Physico-chemical characteristics of some Indian and Yemeni Honey

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Highlights: This study is the first to deal with the physical and chemical composition of Yemeni honey and compare it with Indian honey in detail. It will provide researchers and those interested in food science very important data for these types of honey, especially Yemeni honey, which is world-famous and at the same time there is not enough data about it.

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ABSTRACT - The present study aimed to evaluate the physio-chemical properties of four Indian and Yemeni types of honey Coorg and Kashmiri honey (India), Sidr and Acacia honey (Yemen). The honey samples appeared to conform to the European Legislation (EC Directive 2001) for most of the parameters. The moisture content of the samples varied between 13.5 to 19.5g/100g, Indian honey were the highest moisture content. PH values ranged between 4.7 to 5.6, all tested samples were light acidic. Electrical conductivity ranged from 0.13 to 1.4mS /cm. The color intensity values ranged between 137.3 to 624.7mAU. HMF content fluctuated between 15.4 to 19.9 mg / kg. The ash content varied from 0.05 to 0.68%. The total protein content varied between 0.61 to 1.9 %. The diastase activity values ranged between 9.6 to 11.9 DN. The Kashmiri honey showed the highest value of reducing sugars (64.6 g/100g), while Acacia honey showed the lowest value (59.9 g/100 g) The estimated fructose/glucose ratio for all investigated samples was ranged from 1. 03 to 1.37 and estimated glucose/water ratio was ranged from 1.48 to 1.90. Potassium was found to be the predominant mineral in all honey tested. The highest Potassium content (2176.4 mg/kg) was found in Sidr Yemeni honey. The heavy metals were not detected in all honey samples, that the tested honey was safe for human consumption. The results suggest that Indian and Yemeni honey could be beneficially used as a functional or nutraceutical substance.

Keywords: Honey bee; Physicochemical characteristics; India; Yemen.

Journal of Bioenergy and Food Science. Vol.7: e2832019JBFS, 2020

1

INTRODUCTION

Honey is the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which honeybees collect, transform and combine with specific substances of their own, store and leave in the honeycomb to ripen and mature. This is the general definition of honey in the Codex Alimentarius (2001). The quality of honey, properties, and compositions of bee honey depend on its geographical floral origin, season, environmental factors and treatment of beekeepers (EL-Metwally, 2015). Bee honey is one of the nutritive nonallergic foods and energetic provider Rashman et al. (2010), that body easily assimilates Bogdanov et al. (2004). The important factors related to honey quality are the sum of fructose, glucose, fructose/glucose ratio and glucose/water ratio which indicates the ability of honey to crystallize (Buba et al., 2013). The physicochemical properties of the honey depend on some elements as moisture content (El-Metwally, 2015; El Sohaimy et al., 2015), melissopalynology (Ponnuchamy et al., 2014).

Investigate the Physico-chemical properties of honey provides significant information on the quality of honey (Soria et al., 2004). The primary sugars in honey are monosaccharide fructose and Glucose, during absorption the main carbohydrates fructose and glucose rapidly transport into the blood and can be used for the necessities of the human body. A daily dose of twenty grams of honey will provide about 3% of the required daily energy. Honey contains about 0.5% proteins, primarily compounds, and amino acids. Partly, its contribution to human protein intake (Bogdanove, 2016). The pH and water content, as well as glucose/water (G/W) ratio, are crucial parameters in honey. They can control in fermentation and granulation processes. The low pH and moisture content protect honey from the microbiological activity and thus it might be extended its shelf life (Buba et al., 2013; Akhtar et al., 2014; El-Metwally, 2015). There are many types of commercially available honey in markets but the consumer always tends to prefer one type of honey and without other types, this is the attribute to what it has of the physical, chemical as well as organoleptic characteristics (Al-Khalifa & Al-Arify, 1999). For this, the characterization of honey is necessary in order to better our response to consumer demands. Therefore, the present study aimed to evaluate the physicochemical characteristics of some Indian and Yemeni honey as well as to assess the different types of honey quality.

MATERIAL AND METHODS

a) Honey samples

Four samples of the Indian and Yemeni honey produced in various regions of Yemen and India were collected from local beekeepers in 2019. The samples were stored at 4–6°C until analyzed. All analyses were performed in triplicate. The regions from which the samples of honey were collected are indicated in Table 1.

Table 1. Honey samples from different regions of Indian and Yemen.

Honey code	Local name	location	Geographic origin
SDD	Sidr Dowany	Yemen	Dowan Valley, Hadhramout
ASH	Acacia Shabowah	Yemen	Gardan Valley, Shabowah
K	Kashmiri	India	Kashmir Valley, Kashmir
C	Coorg Honey	India	Bhaghmandala, Coorg Dist., Karnataka

b) Pollen analysis

According to the method of Louveaux et al., (1978) ten grams of honey was dissolved in 20 ml of warm distilled water (40 ºC). 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 put on a slid and spread out over an area about 20 × 20 mm, after drying by slight heating at 40 ºC. The sediment was mounted with glycerine gelatine, liquefied by heating in a 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

Journal of Bioenergy and Food Science. Vol.7: e2832019JBFS, 2020

2

grains constituting 16–45%, "Rare" for grains constituting 3–15% and "Sporadic" for grains constituting less than 3% of the total grains (Maurizio, 1975).

c) Moisture content

Moisture content was determined using an Atago HHR-2N refractometer. The samples were prepared according to the International Honey Commission guidelines (Bogdanov, 1997). Representative samples of each honey were transferred to sterile universal containers, sealed and incubated in a shaking water bath at 50º C for 30 minutes. After incubation, the samples were allowed to cool to 20º C in an airconditioned laboratory. Before testing the sample was thoroughly mixed. A drop of honey was placed on the lens of the refractometer, and the lid closed carefully to ensure an even spread of the sample with no air bubbles on the lens. Then the refractometer was held towards the light and the position of the interface was recorded. Between each sample, the refractometer was cleaned and dried.

d) pH

A pH meter (PH-200, MH Digital Inc., USA) was used to measure the pH of a 10% (w/v) solution of honey prepared in milli-Q water (Bogdanov et al., 1997).

e) Electrical conductivity (EC)

EC (mS/cm) was measured using a COM-100 conductivity meter (MH Digital Inc., USA) and a 20% (w/v) solution of honey was suspended in milli-Q water (Bogdanov et al.,1997).

f) Colour intensity: ABS $_{450}$

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 ºC) distilled 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 UV-Visible spectrophotometer (LMS-UV1900, Labman scientific instruments, India), and the difference in absorbance was expressed as mAU.

g) Hydroxymethylfurfural (HMF)

The HMF content in honey was determined using the White spectrophotometric method based on the determination of the difference between the absorbance at 284 and 336 nm of a honey solution and the same solution after the addition of sodium bisulfate using a UV-Visible spectrophotometer (LMS-UV1900, Labman scientific instruments, India). The results were expressed in mg/kg (Codex Alimentarius Commission, 2001).

h) Ash content

Ash content was determined according to the methods of (AOAC, 1999); five grams of honey were 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 ºC) in a burning muffle for 5 h. After cooling at room temperature, the obtained ash was weighed.

i) 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 with an 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.

Journal of Bioenergy and Food Science. Vol.7: e2832019JBFS, 2020

3

j) Diastase activity

Diastase activity was measured according to (Codex Alimentarius Commission, 2001) the method based on the rate of starch hydrolysis by diastase present in a honey buffer solution at 40ºC. The endpoint for this reaction was established by measuring the absorbance at 660 nm using a UV-Visible spectrophotometer (LMS-UV1900, Labman scientific instruments, India) until it was less than 0.235. The results were expressed in the diastase number (DN).

k) Sugar analysis

The determination of sugars was performed with an Agilent 1260 infinity high-performance liquid chromatograph equipped with a differential refractive index (RID) detector (AOAC, 2000). The separation was performed using a carbohydrate analysis column (250 × 4.6 mm) with a particle size diameter of 10 μm. The column was kept at 25 ºC throughout the analysis. The mobile phase was composed of 80% acetonitrile in water. The injection volumes of the samples were 20 μl, with a flow rate of 1.5 ml/min. Comparing the 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 tested honey samples.

l) Mineral analyses

Thirteen minerals (K, Mg, Ca, P, Na, Fe, S, Zn, Cu, Pb, Cr, Cd, and Ni) were determined in honey samples of known weight (3 replicates/honey type). An atomic absorption spectrophotometer (Model 3300, PerkinElmer Inc., USA) was used according to the method described by Chapman and Pratt (1961).

m) Statistical analysis

All analyses were conducted in triplicate and the data expressed as mean± standard deviation using the SPSS statistical package (IBM SPSS statistics 23 Inc., Chicago, IL.) Data were subjected to analysis of variance (ANOVA) followed by the Tukey test was used to evaluate the significance of difference ($P < 0.05$) between means.

RESULTS AND DISCUSSION

a) Pollen analysis

The percentages of pollen spectra are related to pollens of nectar-producing plants (Table 2). Pollen analysis of honey samples showed a wide variability between samples from different honey geographical origins of India and Yemen. Yemen's flora is species-rich. There are an estimated 3,000 species, by far the greatest diversity in the Arabian Peninsula. Due to diversity in flora and climate in Yemen, bee forage plants are widely spread in most areas of Yemen. There are more than 1,000 species of bee plants in Yemen, more than 75% of these grow wild. These can be subdivided into herbs, shrubs, and trees that provide nectar and pollen for foraging bees (Khanbash & Al-Madani, 2007). The Sidr (*Ziziphus sp.*) three is the main source of pollen 63% in SDD honey, while *Acacias sp*. 60 % was the main source of pollen in ASH honey. According to the results tested Yemeni honey can be considered as uni-floral honey. However, Indian honey (K and C) were more various sources of pollen grains than Yemeni honey. Coorg honey (C) has collected from a forest area in Coorg District, Karnataka, India, which serves as a food source for the bee during the whole year. However, the *Coffea sp.* (28%) was the predominant in this type of honey. The main source of pollen at the Kashmir honey was *Phoneix sp*. (25%), *Thymus sp*. (22%) and Brassica (15%). The tested Indian honey was considered as multi-floral honey. According to the Melissopalynological analysis of honey samples, the examined honey samples were considered as natural bee honey. Moreover, their tested honey was rich in pollen. They could be also suggested that this type of honey was produced from different types of pollen and nectar plant sources. They could be also suggested that these types of honey were produced by centrifuging the honeycombs (El Sohaimy et al., 2015).

Journal of Bioenergy and Food Science. Vol.7: e2832019JBFS, 2020

4

The Kashmiri honey (K) collected from medicinal plants, such as *Thymus sp.*, indicated to the geographical origin of this honey (Ara et al., 2019).

Table 2. Main pollen types of honey samples.

Pollen type	Percentage (%) of pollen in honey samples*			
	SDD	ASH	K	C
Acacia sp.	2	60	3	-
Anogesisus sp.	6	9	-	-
Brassica sp.	-	-	15	-
Cassia sp.	-	3	-	-
Coffeea sp.	-	-	-	28
Cocos sp.	-	-	-	8
Eucalyptus sp.	7	1	-	8
Helianthus sp.	-	-	5	7
Nigella sp.	10	2	-	-
Malus sp.		-	12	-
Peganum sp.	-	5	-	-
Phoenix sp.	12	6	25	-
Prosopis sp.	-	5	-	-
Prunus sp.		-	10	-
Schlecichera sp.	-	-	-	9
Sesamum sp.	-	2	-	-
Sorghum sp.	-	4	6	12
Syzygium sp.	-	-	-	15
Terminelia sp.	-	-	-	13
Thymus sp.	-	-	22	-
Trifulium sp.	-	-	2	-
Ziziphus sp.	63	3	-	-

* SDD: Sidr Dowany honey, Yemen. ASH: Acacia Shabowah honey. Yemen, K: Kashmiri honey, India. C: Coorg honey, India

b) Moisture content

The average of moisture contents of the tested honey samples was 13.5± 0.25 g/100g for SDD honey, 16.2± 0.35 g/100g for ASH honey, 19.1± 0.21 g/100g for K honey and 19.5± 0.20 g/100g for C honey respectively and the differences were significant ($P < 0.05$). The moisture content of all analyzed samples was within the range of 13.5–19.5% recommended by Codex Alimentarius (<20%) as shown in (Table 3). Chirife et al. (2006) stated that moisture content was affected by climate, season, and moisture content of original plant nectar and was considered unripened at the moisture content higher than 20%. However, it is prominent to state that the highest moisture content value was found in Indian (K and C) honey and the differences were not significant among the Indian honey ($P > 0.05$). These results were accepted by the international regulations for honey quality (Codex Alimentations, 2001) and (Council Directive of the European Union, 2001). The moisture content of honey samples is important as it contributes to its ability to resist fermentation and granulation during storage (Singh & 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.

c) pH

The pH values of honey samples were measured, and the obtained results confirmed that, all tested samples were light acidic (pH 4.7 – 5.6) (Table 3) and within the standard limit (pH 3.40–6.10) (Codex Alimentations, 2001) that ensures honey samples' freshness. There was a significant difference recorded between the four studied types of honey concerning pH values ($P < 0.05$). The SDD hone was least acidic (5.6± 0.15) while the ASH was the highest acidic (4.7± 0.16). The average

Journal of Bioenergy and Food Science. Vol.7: e2832019JBFS, 2020

5

pH of Indian (K and C) honey were 4.9± 0.07 and 5.0± 0.01 respectively. pH values were in agreement with the results of Algerian, Egyptian, Saudi, Brazilian, Spanish and Turkish honey (Azeredo et al., 2003; Ouchemoukh et al., 2007; Ozcan & Olmez, 2014; El Sohaimy et al., 2015). The high acidity of honey relates to the fermentation of sugars present in the honey into organic acid, a product of glucose oxidation by glucose oxidase which is responsible for two important characteristics of honey: flavour and stability against microbial spoilage (Bogdanov et al., 2013). Moreover, it might also indicate that honey has a high content of minerals (El-Metwally, 2015).

d) Electrical conductivity (EC)

The values for electrical conductivity found in the four samples of honey ranged from 0.13± 2.01 to 1.4± 1.7mS /cm (Table 3). The largest EC was found in Yemeni honey (SDD and ASH) 1.4± 1.70 and 1.0± 2.95 respectively. While the lowest value was found in Indian honey (K and C) 0.31± 0.97 and 0.13± 2.01 respectively. The differences between means were significant ($P < 0.05$). The Indian honey was within the standard limit (not more than 0.8 mS/cm) but the Yemeni honey was out of the standard limit (Codex Alimentations, 2001). The electrical conductivity of honey is associated with the ash content and acidity, revealing the presence of ions, organic acids and proteins (Da Silva et al., 2016). Thus, this parameter has been used as a honey quality indicator, assisting in the identification and distinction of floral honey. Similar values are reported by Alqarni et al. (2014) and El Sohaimy et al. (2015), ranging from 0.21 to 4.18 mS/ cm, in the honey bee from different origins.

e) Colour intensity AB_{450} (mAU)

Colour is an important characteristic of honey and varied from region to region. It is the physical property that is immediately perceived by the consumer. The color intensities (ABS_{450}) of testing honey samples ranged between 137.3 and 624.7 mAU (Table 3). The results showed that there were significant differences ($P < 0.05$) between studied types of honey in colour intensity. Sidr Yemeni honey (SDD), which showed the highest colour intensity (624. 7 ± 0.32 mAU) followed by Kashmiri honey (K) 485.3± 0.00 mAU and Acacia Shabowah honey (ASH) 405.0 ±0.01 mAU, while Coorg Indian honey showed the lowest colour intensity (137.3 ±0.26 mAU). Higher colour intensity values indicate the higher content of phenolic compounds and flavonoids (Moniruzzaman et al., 2013). Changes in colour might be accredited to the beekeeper's interference and different habits 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 (Moniruzzaman et al., 2013; El-Metwally, 2015).

Table 3. Moisture, pH, Electrical conductivity, Colour intensity of Indian and Yemeni honey*.

Honey code**	Moisture g/100g	pH	EC (ms/cm)	Colour AB_{450} (mAU)
SDD	13.5± 0.25c	5.6± 0.15a	1.4± 1.70a	624.7± 0.32a
ASH	16.2± 0.35b	4.7± 0.16cd	1.0± 2.95b	405.0± 0.01c
K	19.1± 0.21a	4.9± 0.07bc	0.31± 0.97c	485.3± 0.00b
C	19.5± 0.20a	5.0± 0.01b	0.13± 2.01d	137.3± 0.26d

* Results are reported as a means± standard deviation. Means in the same column with different letters are significantly different at $P < 0.05$.
** SDD: Sidr Dowany honey, Yemen. ASH: Acacia Shabowah honey, Yemen. K: Kashmiri honey, India. C: Coorg honey, India

f) Hydroxymethylfurfural (HMF)

Hydroxymethylfurfural (HMF) HMF is, indisputably, an excellent indicator of honey freshness and purity. High concentrations of HMF in honey indicate overheating or poor storage conditions. According to the International Trade Guidelines (European Economic Committee, 2001), honey's HMF content should not exceed 40 mg/kg. In this study, the HMF of the four examined Indian and Yemeni honey ranged from 15.4 to 19.9 mg/kg. The highest HMF was observed in Coorg (C) honey 19.9± 0.28 mg/kg followed by Acacia Shabowah (ASH) honey 16.7± 0.31 mg/kg, while the Sidr Dowaney (SDD) honey and Kashmiri (K) honey were 15.6± 0.42 mg/kg and 15.4± 0.21 mg/kg receptively. There were significant differences ($P < 0.05$) between the means (Table 4). All the tested honey samples had an HMF value lower than the above limit, and none showed values higher than

Journal of Bioenergy and Food Science. Vol.7: e2832019JBFS, 2020

6

40 mg/kg. Hence, the honey samples herein examined were to be deemed as fresh honey. It has been demonstrated that the HMF parameter is related to the honey's quality and its heat processing but not to its origin (Anklam, 1998). There are many factors influence the formation of HMF in honey, such as processing methods and storage temperature (Meda et al., 2005; Fallico et al., 2004). In addition to these facts, the HMF level in honey also depends on the sugar type present in honey itself like fructose: glucose ratio (Doner, 1979). Similarly, it is well known that honey heating results in the formation of HMF, which is produced during acid-catalyzed dehydration of hexoses, such as fructose and glucose (Fallico et al., 2004; Tosi et al., 2008).

g) Ash content

The ash content in honey constitutes a quality parameter reflecting its richness in minerals that is determined by the botanical geographical origin. In the present study, Yemeni (SDD and ASH) honey showed the highest values of ash content (0.68± 0.08 % and 0.51± 0.02%) respectively. On the other hand, Indian (K and C) honey showed the lowest of ash content (0.12± 0.12% and 0.05± 0.01 %) respectively (Table 4). There was no significant difference remarked between Indian honey samples in ash content (P >0.05). On the contrary, there was a significant difference remarked between Yemeni honey samples in ash content (P <0.05). Ash content of Yemeni honey was within the acceptable range (0.6 –1.2 %), while Indian honey samples, which were not accepted by codex range (Codex Alimentations, 2001). These results referred to the rich content of the pollen source surrounding the apiary yard during honey production. Similar findings were made by Abu- Tarboush et al., (1993) who found the highest ash contents in Sidr honey. A similar range of ash content for honey samples from different origins of 0.20–2.33% was reported by El Sohaimy et al. (2015).

h) Total protein content

The total protein content for examined Indian and Yemen honey ranged from 0.61 -1.9 % (Table 4). The Acacia Shabowah (ASH) honey showed the highest total protein content (1.9± 0.08 %) followed by Sidr Yemeni (SDD) honey (1.5± 0.07 %). However, there were significant differences between Yemeni honey types concerning their protein content (P <0.05). The Indian (K and C) honey showed the lowest of total protein content (0.75± 0.12% and 0.61± 0.04%) respectively. There were no significant differences among Indian honey (P > 0.05). This variation may be attributed to the type of flora. It is well known that honey contains a trace amount of protein that usually originated from pollens which are a natural and protein-rich food source and some enzymes such as glucose oxidase, invertase, and diastase (Anklam, 1998). The variability in protein content of different types of honey might refer to the origin of the honey and the type of pollens. The high protein content of honey could be an indication of high pollen content (Nazarian et al., 2010) which indicates natural good-quality honey.

i) Diastase activity

Diastase activity is a honey quality parameter used to determine if honey has been extensively heated during processing. The diastase activity of the tested samples ranged from 9.6± 0.25 DN of Indian (C) honey to 11.9± 0.60 DN of Sidr Yemeni (SDD) honey (Table 4). There were significant differences (P < 0.05) between means. All the samples showed the values within the Codex Standard (>8 DN) which indicated that all the samples were unprocessed and properly stored. Our results were consistent with the reporting of (Aazza et al., 2013).

Table 4. Ash, HMF, Total protein, Diastase activity of Indian and Yemeni honey *.

Honey code**	HMF (mg/kg)	Ash (%)	Protein (%)	Diastase activity (DN)
SDD	15.6± 0.42bc	0.68± 0.08a	1.5± 0.07b	11.9± 0.60a
ASH	16.7± 0.31b	0.51± 0.02b	1.9± 0.08a	10.5± 1.12ab
K	15.4± 0.21c	0.12± 0.12c	0.75± 0.12c	11.2± 0.15ab
C	19.9± 0.28a	0.05± 0.01c	0.61± 0.04c	9.6± 0.25b

* Results are reported as a means± standard deviation. Means in the same column with different letters are significantly different at P < 0.05.
** SDD: Sidr Dowany honey, Yemen. ASH: Acacia Shabowah honey, Yemen. K: Kashmiri honey, India. C: Coorg honey, India

Journal of Bioenergy and Food Science. Vol.7: e2832019JBFS, 2020

7

j) Sugar analysis

Sugars are the main components of honey which depend mostly on floral and geographical origins and less on seasonal, processing and storage conditions. Sugar composition has been used to discriminate honey samples on the basis of floral as well as geographical origin (Gomez-Barez et al., 2000). The results of sugar analysis of the honey samples are presented in (Table 5). The figs. 1–5 illustrated HPLC chromatograms of the sugar analysis of honey samples in different concentrations. The results indicated that there were significant differences between examined honey samples (P <0.05) for fructose and glucose contents. The fructose content of the examined honey samples was 35.4 ± 0.20, 32.5± 0.58, 36.3 ±0.21 and 31.2 ±0.35 g/100 g for Sidr Yemeni (SDD), Acacia Shabowah (ASH), Kashmiri (K), Coorg (C) honey respectively. Furthermore, the Coorg (C) honey recorded the highest glucose content 30.3 ±0.68 g/100 g, followed by Kashmiri (K) 28.3 ± 0.20, Acacia Shabowah (ASH) 27.4 ±0.56 and Sidr Yemeni (SDD)25.7± 0.60 g/100 g. The glucose content was lower than the fructose content which indicated the natural feeding of honey colonies in Indian and Yemeni honey. These values are similar to the values as recorded earlier by (Buba et al., 2013; Manzoor et al., 2013; El Sohaimy et al., 2015). The values of reducing sugars were 61.1 ± 0.85, 59.9± 0.11, 64.6 ±0.73 and 61.5 ±0.76 g/100 g for Sidr Yemeni (SDD), Acacia Shabowah (ASH), Kashmiri (K), Coorg (C) honey respectively (Table 5). All the honey presented a value of glucose plus fructose higher than 60 g/100 g, which is the value, required for all the kinds of honey in the European and Codex standards (Codex Alimentations, 2001).

The content of reducing sugars might vary due to the storage factor, enzyme activity and acid reversion in honey. Besides that, the time of harvest for honey also affects the total amount of reducing sugars in honey. For honey which harvested in the flowering season, the total amount of reducing sugars is expected to be higher. The obtained results clarified that fructose and glucose are the dominant sugars in honey samples (White & Doner, 1980). The sucrose contents of the honey samples studied were in the range of 3.5 to 10.2 g/100 g (Table 5), there were significant differences between examined honey samples (P <0.05). The values obtained for sucrose contents of the Sidr Yemeni (SDD) honey and Kashmiri (K) honey 4.9± 0.26 and 3.5± 0.15 g/100g respectively, were within the limits of international standards that is the international norm established by the Codex Alimentarius Commission requirement that a good quality honey should not contain more than 5 g/100 g sucrose. While the values of sucrose content of Acacia Shabowah (ASH) and Coorg (C) honey 10.2± 0.45 and 6.5± 0.20g/100g respectively, were higher than the standard limit (Codex Alimentations 2001). According to Doner (1977) even though honey contains active sucrose splitting enzymes (sucrase, glucosidase), the sucrose level in honey never reaches zero. The reasons for this high sucrose may due to the variability of the maturity of honey, increase the relative humidity, or collect of nectar from flowers with high sugar content or that the honey was produced in a hot and dry atmosphere or that the bees were overfed sugar solutions during the period of honey overflow the bees could not handle them.

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

The values of Fructose/Glucose ratio and glucose/water ratio were listed in (Table 5). The Fructose/glucose (F/G) ratio for all examined honey samples was 1.37 ± 0.23, 1.17± 0.03, 1.28 ±0.37 and 1.03 ±0.02 for Sidr Yemeni (SDD), Acacia Shabowah (ASH), Kashmiri (K), Coorg (C) honey respectively. However, the glucose/water (G/W) ratio was 1.90 ± 0.12, 1.70± 0.34, 1.48 ±0.05 and 1.55 ±0.09 for Sidr Yemeni (SDD), Acacia Shabowah (ASH), Kashmiri (K), Coorg (C) honey respectively. There were significant differences (P < 0.05) among examined honey samples. The concentration of fructose and glucose, as well as their ratio and G/W ratio, is useful indicators for honey quality (Oddo & Piro, 2004). Fructose/Glucose (F/G) ratio indicates the ability of honey to crystallize. The honey remains liquid when its F/G ratio is high, and vice versa. Moreover, honey crystallization is slower when F/G ratio exceeds 1.3, and it is faster when the ratio is below 1. However, F/G ratio-based crystallization remained not clearly demonstrated, because honey contains other sugars (sucrose, maltose, etc.) and insoluble substances (dextrin, colloids, etc.) able to influence the crystallization process (Amir et al., 2010). According to our results, all honey samples showed F/G ratios higher than 1. Hence, the chance of crystallization less. The Glucose / Water (G/W) ratio is more appropriate than the F/G for honey crystallization prediction. Honey crystallization is slow or null when G/W ratio is less than 1.3, and it is complete and rapid when the ratio is greater

Journal of Bioenergy and Food Science. Vol.7: e2832019JBFS, 2020

8

than 2 (Manikis & Thrasivoulou, 2001; Amir et al., 2010). The results indicated that Indian honey has the lowest ability to crystallize compared with Yemeni honey types. Thus, moisture levels in honey play a crucial role in 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. In addition to F/G and G/E ratios, several factors including dust, pollen grains, agitation, and air bubbles could influence also crystallization process. During honey aging, there is the loss of dextrose and levulose and the significant increase in reducing disaccharides (such as maltose) resulting in F/G ratio change (Amir et al., 2010).

**Table 5.** Sugar analysis of Indian and Yemeni honey samples *

Honey Code**	Fructose g/100g	Glucose g/100g	Estimate reducing sugars	Estimated fructose/glucose ratio	Estimated glucose/ water ratio	Sucrose g/100g
SDD	35.4± 0.20a	25.7± 0.60c	61.1± 0.85b	1.37± 0.23a	1.90± 0.12a	4.9± 0.26c
ASH	32.5± 0.58b	27.4± 0.56b	59.9± 0.11b	1.17± 0.03c	1.70± 0.34a	10.2±0.45a
K	36.3± 0.21a	28.3± 0.20b	64.6± 0.73a	1.28± 0.37b	1.48± 0.05b	3.5± 0.15d
C	31.2± 0.35c	30.3± 0.68a	61.5± 0.76b	1.03± 0.02d	1.55± 0.09b	6.5± 0.20b

* Results are reported as a means± standard deviation. Means in the same column with different letters are significantly different at P < 0.05.
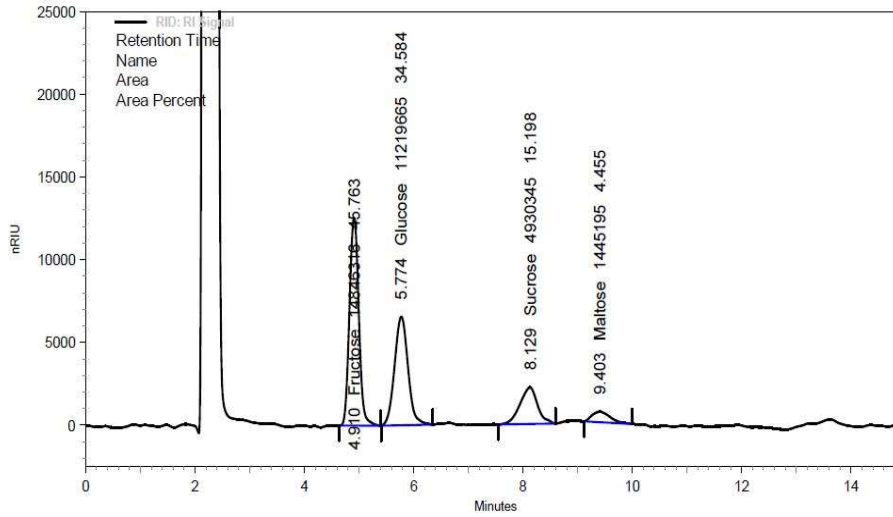** SDD: Sidr Dowany honey, Yemen. ASH: Acacia Shabowah honey, Yemen. K: Kashmiri honey, India. C: Coorg honey, India.
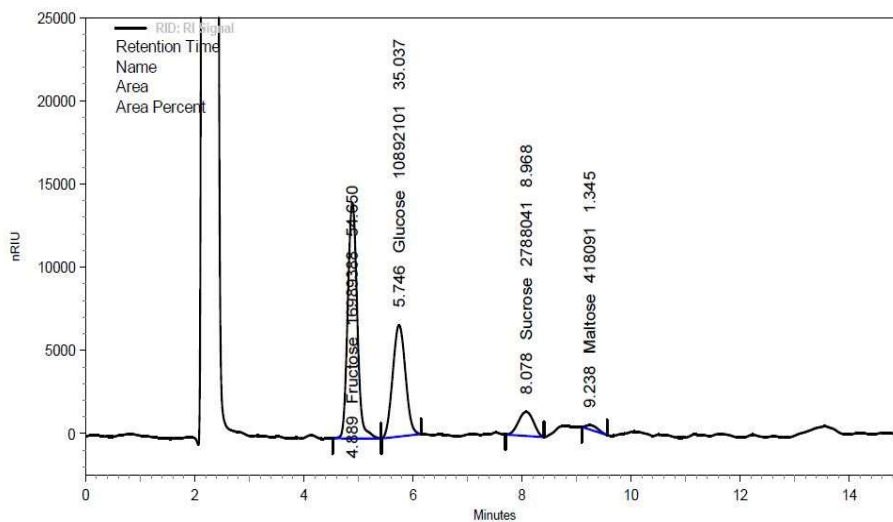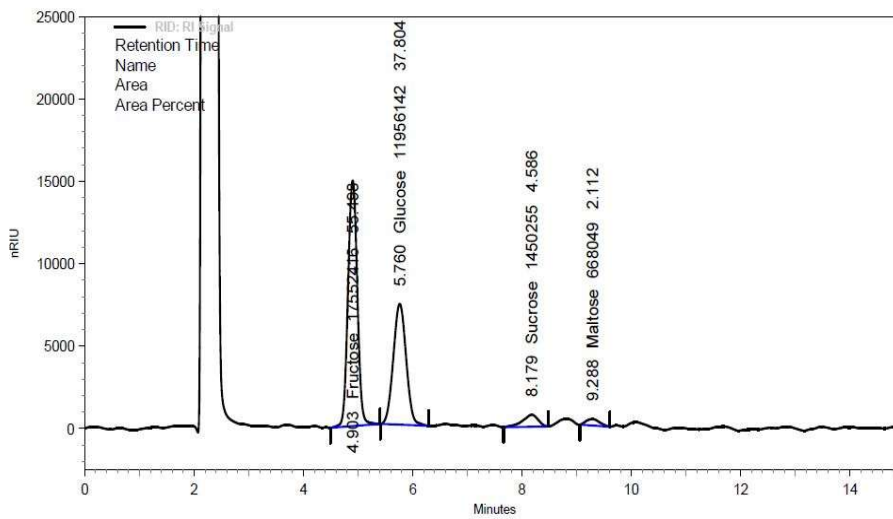

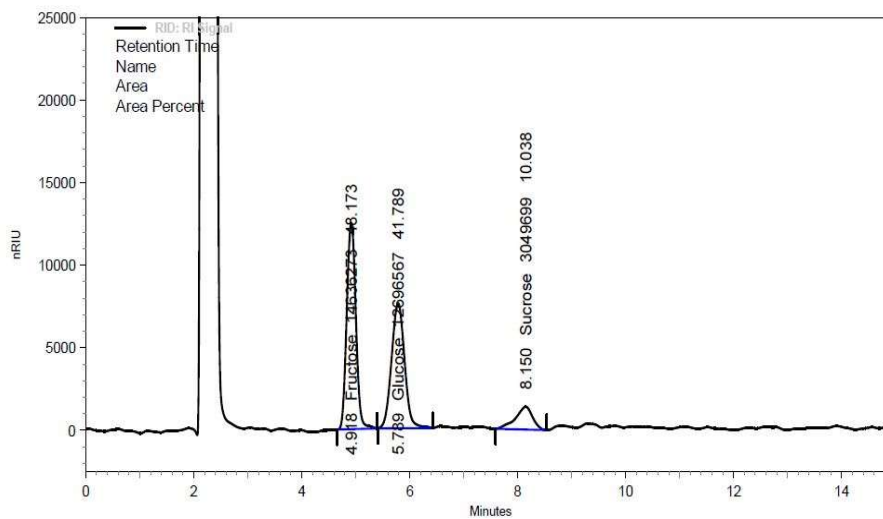**Figure 1.** Chromatogram of sugars standards
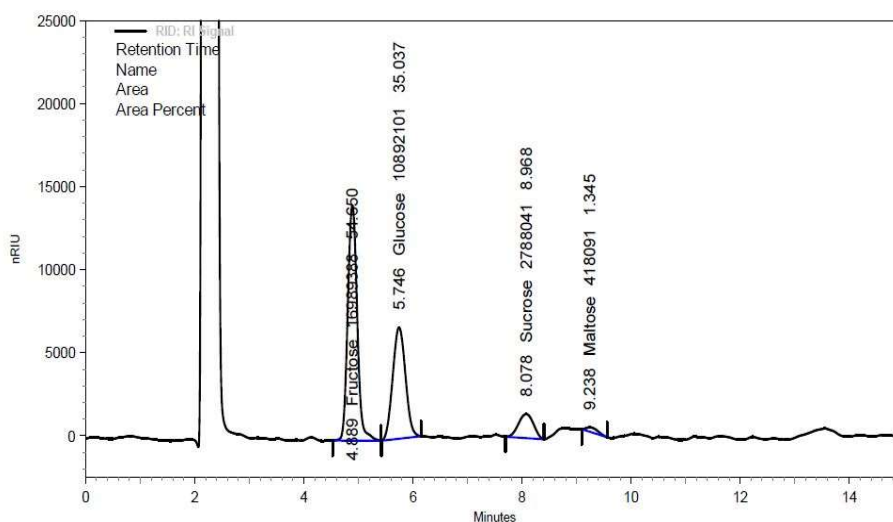
Figure 2 Chromatogram of sugars of (SDD) honey

Journal of Bioenergy and Food Science. Vol.7: e2832019JBFS, 2020

9

Figure 3 Chromatogram of sugars of (ASH) honey

Figure 4 Chromatogram of sugars of (K) honey

Figure 5 Chromatogram of sugars of (C) honey

Journal of Bioenergy and Food Science. Vol.7: e2832019JBFS, 2020

10

l) *Mineral analysis*

The minerals identified in the tested Indian and Yemeni honey are listed in Table 6. These minerals, in the descending order of quantity, were as follows: potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), phosphorous (P), iron (Fe), Sulphur (S) and zinc (Zn). Generally, Yemeni honey was richer with mineral content than Indian honey. Potassium was found to be the predominant mineral in all honey tested. The highest K content (2176.4± 6.57 mg/kg) was found in SDD (Yemeni Sidr honey), and the lowest (123.0±0.98 mg/kg) was found in C (Indian Coorg honey), with a significant difference between all the honey samples ($P < 0.05$). The second most prevalent mineral was Mg, its content was 418±1.94, 371.8±1.70, 223.5±1.96 and 91.3± 1.35 mg/kg for Sidr Yemeni (SDD), Acacia Shabowah (ASH), Kashmiri (K), Coorg (C) honey respectively. There were significant differences ($P < 0.05$) among examined honey samples. The calcium content was ranged between 353.1± 2.25 mg/kg and 77.4± 0.8 mg/kg in SDD and C honey respectively, with a significant difference ($P<0.05$). Sodium content was lower than K, Mg, or Ca content and ranged between 259.3± 4.1 mg/kg and 57.6± 0.94 mg/k in SDD and C honey respectively, with a significant difference ($P<0.05$).

The P values were ranged between 155.0±1.31mg/kg and 36.4±0.76 mg/kg in SDD and C honey respectively. The Fe content was lower than Na and P content and ranged between 114.7± 1.91 mg/kg in Sidr (SDD) Yemeni honey and 14.3± 0.77 mg/k in K (Kashmiri honey), with a significant difference ($P < 0.05$). The Sulphur content was moderate with a significant difference ($P < 0.05$) between the values ranging from 76.0 ± 2.37 mg/kg in Sidr (SDD) Yemeni honey to 14.0± 0.31 mg/kg in Coorg (C) honey. The lowest mineral content in the examined honey was found for Zn and ranged between 6.30 mg/kg in C honey and 2.70 mg/kg ASH honey, with a significant difference ($P < 0.05$) between the two values. The data showed that Coorg (C) honey had the lowest values for K, Mg, Ca, Na, P, and S. These results are in total agreement with the previous studies (Alqarni et al., 2014; Boussaid et al., 2018). On the other hand, The Copper, Lead, Chromium, Cadmium, and Nickel were not detected in all honey samples, that the tested honey was safe for human consumption. The results showed that honey can contribute greatly to the recommended daily intake (RDI) of nutritional minerals (Bogdanov et al., 2013).

Table 6. Mineral analysis of Indian and Yemeni honey samples mg/kg*

Minerals	Honey code**			
	SSD	ASH	K	C
K	2,176.4± 6.57a	1,597.2± 4.89b	414.9± 1.76c	123.0± 0.98d
Mg	418.2±1.94a	371.8± 1.70b	223.5± 1.96c	91.3± 1.35d
Ca	353.1± 2.25a	255.4± 1.90b	174.6± 0.96c	77.4± 0.80d
Na	259.3± 4.10a	208.5± 2.02b	61.5± 1.13c	57.6± 0.94c
P	155.0± 1.31a	121.2± 0.95b	152.5± 1.13a	36.4± 0.76c
S	76.0± 2.30a	54.8± 2.51b	16.1± 0.45c	14.0± 0.31c
Fe	114.7± 1.91a	83.8± 1.55b	14.3± 0.77c	29.2± 0.35d
Zn	5.2± 1.70ab	2.7± 0.52b	2.9± 0.45b	6.3± 0.72a
Cu	Nd	Nd	Nd	Nd
Pb	Nd	Nd	Nd	Nd
Cr	Nd	Nd	Nd	Nd
Cd	Nd	Nd	Nd	Nd
Ni	Nd	Nd	Nd	Nd

* Results are reported as a means± standard deviation. Means in the same line with different letters are significantly different at $P < 0.05$.
** SDD: Sidr Dowany honey, Yemen. ASH: Acacia Shabowah honey, Yemen. K: Kashmiri honey, India. C: Coorg honey, India.
Nd: Not detected

Journal of Bioenergy and Food Science. Vol.7: e2832019JBFS, 2020

11

CONCLUSION

This is the first study on the physicochemical of Yemeni honey. The results of the physicochemical analysis of all the honey varieties were within the limits recommended by the European Commission and the Codex Alimentarius. Some samples did not agree with characteristics established in European and Codex standards relative to the sucrose contact and electrical conductivity, although the other physicochemical parameters were within the range of the allowable limits. The result of pollen analysis indicated the Indian honey were multi-floral honey while Yemeni honey was uni-floral honey. The Yemeni honey was rich with ash content. Indian honey was the highest moisture content compared with Yemeni honey. The carbohydrate profile of studied honey revealed that all the unique honey varieties possessed reducing sugars, mainly fructose and glucose in the largest portion. The Indian and Yemeni honey was rich with minerals, principally potassium, magnesium, and calcium. We recommended that intake honey as food and medicine resulting in high nutritional benefits and therapeutic promise.

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CONTRIBUTIONS of AUTHORS

In this study, both authors contributed effectively. Mohammed Ali designed and achieved experiments and wrote the paper; M. Jayashankar supervised the study and analyzed the data and performed data interpretation.

COMPETING INTERESTS

The authors have declared that no competing interests exist

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12

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13

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15