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Effect of inverted saccharose on some properties of honey

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Abstract

In this study, three groups of honey [natural honey; honey produced by the supplementary feeding of bees with saccharose syrup (SSH) and heat and acid (88 °C, 2 h; 0.1% HCl) treated saccharose syrup honey (ISSH)] were produced and physicochemical (water content, pH, free acidity, ash, HMF, diastase activity, sucrose, protein and viscosity), microbiological and sensory properties of these honeys were determined. Also, mineral contents of the honeys were measured. Moisture and ash contents of SSH were higher, acidity level was lower than those of other honeys. The mineral content of natural honey was higher than that of the others, except for Pb and Zn. Diastase activity of ISSH was below the standard limit and HMF content of this honey was high, but not exceeding the limit. Supplementary feeding of honey bees with inverted (acid and heat treatment) saccharose yielded a honey which had a higher HMF content and a lower diastase activity, moisture content and free acidity than natural honey or SSH.

Keywords: Feeding; Saccharose; Inversion; Honey; Properties

1. Introduction

Composition of honey is affected by contributions of the plant, climate, environmental conditions and the ability of the beekeeper (White, 1978). The diversity of the physical and chemical properties of honey depends on the nectar and pollen of the original plant, colour, flavour, moisture and contents of protein and sugars (Barth, 1989; White, 1978; White & Maher, 1980). Carbohydrates form nearly 95% of honey. Sugars in honey are responsible for properties such as viscosity, hygroscopy, granulation and energy value (Ötleş, 1995). Honey contains mainly glucose and fructose with trace amounts of other sugars (sucrose and maltose) (Siddiqui & Furgula, 1976) and organic acids, lactones, amino acids, minerals, vitamins (vit B1, B2, C and nicotinic acid), enzymes, pollen, wax and pigments are also present (White, 1978). The mineral content and trace elements in honey samples could give an indication of the geographical origin of honey (Rodriguez-Otero, Paserio, Simal, & Cepeda, 1992).

Physical and chemical properties of different types of honey have been reported by many scientists. Effects of principal chemical constituents on the quality of honey have been reviewed by Estupinon and Sanjuan (1998). In another study, Anupama, Bhat, and Sapna (2003) found that viscosity, red component, percent acidity and sucrose content were the major physicochemical variables contributing to the grouping of honey samples.

Azeredo, Azeredo, De Souza, and Dutra (2003) determined protein contents and physico-chemical properties and the mineral content in honey. They found that moisture content, freeacid, lactone and total acidity were physicochemical properties that depended on the flower types used by honey bees for nectar.

Carbohydrates are abundant in the natural diet of the honey bee and are used mainly for the production of energy, but may be converted to body fats and stored. Adult bees can use glucose, fructose, sucrose, trehalose,

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maltose and melezitose. But they cannot use rhamnose, xylose, arabinose, galactose, mannose, lactose, raffinose, dextrin or inulin. Differences in carbohydrate utilization between larvae and adults may be due to the absence of appropriate enzymes. Food enters the alimentary canal by way of the mouth of the bee and passes trough the esophagus to the honey stomach. In the honey stomach, invertase breaks down the sucrose of nectar to the simpler monosaccharides, glucose and fructose, present in honey. Honey bees can be fed various foodstuffs to supplement inadequate supplies of pollen or honey. In early spring, before pollen and nectar are available, or at other times of the year when these materials are in short supply, supplementary feeding may help the colony to survive or make it more populous and productive (Haydak, 1970).

A number of investigations have been related to physical and chemical composition of honey (Anupama et al., 2003; Azeredo et al., 2003; Mendes, Proenca, Ferreira, & Ferreira, 1998; Terrab, Diez, & Heredia, 2002; Üren, serifoğlu, & Sarikahya, 1998; Vorlova & Celechovskà, 2002).

The aim of this investigation was to determine the effects of supplementary feeding of honey bees with saccharose syrup and inverted saccharose syrup on typical quality parameters of honey.

2. Materials and methods

2.1. Bee feeding and honey production

The study was conducted on the bee colonies of Selcuk University, Taskent High College of Beekeeping Programme in Konya (Taskent), from May 10th, 2003–August 15th, 2003. The colonies, containing seven bee frames of 1-year-old mother-bees, were grown in the same region by the Doolithle larva transfer method. Langstroth types of hives were used in the study. Six colonies were determined and these colonies were divided into three groups.

I *Natural honey:* The first group of colonies was not supplementary-fed and was allowed an entirely natural feeding.

II *Saccharose syrup honey (SSH):* Every colony in the 2nd group were fed with 0.8 l of saccharose syrup (1/1 ratio of saccharose/water), once every 3 days, between May 10th and June 15th. Then this supplementary feeding was maintained, every other day, between June 15th and July 30th.

III *Inverted saccharose syrup honey (ISSH)*: The colonies of the third group were fed with 0.8 l of inverted syrup, once every three days, between May 10th and June 15th and every other day between June 15th and July 30th.

The inverted saccharose syrup, which was given to the colonies of 3rd group, was prepared from saccharose syrup by heating at 88 °C for 2 h. Then the syrup was cooled to 70 °C and 0.1% HCl solution was added to adjust the pH to 2.15. Then the acid inverted syrup was neutralized with Na_2CO_3. All groups of honeys were collected and stored in holders and immediately transferred to the laboratory and kept at 4–5 °C until analyzed.

2.2. Physicochemical analysis

pH was assessed by means of a potentiometric WTW 315 I Set Sentix 41 Electrode pH meter. Moisture in honey was determined with a refractometer reading at 20 °C and obtaining corresponding % moisture from table (AOAC, 1990). Ash percentage was measured by calcinations, overnight in a furnace at 550 °C, to constant mass (AOAC, 1990). Diastase activity was measured using a buffered solution of soluble starch and honey which was incubated in a special glass test tube. Results were expressed as ml of 1% of starch hydrolyzed by an enzyme in 1 g honey in 1 h (AOAC, 1995). Saccharose content was determined according to Codex Standard, 1981. Free acidity of aqueous 20% solutions was measured by neutralizing acidic honey components with a standard solution of sodium hydroxide (AOAC, 1995). Protein content was determined by the method of Bradford (1976). Viscosity measurements were carried out using a Lab-Line Viscometer Model No. 4535 and Spindle No. 7, at 30 °C at a shear rate of 5 rpm (Matsuda & Sabato, in press). Hydroxymethylfurfural (HMF) was determined after clarifying samples with Carrez reagents (I and II) and the addition of sodium bisulphate (AOAC, 1990). The absorbance was determined at 284 and 336 nm in a 1-cm quartz cuvette in a spectrophotometer (Milton Roy UV–Vis Spectronic 3000 Array). Mineral elements in honey samples were analyzed by ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectrometer) method. About 0.5 g sample was put into a burning cup and 15 ml of pure HNO_3 were added. The sample was incinerated in a Mars 5 Microwave Oven at 200 °C and the solution diluted to a certain volume with distilled water (Skujins, 1998).

Three replicate analyses were done from each sample to obtain the reported data.

2.3. Microbiological analysis

Moulds–yeasts, total bacteria and Coliform bacteria content were determined with potato dextrose agar, plate count agar and violette red bile agar (Merck), respectively. The results were calculated as cfu/g.

2.4. Sensorial evaluation

The sensorial evaluation was done by untrained panellists ($n = 35$). A 9-point hedonic scale was employed, ranging from 1 (most disliked) to 9 (most liked), for the parameters colour, odour, taste and consistency (ASTM, 1973; Carpenter, Lyon, & Hasdell, 2000; Matsuda & Sabato, in press).

2.5. Statistical analysis

The data from the experimental design were submitted to a statistical analysis, consisting of a *F*-test between treatments and Anova among all data (MINITAB 10.0).

3. Results and discussion

The quality parameters of honey samples are listed in Table 1 and, for the three groups of honey samples, the quality parameters were significantly affected ($p < 0.01$). Moisture contents of all samples were below 20%, the maximum value allowed by Turkish Food Codex, Honey Notification (2000) which means that the fermentation ability is low. Natural honey (15.36%) and ISSH (15.50%) had similar moisture contents while SSH (17.10%) had a higher moisture content. Sugars are dominating contents of the honeys' dry mass ranging from 80% (Maurizio, 1966) to 99% (White, 1976) and the high sucrose content of SSH may be an explanation of its high moisture content. Moisture content depends on the botanical origin of the sample, the degree of ripeness, processing techniques and storage conditions (Instituto Zooprofilattica Sperimentale Dell' Abruzzo E Del Molise, 1991). Also, moisture content is affected by climate, season and moisture content of original plant nectar. Generally, the moisture content of honey ranges from 13% to 25% (Estupinon & Sanjuan, 1998).

All of the honey samples' pH values fell within the normal ranges for pH. pH value of natural honey (pH 3.94) was lower than those of the other two groups of honey. pH is of great importance during honey extraction and storage, due to influence on texture, stability and presentation (Terrab et al., 2002).

Free acidity is highest (22.8 meq/kg) in natural honey. Then SSH (20.6 meq/kg) and ISSH (14.0 meq/kg) follow it. The lowest acidity shown by the ISS honey can be connected with more dissociated organic acids contained it (Popek, 2002). The values for the free acidity ranged from 10.3 to 102 meq/kg in a study of Terrab et al. (2002). The acidity of honey is due to the presence of organic acids, particularly gluconic acid, in equilibrium with their lactones or esters and inorganic ions, such as phosphate and chloride (Echingo & Takenaka, 1974).

The results obtained showed that natural honey is perfectly differentiated from the rest, due to its high protein content which is, on average, 1765 μg/g ($p < 0.01$). Protein content of honey gives an indication of its floral origin of them.

Ash contents of the honey samples were close to each other. Honey normally has a low ash content and this depends on the material collected by the bees during foraging. The higher ash content of ISSH (0.49%) may be related to the residues of acidic and alkaline solutions added to it. However, ash content in each of the honey groups was appropriate for the limit allowed in flower honeys, 0.6%, in Turkish Food Codex, Honey Notification (2000). Also, ash content of honey depends on the floral types used by bees (Abu Tarboush, Al-Kahtani, & Elsarrag, 1993).

Natural honey showed the highest diastase activity (10.9). ISSH had a diastase activity of 5.0 which is below this limit. The heat treatment for this honey may result in denaturation of enzymes naturally present in honey. Variation of enzyme activity, from honey to honey, has been shown to occur for a variety of reasons, including the amount of sucrose in food sources, rate of nectar flow and even age of the bees. The minimum standard value for diastase activity is eight, according to the Turkish Food Codex, Honey Notification (2000).

Table 1
Physicochemical parameters of honey samples[a]

Parameter	Honey type		
	Natural honey	Saccharose syrup honey (SSH)	Inverted saccharose syrup honey (ISSH)
Water content (%)	15.36 ± 0.343b	17.11 ± 0.277a	15.50 ± 0.141b
pH	3.94 ± 0.040b	4.04 ± 0.040ab	4.06 ± 0.035a
Free acidity (meq/kg)	22.8 ± 2.32a	20.6 ± 2.52b	14.021 ± 1.812c
Ash (%)	0.177 ± 0.003b	0.216 ± 0.012b	0.498 ± 0.006a
HMF (mg/kg)	1.75 ± 0.241b	1.347 ± 0.30b	28.22 ± 0.78a
Diastase activity (Gothe)	10.9 ± 0.90a	8.3 ± 0.82b	5.0 ± 0.73c
Sucrose (%)	0 ± 0c	7.9 ± 1.03a	4.75 ± 0.69b
Protein (μg/g)	1765 ± 81a	1014 ± 15b	964 ± 18c
Viscosity (cP)	25,200 ± 1600a	10,200 ± 1100c	16,600 ± 800b

Mean values followed by different letters in the same column are significantly different ($p < 0.01$).
[a] Mean ± SD.

Generally the sucrose content does not exceed 8% for authentic honey samples. The sucrose content of SSH (7.9%) was the closest one to this limit which can be related to the supplementary feeding of honey bees with sucrose syrup.

All of the samples showed an appropriate HMF content, below the allowable limit of 40 mg/kg, with the exception of honey coming from countries or regions with tropical ambient temperatures, where HMF content must not exceed 80 mg/kg (Turkish Food Codex, Honey Notification, 2000; 2001/110 EC). Table 1 shows the differences among samples in HMF content. ISSH showed the highest HMF content (28.2 mg/kg), while natural and SSH showed closer HMF values ($p < 0.01$). The high HMF content of ISSH may be attributed to the heating process of sucrose syrup to 88 °C for 2 h. Donner (1977) reported that honeys from different sources vary in their fructose/glucose ratio, HMF formation results from the acid-catalysed dehydration of hexose sugars with fructose being particularly susceptible to this reaction. Stability of sugar molecules explains the faster rate of HMF formation from fructose than from glucose because fructose enolizes rapidly (Isbell, Frush, Wade, & Hunter, 1969; Kuster, 1990; Lee & Nagy, 1990). The chemical properties of honey, such as pH, total acidity and mineral content, influence the formation of HMF (Anam & Dart, 1995; Bath & Singh, 1999; Hase, Suzuki, Odate, & Suzuki, 1973; Singh & Bath, 1997).

Natural honey exhibited the highest viscosity, 25,200 cP, and ISSH and SSH followed it with values of 16,600 and 10,200 cP, respectively. The lowest viscosity measured was in SSH. The low viscosity of SSH could be a result of its higher moisture content. Viscosity is one of the important properties of honey and depends on water and sugar quantities. Also temperature affects the viscosity of honey (Bhandari, D'arcy, & Chow, 1999).

The mineral contents of the separate groups of honey samples, with the means and standard deviationa are shown in Table 2. The differences were highly significant for all minerals ($p < 0.01$). Calcium which was an average, for 532 (μg/g), 436 (μg/g) and 459 (μg/g) in natural, SSH and ISSH honeys, respectively, was the most abundant element determined. In addition, all the assayed honeys also had high P, Na and Mg contents. The concentrations of minerals in natural honey were higher than the concentrations in SSH and ISSH honeys, except for Pb and Zn. Pb (0.715 μg/g) and Zn (7.43 μg/g) contents of SSH honey were higher than those of the others' (Table 2). The concentration of minerals found in our study were higher than those found by Üren et al. (1998), who determined the distribution of elements in Turkish honeys. Ca, Na, Fe and Mg contents of honeys determined in this study were high with respect to results of Nanda, Sarkar, Sharma, and Bawa (2003) and Conti (2000). The mean Zn level in natural honey (2.94 μg/g), SSH (7.43 μg/g) and ISSH (3.26 μg/g) were very similar to that reported by Al-Khalifa and Al-Arify (1999) while P, B, Al, Mn and Pb values were found to be higher. The difference in mineral content of natural honey can be attributed to the origin of food of honey bees.

The microbiological results are presented in Table 3. Generally, total bacteria counts were very low ranging from 1×10^3–8×10^3 cfu/g. Coliform bacteria was not detected, except in SSH with a population of 1×10^2 cfu/g. Natural honey and SSH had similar microbial populations.The SSH honey had the highest total bacteria and moulds–yeasts number. The higher

Table 2
Mineral contents of honey samples[a]

Minerals	Honey type		
	Natural honey (μg/g)	Saccharose syrup honey (SSH) (μg/g)	Inverted saccharose syrup honey (ISSH) (μg/g)
Al	13.7 ± 5.47a	9.01 ± 3.22b	9.94 ± 2.25b
B	30.5 ± 12.2a	23.5 ± 4.68b	25 ± 8.30ab
Ca	532 ± 228a	436 ± 61.0b	459 ± 173b
Cr	3.12 ± 1.56a	2.42 ± 1.16b	2.54 ± 0.204b
Fe	80.30 ± 34.7a	61.8 ± 9.65c	69.8 ± 27.5b
Li	3.70 ± 1.42a	3.04 ± 0.238b	3.18 ± 1.33b
Mg	212 ± 80.6a	183 ± 25.4b	184 ± 70.6b
Mn	0.848 ± 0.227a	0.536 ± 0.119b	0.583 ± 0.149b
Na	354 ± 97.4a	287 ± 71.6c	327 ± 109b
Ni	1.93 ± 0.529a	1.52 ± 0.226c	1.63 ± 0.585b
P	436 ± 53.2a	360 ± 140b	286 ± 94.8c
Pb	0.451 ± 0.110b	0.715 ± 0.573a	0.020 ± 0.625c
Se	0.957 ± 0.320	–	–
Sr	1.68 ± 0.743a	1.42 ± 0.267 c	1.52 ± 0.583b
Zn	2.94 ± 0.918b	7.43 ± 1.29 a	3.26 ± 0.007b

Mean values followed by different letters in the same column are significantly different ($p < 0.01$).
[a] Wet weight.

Table 3
Microbiological analyses of honey samples

Microorganisms (cfu/g × 10^3)	Honey type		
	Natural honey	Saccharose syrup honey (SSH)	Inverted saccharose syrup honey (ISSH)
Total bacteria	2	1	8
Moulds–Yeasts	3	35	3
Coliform bacteria	–[a]	0.1	–

[a] No growth.

Table 4
Sensorial evaluation results of honey samples[a]

	Colour	Odour	Taste	Consistency
Natural honey	6.57 ± 1.272a	6.57 ± 1.512a	6.86 ± 0.690a	7.14 ± 0.900a
SSH	5.86 ± 1,215b	5.43 ± 1.813b	6.57 ± 0.976ab	5.43 ± 1.27c
ISSH	4.57 ± 1.397c	5.14 ± 2.27b	6.43 ± 1.90b	6.14 ± 1.68b

Mean values followed by different letters in the same column are significantly different ($p < 0.05$).
[a] Mean ± SD.

moisture content of SSH may be the reason of its high moulds–yeast content. When moisture content of honey is >17.1, fermentation is dependent on the number of osmotic yeasts (>1000/g of honey for 17.1 < moisture < 18% and 10/g honey for 18.1 < moisture<19%) (Conti, 2000; Nicoletti, 1988).

The sensorial evaluation results are presented in Table 4. The colour of ISSH showed a lower mean compared to other types of honeys and natural honey had the highest mean for colour. The highest odour value was obtained for natural honey. Odour values of SSH and ISSH were found to be close to each other. The panellists evaluated the tastes of all honey types, with close values. More than 60% of the panellists graded tastes of all honeys with values higher than 6. The highest values of consistency were found for natural honey; SSH and ISSH followed. Natural honey presented scores for colour, odour and consistency characteristics significantly higher than were the other groups of honeys ($p < 0.05$).

4. Conclusions

It could be concluded, from the above results, that supplementary feeding of honey bees with inverted acid and heat-treated saccharose yielded a honey which had a higher HMF content and a lower diastase activity, moisture content and free acidity than had natural honey and SSH. Supplementary feeding of honey bees with saccharose syrup caused a higher saccharose content in honey. The mineral contents of these two groups of honeys were lower than those of the natural honey. However, free acidity was higher in natural honey. Colour and consistency were significantly affected ($p < 0.05$) in all the honey samples. The highest viscosity and colour values were for natural honey. The lowest colour and viscosity values were for ISSH and SSH, respectively. The taste of honey was not significantly affected by feeding inverted saccharose syrup.

Extensive research is required to determine the effects of different norms of acid and heat treatments, of the supplementary foods for honey bees, on honey quality.

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