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Physicochemical characteristics of honey from different origins

S.A. El Sohaimy [a,*], S.H.D. Masry [b], M.G. Shehata [a]

[a] *Food Technology Department, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Application, Alexandria, Egypt*

[b] *Department of Plant Protection and Molecular Diagnosis, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Application, Alexandria, Egypt*

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Abstract Honey is a natural sweet substance produced by honey bees, from the nectars of plant flowers and honey dew. The present study aimed to evaluate physicochemical characteristics and quality of honey from different origins. Melissopalynological analysis of honey samples showed a wide variability, with samples from different honey sources being collected from different geographical origins. The colour ranged from light amber for Egyptian and Yemeni samples to amber for Saudi and Kashmiri samples. Egyptian and Yemeni samples recorded the higher acidity than Saudi and Kashmiri honey, but all samples are still within the standard limit (pH 3.40 ± 0.002–6.10 ± 0.003). The electrical conductivity (EC) ranged from 0.53 ± 0.03 to 4.18 ± 0.05 ms/cm. The moisture content of honey samples was ranged from 14.73 ± 0.36% to 18.32 ± 0.67%. Ash content ranged from 0.23 ± 0.02% to 2.33 ± 0.02%. Kashmiri honey showed the highest protein content (4.67 ± 0.171 mg/g) while the lowest value of protein content was registered in Egyptian honey (1.69 ± 0.015 mg/g). Samples of Saudi honey showed the highest value of reducing sugars (72.36 ± 0.32 g/100 g), while Kashmiri honey showed the lowest value (15.11 ± 0.25 g/100 g). The estimated fructose/glucose ratio for all investigated samples was ranged from 0.42 ± 0.02 to 2.35 ± 0.02 and estimated glucose/water ratio was ranged from 0.72 ± 0.025 to 1.56 ± 0.025. It is noteworthy that, the crystallization of Kashmiri honey was faster than other types of studied honey samples. The quality of honey was varied based on the botanical origins, handling, transportation and storage conditions.

Introduction

Bee honey is the most well-known and economically important honey bee (*Apis mellifera*) colony product. It is defined as the natural sweet substance produced by honey bees, from the nectars of plant flowers and honey dew (Codex Alimentations,

* Corresponding author. Tel.: +20 34593420; fax: +20 34593423.
E-mail address: elsohaimys@gmail.com (S.A. El Sohaimy).

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2001). Properties and compositions of bee honey depend on its geographical floral origin, season, environmental factors and treatment of beekeepers (Da Costa Leite et al. (2000), Kaškonienė et al. (2010) and El-Metwally 2015). Bee honey is one of the few virtually totally non-allergic foods that body easily assimilates. It contains nutrients especially as energy provider Rahman et al. (2010), it is a high-energy carbohydrate food (80–85%) and the honey sugars are easily digestible as those in many fruits (White and Doner, 1980). Bogdanov et al. (2004) found more than 22 sugars in honey; however, fructose and glucose are the major sugar content. Primary sugars existed in honey are fructose and glucose, and in nectar honey the fructose content should exceed that of glucose Zafar et al. (2008). Furthermore, the sum of fructose, glucose, fructose/glucose ratio and glucose/water ratio are other important factors related to honey quality. Fructose/glucose ratio indicates the ability of honey to crystallize (White and Doner (1980), Manikis and Thrasivoulou (2001), Kaškonienė et al. (2010) and Buba et al. 2013). Honey contains more than 180 substances, including amino acids, enzymes, protein, vitamins, minerals, ash, organic acids and phenol compounds Ouchemoukh et al. (2007). Moisture content of bee honey represents a major importance to its stability against fermentation and granulation. The low moisture content protects honey from microbiological activity and thus it can be preserved for longer periods (AL-Naji and Hujazy, 1982; Cantarelli et al., 2008; Bogdanov, 2009; Buba et al., 2013; Akhtar et al., 2014 and El-Metwally, 2015). Melissopalynology is the most frequently used method for the determination of honey botanical and geographical origin (Vorwhol, 1981; Cotte et al., 2004 and Ponnuchamy et al., 2014). Melissopalynological analysis remains nowadays as the only technique, which allows a direct botanical origin characterization, while physicochemical parameters afford quantitative results and allow an approximate estimation of the presence of honey blends Soria et al. (2004). This study aimed to evaluate physicochemical characteristics of local and imported honey in Egypt to assess the different types of honey quality.

Materials and methods

Honey samples

Honey samples were collected from different markets in Alexandria, Egypt, representing Yemeni, Saudi and Kashmiri honey. While, one honey sample was collected from *Rhamnus* sp. (Sidr trees) farm at El-Nobareya city, El-Beheira governorate represented Egyptian honey sample. All samples were stored at (−28 ± 2 °C) till further analysis to avoid the effect of laboratory conditions on the chemical composition and physical properties of honey samples (El-Metwally, 2015).

Determination of sediment content

Based on the method of Louveaux et al. (1978) ten grams of honey was dissolved in 20 ml of warm distilled water (40 °C). The solution was centrifuged for 10 min at 2500*g*. The solution was poured into a small tube and centrifuged again for 10 min. The entire sediment was putted on a slid and spread out over an area about 20 × 20 mm, after drying by slight heating at 40 °C. The sediment was mounted with glycerine gelatine, liquefied by heating in water bath at 40 °C. Melissopalynology was used as a reference. However, terms used in estimates of pollen grain Frequencies are as follows: "Very frequent" for grains constituting more than 45%, "Frequent" for grains constituting 16–45%, "Rare" for grains constituting 3–15% and "Sporadic" for grains constituting less than 3% of the total grains Maurizio (1975).

Moisture content

Moisture content was determined from the refractive index of the honey. A digital refractometer (NR 101 Spain), that can be thermostated at 20 °C, regularly calibrated with distilled water or with another certified reference material (Bogdanov, 2009).

pH

A pH metre (HI 98127, Hanna instruments, Mauritius) was used to measure the pH of a 10% (w/v) solution of honey prepared in milli-Q water (Millipore Corporation, Billerica, Massachusetts, USA) Bogdanov, 2009.

Electrical conductivity (EC)

EC was measured using an HI 98311 conductivity meter (Hanna Instruments, Mauritius) and a 20% (w/v) solution of honey was suspended in milli-Q water Bogdanov et al. (1999). The electrical conductivity of the milli-Q water was determined to be less than 10 μS/cm.

Colour analysis

The colour intensity of honey samples was measured according to the Pfund classifier. Briefly, homogeneous honey samples devoid of air bubbles were transferred into a cuvette with a 10 mm light path until the cuvette was approximately half full. The cuvette was inserted into a colour photometer (HI 96785, Hanna Instruments, Cluj County, Romania). Colour grades were expressed in millimeter (mm) Pfund grades when compared to an analytical-grade glycerol standard. Measurements were performed in triplicate for each sample using the approved colour standards of the United States Department of Agriculture USDA (1985).

Colour intensity

The mean absorbance of honey samples was determined using the method of Beretta et al. (2005). Briefly, honey samples were diluted to 50% (w/v) with warm (45–50 °C) milli-Q water, and the resulting solution was filtered using a 0.45 μm filter to remove large particles. The absorbance was measured at 450 and 720 nm using a spectrophotometer (T80 UV/VIS England), and the difference in absorbance was expressed as mAU.

Optical density (OD)

One gram of honey was diluted with 9 ml of distilled water and centrifuged for 10 min at 3000*g*. The absorbance of the filtrate supernatant was measured at 530 nm against distilled water as

a blank using a spectrophotometer (T80 UV/ VIS England) Wakhle (1997).

Ash content

Ash content was determined according to the methods of (AOAC, 1999); 5 g of honey was placed in combustion pots, which required preheating to darkness with a gas flame to prevent honey foaming. Then, the samples were incinerated at high temperature (550 °C) in a burning muffle for 5 h. After cooling at room temperature, the obtained ash was weighed.

Total protein content

Total protein content was measured using the Kjeldahl method as described in (AOAC, 2005), based on the conversion of the organic nitrogen present in the sample to $(NH_4)_2SO_4$. Dried sample (1 g) was subjected to two processes: digestion and distillation. The sample was mixed with a selenium catalyst and H_2SO_4 (15 ml, 95–98%). The resulting solution was distilled after adding NaOH, and the distillate was collected in a flask with H_3BO_3 (4%) and mixed indicator. Finally, the mixture was titrated with HCl (0.1 N). The percentage of nitrogen quantified was transformed into protein content by multiplying by a conversion factor of 6.25.

Sugar analysis

Determination of sugars was performed with a Waters 2690 high-performance liquid chromatograph equipped with a differential refractive index (DRI) detector (Waters model 2414) (AOAC, 2000). The separation was performed using carbohydrate analysis column (3.9 × 300 mm) with a particle size diameter of 10 µm. The column was kept at 25 °C throughout the analysis. The mobile phase was composed of 80% acetonitrile in water. The injection volumes of the samples were 25 µl, with a flow rate of 2 ml/min. Comparing a retention times obtained by standards identified the sample peaks. The honey samples were also spiked with standards in order to verify the identity of the chromatographic peaks. Duplicate injections were performed and average peak areas were used for the peak quantification. Glucose, fructose, sucrose and maltose were used as standards to determine the sugar content of honey.

Statistical analysis

All analyses were carried out in triplicates and the data were presented as means ± standard deviations. Analysis of variance (ANOVA) was used to compare the quantified variables in the samples of honey. The significance was calculated for $P < 0.05$. The statistical analyses were performed with the SPSS Statistic.

Results and discussion

Melissopalynological analysis

Main pollen contributions for the studied honeys are listed according to their importance in (Table 1). Melissopalynological analysis of honey samples showed a wide variability between samples from different honey geographical origins. Sesame (*Sesamum indicum*) the frequent grain is the main source of nectar 41% followed by the buckthorn (*Rhamnus* sp.) 14% as rare grain for Egyptian honey. However, Yemen honey has the highest content of *Rhamnus* sp. 33% followed by the pollen of black cumin (*Nigella sativa*) (20%) and Eucalyptus (*Eucalyptus* spp.) 15% respectively, indicating the source of nectar. The main source of nectar at the Saudi honey was *S. indicum* and clover (*Trifolium* sp.) with the same percentage 12% followed by *Rhamnus* sp. 5%. For the Kashmiri honey the main nectar source was thymes (*Thymus* sp.) 28% followed by *Eucalyptus* spp. 11% but *Rhamnus* sp. and opium poppy (*Papaver* sp.) were found with only 5%. Furthermore, pollen of date palm (*Phoenix dactylifera*) was 61%, 31%, 20% and 5% for Saudi, Kashmiri, Egyptian and Yemeni honey samples respectively. These pollens were considered as pollen sources only. The result of pollen analysis indicated that the honey samples were rich in different pollen types but in low percentage. Moreover, Yemen honey is richer in pollen than the other honey samples. It could be also suggested that this type of honey was produced from different types of pollen and nectar plant sources. It could be also suggested that this type of honey was produced by pressing the honey combs (Louveaux et al., 1978 and Vorwhol, 1981). On the other hand, Saudi and Kashmiri honey contained sugar feeding; this might be affected by the physicochemical and granulation characteristics of these types of bee honey (El-Metwally, 2015). The Kashmiri honey collected from medicinal plants, *Thymus* sp., *Eucalyptus* spp. *Rhamnus* sp. and *Papaver* sp. indicated to the geographical origin of this honey. According to Melissopalynological analysis of honey samples, the examined honey samples were considered as natural bee honey, while, pollen analysis indicated that, Kashmiri and Saudi honey might be produced from bee colonies fed partially with sugar syrup Abd Alla et al. (2014).

Table 1 Main pollen types of honey samples.

Pollen type	Percentage (%) of pollen			
	Egyptian	Yemeni	Saudi	Kashmir
Sesamum indicum	41	–	12	–
Rhamnus sp.	14	33	5	5
Eucalyptus spp.	1	15	4	11
Trifoliumm sp.	3	3	12	1
Phoenix dactylifera	20	5	61	31
Nigella sativa	–	20	–	–
Fam.: Compositae	7	8	2	2
Fam.: Cucurbitaceae	4	4	1	2
Fam.: Cyperaceae	3	2	–	1
Casuarina sp.	3	1	–	–
Acasia sp.	2	1	–	2
Fam. Umbelliferae	1	–	3	–
Fam.: Chenopodiaceae	–	3	–	7
Medicago sativa	1	–	–	2
Salix sp.	–	5	–	–
Thymus sp.	–	–	–	28
Papaver sp.	–	–	–	5
Fam: Rosaceae	–	–	–	3

All results in table show the mean of triplicates ± SD, $P > 0.05$.

Colour and colour intensity

Table 2 presented the colour of examined samples, classified according to USDA-approved colour standards 1985. The colour of honey usually ranges from light yellow to amber, dark amber and black in extreme cases and sometimes even green or red hues Bogdanov et al. (2008). In studied honey samples, the colour ranged from light amber for Egyptian and Yemeni honey to Amber for Saudi and Kashmiri honey. The highest Pfund value was registered with Saudi honey (113.82 ± 2.19). On the other hand the lowest Pfund value was registered with Yemeni honey (56.40 ± 2.32). The Pfund values of Egyptian and Kashmiri honey were 73.88 ± 2.29 and 89.45 ± 1.17 respectively. There are no significant differences in colour remarked between all studied types of honey. Changes in colour might be attributed to beekeeper's interventions and different ways of handling the combs such as the use of old wax combs for producing honey, minerals content, contamination of heavy metals, and exposure to either high temperatures or light (El-Banby et al., 1989; Moniruzzaman et al., 2013 and El-Metwally, 2015). Colour classification of monofloral honeys is very important for commercial activities. The Pfund value of Saudi and Kashmiri honey is similar to Gelam and Manuka honeys, which were amber, with Pfund values of 122 and 110, respectively Moniruzzaman et al. (2013). Colour intensity of the honey is represented by the AB_{450}. In the present study, AB_{450} values ranged from 246 to 722 mAU (Table 2). The results showed that there is no significant difference between studied types of honey in colour intensity. Saudi honey, which showed the highest Pfund value, also showed the highest colour intensity (722.67 ± 1.53 mAU) followed by Kashmiri honey (658.67 ± 2.08) and Egyptian honey (414.00 ± 1.00 mAU), while Yemeni honey showed the lowest colour intensity (246.67 ± 1.53 mAU). Higher Pfund and colour intensity values indicate the higher content of phenolic compounds and flavonoids Moniruzzaman et al. (2013).

pH

The pH values of four honey samples were measured and the obtained results confirmed that, all tested samples were acidic (pH 4.114–4.637) (Table 2) and within the standard limit (pH 3.40–6.10) (Codex Alimentations, 2001) that insures honey samples' freshness. Among all honey types, Yemeni honey was the most acidic (pH 4.114 ± 0.02) followed by Egyptian (4.415 ± 0.09) and Saudi honey (4.460 ± 0.02). The lowest acidity was detected in Kashmiri honey (4.637 ± 0.03). Egyptian and Yemeni samples recorded the higher acidity than Saudi and Kashmiri honey. There was no significant difference recorded between the four studied types of honey concerning pH values ($P > 0.05$). The pH values of four tested types of honey samples were close to those previously reported in Indian, Algerian, Brazilian, Spanish and Turkish honeys (between pH 3.49 and 4.70) (Azeredo et al., 2003; Ouchemoukh et al., 2007; Kayacier and Karaman, 2008 and Saxena et al., 2010). The high acidity of honey correlates with the fermentation of sugars present in the honey into organic acid, which is responsible for two important characteristics of honey: flavour and stability against microbial spoilage Bogdanov et al. (2008). Furthermore, it might also indicate that the honey samples have high content of minerals (Mohammed and Babiker (2009) and El-Metwally, 2015).

Electrical conductivity (EC)

In the examined samples the Yemeni and Egyptian honey samples showed the highest EC (4.18 ± 0.05 and 1.98 ± 0.03 ms/cm) respectively (Table 2). On the other hand, Saudi and Kashmiri honey samples showed the lowest EC (0.53 ± 0.03 and 0.67 ± 0.07 ms/cm) respectively. EC is a good criterion of the botanical origin of honey and it is determined in routine honey control instead of the ash content (Adenekan et al., 2010). This measurement depends on the ash and acid content of honey; the higher ash and acid content, the higher the resulting conductivity. There is a linear relationship between the ash content and the EC but there was no significant difference between examined samples ($P < 0.05$). The Saudi and Kashmiri honey was within the standard limit (not more than 0.8 mS/cm) but the Egyptian and Yemeni samples are out of the standard limit (Codex Alimentations, 2001). Obtained results indicated that, the quality of Saudi and Kashmiri honey was better than Egyptian and Yemeni honey. A correlation coefficient was found between the ash content of honey and the EC ($P < 0.05$) (Table 4) Vorwhol (1984a,b).

Moisture content

In the present study, the moisture contents of the examined honey samples were 18.32 ± 0.67 g/100 g for Egyptian, 16.28 ± 0.22 g/100 g for Yemeni, 15.64 ± 0.30 g/100 g for Saudi and 14.73 ± 0.3 g/100 g for Kashmiri respectively (Table 3). Moisture content of honey is a limiting factor in determination of its quality, stability and spoilage resistance against yeast fermentation. The higher the moisture content is the higher probability of honey fermentation during storage. Lower moisture limits (<20%), elongates honey shelf life which would be met by a large majority of the commercial honeys, have been proposed by some countries for the revision of the (Codex Alimentations 2001). These results were accepted by the international regulations for honey quality

Table 2 Physical characteristics of honey.

Honey type	pH	EC (ms/cm)	Colour (Pfund)	Colour intensity AB_{450} (mAU)
Egyptian	4.415 ± 0.09	1.98 ± 0.03	73.88 ± 2.29	414.00 ± 1.00
Yemen	4.114 ± 0.02	4.18 ± 0.05	56.40 ± 2.32	246.67 ± 1.53
Saudi	4.460 ± 0.02	0.53 ± 0.03	113.82 ± 2.19	722.67 ± 1.53
Kashmir	4.637 ± 0.03	0.67 ± 0.07	89.54 ± 1.17	658.67 ± 2.08

All results in the table show the mean of triplicates ± SD, $P > 0.05$.

Table 3 Chemical characteristics of honey samples.

Honey samples	Moisture (g/100 g)	Ash (g/100 g)	Total protein mg/g
Egyptian	18.32 ± 0.67	1.07 ± 0.02	1.69 ± 0.015
Yemeni	16.28 ± 0.22	2.33 ± 0.02	2.64 ± 0.045
Saudi	15.64 ± 0.30	0.23 ± 0.02	2.42 ± 0.172
Kashmiri	14.73 ± 0.36	0.30 ± 0.03	4.67 ± 0.171

All results in table show the mean of triplicates ± SD, $P > 0.05$.

Table 4 Correlation coefficient between Ash and EC.

Corr (r)	S.E or r	P ($r = 0$)	n
0.99997926	0.00455461713	.0000***	4

*** Highly significant.

(Codex Alimentations 2001) and (Council Directive of the European Union, 2001). There were significant differences in the moisture content between the four types of honey especially between Egyptian and Kashmiri samples ($P < 0.05$). Generally, the moisture contents for Kashmiri honey recorded the lowest moisture content (14.73 ± 0.36 g/100 g) among tested samples, followed by Saudi (15.64 ± 0.30 g/100 g) and Yemeni (16.28 ± 0.22 g/100 g), while the Egyptian honey showed the highest moisture content (18.32 ± 0.67 g/100 g). The moisture content of honey samples is important as it contributes to its ability to resist fermentation and granulation during storage (Singh and Bath, 1997). Low moisture content also helps to promote longer shelf life during storage Terrab et al. (2003). However, moisture content depends on the temperature and relative humidity in the geographical origin during honey producing in honey colonies (Crane, 1979).

Ash content

Ash content is a quality criterion for botanical and geographical origin of honey. In the present study, Saudi and Kashmiri honey samples showed the lowest ash content (0.23 ± 0.02 and 0.30 ± 0.03 g/100 g) respectively. On the other hand Egyptian and Yemeni samples showed the highest values of ash content (1.07 ± 0.02 and 2.33 ± 0.02 g/100 g) respectively. There was no significant difference remarked between samples in ash content ($P > 0.05$). Ash content of all samples was within the acceptable range (0.6–1.2 g/100 g), except for Yemeni honey, which was not accepted by codex range (Codex Alimentations, 2001). These results referred to the rich content of pollen source surrounding the apiary yard during honey production. Furthermore, the results revealed that, the honey produced from colonies fed with sugar syrup was showed low ash content (Sahinler et al., 2004 and Buba et al., 2013).

Total protein

The protein content of honey samples ranged from 1.69 ± 0.015 mg/g of Egyptian honey to 4.67 ± 0.171 mg/g of Kashmiri honey (Table 3). However, there were significant differences between honey types concerning their protein content ($P > 0.05$). Kashmiri honey showed the highest protein content (4.67 ± 0.171 mg/g) followed by Yemeni (2.64 ± 0.045 mg/g) and Saudi (2.42 ± 0.172 mg/g), while the lowest value of protein content was registered in Egyptian honey (1.69 ± 0.015 mg/g). It is well known that honey contains a trace amount of protein usually originated from pollens which is a natural and protein-rich food source Schäfer et al. (2006), and some enzymes such as glucose oxidase invertase and diastase (Anklam, 1998; Subramanian et al., 2007). The variability in protein content of different types of honey might refer to the origin of honey and the type of pollens.

Sugars in honey

Figs. 1–5 illustrated HPLC chromatogram of the sugar analysis of honey samples in different concentrations. The results indicated that there were no significant differences between examined samples ($P > 0.05$) for fructose and glucose contents (Table 5). Fructose content of the examined honey samples was 50.78 ± 0.41, 43.30 ± 0.24, 38.76 ± 0.20 and 4.48 ± 0.31 g/100 g for Saudi, Egyptian, Yemeni and Kashmiri honey respectively. Furthermore, the Egyptian honey recorded the highest glucose content 26.54 ± 0.31 g/100 g, followed by Yemeni 25.45 ± 0.22, Saudi 21.58 ± 0.18 and Kashmiri 10.63 ± 0.32 g/100 g. The glucose content was lower than the fructose content which indicated the natural feeding of honey colonies in Saudi, Egyptian and Yemeni honey and confirmed the high quality of studied types of honey. These obtained results supported the previous several studies on different honey types (Buba et al., 2013; Manzoor et al., 2013 and EL-Metwally, 2015). Saudi honey showed the highest value of reducing sugars (72.36 ± 0.32 g/100 g), while Kashmiri honey showed the lowest value of reducing sugars (15.11 ± 0.25 g/100 g). Egyptian and Yemeni honey showed 69.84 ± 0.31 and 64.21 ± 0.18 g/100 g respectively (Table 5). Reducing sugars value of all samples was accepted by Codex Alimentations except Kashmiri honey showed the value lower than standard limit (15.11 ± 0.25) (Codex Alimentations, 2001). The obtained results clarified that fructose and glucose are the dominant sugars in honey samples (White and Doner, 1980), which although no limits have been fixed for their individual values, their sum (Fructose + glucose) have the values corresponding to the limits required of the international standard for honey established by Codex Alimentations Commission (not less than 60 g/100 g) (Codex Alimentations, 2001). Furthermore, sucrose content of honey samples listed in Table 5 showed high significant differences between examined samples ($P < 0.05$). Sucrose content was varied from 1.34 ± 0.19 to 3.59 ± 0.20 g/100 g. All honey samples were accepted by national and international regulations, which should be not more than 5 g/100 g (Codex Alimentations, 2001 and EOSC, 2005).

Fructose/glucose (F/G) ratio and glucose/water (G/W) ratio

Fructose/glucose ratio and glucose/water ratio were listed in Table 5. The F/G ratio for all investigated samples was 0.42 ± 0.02, 1.52 ± 0.04, 1.63 ± 0.05 and 2.35 ± 0.02 for Kashmiri, Yemeni, Egyptian and Saudi honey respectively. However, G/W ratio was 0.72 ± 0.025, 1.38 ± 0.025, 1.45 ± 0.025 and 1.56 ± 0.025 for Kashmiri, Saudi, Egyptian and Yemeni honey respectively. The concentration of fructose

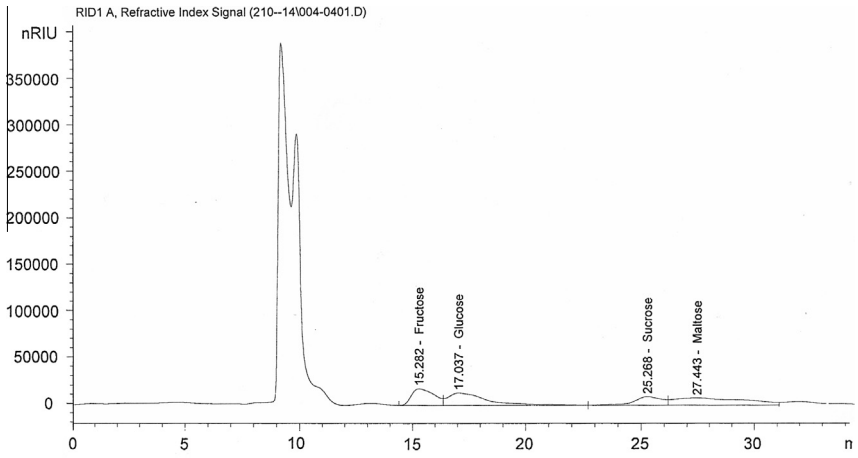

Fig. 1 Chromatogram of sugar standards.

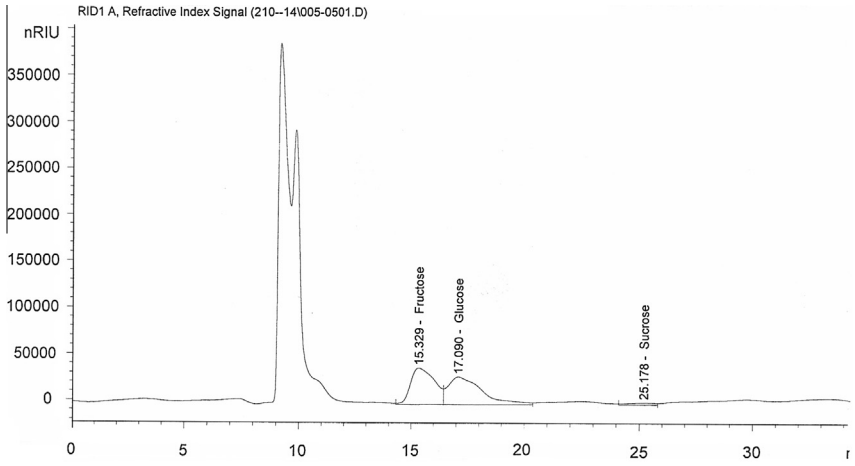

Fig. 2 Chromatogram of sugars of Egyptian honey.

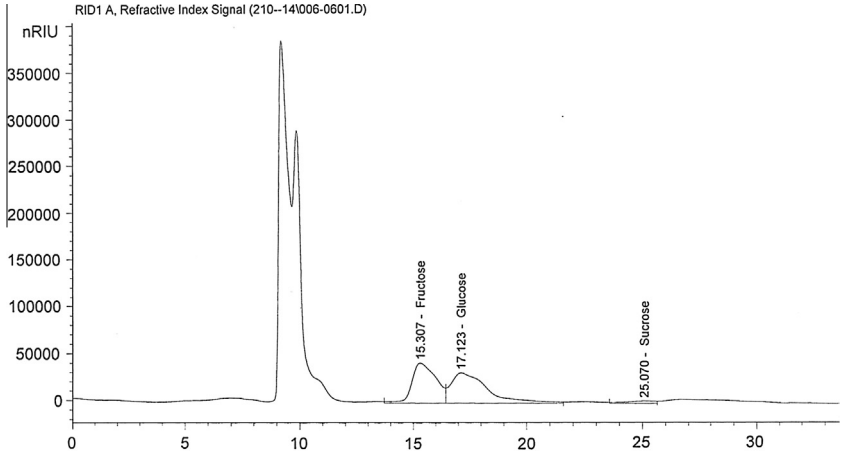

Fig. 3 Chromatogram of sugars of Yemeni honey.

and glucose as well as their ratio and G/W ratio is useful indicators for honey quality (Nour, 1988; Oddo and Piro, 2004; Soria et al., 2004 and Buba et al., 2013). F/G ratio indicates the ability of honey to crystallize, since the glucose is less soluble in water than fructose (Amir et al., 2010). Honey crystallization is faster when the F/G ratio is below 1.0 and it slows when this ratio is more than 1.0 (Draiaia et al., 2015). Accordingly, Kashmiri honey was crystallized faster than other types

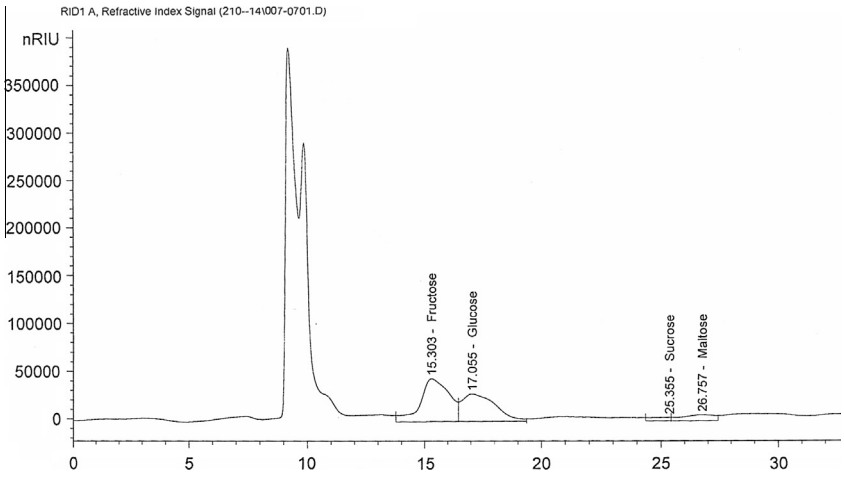

Fig. 4 Chromatogram of sugars of Saudi honey.

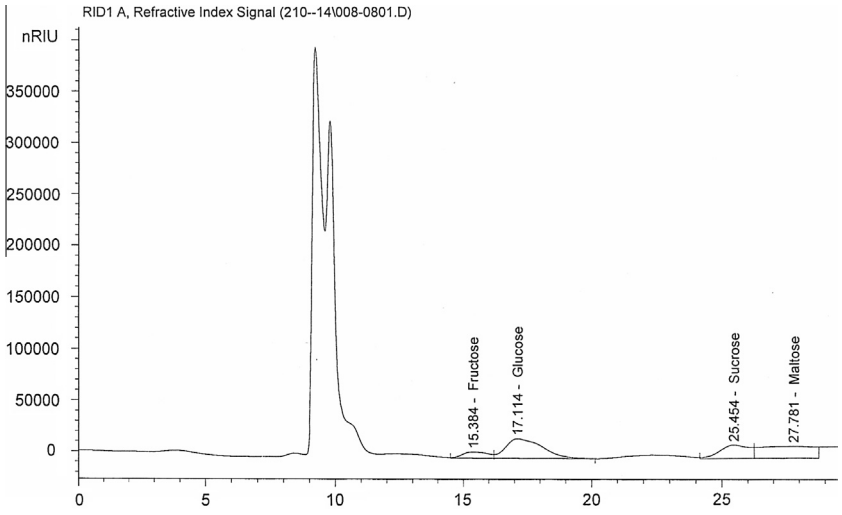

Fig. 5 Chromatogram of sugars of Kashmiri honey.

Table 5 Sugar analysis of honey samples.

Honey types	Glucose g/100 g	Fructose g/100 g	Estimated reducing sugars	Sucrose g/100 g	Estimated fructose/glucose ratio	Estimated glucose/water ratio
Eg	26.54 ± 0.31	43.30 ± 0.24	69.84 ± 0.31	3.31 ± 0.23	1.63 ± 0.05	1.45 ± 0.025
Yem	25.45 ± 0.22	38.76 ± 0.20	64.21 ± 0.18	3.43 ± 0.12	1.52 ± 0.04	1.56 ± 0.025
Sau	21.58 ± 0.18	50.78 ± 0.41	72.36 ± 0.32	3.59 ± 0.20	2.35 ± 0.02	1.38 ± 0.025
Kash	10.63 ± 0.32	4.48 ± 0.31	15.11 ± 0.25	1.34 ± 0.19	0.42 ± 0.02	0.72 ± 0.025

All values are represented as the mean of triplicates ± SD.

of honey and Saudi honey was the lowest. (White and Doner, 1980 and Crane, 1979) mentioned that in nearly all honey types, fructose predominates; a few honeys appeared to contain more glucose than fructose. Honey, which contains less glucose than fructose has ability to fluid (Ouchemoukh et al., 2007). Furthermore, honey crystallization depending on other factors such as other sugar contains (e.g. sucrose, maltose), insoluble substance (e.g. dextrin, colloids, pollen) and storage temperature that can influence the crystallization process (Buba et al., 2013 and EL-Metwally, 2015). The G/W ratio is considered an appropriate indicator than F/G ratio for the prediction of honey crystallization. The least ability of honey crystallization is obtained, when the glucose/water ratio is less than 1.0, while it is faster or completely crystallize when that ratio is more than 2.0 (Manikis and Thrasivoulou, 2001 and Amir et al., 2010). The results indicated that, Kashmiri honey has lowest ability to crystallize but the rest of honey types were moderate. Thus, moisture levels in honey play crucial role for

honey crystallization. According to Buba et al. (2013), fructose/glucose ratio and glucose/water ratio could be used to predict and control granulation tendencies in honey.

Conclusion

This study aimed to investigate and evaluate a physicochemical characterization of different honey samples from different origins to confirm its economical and nutritional quality. The result of pollen analysis indicated that, all investigated samples of honey were rich in pollen types but with low percentages. It could be also suggested that these types of honey were produced from different types of pollen and nectar plant sources. This might affect the physicochemical and granulation characteristics of the type of honey. There is no significant differences in colour remarked between all studied samples of honey. Changes in colour might be attributed to the beekeeper's interventions and different ways of handling the combs such as using of old honeycombs, contact with metals and exposure to either high temperatures or light. The higher Pfund and colour intensity values might indicate higher phenolic compounds and flavonoids. All investigated types of honey were acidic and were within the standard limit that indicates freshness of all investigated samples. All studied types of honey were within the standard limit of moisture content (<20%), which can elevate the honey ability to resist fermentation and granulation and promote longer shelf life during storage. Ash content of all samples was in acceptable range except Yemeni honey was out of codex range. There is a linear relationship between the ash content and the electrical conductivity (EC), while there were no significant differences between examined samples ($P < 0.05$). The Saudi and Kashmiri honey was in the standard limit but the Egyptian and Yemeni samples are out of the standard limit. A correlation coefficient was found between ash content of honey and EC ($r = 0.999$). Kashmiri honey showed the highest protein content followed by Yemeni and Saudi, while the lowest value of protein content was registered in Egyptian honey. It might be attributed to the type of pollen and bee feeding. The high sugar content of the investigated honey samples could be attributed to its high acidity and low moisture content, which inhibits the formation of Hydroxy Methyl Furfural (HMF) from sugars, especially glucose and fructose. Fructose/glucose ratio and glucose/water ratio might be used to predict and control granulation tendencies in honey. Finally the present study concludes that, the quality and physicochemical properties of honey were varied based on the geographical and botanical origins, handling, transportation and storage conditions.

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