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COMPARISON OF PHYSICAL AND BIOCHEMICAL CHARACTERISTICS OF DIFFERENT QUALITY OF TURKISH HONEY

Farklı Kalitede Türk Ballarının Fiziksel ve Biyokimyasal Özelliklerinin Karşılaştırılması

(Genişletilmiş Türkçe Özet Makalenin Sonunda Verilmiştir)

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ABSTRACT

Honey adulteration is a serious ethical problem and results in many losses such as in nutrition, health and economy. While adulteration of honey is very easy, it is difficult to determine it and requires troublesome techniques. The aim of the present study was to determine some physical and biochemical to differentiated parameters between the natural and adulterated with saccharose syrup honeys. Therefore, moisture, color, optical rotation, fructose, glucose, maltose, ribose, arabinose, proline, 5-hydroxymethlfurfural (HMF), total phenolic substances and total antioxidant capacities were measured to find any difference. Proline content, total amount of phenolic substances were found as important parameters that can be used to distinguish natural honey from that produced by over-feeding of bees with saccharine.

INTRODUCTION

Honey is a natural product mainly consisting of fructose and glucose and the minor amount of saccharides and other compounds are phenolics, proteins, enzymes, amino acids, minerals, vitamins, organic acids and Maillard reaction products, and possible other minor components (Anklam, 1998, Gheldof et al., 2002, Ahn et al., 2007). The quality and biological properties of honeys are related with many factors such as maturity, processing, storage conditions, production methods, climatic and botanical conditions (Abdel-Aal, et al., 1993; Guler et al., 2007, Meda et al., 2005). Because honey composition is highly variable, the adulteration is very easy with overfeeding with inexpensive sweeteners such as saccharose syrups, corn syrups, high fructose corn syrups, invert syrups and saccharide variants. Overfeeding bees with saccharide or invert saccharide derivatives to increase the amount of honey produced has been commercially practiced by beekeepers (Guler et al., 2007; Cordella et al., 2005; Ruiz- Matuta et al., 2010). Therefore, for centuries the purity and naturality of the commercialized honey has always been questioned. Saccharide analysis has been frequently used to determine the adulteration, but the test is not adequate, because of worker bees convert saccharose to glucose and fructose by digestive enzymes (White, 1998). However, some researchers have reported that saccharose, fructose, proline, mineral contents, and some physical parameters can be used to distinguish pure honey from adulterated honey (White, 1979; Guler et al., 2007; Ruiz- Matuta et al., 2010; Silici et al., 2008). Many researches have used pollen analysis to distinguish honey types based on its floral origins (Mendes et al., 1998; Silici et al., 2010). Some chromatographic methods for the detection

of adulteration in honeys have been reported (White et al., 1975; Doner et al, 1979; Abdel-Aal, et al., 1993). Paradkar and Irudayaraj (2001) have used FT-Raman spectroscopy to discriminate adulteration with beet and cane saccharides. Cordella et al. (2005) has developed an anion exchange chromatography (HPAEC-PAD) for honey analyses and adulteration detection. During the last decades, many researchers did investigations to distinguish pure honey samples from adulterated honey by the method of stable carbon isotopic ratio analysis (SCIRA) (White, 1998; Kerkvielt and Meijer, 2000 and Martin et al., 1998). This technique is based on $_{12}C$ /$_{13}C$ ratio determination for both of saccharides and internal protein content. But the method was suitable only for saccharides from C_4 plants (cane and corn) instead of C_3 plants (beet) (Anklam, 1998). Because these sophisticated methods are required high technology and are generally not economical, there is a need for development of more practical and less costly method to detect honey adulteration. Therefore, this research group intends to distinguish adulteration in some authentic Turkish honey samples, documenting their physico-chemical, chemical and biochemical properties.

MATERIALS AND METHODS

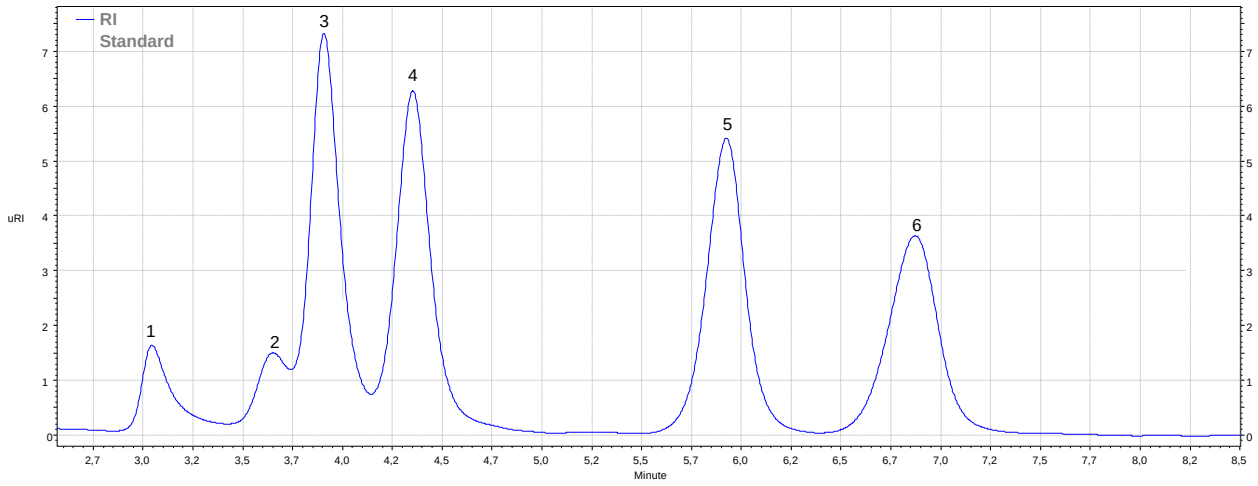
Honey samples

For this study, four different group floral honey samples were supplied by experienced beekeepers from different areas of Turkey aiding of chairmanship of Trabzon Honey Agricultural Cooperative (Trabzon, Turkey) in 2008. The pure honeys are; multifloral blossom honeys (11 sample), chestnut (10 sample) (*Castania sative* L.), rhododendron (8 sample) (*Rhododendron ponticum* L.), pine (8 sample) (*Pinus brutia* Ten), and the honeys adulterated with saccharose syrup (13 sample) were collected and studied. The honey adulterated with saccharose syrup was obtained by give water: saccharose (about, 1:1.5) (w/w) solution to each colony as randomly.

Chemical analysis

Moisture in honey was measured with a refractometer (Atago, Tokyo, Japan) reading at 20 ºC and the corresponding % moisture determined from refractive index's table from in AOAC 969.38 (AOAC, 1990). HMF was determined by RP-HPLC method in aqueous honey solution by using an external calibration curve (5-hydroxymethlfurfural, Sigma-Aldrich, Milano, Italy), and the detector was set to 285 nm (Jeuring and F. Kuppers, 1980). Optical rotation was measured in a polarimetry (Beta PPP7 Optical Activity, Cambridge, United Kingdom) as follows: 12 g honey sample and 10 ml Carrez reagents (I and II) were mixed 30 min, and the volume was completed to 100 ml. Then this solution was inserted into the polarimetry and the results were stated in angular on a 200 mmol basis (Junk and Pancoast, 1973).The colour index was measured as Pfund measurement as the optical density at 560 nm (Fell, 1978). The carbohydrate contents were determined by HPLC-RI (Shimadzu, Tokyo, Japan) to evaluate the monosaccharides; glucose, fructose, arabinose and ribose, and the disaccharides; saccharose, and maltose (Fig 1.) (Bogdanov and Baumann, 1998).

Fig 1. The standard chromatogram of six individual sugar component at RI dedector. (1) Ribose, (2) Arabinose, (3) Fructose, (4) Glucose, (5) Saccharose, (6) Maltose



The content of total phenolic compounds was determined by the Folin- Ciocalteu reagent (Singleton and Rossi, 1965), and the results were expressed in mg GAE per kg of honey (GAE–gallic acid equivalent). Total antioxidant capacities of the honeys were determined in terms of ferric reducing antioxidant power (FRAP) (Benzie and Strain, 1996).FRAP values were expressed as mmol Fe (II) of kg honey.

Statistical analysis

The results were presented as mean values and standard deviations (mean ±SD). Data and regression analyses were performed with Microsoft Office Excel 2003 (Microsoft, Redmond, Washington, USA). Data were tested using SPSS (version 9.0 for Windows 98, SPSS, Chicago, Illinois, USA). Statistical analyses of the results were based on Kruskal Wallis, Mann-Whitney U tests and Pearson correlation analysis, a nonparametric test. The significance of the differences was statistically considered at the level of $p<0.05$, or otherwise given.

RESULTS AND DISCUSSION

The chemical, physico-chemical and biochemical properties of the five groups honey samples are listed in Table 1. Statistical analyses showed that there are no significant differences between the pure and adulterated honey samples based on moisture, HMF, glucose, ribose and arabinose ($p>0.05$). The moisture contents of all the samples were below 20%, the maximum value allowed by Turkish (TSE) and European (CEU) standards that indicate harvesting time is enough. Moisture content of honey is an important factor, contributing to its stability against fermentation and granulation during storage (Anklam, 1998, White and Winters, 1989).

Optical activity is a physical property, which is the ability of a chiral molecule to rotate the plane of plane-polarized light measured using a polarimetry. Determination of specific rotation by means a polarimetry is mainly used to distinguish between honeydew honeys (dextrorotatory, positive values) from blossom honeys (laevorotatory, negative values). The overall optical rotation depends on the content of various saccharides in honey and is the sum of rotations of individual saccharide compounds present in a sample. Except pine honey, all of the honeys have a negative optical activity. The pine honey classified as honeydew or secretion honey, and showed positive optical activity. These values are in agreement with those reported several researches (Beretta et al., 2005; Al-Khalifa & Al-Arity, 1999; Nanda, et al., 2003).

The glucose contents varied from 22.0 to 35.0 g per 100g of honey. The highest glucose values were found in adulterated honeys, but the differences were not statistically significant ($p<0.05$). The mean fructose values of all the honey samples varied from 23.0 to 42.6 g per 100 g. While the adulterated honeys had the lowest fructose value, the pure honeys had higher fructose amounts ($p<0.05$). The blossom, chestnut and rhododendron honeys had similar levels of fructose values, which ranged from 38.8 and 39.9 g per 100 g. The pine honey had lowest fructose content among the pure honeys. F/G ratio of the five group honey samples in the study ranged between 1.15 and 1.62. The F/G (Fructose/Glucose) ratio was found the lower in adulterated honeys ($p<0.05$). F/G ratio is a substantial indicator for honeys and fruit juice, and the ratio should be taken into account to evaluate honey adulteration (Manzanares, et al., 2011; Tosi et al., 2004; Kolayı et al., 2010). Because saccharose has a 1:1 ratio of fructose and glucose, worker bees convert nearly all available saccharose to invert glucose and fructose, by invertase enzyme. The actual proportion of fructose to glucose in any particular honey depends largely on the source of the nectar (Anklam, 1998). In addition, saccharide composition, moisture and pH are related to crystallization of honeys (Cavia et al., 2002; Tosi, et al., 2004). It is reported that the F/G ratio of 1.14 or less would indicate fast granulation, while values over 1.58 are associated with no tendency to granulation (White, 1979; Tosi, et al., 2004). The chestnut honeys have the highest F/G ratio, and, thus, these honeys are not prone to crystallization. The results indicate that adulterated honeys with saccharose syrup have higher tendency to crystallization. For comparison, F/G ratios of honeys from different studies were reported to be 1.11–1.36 in thirteen different floral Algerian honeys (Oucemoukh et al., 2010) and 1.19–1.34 in Venezuelan multifloral honeys (Rodrìguez et al., 2004). Maltose is a disaccharide source from malt and starch. Although the rhododendron and the chestnut honeys showed the lowest maltose content, the pine, the blossom and the overfeeding honeys showed higher maltose content. We also measured two individual pentose saccharides, ribose and arabinose in the five group honey samples to find any differences. Ribose content was ranged from

0.18% to 1.00% in the five groups. High ribose values were detected in the rhododendron and the chestnut honeys and, the lower ribose were in over-feeding honey (Table. 1), but the differences were not significant ($p > 0.05$). We also could not find a regular distribution with respect to ribose in the honey samples, except for the chestnut and pine honeys. We have not found enough study in the literature that measured ribose and arabinose content in honey. Thus, it is almost impossible to compare the ribose and arabinose values with other honey samples. Saccharide composition has been used to determine honey adulteration and botanical origin, but is not enough to discriminate honeys (Cavia et al., 2002; Manzanares et al., 2011).

We have measured total phenolic content and in vitro antioxidant activity of methanolic extracts to discriminate of the five types honey samples. Total phenolic content was determined in comparison with gallic acid and the results expressed in terms of mg GAE per kg of honey and all of the studied honey samples showed a linear positive relationship with the extract content. As seen from Table. 1, the lowest phenolic content value was determined in adulterated honey, where the average results of thirteen samples was 118 mg/kg, rising further in blossom, rhododendron, pine and chestnut. The highest phenolic content values were obtained for chestnut and pine, 1074 mg and 596 mg per kg honey, respectively, and were approximately 5-10 folds higher than adulterated honey. The higher total phenolic content was in close agreement with the results reported by some researchers for chestnut honey (Küçük et al., 2007; Bertoncelj et al., 2007).

Table.1. Physical parameters, carbohydrate, antioxidant capacity, and total polyphenolic contents of the tested honeys*

	Blossom	Chestnut	Pine	Rhododendron	Adulterated with sucrose syrup	*p* value
Samples (n)	11	10	6	6	13	
Moisture (g/100 g)	18.19± 0.96	17.64±0.94	17.26±0.93	17.41±1.13	16.66±1.10	**0.055**
Color Abs (560 nm)	0.39±0.20	2.48±0.49^{a}	1.51±0.15^{a,b}	0.72±0.13^{a,b,c}	0.50±0.46^{b,c}	**0.001**
Optical Rotation	-1.79±1.38	-2.09±0.86	2.42±0.92^{b}	-1.13±0.36^{a,c}	-0,97±0.65^{b,d}	**0.001**
HMF mg/kg	5.75±4.45	7.16±6.63	6.46±2.93	10.97±8.64	9.85±7.80	**0.709**
Glucose (g/100 g)	29.97±2.50	25.30±1.65	27.66±2.69	29.60±2.00	31.01±2.23	**0.133**
Fructose (g/100 g)	39.05±1.68	40.81±1.92	38.99±1.83^{b}	40.05±1.48	35.79±4.57^{a,b}	**0.022**
Fructose/Glucose ratio	1.31±0.11	1.62±0.10^{a}	1.48±0.16	1.36±0.09	1.15±0.12^{a,b}	**0.001**
Glucose/Moisture ratio	1.50±0.18	1.65±0.55	1.46±0.09	1.7±0.20	1.83±0.20	**0.001**
Sucrose (g/100 g)	0.13±0.20	0.05±0.03	0.45±0.52^{a,b}	0.38±0.37	1.23±0.44^{a,b}	**0.001**
Maltose (g/100 g)	1.66±0.87	0.07±0.02	2.40±1.33^{b}	0.51±0.59^{a,b,c}	1.89±0.64^{b,c,d}	**0.001**
Ribose (g/100 g)	0.21±0.16	0.68±1.15	0.23±0.10	1.00±1.13	0.18±0.19	**0.782**
Arabinose (g/100 g)	0.06±0.04	-	-	-	0.09±0.13	**0.517**
Proline (mg/kg)	696±227	704±177	436±66^{a}	526±45.77^{b}	258±66.52^{a,b,c,d}	**0.001**
Total phenolic content (mg GAE/kg honey)	466±265	1074±242^{a}	496±148^{b}	580±199^{b}	118±82 ^{a,b,c,d}	**0.001**
FRAP mM Fe(II)/kg honey	**270±118**	**513±126^{a}**	**311±47^{b}**	**435±71^{a,c}**	**165±105 ^{a,b,c,d}**	**0.001**

*Statistical analysis by Kruskal Wallis test. Values are mean ± SD.

a – values are significantly different from those of blossom ($p<0.05$), b – values are significantly different from those of chestnut ($p < 0.05$), c– values are significantly different from those of erica ($p < 0.05$), d–values are significantly different from those of rhododendron ($p < 0.05$), e – colour values are expressed as Pfund index of 560 nm absorbance, f – total phenolics are expressed as mg of gallic acid equivalent per 1 kg of honey, g – FRAP values are expressed as µmol of Fe(II) per 1 l of honey solution.

For determination of the antioxidant capacity, we used the FRAP assay (ferric reducing/antioxidant power), a simple test that is widely used for determination antioxidant capacity in many natural samples, the test is considered to be a good indicator for total antioxidant power (Küçük et al., 2007 and Bertoncelj et al., 2007). The increased absorbance is an indication of higher reducing power in this method. As shown Table. 1, there were significant differences among the types of honey ($p < 0.05$). The FRAP values of the honey samples varied from 165-513 millimoles of ferrous equivalent (Fe [II]) per kg honey. The FRAP value for five different types increased in the order; adulterated < blossom < pine < rhododendron < chestnut. Adulterated honey had an average FRAP value of 165 mM Fe (II) per kg honey, while the highest FRAP values were obtained in chestnut and rhododendron honey. Because of the adulterated honeys have lower total phenolic contents than natural honey; the antioxidant capacity was relatively lower. Phenolic compounds are plant derived secondary metabolites, mainly sourced from nectars and pollens into honey by *Apis mellifera* (Bogdanov, et al., 2004). The adulterated honey includes lower value of phenolics, lack of nectars and pollens. On the other hand, the average total phenolic contents were in close agreement with the results reported by for chestnut and rhododendron and multifloral honeys (Küçük et al., 2007; Silici et al., 2010). A positive linear correlation between the total phenolic content and total antioxidant capacity was determined (r^2 = 0.76). This positive correlation has been reported in several investigations (Silva et al., 2006, Socha et al., 2009; Bertoncelj et al., 2007; Tezcan et al., 2011). Therefore, the results showed that honey has highly biologically active substances, and its phenolic composition is mostly responsible its antioxidant power (Kolayli et al., 2010; Meda et al., 2005; Bertoncelj et al., 2007).

There are a few different methods to measurement colour of honey; the most commoly used methods are based on optical comparison (Bogdavov, et al., 2004). In this study, we used Pfund scale, a simple method, for determine and comparison of the honey colour characteristic as physical parameters (Fell, 1978). The colour characteristics are presented in Table. 1. The colours of the honey samples varied from almost colourless to dark brown. The blossom and adulterated honeys were the brightest honeys, while chestnut and pine honeys were the darkest honeys ($p<0.05$). No statistically significant differences existed between pure blossom honeys and adulterated honeys that both of the colours were extra light amber ($p>0.05$). In general, colour of chestnut and pine honeys were in a similar range of as previously reported data (Bertoncelj et al., 2007). The colour of honey is related to the content of pollen, total phenolics, mineral composition, HMF and is characteristic of floral origin (Gonzales- Miret et al., 2005 and Bertoncelj et al., 2007). HMF values in all the honey samples were measured ranged from 5.75 mg to 14.10 mg per kg honey (Table. 1). HMF content is also related in freshness and heating of honey (Yildiz et al., 2010) and in Codex Alimentarius (Codex Alimentarius Commission-1981) limit for HMF content in honey to 40 mg per kg honey. All of the HMF values were below the 40 mg per kg honey that is the recommendation values of Honey Codex. We have not found any correlation between the HMF values and the pfund values (A_{560}) of colors (r^2=0.02, $p>0.05$) in the 46 honey samples. Since the standard deviation of HMF values were very high, a significantly correlation was not observed between HMF and color parameters. There is a positive correlation between pfund values (Abs_{560}) of colour and total phenolic content (r^2=0.70, $p<0.05$). Similar to our results, dark colored honeys are reported to contain more phenolic acid derivatives and consequently a higher antioxidant capacity (r^2=0.65) (Bogdanov, et al., 2004; Bertoncelj et al., 2007; Beratta et al., 2005 and Frankel et al., 1998). There are some studies that HMF content changed with effect of heating and some of them not changed in honey and other sweet food (Fallico et al., 2004; Ajlouni & Sujirapinyokul, 2010 and Yildiz and Alpaslan, 2012).

The proline content varied from 258±66.52 mg to 704±177 mg per kg honey using the standard curve of proline with HPLC analysis. The highest proline content was observed in chestnut honey among the five different types honeys. The proline values of the adulterated honey with saccharide syrup varied from 192 mg to 324 mg per kg honey. Proline content of the adulterated honey was found significantly lower than the pure honeys ($p < 0.05$). Proline comes mainly from salivate secretions of *Apis mellifera* during the conservation of nectar into honey (Turhan et al., 2008). Proline content is considered an important quality parameter for honey that can serve as an additional determinant of purity and maturity of honeys. The proline contents of all the samples were above 180 mg per kg honey the minimum value allowed by the Turkish Standards Insti-

tute (TSE) and Council of the European Union (CEU), all of the proline values found to be within accepted ranges (Bogdanov and Baumann, 1997).

CONCLUSION

Four different types of authentic Turkish honey and a group of honey adulterated with saccharose syrup were investigated in terms of moisture, color, rotation, fructose, glucose, maltose, ribose, arabinose, proline, HMF, total polyphenolic substances, and total antioxidant capacities. Honey adulterated with saccharose syrup were found to meet all major national and international honey specifications. All types of honey contained phenolic compounds and possessed antioxidant activity, while the adulterated honeys showed low total phenolic and antioxidant capacity. The total phenolic contents and antioxidant activity were found to be the highest in darker honeys, namely chestnut and pine. Proline content proved to be the best marker of honey adulteration in the studied parameters.

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GENİŞLETİLMİŞ ÖZET

Özet

Bu çalışmada deneyimli arıcılardan toplanan 4 grup farklı floral balların ve kontrollü şartlarda şeker beslemeli olarak üretilen balların fiziksel ve biyokimyasal bazı parametreleri kıyaslanarak bu ballarda hilenin tespit edilmeye çalışılmıştır.

Materyal ve metot

Çalışmada dört grup floral orjinli saf bal numunesi deneyimli arıcılardan temin edildi. Saf ballar çiçek balları (11 adet), kestane balları (10 adet), orman gülü balları (8 adet), çam balları (8 adet) idi. Ayrıca 13 adet şeker beslemeli bal üretildi ve çalışmada kullanıldı.

Kimyasal analizler

Balların nemleri refraktometre ile AOAC 969.38'e göre; HMF içeriği RP-HPLC metodu ile; optik çevirme polarimetre ile; renk indeksi spektrofotometre ile; şeker içeriği HPLC-RI ile; toplam fenolik madde Folin- Ciocalteu metodu ile; antioksidan kapasite FRAP metodu ile yapıldı, sonuçlar SPSS istatistik yöntemi ile değerlendirildi.

Sonuçlar

Hileli bal üretimi ciddi bir etik problem olup ekonomik, sosyal ve tıbbi açıdan pek çok sorunlara yol açmaktadır. Balın bileşimi oldukça kompleks olmasından dolayı hileli bal üretimi oldukça kolay; fakat hileli balların ayırt edilebilmesi oldukça zordur. Günümüzde ballardaki hilelerin ortaya çıkarılmasına yönelik değişik analiz yöntemleri kullanılmaktadır. Yöntemlerin çoğunluğu ülkemizdeki ve dünyadaki bal standartları ve kodekslerinde geçen parametrelerin tespitine ve kıyaslanmasına yönelik çalışmalardır. Ancak mevcut analizlerle bir baldaki hilenin tam olarak ortaya çıkarılması oldukça zordur. Bilhassa günümüzde nişasta bazlı şekerlerin arı beslemesinde kullanılması ile üretilen hileli ballarda daha detaylı analizlere ihtiyaç duyulmaktadır. Bunların yanında floral orjinleri değişik bal standartları kıyaslama yapılan parametreler bazında detaylandırılmadığı için hileli balların tespitinde standartların kullanılması zorlaşmaktadır.

Yapılan çalışmanın amacı değişik floralara ait kaliteli ve hileli balları fiziksel, kimyasal ve biyokimyasal yönlerden analiz edip, aralarındaki farklılıkları ortaya çıkarmaktır. Balların nem, renk, optik çevirme, fruktoz, glukoz, maltoz, riboz, arabinoz, prolin, hidroksimetil furfural (HMF), toplam fenolik madde ve toplam antioksidan kapasitelerinin ölçülmesi ile hileli balların ayırt edilmesine yönelik testler ve test birliktelikleri çalışmada araştırılmıştır. Çalışılan ballarda prolin ve toplam fenolik madde miktarlarının kaliteli ve hileli ballar arasında en ayırt edici parametreler olduğu tespit edilmiş, sonraki çalışmalarda nişasta bazlı şeker beslemeli ballar da üretilerek sonuç kıyaslamasına gidilmesi, karbon 13 izotop analizleri ile kıyaslama yapılması gerekliliği vurgulanmıştır.