main food source of honey bee queen (*Apismellifera*). It is believed that to play a key role in queen bee development. The long life and fertilizes of the queen honeybee as the opposite of worker bees is attributed to royal jelly. The raw RJ is an effective mixture in the form of a gel and creamy-white color, which includes honey bee enzymes, proteins, peptides, amino acids, fatty acids, vitamins, sugars, minerals and hormones.

Although queen bees and worker bees normally have the same genetic structure when they hatch, significant differences occur in their anatomical, physiological and morphological structures as larvae are fed with queen milk (Sabatini et. al., 2009; Tamura et al., 2009). As a result of this six daily feeding, queen bees are resistant to diseases, can produce eggs twice their weight (1500-3000) per day and live for 3-5 years. On the other hand,

other worker bees are more easily ill due to their weak immune immunity, cannot ovulate and live only 2-3 months. It is suggested that the main reason for this difference between the two individuals is that they are fed with royal jelly (Albert, Klaudiny & Šimúth, 1999; Gua, Kouzuma&Yonekura, 2009; Kolayli et al., 2016;Tamura et al., 2009). Therefore, royal jelly is used as a functional food for various purposes (Pasupuleti et al., 2017; Ramadana & Al-Ghamdi, 2012; Tamura et al., 2009; Viuda-Martoset al., 2008). For this reason, royal jelly attracts the attention of the scientific world.

The composition of royal jelly varies according to the bee breed and the productivity of the region, but fresh RJ contains approximately nearly 60-70 % water, 27- 40 % protein, 3-7 % lipids, 10-12 % carbohydrates, and other are minerals, vitamins (Kolaylı et al., 2016; Scarselli et al., 2005).

Recently, it is reported that royal jelly, which is consumed especially as food supplement for strengthens of immune system, increases fertilization and renewal cells (Pasupuletiet al., 2017). Our purpose of this study is protein pattern of royal jelly by basic SDS-PAGE electrophoresis for basic characterization.

Royal jelly proteins are mostly soluble (about 80) in water. Until now, eight major RJ proteins (MRJP1, MRJP2, MRJP3, MRJP4, MRJP5, MRJP6, MRJP7 and MRJP8) have been characterized by the cloning and sequencing of their respective cDNAs (Albert &Klaudiny, 2004). They are also named as apalbumine, and apart from these proteins, apimisin, royalisin, royalactin, jelleines, glucose oxidase and apalipophorin III like proteins were also present in royal jellies. It was reported that MRJPs are thought to be the major factors responsible for the specific physiological role of RJ in queen honeybee development, as MRJPs include numerous essential amino acids, similar to ovalbumin and casein (Schmitzova et al., 1998).

2. MATERIAL AND METHODS

2.1. Materials

Nine different royal jelly samples were collected different region of Anatolia, Turkey. The samples were transported by cold chain and stored in a freezer at -20 °C.

2.2. SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel) Electrophoresis

The supernatant fraction was used for protein profiles of royal jelly samples by classical SDS-PAGE. Royal jelly (10 mg/mL) was dissolved in 0.9 % NaCI, and centrifuged at 12.000 xg for 20 min. For this analyse classical sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) protocol was used in this study. BIO-RAD Mini PROTEAN©Tetra Cell 4-gel hand casting system (California, USA) apparatus was used. Samples were loaded to a 5% stacking and 8% resolving PAGE under non-reducing and reducing conditions. Gel electrophoresis was performed at room temperature (RT), at 40-50 mA and approximately for 3h. After electrophoresis, the gel slabs were stained with the solution Coomassie brilliant blue R-25. Protein marker (Promega Corporation, Cat No. V8491, Wisconsin, US) wasloaded inparallel with bee venom sample determined by Sirin et al. (2016) (Table 1).

Table 1. Gel components of SDS-PAGE electrophoresis

Components	Stacking gel 5% (mL)	Resolving gel %10 (ml)
Distilled water	2.7	4.6
Acrylamide/Bis-acrylamide30%	0.67	2.7
1.5 M Tris Buffer (pH 8.8)	--	2,5
1.5 M Tris Buffer (pH 6,8)	0.5	--
Ammonium persulfate 10%	0.04	0.1
SDS (10% w/v)	0.04	0.1
Tetramethylethylenediamine (TEMED)	0.005	0.006

3. RESULTS AND DISCUSSION

Royal jell containsnearly (27–41%) proteins and it is not clear how many different proteins, peptides, royal jellies contain, but some sources inform that eight members of the MRJPs family (MRJP1-8), which are nearly 11% of the total proteins and apismin, royamisin, royalactinetc. (Albert &Klaudiny, 2004; Ramadana & Al-Ghamdi et al., 2012; Scarselli et al., 2005;). Royalisin, a 5.5 kDa protein, was found to have potentanti bacterial activity against Gram-negative bacteria. Protein profile of RJ is determined by different techniques such as two dimensional electrophoresis, LC-MS, MALDI-

TOFF MS. SDS-PAGE and native PAGE electrophoresis were also used to characterize RJ (Gua, Kouzuma&Yonekura, 2009; Ramadana& Al-Ghamdi 2012; Sano et al., 2004; Tu et al., 2018). The aim of this study is to search a simple technique to display the royal jelly protein profile. For this reason, SDS-PAGE electrophoresis was tested preliminary for royal jelly analysis.

In this study, SDS-PAGE electrophoresis profile of royal jelly proteins was investigated. Aqueous solution of ten different royal jelly samples was analyzed by conventional SDS-PAGE using 5% and 8% gels. Them spots obtained were compared with known marker proteins with molecular weights. Fig I is the obtained chromatogram of SDS-PAGE electrophoresis. There is not a very good separation, but only two large spotsare formed, indicating that the set wospots are collected in the same area in all samples and between 40 and 70 kDa.

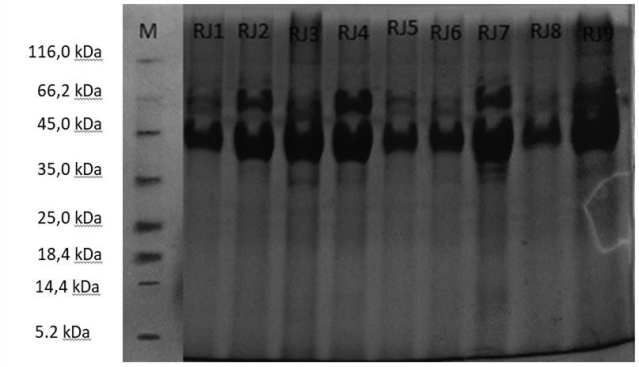

Figure 1. SDS-PAGE Electrophoresis of RoyalJelly

In a study of reducing and non-reducing SDS-PAGE Electrophoresis, similar to our work in the two large spots and collected in the same region is shown (Ramadana& Al-Ghamdi, 2012). In another size-exclusion HPLC on a Superose 12 column study, it was found five large spots are formed (Ramadana& Al-Ghamdi, 2012). In an old study supporting our study, that major royal jelly proteins are collected 47-89 kdal by SDS-PAGE electrophoresis (Schmitzova et al., 1998). In the two-dimensional two-dimensional gel electrophoresis study, MRJP 3 shows polymorphism between Africanized and European Honeybees (*Apis mellifera*), 87 kdal glucose oxidase shows (Sano et al., 2004).

In conclusion, SDS-PAGE electrophoresis of royal jelly proteins needs to be applied by modifying the buffer and gel systems for a complete characterization in terms of showing the profile.

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