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Investigation of The Effect of Creamed Honey Production Process on The Sugar Profile of Honey

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Honey, Crystallization, Creamed Honey, Sugar Profile, HPLC-RID

Abstract: Honey is a supersaturated solution with more than 70% sugar and less than 20% water content. Crystallization of supersaturated solutions is expected from a physicochemical point of view. However, since the reasons for the crystallization of honey are not known by the consumers, crystallized honey is generally not liked. Beekeepers and/or marketers of bee products, liquefy crystallized honey by applying high degrees of heat treatment to convert it into the form preferred by consumers. Honey has been used for both healing and nutritional purposes from past to present. Unfortunately, some processes applied to honey, consciously or unconsciously, can make honey harmful for health, although it may lose its healing value. The new product, which is formed as a result of controlled crystallization to improve some sensory and physical characteristics of honey, is called creamed honey. Creamed honey is an alternative product to crystallization and its effects. In this study, creamed honey was produced from filtered honey and the effect of creamed honey production process on the sugar profile of honey was investigated by High Performance Liquid Chromatography- Refractive Index Detection (HPLC-RID) method. In the analyzes made, it was determined that the creamed honey production process did not have a significant effect on the sugar profile of honey ($p>0.05$).

Krem Bal Üretim Prosesinin Balın Şeker Profili Üzerindeki Etkisinin Araştırılması

Anahtar Kelimeler
Bal, Kristalizasyon, Krem Bal, Şeker Profili, HPLC-RID

Öz: Balda bulunan baskın bileşikler esas olarak fruktoz ve glukozdan oluşan karbonhidratlardır. Bal % 70'den fazla şeker, % 20'den az su içeriği itibariyla aşırı doygun bir çözeltidir. Aşırı doymuş çözeltilerin kristalizasyonu, fizikokimyasal açıdan beklenen bir durumdur. Ancak balın kristalleşmesinin sebepleri tüketiciler tarafından bilinmediği için kristalize bal genel olarak beğenilmemektedir. Arı yetiştiricileri ve/veya arı ürünleri pazarlamacıları, kristalize balı tüketicilerin tercih ettiği forma dönüştürmek için yüksek derecelerde ısıl işlem uygulayarak sıvılaştırmaktadır. Bal, geçmişten günümüze hem şifa hem de beslenme amacıyla kullanılmaktadır. Ne yazık ki bala bilinçli veya bilinçsiz olarak uygulanan bazı işlemler, balın şifa değerini kaybettirmekle birlikte, balı sağlık için zararlı hale dönüştürebilmektedir. Balın duyusal ve fiziksel özelliklerinin geliştirilmesi için yapılan kontrollü kristalizasyon sonucu oluşan yeni ürüne krem bal adı verilmektedir. Krem bal, kristalizasyon olayına ve etkilerine alternatif bir üründür. Bu çalışmada süzme baldan krem bal üretilmiş ve krem bal üretim prosesinin balın şeker profili üzerindeki etkisi HPLC-RID metodu ile incelenmiştir. Yapılan analizlerde krem bal üretim prosesinin, balın şeker profili üzerinde anlamlı bir etki göstermediği ($p>0,05$) tespit edilmiştir.

1. INTRODUCTION

Codex Alimentarius [1] defines honey as follows; It is a viscous, naturally crystallable natural sweet product obtained by the honey bee (*Apis mellifera L.*) from the nectar of plants, the secretions of living parts or the secretions of plant-sucking insects living in the living parts of plants, which the bees transform by treating with their own substances, accumulate and leave in the honeycombs for maturation. Honey has been used for both healing and nutritional purposes throughout history. For example, the Ancient Egyptians, Assyrians, Greeks, Romans and Chinese used honey both for nutritional purposes and for the treatment of wounds [2]. Although the structure of honey varies depending on its botanical source, it contains more than 200 bioactive components. Honey is composed of vitamins, minerals, organic acids, flavonoids, phenolic compounds, amino acids, enzymes and other bioactive substances thus, it is not difficult to digest, nutritious and has many biological activities [3].

Due to its geographical location, Türkiye is known as a country rich in biodiversity and ecological differences. This ecological difference and biodiversity can be considered an important factor for other bee products, especially the richness and quality of honey production [4, 5]. In the literature, studies have shown that bioactive components in honey generally have antibacterial, antioxidant, anticarcinogenic, antiviral, antifungal, antitumoral, anti-inflammatory and anti-insecticidal effects. [6, 7]. The main sugars of honey are known as glucose and fructose. In addition, disaccharides such as sucrose, maltose, lactose, and some oligosaccharides are other sugars found in honey, albeit in small amounts. Honey is a supersaturated solution with more than 70% carbohydrates and less than 20% water content.

Crystallization of supersaturated solutions is expected from a physicochemical point of view. Honey is a supersaturated food with glucose molecules, which are usually less soluble than fructose. Therefore glucose tends to crystallize in the monohydrate form at certain temperatures [8, 9]. Honey crystallizes fastest in the temperature range of 10-15°C [8].

The water in honey binds carbohydrates with hydrogen bonds. Fructose gives weak energy to the hydrogen bonds between water molecules, and the water molecules move around them to hydrate the fructose molecules. Crystallization occurs as a result of the release of glucose by water molecules. In other words, crystallization is caused by the change of glucose and fructose ratio in honey due to this instability of fructose [10]. Since honey is a saturated solution in terms of carbohydrates, crystallization under certain conditions is a very natural phenomenon. However, since some consumers do not know exactly what crystallization is, crystallized honey is often not liked. Beekeepers and/or marketers of bee products bring the honey into liquid form and market it by applying a high degree of heat treatment to the crystallized honey in order to meet the preferences of the consumers. Although applying heat treatment to honey seems to improve some physical properties of honey, it creates various negative effects. The most important of these risks is the increase in the 5-hydroxymethylfurfural (HMF) content of honey. HMF is formed as a result of hexoses losing water molecules at low pH and suitable temperature (usually high temperatures) or Maillard (non-enzymatic browning) reaction. As a result of the reactions in which acids act as catalysts, water is separated from the monosaccharide molecule; furfural pentoses, HMF consists of hexoses [11]. The reaction rate varies depending on pH, water activity (a_w), reducing sugar and amino acid content and ambient temperature. It is reported that every approximately 10°C increase in temperature increases the reaction kinetics by approximately four times [12]. The results of studies on the effects of HMF compound on human health show that HMF has mutagenic, genotoxic, cytotoxic and tumor formation effects [13]. Another negative effect of heat treatment applied to honey is that it reduces the activity of enzymes in honey. In honey, high levels of α-amylase, glucose oxidase, β-amylase α-glucosidase; low levels of catalase and acid come from bees' pharyngeal secretions, salivary fluids, and nectar source [14]. Diastase enzyme provides hydrolysis of starch. Since the diastase enzyme denaturates depending on the storage time and temperature of the honey, it can be considered as a freshness indicator of honey [15]. Unfortunately, some processes applied to honey consciously or unconsciously can have the opposite effect, although they lose the healing value of honey. Controlled crystallization is a good alternative in order to prevent the undesired crystallization phenomenon in honey and the application of heat treatment to honey, which is seen as a solution to improve this phenomenon.

Creamed honey is a new product formed as a result of controlled crystallization made to develop the some sensory and physical characteristics of honey (e.g. giving natural honey a spreadable feature), with crystals in very small sizes that cannot be perceived by the palate. In short, it is the controlled crystallization process to ensure that the honey is spread on the bread like butter and to prevent it from dripping [16]. The chemical content of Creamed honey is almost indistinguishable from the chemical content of raw honey. Creamed honey contains many small crystals that prevent the formation of larger crystals that would normally occur in raw honey. The main difference is physical and sensory differences. Many factors determine the crystal size formed in the product. However, fructose/glucose ratio and storage temperature are reported as the most important among these determining factors [16]. Creamed honey provides various advantages in consumption as it is more spreadable than strained honey. It is expected that the negative factors such as the appearance and dripping of strained honey, which is very important in the balanced diet of people, especially for children in development, disappear with the production of Creamed honey, and it is expected to have a soft taste, spread like butter at normal room temperature, and encourage people to consume honey. It is expected to contribute to the consumption of other bee products (royal jelly, propolis, perga, and pollen) during the production phase of

creamed honey, by mixing them with creamed honey in a homogeneous manner and presenting them to the consumer. In many countries, the Creamed form of honey is more common and preferred than the liquid form [17]. Although the production and consumption of creamed honey is popular in other countries, it is not very common in Türkiye yet. There is a lot of research in the literature investigating honey crystallization, creamed honey production and production optimization.

Karasu et al. (2015) carried out a study on determining the structural changes and thermal stability of creamed honey. For this, they studied the thermal stability of the test at a certain temperature range, that is, its rheological characterization [16]. In another study, different parameters were optimized to produce creamed honey from blossom, canola and honeydew honey [18]. D'arcy (2007) conducted research on the development of an alternative process based on the use of ultrasound technology to the current heating method used in the honey industry to liquefy crystallized honey [15]. Chen et al. (2009) transformed liquid honey into crystallized honey by adding a new nuclei material to give honey good spreadability. Moreover; they carried out a study to test the rheological properties and spreadability of the product they developed [17].

However, did not encounter studies investigating the effect of the creamed honey production process on the sugar profile of honey. It has been shown that in studies, various processes applied to honey caused changes in its physical and chemical properties. It is possible that these changes can also be seen in the Creamed honey production process. Therefore, it is important to detect these changes in the production process of creamed honey, which will be produced from honey with high sugar content. In this study, it was aimed to produce creamed honey and to determine the effect of this production process on the sugar profile of honey by HPLC.

2. MATERIAL AND METHOD

2.1. Sample

Raw honey sample were obtained from local beekeepers at Bingöl, Türkiye, in 2021. The sample was stored at room temperature to the time of next process.

2.2. Chemicals and Reagents

The fructose, glucose, maltose and sucrose standards were of high purity and were obtained from Sigma Aldrich (Darmstadt, Germany). All reagents used were analytical grade, Acetonitrile and methanol were HPLC grade Sigma Aldrich (Darmstadt, Germany).

2.3 HPLC System

The HPLC system; an auto sampler, pump, sample loop and a Refractive Index Detector (RID), all from Agilent (Santa Clara, United States).

Analyses were performed on an NH_2 column (250mm×4.6mm, 5µm particle size-Zorbax). Data were analyzed using Agilent ChemStation Software (Agilent, Santa Clara, United States). All samples were prepared and injected in triplicate.

2.4. Creamed Honey Production Process

Creamed honey production was successfully carried out in the Food Analysis and Research Laboratory in the Food Engineering Department of the Faculty of Engineering and Architecture of Bingöl University-Türkiye. Raw flower honey produced in Bingöl province was used. Creamed honey production was carried out by modifying according to the methods in the literature [18-21]. Room temperature honey was filled into the creamed honey making machine. Then, creamed honey was produced in a creamed honey making machine with a constant mixing speed of 60 rpm, at a temperature of 14ºC, and a mixing time and number of 4 minutes and 5 times, respectively, by adding 10% of the creamed honey purchased from a local company to act as a seed crystal. Figure 1 shows the creamed honey produced in our laboratory.

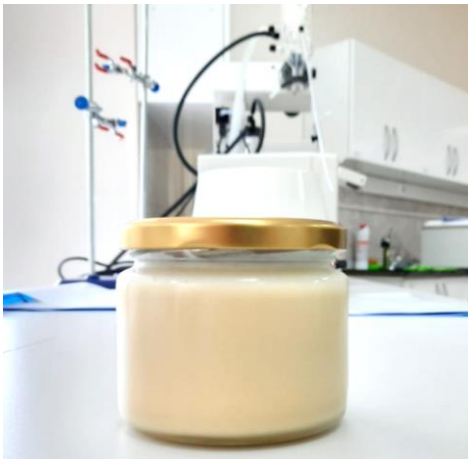

Figure 1. Creamed honey produced in our laboratory

2.5. Honey Sugar Profile Analysis by HPLC

For sugar profile analysis of honey, both raw honey and creamed honey samples were analysed according to the Turkish Standardization Institute method [22]. Standard solutions of glucose, fructose, sucrose, maltose at different concentrations were prepared for calibration. After the prepared standard solutions were transferred to vials, they were read in the HPLC device. The calibration curve was drawn by peak areas obtained from the read standard solutions were drawn with Agilent ChemStation Software. 5 g honey was weighed with 0.001 g precision and dissolved in approximately 40 mL ultrapure water. Then, 25 mL of methanol was added to it, and it was taken into a metered balloon and the volume was adjusted to 100 mL with adding ultrapure water. Approximately 1.5 mL of this solution was transferred to the HPLC vials with a sterile disposable syringe and a 0.45 µm syringe filter. Then analyzed by the HPLC device. Sugar concentrations were calculated as % by using the formulas in the obtained peak area

calibration curves. An example of a chromatogram of the sugar standards solution is shown in Figure 2. During HPLC analysis ultrapure water: acetonitrile (20:80, v/v) mixture was the mobile phase, flow rate 1.3 mL/min, injection volume 10 µL, and detection temperature 30°C.

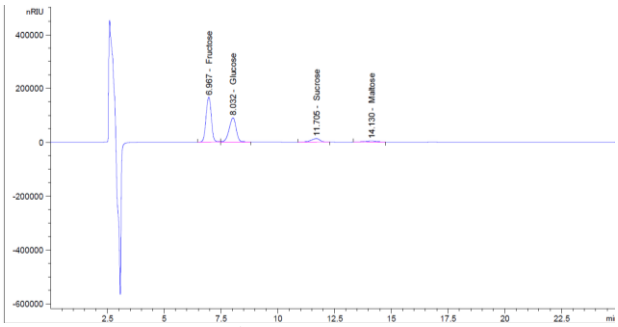

Figure 2. Chromatogram of the sugar standards solution

2.6. Statistical Analysis

In order to examine the effect of the creamed honey production process on the sugar profile of honey, the differences between them were analysed with the Mann-Whitney U Test, which is a non-parametric test in the IBM SPSS Statistics (Chicago, USA) program.

3. RESULTS and DISCUSSION

Sugar profile analysis is one of the important parameters like other parameters used to determine the quality of honey. Generally, the amount of fructose contained in honey is higher than glucose. The fructose content of honey is about 38-40%, and the glucose content is around 30-32%. However, this ratio may vary depending on storage time and temperature, as well as other factors such as the nectar source.

Fructose (F), glucose (G), sucrose, maltose, fructose/glucose (F/G) and fructose+glucose (F+G) results of raw honey and creamed honey samples are given in Table 1 and Table 2 respectively.

Table 1. Sugar amount of raw honey

Descriptive Statistics				
Parameters	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Fructose	39.998	40.322	40.171	0.164
Glucose	33.790	33.864	33.819	0.039
Sucrose	0.040	0.041	0.040	0.000
Maltose	1.460	1.486	1.472	0.013
F/G	1.184	1.191	1.188	0.004
F+G	73.787	74.186	73.990	0.200

The fructose amount of raw honey used in making creamed honey is between 39.998-40.322 $100g^{-1}$ and the average is 40.171 g $100g^{-1}$. The amount of glucose is between 33.790-33.864 g $100g^{-1}$ and the average is 33.819 g 100g-1. This is expected because in general, the fructose ratio of honey is higher than glucose. The fructose content of honey is about 38-40%, and the glucose content is around 30-32% [23]. The lowest amount of sucrose is 0.040 $100g^{-1}$, the highest amount is 0.041 g $100g^{-1}$, and the average is 0.040 g $100g^{-1}$. The amount of maltose is between 1.460-1.486 $100g^{-1}$ and on average 1.472 g $100g^{-1}$. The average of F/G and F+G is 1.188 $100g^{-1}$ and 73.990 $100g^{-1}$, respectively.

Table 2. Sugar amount of creamed honey

Descriptive Statistics				
Parameters	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Fructose	39.579	39.616	39.599	0.019
Glucose	33.659	33.701	33.686	0.024
Sucrose	0.030	0.037	0.033	0.004
Maltose	1.042	1.052	1.048	0.005
F/G	1.175	1.176	1.176	0.000
F+G	73.238	73.317	73.285	0.042

The fructose amount of creamed honey is between 39.579-39.616 $100g^{-1}$ and the average is 39.599 g $100g^{-1}$. The amount of glucose is between 33.659-33.701 g $100g^{-1}$ and the average is 33.686 g $100g^{-1}$. This is expected because in general, the fructose ratio of honey is higher than glucose. The lowest amount of sucrose is 0.030 $100g^{-1}$, the highest amount is 0.037 g $100g^{-1}$, and the average is 0.033 g $100g^{-1}$. Sucrose is generally present in honey in low amounts, ideally no more than 5%. Because pure honey contains a low amount of sucrose due to the presence of the enzyme invertase, which breaks down sucrose. For this reason, the high rate of sucrose in honey may raise suspicion of adulteration [24]. The amount of maltose is between 1.042-1.052 $100g^{-1}$ and on average 1.048 g $100g^{-1}$. The average of F/G and F+G is 1.176 $100g^{-1}$ and 73.285 $100g^{-1}$, respectively. According to the obtained analysis results, fructose and glucose are present in all honey samples. Moreover; as expected in natural honey, the amount of fructose was found to be higher than the amount of glucose in all samples [23].

According to the Turkish Food Codex [25], the limits that honey should carry are shown in Table 3.

Table 3. Turkish Food Codex limits of sugar in honey

Parameters	**Limits**
Humidity (%, Max.)	% 20
Sucrose (Max.)	5 g $100g^{-1}$
Fructose+ Glucose (Min.)	60 g $100g^{-1}$
Fructose / Glucose	0.9 – 1.4
Maltose (%, Max.)	4

All sugar parameters of both the raw honey used and the creamed honey are in compliance with the Turkish Food Codex limits.

The sugar results found in a study [16] on the determination of the rheological properties of creamed honey are in a similar range with the results of our study. Kamal et al. [26] conducted a study on the determination of sugars in honey by liquid chromatography. The findings of our study are in the range of the findings of their study. Alghamdi et al. [23] determined the sugar composition in honey samples collected from Saudi Arabian markets using HPLC technique. The results they found are very close to the results of our study.

The results found in the study [24] on the determination of the physical and chemical properties of different honey samples are similar to the results we found.

The differences between the findings were analysed with the Mann-Whitney U Test, which is a non-parametric

test in the SPSS (IBM, Chicago, USA) program. As a result of the analysis, it was observed that the creamed honey production process did not affect the sugar profile of the raw honey used in production significantly ($p>0.05$). The reason for this is that, as mentioned above, the creamed honey production process is not a chemical process, but a physical process.

4. CONCLUSION

In general, honey can be consumed directly for healing purposes as well as being used as a food supplement. With the recent studies, honey processing processes are developed and new functional products are tried to be produced. Creamed honey has an important place among these new products. Sugar content is very important in the selection of honey to be creamed. Because the sugar content is directly effective in the controlled crystallization of natural honey, which also affects the creamed honey production process. Therefore, determining the sugar profile of honey is of great importance for the honey industry in terms of both quality parameter and processing. The study in question is an original study to determine the effect of the Creamed honey production process on the sugar profile of honey. It is expected that the results of creamed honey production process does not affect the sugar profile, which is one of the important quality parameters of honey will contribute to both the food industry and the literature. Moreover; it is expected that will contribute to breaking the prejudices and drawbacks of both consumers and the industry against creamed honey.

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