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Bingöl Arı Sütünün Şeker Bileşiminin Yüksek Basınçlı Sıvı Kromatografisi - Kırılma İndisi Detektörü (HPLC-RID) ile Belirlenmesi

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Özet

Arı sütü, 5-10 günlük işçi arıların üst çene ve boğaz bezlerinden ana arı ve larvaları beslemek için salgılanan, beyaz veya krem renginde olabilen çok önemli bir doğal üründür. Aynı zamanda yaygın olarak tüketilen ve birçok hastalığa iyi geldiği bilinen önemli bir fonksiyonel gıdadır. Bu nedenle tüketiminde kalite parametrelerinin sağlandığı, taklit ve tağşişten uzak olduğu tespit edilmelidir. Arı sütünün önemli bileşenlerinden biri karbonhidratlardır. Şeker içeriğinin miktarının güvenilir ve hassas yöntem ve teknolojilerle belirlenmesi çok önemlidir.

Bu çalışmada Bingöl ilinden elde edilen arı sütünün dört farklı şeker içeriği Yüksek Basınçlı Sıvı Kromatografisi - Refraktif İndeks Dedektörü (HPLC-RID) teknolojisi ile belirlenmiştir. Fruktoz (m/m) 4.87 ± 0.76, glukoz (m/m) 6.13 ± 0.81, sakkaroz (m/m) 1.13 ± 0.57 ve maltoz (m/m) 0.32 ± 0.80 olarak belirlenmiştir. Bulunan değerlerin Türk Gıda Kodeksi Arı Ürünleri Tebliği'nde yer alan arı sütü şeker içeriği değerlerine uygun olduğu tespit edilmiştir. Özellikle fonksiyonel bir gıda olan arı sütünün taklit, tağşiş ve sahtecilikten ari olması için bu analizlerin düzenli olarak yapılması önem arz etmektedir.

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Determination of Sugar Composition of Bingöl Royal Jelly by High Pressure Liquid Chromatography - Refractive Index Detector (HPLC-RID)

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Abstract

Royal jelly is a very important natural product secreted from the upper jaw and throat glands of 5-10-day-old worker bees to feed the queen and larvae and can be white or cream colored. It is also an important functional food that is widely consumed and known to be good for many diseases. For this reason, it should be determined that quality parameters are met in its consumption and that it is free from counterfeiting and adulteration. One of the significant components of royal jelly is carbohydrates. It is very important to determine the amount of sugar content with reliable and sensitive methods and technologies.

In this study, four different sugar contents of royal jelly obtained from Bingöl province were determined by High Pressure Liquid Chromatography - Refractive Index Detector **(**HPLC-RID) technology. Fructose (m/m) 4.87 ± 0.76, glucose (m/m) 6.13 ± 0.81, sucrose (m/m) 1.13 ± 0.57, and maltose (m/m) 0.32 ± 0.80 were determined. It was determined that the values found were in accordance with the royal jelly sugar content values in the Turkish Food Codex Bee Products Communiqué. It is important to carry out these analyses regularly, especially for royal jelly, which is a functional food, to be free from imitation, adulteration and counterfeiting.

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Introduction

Royal jelly is a creamy, viscous liquid secreted from the cranial exocrine (mandibular and hypopharyngeal) glands of *Apis mellifera* L. [1]. It plays a critical role as a staple food for queen bees and larvae [2]. Its unique biological composition and nutritional value make it more valuable than other bee products. Not only its raw form but also its addition to various foods with different formulations continues to increase its consumption and prevalence worldwide [3, 4]. From a biomedical point of view, the fact that it is an antioxidative [5] and vasodilator agent, microbial and inflammation inhibitor, and immunomodulator shows that it is a potential chemopreventive and protective [6-8].
Recently, the production and consumption of functional foods in the promotion and protection of human health has become an important market for the food industry. It is understood that a link has been established between healthy nutrition and the treatment or control of various diseases [9]. In this context, “superfoods,” which are defined as functional foods, attract attention in terms of being formed both naturally and by adding certain ingredients to certain substances [10]. Royal jelly, which is among the functional foods obtained by natural means, is of scientific, therapeutic and economic importance [11].
Apart from its nutritional value, royal jelly is used as a medicinal product in many countries as a medical support in a wide age range, including pediatric and geriatric areas [12]. Apart from the food and health sector, this product is also used effectively in cosmetic applications. Royal jelly production is increasing worldwide to meet the demand every day. In particular, China is the world's largest royal jelly producer and exporter, accounting for about 90% of global production with an annual capacity of 4000 tons [13, 14].
In the chemical composition of royal jelly, the majority of the secretion consists of 60-70% water, with the remaining parts consisting mainly of proteins, carbohydrates and fats. Twenty-three different amino acids, albumin, globulin types, lipoproteins and glycoproteins are included in the protein amount between 9% and 18%. The fats in royal jelly are mostly composed of medium-chain fatty acids. The total fat content in royal jelly is approximately 3-8%. Especially in this group, 10-hydroxy-2-decanoic acid (10-HDA) and phenolic lipids are important. It is very rich in mineral content (zinc, potassium, calcium, manganese, iron and sodium) and vitamins (B group, A, C, D, E and K). The total proportion of simple sugars in royal jelly varies between 7% and 18%. Sugar content is mostly composed of monosaccharides such as glucose and fructose. Apart from these, trace amounts of sugars such as inositol, trehalose, ribose and isomaltose are also found [15-18].
These sugars in royal jelly not only contribute to energy and nutrition but also play a differentiating role in the developmental differentiation of queen and worker bees for the continuity of the colony's life cycle [19]. Differentiation of the colony into worker or queen bees is influential in hive dynamics by providing nutrition-triggered epigenetic pathways such as IGF-1/mTOR [4,20].
The sugar profile has been found to vary depending on climatic and geographical factors, the type of bee population, and floral sources [21, 22]. These monosaccharides are among the components that need to be carefully monitored in terms of food safety. In particular, the glucose/fructose ratio, which hovers around 1:1, can be used as a marker to understand the authenticity of the source of the product and possible environmental contamination [18, 23].
In particular, research on the bioavailability of sugars in royal jelly, their effects in the gastrointestinal system, and their activities on metabolism at the cellular level indicate that this component has biological functions beyond being a source of energy metabolism [24]. Therefore, analyzing the structural and functional properties of monosaccharides (glucose and fructose) in royal jelly in a holistic manner contributes to overcoming the lack of information in the functional foods discipline of food technology [25].
In chromatographic-based analyses, mainly with the help of the HPLC-RID method, it is used in the separation and quantitative analysis of sugars in royal jelly because it provides high sensitivity and accuracy [26].
In this context, HPLC-RID method is of critical importance for the determination and concentration of sugars in royal jelly with sensitive methods, control of product quality and prevention of adulteration (fraudulent product production). Studies with chromatographic-based analytical approaches allow comprehensive characterization of carbohydrates in royal jelly [27,28].

Material and Methods

Chemicals

We bought the chemicals and standards needed for HPLC-RID analysis from Sigma-Aldrich (Steinheim, Germany).

Supply of royal jelly

Royal jelly was obtained from beekeepers registered with the Bingöl Beekeepers Association. Royal jelly collected with an injector was kept in a cold chain.

Figure 1. Royal Jelly

Preparation of royal jelly for analysis
1 g of fresh royal jelly (RJ) was diluted in water: methanol (3:1) in a 10 ml volumetric flask. Proteins were precipitated by adding 0.1 ml Carrez I and 0.1 ml Carrez II reagent and then homogenized. The solution was transferred to a glass centrifuge tube and centrifuged at 3800 rpm for 20 min to remove the protein fraction. 2 ml of the supernatant was transferred to a capped glass tube and prewashed with dichloromethane. The sugar fraction was then transferred to vials using a 0.45 µm syringe filter [26, 29].

Determination of sugar contents by HPLC-RID
Royal Jelly (RJ) sugars were determined by the HPLC method described by Sesta (2006). The following modules were used for HPLC analysis (Agilent 1260 Infinity II): autosampler/injector, pump, column oven, refractive index detector (RID), software (Agilent OpenLab ChemStation), and column (Cosmosil NH2, 5 µm, 250 × 3.2 mm). Elution was performed at an isocratic flow rate of 1.4 mL/min using acetonitrile/water (85:15) as the mobile phase. The column and RID were kept at 30 °C. The injection volume was 5 µL. The assay was based on the injection of an external standard prepared as a 100 mL water/methanol solution (3:1) containing 1.1 g fructose, 1.1 g glucose, 0.3 g sucrose, and 0.3 g maltose. Results were expressed as a percentage (g/100 g) of each sugar on RJ [26, 29, 30].

Results and Discussion

Sugar content by HPLC-RID
The determination of RJ sugars is a crucial step towards quality standardization. This method allows the determination of the three main RJ sugars (fructose, glucose, and sucrose) and the rare maltose. Figure 1 shows the standard chromatogram obtained in the analysis of a mixture of sugar standards (fructose, glucose, sucrose and maltose). Peak identification was done by comparison with a standard mixture solution based on retention time similarity. The mean and standard deviation values of RJ as a result of this study are given in Table 1.

Table 1. Sugar contents of Bingöl Royal Jelly by HPLC-RID

Parameters	Quantity
Fructose (% m/m)	4,87 ± 0,76
Glucose (% m/m)	6,13 ± 0,81
Sucrose (% m/m)	1,13 ± 0,57
Maltose (% m/m)	0,32 ± 0,80

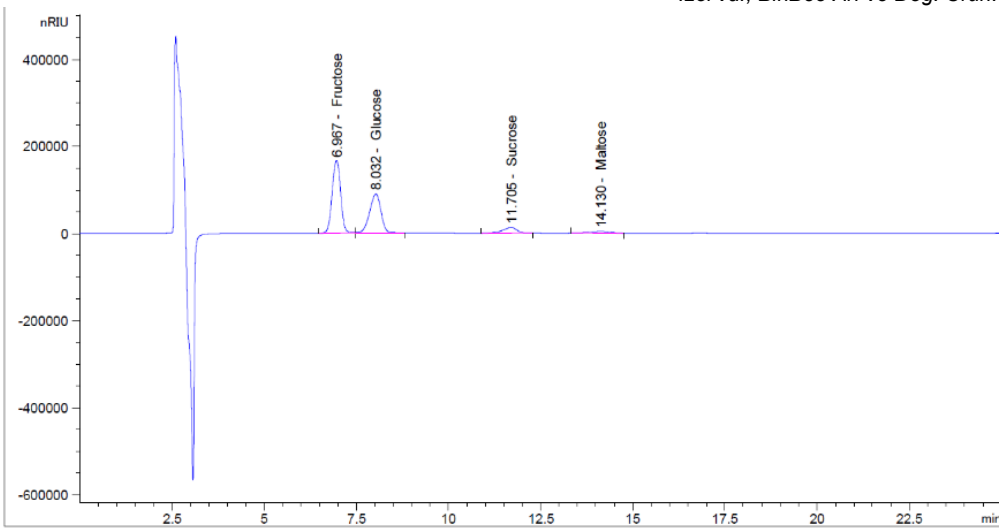

Figure 2. Chromatogram of mixture sugars standards

The highest sugar content is glucose, followed by fructose, sucrose and maltose. In the Turkish Food Codex Communiqué on Bee Products (Communiqué No: 2024/6), the sugar limits that RJ should carry are shown in Table 2.

Table 2. Turkish Food Codex Royal Jelly(RJ) specifications

Parameters	**Quantity**
Fructose (% m/m)	2-9
Glucose (% m/m)	2-9
Sucrose (% m/m max)	3
Maltose (% m/m max)	1,5

As a result of the study, it was determined that the royal jelly obtained from the Bingöl region complies with the values in the Turkish Food Codex Bee Products Communiqué. This is an indication that royal jelly is not supplemented with any sugar from outside. The amount of carbohydrate in its content is important to show that royal jelly is consumed for many diseases and especially for diabetic patients. It is especially important for diabetics that glucose and fructose, which cause an increase in blood sugar, are not in high amounts.

Sesta et al. (2009) determined the sugars of 97 different RJ samples by HPLC. The average sugar contents of 97 samples were fructose 4.6%, glucose 5.8%, sucrose 1.0%, and maltose 0.4% [26].

In another study, 10 different RJ samples were analyzed. The average sugar content of 10 different RJ samples was 4.83% fructose, 6.61% glucose, 1.87% sucrose, and 0.3% maltose, respectively [29].

Balkanska and Kashamov (2011) conducted a study to determine the chemical content of 6 different lyophilized RJ samples produced in Bulgaria. As a result of the study, they detected 11.85% fructose, 9.9% glucose, and 7.65% sucrose, including 29.4% total sugar in RJ samples [31].

Šimúth (2001) found the amounts of fructose, glucose, and sucrose to be 14.0%, 18.8%, and 1.0%, respectively, in his study on RJ.

In a study by Zhu et al. (2019), 19 types of sugar compounds were analyzed in royal jelly samples produced in China using the HPLC-RID method. This study revealed the high sensitivity of the method and comprehensive sugar analysis.

In a study conducted by Şenel et al. (2010), it was reported that glucose (3.2-4.3%) and fructose (3.8-5.1%) levels were significant in royal jelly samples by the HPLC-RID method, while sucrose was generally detected at <1% levels. The data obtained in this study are consistent with the sugar levels reported in the literature and Turkish Food Codex [26, 27, 29, 31]. There are some differences in the values of RJ samples; this difference depends on factors such as geographical location, climate, etc. Sabatini et al. (2009) reported that fructose was more prevalent than glucose. Sucrose is present in almost all RJ samples, but its concentrations are highly variable [33].

Conclusion

Royal jelly is a significant product that is a pioneer among alternative natural products and has uses for many diseases. The need for quality standards for bee products other than honey is widely recognized, both for consumer safety and for the development of beekeeping through value-added products. Royal jelly should be unaffected by the sugar

syrups used in beekeeping and should not have external additives. Sugars are among the main components of royal jelly, and their quantification is crucial for quality control, including the detection of possible adulteration of royal jelly with honey or sugar. Fructose, glucose and sucrose are the main sugars in royal jelly. In this study, royal jelly produced in Bingöl province, which is one of the important beekeeping centers of Turkey, was profiled for sugars at the Bee and Natural Products R&D and P&D Application and Research Center at Bingöl University, a specialized university in bees and bee products. The results were found to be in accordance with the data in the Turkish Food Codex Bee Products Communiqué.

Author Contribution
E.İ.: investigation, methodology, formal analysis, writing – original draft, writing – review & editing. D.K: methodology, formal analysis, writing – original draft. E.K.: investigation, methodology, writing – original draft

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