

## **Bingöl İlçelerinden Elde Edilen Süzme Çiçek Ballarında İnvert Şeker Miktarının Belirlenmesi**

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### **Özet**

Bu çalışmada Bingöl ili Adaklı, Genç ve Karlıova ilçelerinden temin edilen sekiz adet süzme çiçek bal örneğinin invert şeker oranlarının tespiti ve Türk Gıda Kodeksi Bal Tebliği'ne uygunluklarının belirlenmesi amaçlanmıştır. Yapılan çalışmada bal örneklerinin hepsinin Türk Gıda Kodeksi Bal Tebliği'nde çiçek balları için belirtilen yasal limite uygun olduğu belirlenmiştir.

**Anahtar Kelimeler:** Bal, İnvert şeker, Bingöl, Türk Gıda Kodeksi Bal Tebliği

## **Determination of Invert Sugar Amount in Filtered Blossom Honey Obtained from Bingöl Districts**

### **Abstract**

In this study, it was aimed to determine the invert sugar ratios of eight filtered blossom honey samples obtained from Adaklı, Genç and Karlıova districts of Bingöl province and to determine their compliance with the Turkish Food Codex Honey Communiqué. In the study, it was determined that all honey samples comply with the limit value specified for blossom honey in the Turkish Food Codex Honey Communiqué.

**Keywords:** Honey, Invert sugar, Bingöl, Turkish Food Codex Honey Communiqué

### **1. INTRODUCTION**

Türkiye has four different seasons, topographic structure, pine forests, extensive vegetation, industrial plants such as cotton and sunflower, plateaus, pastures, forage crops, and the presence of many different tree species such as acacia, eucalyptus, linden, chestnut, and oleaster. It is an extremely advantageous country in terms of natural resources. Having such extensive vegetation is very important for the diversity and production of honey. Thanks to these geographical features, beekeeping have become one of the oldest and most widespread production sectors in Anatolia [1]. Honey, the most well-known and most consumed beekeeping product, has antioxidant and antimicrobial properties due to the components which contains and prevents the growth of fungi and bacteria [2]. There are 200 different types of compounds in the structure of honey. Honey is an easy-to-digest, nutritious, protective and therapeutic food against various diseases, thanks to the minerals, organic acids, vitamins, flavonoids, amino acids, phenolic acids and enzymes [3,4].

According to the 2021 data of the Food and Agriculture Organization of the United Nations (FAO), Turkey ranks second with its annual honey production of 96 344 tons and third with the number of colonies as 8 733 394 in the world [5]. This information is in **Table 1.1.** presented.

**Table 1.1.** Countries that rank in the top five in world honey production and number of colonies

<b>Countries</b>	<b>Honey Production (Ton)</b>	<b>Countries</b>	<b>Number of Colonies (Pieces)</b>
<b>China</b>	472 700	India	12 848 197
<b>Türkiye</b>	96 344	China	9 216 664
<b>Iran</b>	77 152	Türkiye	8 733 394
<b>Argentina</b>	71 318	Iran	7 527 258
<b>Ukraine</b>	68 558	Ethiopia	7 105 876
<b>World</b>	1771944,36		101 624 052

In addition to being used as a food source, honey has also been used as medicine throughout human history [6]. Apitherapy, a field of complementary medicine based on honey and other bee products, has gained popularity recently and has offered developments that provide treatment against a wide range of diseases.

Kukurova et al. (2008), the climate of the region where honey is harvested, plant species, bee species, environmental variables, honey harvest technique and post-harvest storage conditions affect the composition, quality, color and taste of honey. Honey consists of approximately 80% sugar, 17% water, 3% enzymes and other components [7].

Sugar is one of the most important elements in the composition of honey .Sugars have an impact on physical quality indices such as viscosity, crystallization and moisture absorption [8]. The sugar profile of honey may vary depending on its botanical source, processing and storage conditions, and the climate of the place where it is produced . This sugar profile is important to determine the qualities of honeys of different origins [8]. The type and amount of sugar in honey affects the hygroscopic properties, energy content, crystallization tendency and viscosity of honey [8]. The sugar content of honey is also an important factor in determining adulterated honey [9] .

95-99% of the dry matter content of honey consists of sugars, the most abundant simple sugars in its structure are fructose and glucose. Glucose and fructose are formed as a result of honey bees breaking down the sucrose in nectar by taking in water with the help of acid and invertase enzymes. The glucose and fructose formed are called invert sugar [10,11]. The amounts of these sugars and their ratios can be used as determinants when categorizing monofloral honeys. However, the fructose:glucose ratio is also taken into account in the crystallization stage of honey because the solubility of glucose in water is less than fructose [12].

Many studies have been published in the literature on the sugar concentration of honey, and sugar compositions can vary significantly depending on nectar sources and production region [13]. Although the amount of sugar in honey varies depending on the variety, fructose is generally found in higher amounts than other sugars [12]. According to the Turkish Food Codex Honey Communiqué, the fructose + glucose ratio in blossom honey should be at least 60% [14].

## 2. MATERIALS AND METHODS

In 2021, eight honey samples harvested from Adaklı, Genç and Karlıova districts of Bingöl province were taken. After each honey sample was coded, it was stored in dry glass jars at room temperature, away from direct light, during the analysis process. Honey samples were homogenized before analysis.

### 2.1. Determination of the Amount of Invert Sugar

The determination of invert sugar in honey was made according to the methods specified by TS3036 [15].

A certain volume of Copper (II) sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) solution, which equivalent in invert sugar is known, was titrated against the methylene blue indicator with the aqueous solution prepared from honey in a basic environment. While the glucose and fructose (invert sugar) in honey are oxidized by losing two electrons per molecule, copper (II) ions are reduced to copper (I). The percentage of reducing sugar (invert sugar) in honey was calculated from the volume of honey solution spent in this titration. The solutions used are presented in **Table 2.1**.

**Table 2.1.** Solutions used in the determination of invert sugar

<b>Carrez I solution</b>	<b>Stock invert sugar solution</b>
<b>Carrez II solution</b>	<b>Standard invert sugar solution</b>
<b>2% methylene blue solution</b>	<b>5 M sodium hydroxide (NaOH) solution</b>
<b>1% phenolphthalein solution</b>	

#### 2.1.1. 5 M Sodium hydroxide (NaOH) solution

50 g NaOH was weighed into a 250 mL volumetric flask, 180 mL ultrapure water was added and dissolved. After the resulting solution was cooled in a cold water bath, it was completed up to the marking line with ultrapure water and the mouth of the volumetric flask was tightly closed.

#### 2.1.2. 2% methylene blue solution

In a 1000 mL volumetric flask, 2 g of methylene blue ( $\text{C}_{16}\text{H}_{18}\text{ClN}_3\text{S} \cdot 3\text{H}_2\text{O}$ ) was dissolved with some ultrapure water and then completed up to the mark line with ultrapure water.

### 2.1.3. 1% phenolphthalein solution

0.5 g of phenolphthalein was weighed into a 100 mL volumetric flask and prepared by dissolving it in a 50% ethyl alcohol-ultra pure water mixture.

### 2.1.4. Stock invert sugar solution (10g/L)

9.5 g of pure sucrose was dissolved in a suitable conical flask with 40 mL of water. After adding 5 mL of concentrated hydrochloric acid ( $\text{HCl} = 1.19 \text{ g/mL}$ ), it was kept in a water bath set at  $60^\circ\text{C}$  for 20 minutes, stirring at regular intervals. The hydrolysis process that took place during the heating process was completed by keeping the solution at room temperature for 24 hours. The invert sugar solution resulting from hydrolysis was placed in a 1000 mL volumetric flask, completed with ultrapure water up to the mark line, and mixed well.

### 2.1.5. Standard invert sugar solution

125 mL of the prepared stock invert sugar solution was taken into a 500 mL volumetric flask and 5–6 drops of phenolphthalein solution were added and mixed. 5 M sodium hydroxide solution taken into the burette was carefully dropped into the resulting solution and titrated until the first drop produced a stable pink color. The volume of the resulting neutral light pink mixture was completed to 500 mL with ultrapure water, its mouth was tightly closed and mixed thoroughly.

### 2.1.6. Adjustment of fehling solution

5 mL Fehling A solution, 5 mL Fehling B, 10 mL pure water and 15 mL standard invert sugar solution were added to the erlen mayer flask and mixed. The solution was mixed with a magnetic stirrer on the heated table and heated until boiling occurred. From the moment the solution started to boil, the boiling process was continued for another two minutes. At the end of two minutes, the solution was taken from the heated table and 10–12 drops of methylene blue solution were added. The resulting solution was titrated until the color changed from blue to red, reaching the end of the titration within 3 minutes. By adding the volume of standard invert sugar solution spent in the titration with the 15 mL added at the beginning, the volume of invert sugar solution (V), which is equivalent to 5 mL Fehling A, was found. The mg amount of invert sugar, equivalent to 5 mL Fehling A, that is, the factor (F), was calculated with the following equation:

$$F = V \cdot 2.5 \quad (1)$$

In this equation;

F = Amount of invert sugar equivalent to 5 mL Fehling A, mg

V = The volume of invert sugar solution equivalent to 5 mL Fehling A, as a result of adding the volume of standard invert sugar solution spent in the titration with the 15 mL added at the beginning, mL

2.5 = It refers to 2.5 mg of invert sugar in 1 mL of the prepared standard reducing sugar solution

### 2.1.7. Preparation of honey solutions

2 g of honey was weighed to the nearest 0.001 in a 250 mL conical flask, and 80–100 mL of ultrapure water was added and mixed to dissolve the honey sample. 1 mL Carrez I and 1 mL

Carrez II solutions were added to the resulting mixture and shaken. The volume was completed to 250 mL with ultrapure water and homogeneity was achieved by mixing. The precipitates formed by the addition of Carrez I and Carrez II solutions were filtered with the help of coarse mesh filter paper. 50 mL of the resulting filtrate was taken and added to a 100 mL conical flask.

### 2.1.8. Titration

The volume of the solution to be used in the determination of invert sugar was completed to 100 mL with ultrapure water. Shaking was done by turning the conical flask upside down to ensure homogenization. 15 mL of the homogenized solution was taken and added to a clean and dry burette.

#### 2.1.8.1. Pre-titration

5 mL Fehling A, 5 mL Fehling B, 10 mL ultrapure water and 5 mL honey solution were added to the 150 mL conical flask. The boiling moment was determined by mixing the conical flask placed on the heated table with the help of a magnetic stirrer. From the moment boiling started, the heating process continued for another two minutes. At the end of two minutes, 10-12 drops of methylene blue were added to the mixture and mixed. After this process, the titration process was continued with the solution in the burette until the color changed from blue to brick red, with the titration ending within 3 minutes. The total volume of invert sugar solution spent in the pre-titration is the sum of the 5 mL added at the beginning and the volume added by dropping from the burette ( $V_s$ ).

#### 2.1.8.2. Final titration

The preliminary titration process was carried out as a trial. While repeating the titration process, the same solutions were added into the flask, but 5 ml less than the  $V_s$  given above was added to the flask. The same titration was repeated once more and the total standard invert sugar solution volume ( $V_n$ ) in this last titration was found.

### 2.1.9. Calculation

Total invert sugar (IS) in honey was calculated as invert sugar as a percentage by mass with the following equation.

$$IS = \frac{250}{m \cdot V_n} \cdot \frac{100}{50} \cdot \frac{F}{1000} \cdot 100 = \frac{50 \cdot F}{m \cdot V_n} \quad (2)$$

In this equation:

IS: Invert sugar in the sample (% by mass),

F: Amount of invert sugar equivalent to 5 mL Fehling A (mg sugar/5 mL solution),

m: Honey sample, (g),

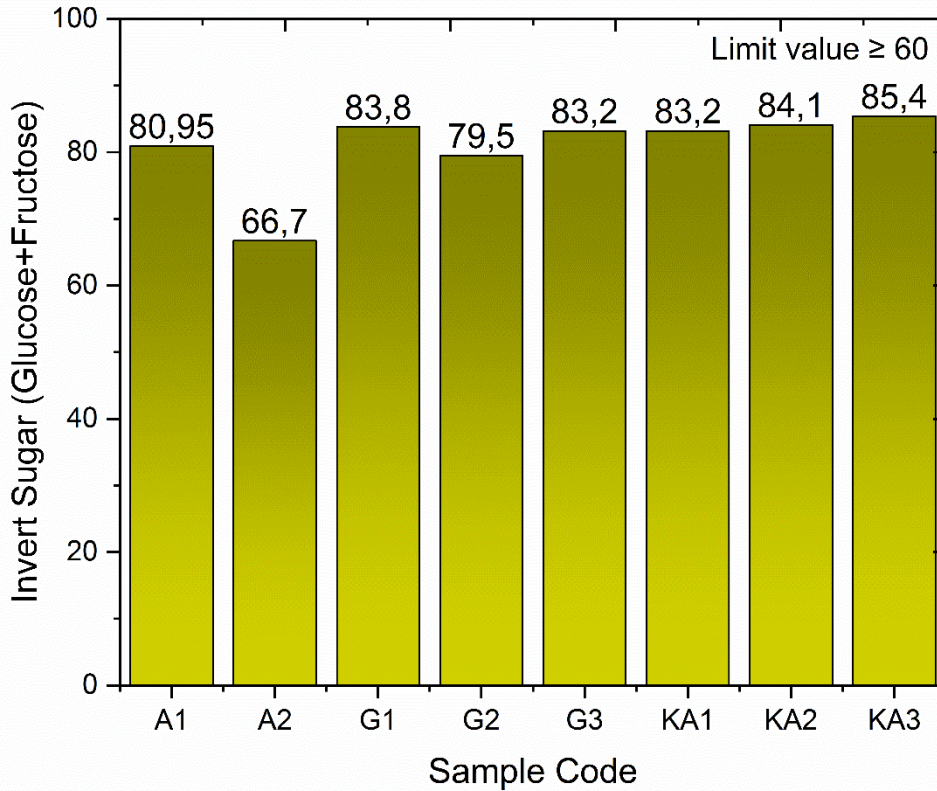
$V_n$ : Volume of standard invert sugar solution spent in the last titration, (mL)

## 3. RESULTS AND DISCUSSION

The mg amount (factor) of invert sugar, which is equivalent to 5 mL Fehling A, was calculated with the equation below and the result was found to be 52.5.

$$F = (15 + 6) \cdot 2.5 = 52.5$$

In the study, invert sugar values ranged between 85.4% and 66.7%, and the average was found to be 80.9%. The distribution of invert sugar values of honey samples is given in **Figure 3.1**. The invert sugar values obtained as a result of the study were found to be above the 60% lower limit value determined for blossom honey in the Turkish Food Codex Honey Communiqué (2020/7) [14].



**Figure 3.1.** Distribution of invert sugar values of honey samples

Merin et al. [16] reported invert sugar values between 70.1% and 79.2% in their study on honey used to sweeten black tea in Israel, Al-Khalifa and Al-Arifly [17] reported 16.7% to 73.3% in their study on Saudi Arabian honey, Yılmaz et al. [18] The invert sugar content of 30 honey samples obtained from the Southeastern Anatolia region was between 64.1% and 76.7%, Şahinler et al. [19] found the average invert sugar value as 57.83% as a result of biochemical analysis of 50 honey samples in Hatay province, Ünal et al. [20] in their study in Ankara, found the invert sugar value in flower and pine honeys to be 23.47%, Ouchemoukh et al. [21] in their study with Algerian honey, between 67.83% and 80.25%, Akbulut et al. [22] in their study with honey obtained from Western Anatolia, 77.10%, Kahraman et al. [23] found the average invert sugar value to be 71.9% in their study, while Saxena et al. [24] found the invert sugar value in Indian honey to be between 43.3% and 65.5%, while Derebaşı et al. [25] found the invert sugar value to be  $67.54 \pm 0.49\%$  in honey samples produced in 18 provinces of the Black Sea region. When all these studies were examined, it was seen that our study results were higher than the results of these studies.

Although it is easy to counterfeit honey, it can be difficult to detect fraud. The main factors used to evaluate the quality of honey are its chemical composition and botanical origin. The compositions of honey obtained from various plant sources and produced in different regions also vary. Although Turkey is in the top five in the world in honey production, it faces serious problems in quality, production and export.

Besides pure honey, there are also many fake and adulterated honey in the market. Today, in order to prevent this fraud, honey must be properly controlled using the right techniques. The taste, appearance or smell of honey alone cannot be used to determine whether honey is adulterated or fake. The most accurate approach is to examine the chemical structure of honey. Honey that meets all the conditions specified in the Turkish Food Codex Honey Communiqué is considered real honey. In this context, the sugar components (sucrose, glucose, fructose and maltose) in the honey samples examined must meet the values specified in the notification. In order to protect consumer health, honey that does not comply with the Turkish Food Codex Honey Communiqué must be identified and withdrawn from the market.

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