Differentiation of Omani Acacia and white Acacia honey by botanical and physicochemical analysis

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Abstract

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Introduction. Consumer in the Middle East market can be confused with two types of Acacia honeys i.e., ‘true Acacia’ honey from *Acacia tortilis* and ‘White Acacia’ honey from *Robinia pseudoacacia*.

Materials and Methods. In this study, we evaluated honey samples to characterize and differentiated true Acacia from Robinia honey using botanical and physicochemical characteristics. Acetolysed pollen from honey was used to determine pollen concentration to establish the purity and floral origin.

Results and Discussion. Acacia honey showed mean pH 4.83, moisture content 16.75%, EC 1.82 mS/cm, free acidity 95.30 meq/kg, diastase activity 12.00 DN and invertase activity 141.07 U/kg which are on par with the permitted specifications except EC and free acidity, being relatively higher. Robinia honey samples have pH 3.80, moisture content 17.41%, EC 0.33 mS/cm, free acidity 8.03 meq/kg, diastase activity 8.00 DN and invertase activity 17.53 U/kg. These values meet the standards. The EC of Acacia honey correlated with pH, moisture content, free acidity, diastase and invertase activities indicating richness in minerals and organic acids. All parameters were relatively higher in Acacia than in Robinia honey.

Conclusion. We recommend that honey be labelled with specific names. We suggest the name “Acacia” for *Acacia* spp. honeys and “Robinia” for *Robinia pseudacacia* honey.

Abbreviations

AOAC – Association of Analytical Communities,
CAC – Codex Alimentarius Commission,
DN – Diastase number,
EC – Electrical conductivity,
EUC – European Union Council,
GSO – Gulf Cooperation Council Standardization Organization,
ICBB – International Commission for Bee Botany,
IHC – International Honey Commission,
mS/cm – milliSiemens per cm,
meq/Kg – Milliequivalents per liter,
U/Kg – Units/kg.

1. Introduction

Oman in Eastern Arabian Peninsula has many agro-climatic regions and diverse flora that provides potential for different types of honeys. Omani honey is well known for its characteristic aroma, taste and texture and has been awarded a gold medal at National honey show in London in 2012 (National honey show, 2012). In Middle East countries, the most popular unifloral honeys are *Acacia* (*Sumr*), *Ziziphus* (*Sidir*) and *Prosopis* (*Ghaf*) honeys (Sajwani et al., 2007). In northern Oman, true *Acacia* honey is produced in summer by bees from *Acacia tortilis* (Forsskal) Hayne., which is the most common wild tree distributed throughout the country (Sajwani et al., 2007 and Sajwani et al., 2014). *Acacia* honey is very famous in this region due to its nutritional and medicinal properties. It is characterized by strong aroma and flavor; has a thick texture and does not crystallize. The color ranges from light amber to dark amber with moderate amount of sugar (Sajwani et al., 2007 and Sajwani et al., 2014). White *Acacia* honey is produced from the nectar of *Robinia pseudoacacia*, which is a typical European honey and studied well (Persano Oddo and Piro, 2004). The consumer can be confused with the name *Acacia* and White *Acacia*, so the present study was carried out to evaluate and characterize *Acacia* honey using melissopalynological and physicochemical analysis. Physicochemical parameters have been considered as quality criteria for the characterization of honey types (Lazarević, et al., 2012) that are strictly regulated by legislative standards by IHC, CAC and AOAC (Persano Oddo and Bogdanov, 2004). There are several reports on the use of physical and chemical parameters to characterize honey from different countries (Juszczak, et al., 2009; Khalil, et al., 2010; Saxena, et al., 2010). However, the physicochemical characterization of honey from Arabian countries is not comprehensively determined and more so little information is available on the physicochemical properties of Omani *Acacia* honey. This work aimed to establish values for authentic Omani *Acacia* honey and to determine whether it meets national and international standards of honey and to differentiate it from European *Robinia* honey.

2. Materials and methods

2.1. Honey samples collection

Eighty samples of *Acacia* honey (Ac1 - Ac80) were collected during summer season from nine governorates of Oman (Figure 1). The honeys from *Apis florea* and traditional (*tubl*) combs were collected by pressing them manually, while the honeys from *A. mellifera* frame hives were extracted by centrifugation. Authenticated pure honey samples were obtained directly from honey cells to ensure that the sample was not mixed with pollen stores in the comb. It was made sure that beekeepers did not feed the bees with any external supplement of pollen pellets and the honey samples were not heated and filtered. Fourteen *Robinia* honey samples (Rb1 – Rb14) were purchased from local markets in Muscat that were imported from different European countries and China. All honey samples were subjected to quantitative and qualitative pollen analysis to determine their floral origin.

Figure 1. The location of sites (marked with red dots) of sample collection of *Acacia* honey in Sultanate of Oman:

1. Muscat, 2. Al Batinah South, 3. Al Batinah North, 4. Ash Sharqiyah South, 5. Ash Sharqiyah North, 6. Ad Dakhiliyah, 7. Ad Dahirah, 8. Al Buraimi, 9. Musndam, 10. Al Wusta, 11. Dhofar

2.2. Melissopalynological studies

The polyfloral character of honey samples was confirmed according to melissopalynological methods reported by Erdtman, (1960); Nair, (1970); Louveaux et al. (1978) and Jones and Bryant, (2004).

2.2.1. Quantitative analysis. Pollen counts were made from slides prepared from the honey samples following the method recommended by ICBB (Louveaux et al., 1978). For determining the honey type, 300-500 pollen were counted from each sample. Honey with predominant (> 45%) pollen type classified as unifloral honey, while honey with several pollen types in considerable number is classified as multifloral honey. Since Poaceae and Chenopodiaceae pollen are airborne, their numbers were subtracted from the total number of pollen grains prior to calculating the frequency of pollen from nectar-producing plants.

2.2.2. Qualitative analysis: Pollen Concentration Count. The pollen concentration count in a honey can form a basis for determining the floral source, as well as assessing the purity of the product. Addition of a known number of exotic pollen or spores to a known volume of honey sample is often used in determination of the pollen concentration (Maurizio, 1975). In this study, *Lycopodium clavatum* spores (Figure 2A) were used as common markers in absolute pollen analysis. One tablet of *L. clavatum* spores (Lund University, Batch 1031, containing $20,848 \pm 1546$ spores) was dissolved in 5 ml of 5% hydrochloric acid and added to each 10 g of honey sample following Stockmarr, (1971). Then each sample was processed using acetolysis (Erdtman, 1960). Counts of 200-300 pollen grains including anemophilous plant pollen were made for all samples unless 1,000 *L. clavatum* spores were encountered first. These spores were also counted but their number was kept separate from the pollen count. Pollen concentration values per 10 g of p-nitrophenyl- α -D-glucopyranoside of honey sample were calculated by computing the ratio of marker spores to counted pollen grains using the following formula:

$$\text{Pollen concentration} = \frac{(\text{Number of pollen grains counted} \times \text{Number of } Lycopodium \text{ spores added})}{\text{Number of } Lycopodium \text{ spores counted}}$$
 (Maurizio, 1975).

Samples were classified into groups according to the pollen grain content of 10 grams of honey following the scheme of Maurizio, (1975); (Group I: < 20 000; Group II: 20 000–100 000; Group III: 100 000–500 000; Group IV: 500 000–1 000 000; Group V: >1 000 000).

2.3. Physicochemical analysis

Standard physicochemical analytical methods were used for honey classification which are validated and harmonized by the IHC (Bogdanov, 2002) and are within the scope of the CAC honey standards (Codex Alimentarius Commission, 2001), EUC (European Commission, 2002) and GSO (2008). Analysis of each sample was carried out in triplicate for each test.

The pH was measured by a pH meter (Mettler Toledo, USA) in a solution containing 10 g honey in 75 mL of freshly boiled warm, distilled water as per the method 962.19 of AOAC (1990). Moisture content was determined following the method of Codex Alimentarius Commission (2001) using a refractometer (Atago 3840 PAL-22S) and the percentage of moisture was measured at room temperature ($25 \pm 1^\circ\text{C}$). Electrical conductivity (EC) of each honey solution with 20% dry matter was determined by a conductivity meter (Accumet Ap85) following protocol of the IHC (Bogdanov, 2002). Free acidity was determined using the titrimetric method of AOAC (1990). The honey solution in distilled

water was titrated to an end point of (pH 8.5) with an alkali solution. The results are expressed in milliequivalents acid per 1000 g (meq/kg).

Diastase activity was determined photometrically by Phadebas method (Bogdanov, 1984). The absorbance of the solution was read at 620 nm. Results of diastase activity were expressed as a diastase number (DN) in Gothe or Schade units per gram of honey (Serrano, Espejo, Villarejo, and Jodral, 2007). To measure the invertase activity Siegenthaler's (1977) method was used. It is based on the spectrophotometric measurement of p-nitrophenol at 400 nm, which is formed by the reaction of honey invertase with p-nitrophenyl- α -D-glucopyranoside (pNPG), used as a substrate. Results were expressed as units/kg (U/kg).

2.4. Statistical analysis

Statistical analysis for calculating the means and the standard deviation of the mean were performed using Microsoft Excel software. All results are expressed as the Mean $\pm$ Standard Deviation (SD). Furthermore, SPSS 22.0 was used for the evaluation of correlation matrix, one-way ANOVA and t-test, as well as the post hoc Tukey's HSD, at the $P < 0.05$. For representation in the tables, the data of 10 samples each is pooled in this publication.

3. Results and discussion

3. 1. Melissopalynological studies: the floral origin of the honey

Among Omani *Acacia* honey samples, the pollen (Figure 2B) percentage ranged from 45.06% in Ac4 to 96.32% in Ac74 (Table 1). These honey samples were dark amber colored, which is typical of unifloral *Acacia* honey. All the *Robinia* honey samples were unifloral with predominant ($> 20\%$) *Robinia* pollen (Figure 2C). These honeys were light amber colored as usually seen in typical unifloral *Robinia* honey. The pollen percentages in the *Robinia* honeys ranged from 20.41% in Rb2 to 61.44.32% in Rb11 (Table 2).

Figure 2. Light micrographs of some significant pollen types and spore recorded in the present study:

(A) *Lycopodium clavatum* (12 μ m); (B) *Acacia tortilis* (15 μ m); (C) *Robinia pseudoacacia* (10 μ m).

Table 1

Pollen and Physicochemical properties of Omani *Acacia* honey samples

No. Of Samples	Pollen (%) Mean	Pollen Conc. Mean	pH Mean±SD	Moisture content (%) Mean±SD
10 (Ac1-Ac10)	58.9	33084.1	4.69±0.07	17.27±0.23
10 (Ac11-Ac20)	56.5	39239.8	4.72±0.08	16.24±0.23
10 (Ac21-Ac30)	61.1	99039.9	4.83±0.04	16.39±0.05
10 (Ac31-Ac40)	69.6	134011.0	4.81±0.03	16.99±0.20
10 (Ac41-Ac50)	64.2	116404.7	4.74±0.03	16.75±0.13
10 (Ac51-Ac60)	67.6	39415.4	4.97±0.04	16.59±0.06
10 (Ac61-Ac70)	67.2	43640.9	5.02±0.03	16.85±0.12
10 (Ac71-Ac80)	67.6	70878.0	4.89±0.05	16.94±0.16
Average of 80 samples	64.1	71964.2	4.83±0.05	16.75±0.15
Min. of 80 samples	45.1	2690.1	4.11±0.04	14.70±0.09
Max. of 80 samples	96.3	499640.1	5.34±0.03	19.20±0.13

Continue of Table 1

No. Of Samples	EC (mS/cm) Mean±SD	Free acidity (meq/kg) Mean±SD	Diastase activity Mean±SD	Invertase activity (U/kg) Mean±SD
10 (Ac1-Ac10)	1.78±0.03	116.96±2.22	14.87±0.33	148.24±0.26
10 (Ac11-Ac20)	1.71±0.02	89.71±4.86	12.31±0.29	144.17±0.49
10 (Ac21-Ac30)	1.86±0.01	88.05±1.27	15.45±0.33	170.20±4.44
10 (Ac31-Ac40)	1.82±0.01	94.94±1.08	9.13±0.26	97.12±2.43
10 (Ac41-Ac50)	1.58±0.02	83.27±0.74	9.30±0.23	107.80±2.48
10 (Ac51-Ac60)	1.83±0.02	88.74±1.43	13.80±0.27	139.81±1.98
10 (Ac61-Ac70)	1.90±0.01	93.01±0.84	10.10±0.29	172.96±2.74
10 (Ac71-Ac80)	2.11±0.07	107.72±0.34	10.05±0.21	148.27±1.37
Average of 80 samples	1.82±0.02	95.30±1.60	12.00±0.28	141.07±2.02
Min. of 80 samples	0.54±0.04	29.46±0.26	3.39±0.09	39.95±0.40
Max. of 80 samples	2.81±0.38	141.36±0.49	30.13±0.93	312.37±0.73

Table 2

Pollen and physicochemical properties of *Robinia* honey samples

No. of Samples	Pollen (%) Mean	Pollen Conc. Mean	pH Mean±SD	Moisture content (%) Mean±SD
3 (Rb1-Rb7)	34.68	40041.60	3.84±0.02	17.63±0.19
3 (Rb8-Rb10)	32.69	24303.67	3.68±0.02	16.97±0.05
3 (Rb11-Rb13)	40.96	21457.82	3.71±0.01	17.33±0.05
3 (Rb14-Rb16)	52.36	29559.13	3.90±0.04	17.60±0.00
2 (Rb17-Rb18)	36.96	23887.25	3.90±0.02	17.60±0.00
Average of 14 samples	39.7121	28132.9	3.80±0.02	17.41±0.06
Min. of 14 samples	20.41	4586.56	3.32±0.00	16.40±0.00
Max. of 14 samples	61.44	50214.5	4.05±0.07	18.25±0.21

Continue of Table 2

No. of Samples	EC (mS/cm) Mean±SD	Free acidity (meq/kg) Mean±SD	Diastrase activity Mean±SD	Invertase activity (U/kg) Mean±SD
3 (Rb1-Rb7)	0.34±0.01	5.91±0.17	8±0.09	23.05±0.67
3 (Rb8-Rb10)	0.29±0.00	7.94±0.17	8±0.08	28.43±0.45
3 (Rb11-Rb13)	0.29±0.00	8.15±0.02	8±0.12	10.73±0.34
3 (Rb14-Rb16)	0.35±0.01	8.09±0.12	10±0.16	13.54±0.87
2 (Rb17-Rb18)	0.40±0.04	11.05±0.16	6±0.12	9.06±0.56
Average of 14 samples	0.33±0.01	8.03±0.12	8±0.11	17.53±0.58
Min. of 14 samples	0.11±0.00	4.75±0.21	5±0.13	3.26±0.24
Max. of 14 samples	0.48±0.09	15.20±0.31	12±0.17	55.26±0.40

3.1.1. Qualitative pollen analysis. Pollen concentration values in 80 *Acacia* honey samples ranged from 2690.06 in sample Ac5 to 499640.00 in sample Ac26 (Table 1). Fourteen of them (17.50%) classified in group I (< 20 000 grains) (Figure 3). These samples may have been pressure-filtered, derived from floral sources that produced little pollen or partly produced by sugar-feeding of bees following the method of Maurizio (1975). Fifty one honey samples (63.75%) were categorized in group II (20,000-100,000 grains). In this respect these samples were regarded as "normal" because they were produced from normal floral sources (Crane, 1975). Fifteen samples (18.75%) were classified in group III (100,000-500,000 grains), indicating that floral sources are high pollen producers, or indicating that combs containing pure pollen were included in the extraction process. Honeys of group IV and V were not present in the samples. These are the ones with high pollen concentrations between 500,000 and 1,000,000 grains/10g, or higher respectively. The absence of such groups in the current study may be due to the authenticated pure honey samples, which were obtained directly from honey cells to ensure that the sample was not mixed with pollen stores in the comb (Maurizio, 1975).

Pollen concentration values in 14 *Robinia* honey samples ranged from 4586.56 in sample Rb8 to 50214.54 in sample Rb3 (Table 2). Three samples (21.43%) classified in group I (<20,000 grains). Most *Robinia* honey samples (11 samples, 78.57%) fall within the "normal" category (20,000 to 100,000 pollen grains in a standard 10 g sample) (Figure 3). No samples were categorized in group III, V and VI. Pollen concentration values were significantly ($p > 0.05$) greater in *Acacia* honey than in *Robinia*. In nature *R. pseudoacacia* produces low number of pollen, therefore the frequency of 20% - 30% of its pollen in honey sample are adequate to classify it as unifloral *Robinia* honey (Louveaux et al., 1978; Von der Ohe, et al., 2004).

Figure 3. Distribution of samples (%) for pollen concentration in honey according to Maurizio's classes:
Group I (<20,000 pollen grains);
Group II (20,000-100,000 grains);
Group III (100,000-500,000 grains per 10 g honey)

3.2. Physicochemical properties

One of the main objectives of this study was to differentiate between Omani *Acacia* honey ($n = 80$) and *Robinia* ($n = 14$) honey according to their physicochemical characteristics which are the major honey quality indicator parameters that include pH, moisture content, EC, free acidity, diastase activity and invertase activity.

3.2.1. pH. All *Acacia* and *Robinia* honeys analyzed were found to be acidic irrespective of their diverse geographical origin. The mean pH of *Acacia* honeys was 4.83 and its range was from 4.11 in sample Ac49 to 5.34 in sample Ac74 (Table 1). The mean pH of *Robinia* honeys was 3.80 and its range was from 3.32 in sample Rb8 to 4.05 in sample Rb14 (Table 2). The pH value range was larger in *Acacia* samples than in *Robinia* samples (Table 1 and Table 2). The range of both honey types complied with requirements (3.0–5.6) of GSO that were followed by the Directorate General for Specifications and Measurements, Ministry of

Commerce and Industry, Sultanate of Oman (GSO, 2008). They were also found to be within the general typical range of (3.42–6.10) as stated by (White, 1978). The current pH range of *Acacia* honeys were in agreement with the Saudi honey pH range (3.51–5.27) reported by Al-Doghairi, et al. (2007) and Alqarni, et al. (2012). It was also closer to the pH values of the Spanish honeys (3.63–5.01) (Cui, et al., 2008). Fairly high pH values of *Acacia* honey might be due to the nature of the nectar, which is produced in hot and humid summer conditions in Oman (Sajwani et al., 2007). The acidic pH prevents the growth of many bacteria in honey (Gomes, et al., 2010). In this study, the pH of *Robinia* honeys ranged from 3.32 to 4.05. It was more or less similar to the pH values of European *Robinia* (3.7–4.2) reported by Persano Oddo and Piro (2004) and Serbian *Robinia* (3.89–5.05) honey (Lazarević et al., 2012).

The pH and acidity of honey may be due to the presence of some weak organic acids, such as gluconic acid, ascorbic acid, pyruvic acid, malic acid, citric acid and even acetic acid in equilibrium with their corresponding lactones or internal esters, and also to the inorganic ions, such as phosphate, sulphate and chloride (Echigo and Takenaka, 1974; Nigussie et al., 2012). These aliphatic acids, particularly gluconic acid, contribute greatly to the flavor of honey by interacting with the flavors of other ingredients. In Omani honey, the most important organic acids found were gluconic acid, formic acid and acetic acid (Al Baz and Sabagh, 2000). Honey pH is of great importance during extraction and storage as it influences texture, stability and shelf-life (Baroni et al., 2009).

3.2.2. Moisture content. The moisture content of honey is another important factor for the shelf-life of honey contributing to its stability against fermentation and granulation during storage (Cereser Camara and Laux, 2010). It is well known that this parameter is related to the maturity and freshness of samples, regardless of their botanic origin or type of honey (Bentabol Manzanares et al., 2011). This factor is affected by climate, harvest season, degree of maturity/ripeness reached in the hive, processing techniques, storage conditions, botanical origin of the sample and moisture content of original plant nectar (Özcan, Arslan et al., 2006). Honey moisture content varies from year to year. High moisture content could accelerate crystallization in certain types of honey and increase its water activity to values where certain yeasts could grow. The undesirable honey fermentation during storage is caused by the action of osmotolerant yeasts resulting in the formation of ethanol and carbon dioxide. The alcohol can be further oxidized to acetic acid resulting in a sour taste and a runny texture with small bubbles, surface heaving or foaming (Sanford, 2009; Saxena et al., 2010). Moisture was not taken into account, as it is only related to the quality of honey and not to the botanical origin and therefore not useful for the differentiation among honeys.

Acacia honey samples showed the average moisture content of 16.75% and the range from 14.70 to 19.20% (Table 1). *Robinia* honey showed an average moisture content of 17.41% and ranged from 16.40 to 18.25%. In comparison, moisture content range of *Acacia* honey was wider than *Robinia* honeys nevertheless, both honey types meet the specification of CAC (2001) and GSO (2008) (Table 2). Acceptable levels moisture content of *Acacia* and *Robinia* honey samples were found in this study that reflect optimum harvesting and proper degree of maturity similar findings were reported for Saudi *Acacia* honeys (Alqarni et al., 2012). Relatively low moisture level of the current *Acacia* honey samples is at comparable levels of Egyptian honeys (20.12%) (Alqarni et al., 2012). This may be attributed to the hot and dry weather during April and May in the region of honey production. These results are in agreement with Alqarni et al., (2012) and Al-Doghairi et al. (2007). Viuda-Martos et al. (2010) reported high moisture content ($\geq 23\%$) in some Mexican honeys and Terrab, et al. (2002) found similar trend in Moroccan honeys which is higher than the prescribed limit for the moisture content standards of CAC (2001). The high moisture values obtained were

probably because of the high rainfall recorded in these regions. In the present study, the water content range of Robinia honey (16.40 - 18.25%) was narrower than Acacia and was within the European Robinia range (14.7 - 19.6%) (Persano Oddo and Piro, 2004). Whereas Lazarević et al. (2012) recorded a wider range (13.90 - 20.57%) for Serbian Robinia honeys and Alqarni et al. (2012) reported moisture content of 21.68% for German commercial Robinia honey.

3.2.3. Electrical conductivity. The average EC values of Acacia honey was 1.82 mS/cm and the range was from 0.54 mS/cm in sample Ac49 to 2.81 mS/cm in sample Ac75. Only the EC values of samples Ac47 and Ac 49 were within the standard (not > 1.2 mS/cm) of GSO (2008) and CAC (2001). In the rest of the 97.5% samples these values were recorded out of the limits (Table 1). This could be due to two reasons: The first being the richness of Acacia honey in the pollen content as 63.75% of these samples belong to Group-II with 20,000-100,000 pollen grains per 10 g sample and these samples were regarded as "normal" following the terminology of Crane, (1975). The second reason might be due to the high content of minerals and organic acids in summer Omani honeys as found by Al Baz and Sabagh, (2000). Higher EC has been reported by El Sohaimy et al. (2015) in Yemen and Egyptian honeys (4.18 ± 0.05 , 1.98 ± 0.03 , respectively) which were also out of the GSO limits. They attributed this to the rich pollen and ash contents of these honey samples.

In *Robinia* honeys the average EC value was 0.33 mS/cm and it ranged from 0.11 mS/cm in sample Rb7 to 0.48 mS/cm in sample Rb13 (Table 2). All *Robinia* samples were within the limits of the CAC standards. *Acacia* honeys had a higher mean value than the *Robinia* honeys (Table 1 and Table 2). Saudi *Acacia* honey was similar to Omani's in the mineral contents as well as the range of EC, which was also higher (2.1–3.1 mS/cm) than the standard value (Alqarni et. al., 2012). They also noted that dark honeys (e.g., *Acacia* and linden) contain higher levels of microelements than light honeys (e.g., rape and *Robinia*) indicating *Robinia* honey is less ionic than *Acacia* honey. This explains its low EC value, in comparison to *Acacia*, as set in the limits of the CAC legislation. This range is similar to EC data recorded by Persano Oddo and Piro, (2004) and Lazarević et al. (2012) (0.09–0.230 mS/cm) and (0.10–0.690 mS/cm), respectively.

The EC of *Acacia* honey samples correlated positively and significantly ($P < 0.05$) with all other parameters studied (Table 3). Relatively weak correlations ($r = 0.240$ and 0.153) respectively were noticed for the moisture content and diastase activity, while moderate correlations ($r = 0.339$ and $r = 0.350$) respectively were obtained for the pH and invertase activity. EC showed a strong correlation ($r = 0.615$) with free acidity (Table 3) but there was a weak correlation between EC of *Robinia* honey samples and diastase activity ($r = 0.151$) and moderate correlation with pH ($r = 0.573$, $P < 0.05$) (Figure 4). No correlations were found between the EC of *Robinia* samples for the moisture content and invertase activity (Table 4).

The EC of honey is closely related to the concentration of mineral salts, organic acids, proteins, complex sugars and polyols content, the higher their content the higher the resulting conductivity (Bogdanov and Gallman, 2008). Since minerals are introduced into honey primarily with pollen, their content depends on the predominant pollen present in honey. Therefore, electric conductivity is a parameter which is very often used in routine honey quality control instead of the ash or mineral content. It can be considered a valid criterion for determining the botanical origin of a honey sample and differentiating honeys of different floral origins (Bogdanov et al., 2004; Ruoff et. al., 2006). EC value decreases with a decrease in the amount of pollen (Kaškonienė and Venskutonis, 2010). It is also a stable value for the same variety of honey, within a range of variability that remains practically invariable with treatments or storage (Krauze and Krauze, 1991).

Figure 4. Correlation between pH and the electrical conductivity of 14 *Robinia* honey samples

3.2.4. Free acidity. All tested honeys were acidic; the range of free acidity for Acacia was from 29.46 meq/kg in Ac49 to 141.36 meq/kg in Ac4 with the average of 95.30 meq/kg (Table 1). Excluding Acacia honey samples Ac47 and Ac49, all the other 97.5% samples' free acidity values were > 50 meq/kg. These values were beyond the limits of GSO (2008). In Robinia honey samples, the free acidity ranged from 4.75 meq/kg in Rb3 and 15.20 meq/kg in Rb13. The mean was 8.03 meq/kg. The wide range may be due to variable flora in different production regions (Table 2). All the samples were within the limits of GSO, (2008) (Table 2) and within the range of the CAC criterion of European Robinia (4.5–17.9 meq/kg) (Persano Oddo and Piro, 2004). Table 1 and Table 2 showed wider free acidity range for Acacia honey than Robinia. Significant correlations were found between the Acacia honey free acidity and both the moisture content and EC ($r = 0.558$ and $r = 0.615$, $P < 0.05$), respectively (Figure 5, Figure 6 and Table 3).

Figure 5. Correlation between free acidity and the moisture content of 80 *Acacia* honey samples

Figure 6. Correlation between free acidity and the electrical conductivity of 80 *Acacia* honey samples

Table 3

Correlation matrix showing the interrelation among physicochemical properties of 80 Omani *Acacia* honey samples

Parameters **	pH	Moisture content (%)	Electrical conductivity (mS/cm)	Free acidity (meq/kg)	Diastase activity (DN)	Invertase activity (U/kg)
pH	1.00	—	—	—	—	—
Moisture content (%)	NC	1.00	—	—	—	—
Electrical conductivity (mS/cm)	0.339	0.240	1.00	—	—	—
Free acidity (meq/kg)	NC	0.558	0.615	1.00	—	—
Diastase activity (DN)	NC	NC	0.153	0.160	1.00	—
Invertase activity (U/kg)	0.213	NC	0.350	0.270	0.688	1.00

** All the correlations were significant at 0.05 level (2-tailed). (NC) no correlation.

In *Robinia* honey samples, free acidity correlated significantly with EC ($r = 0.576$, $P < 0.05$) (Figure 7 and Table 4). All honeys are acidic with a pH value generally between 3.5 and 5.5, due to the presence of organic acids that contribute to honey flavor and stability against microbial spoilage. In honey the main acid is gluconic acid, which is found together with the respective glucono-lactone in a variable equilibrium (Bogdanov et al., 2004).

Figure 7. The correlation between free acidity and the electrical conductivity of 14 *Robinia* honey samples

Table 4

Correlation matrix showing the interrelation among physicochemical properties of 14 *Robinia* honey samples analysed

Parameters **	pH	Moisture content (%)	Electrical conductivity (mS/cm)	Free acidity (meq/kg)	Diastase activity (DN)	Invertase activity (U/kg)
pH	1.00	— [—]	— [—]	— [—]	— [—]	— [—]
Moisture content (%)	NC	1.00	— [—]	— [—]	— [—]	— [—]
Electrical conductivity (mS/cm)	0.573	NC	1.00	—	—	—
Free acidity (meq/kg)	NC	0.136	0.576	1.00	— [—]	— [—]
Diastase activity (DN)	0.275	0.331	0.151	NC	1.00	— [—]
Invertase activity (U/kg)	NC	0.343	NC	NC	0.231	1.00

** All the correlations were significant at 0.05 level (2-tailed). (NC) no correlation.

The pH of a honey is not directly related to the free acidity because of the buffering action of the various acids and minerals present (Abu-Tarboush et al., 1993). Other factors can affect honey acidity, e.g. harvest seasons, floral types, content of enzymes and storage conditions (Perez-Arquillue, Conchello, A. Arino, and Herrera, 1994). Humidity and free acidity were found to be the most important parameters for classification of honey (Anklam, 1998). The free acidity of only samples Ac47 and Ac49 were within the limits of both CAC and GSO (not more than 50 meq/kg). Rest of the 97.5% *Acacia* samples were in the range of (65 - 141 meq/kg) (Table 1), although these samples were fresh and lacking undesirable fermentations.

Food control laboratories in Oman also recorded a wide range and high values of free acidity of *Acacia* honey which may reach to 100 meq/kg. Studies from Yemen and Saudi Arabia documented high free acidity of this type of honey, which are outside of the range of the current standards (AL-Zoreky et al., 2001; Alqarni et al., 2012). Al-Doghairi et al. (2007) found a wide range of total acidity between 9.12 - 93.02 meq/kg for Saudi honeys. The values for the free acidity ranged from 10.31 to 102 meq/kg as reported by (Terrab et al., 2002) in Moroccan honeys. Berg et al. (2008) found that the relatively low pH and high free acid content for New York buckwheat honey that may contribute to its ability to heal chronic wounds.

The high acidity in *Acacia* honey may be the result of the type of nectar produced in summer in plants growing in high salinity soils. It was observed that the honey obtained during the spring flowering season (February to June), is often more acidic than in the autumn season (personal observations; unpublished data). Al Baz and Sabagh, (2000) attributed the high acidity in *Acacia* honey to high humidity during the production season and the nature of the nectar produced by the *Acacia tortilis* plant. On the other hand, Tilbury (1980) and Costa et al. (1999) believe the high acidity in Brazilian honey is due to the hot climatic conditions, which favor the rapid growth of xerotolerant yeasts (e.g. *Saccharomyces rouxii*) that produce organic acids in honey as secondary metabolites, thereby raising the acidity. Missio et al. (2016) and Alvarez-Suarez et al. (2017) reported that the high acidity can be an indicator of fermentation of sugars into organic acids which can affect the organoleptic

characteristics of honey and its quality. While Vranić et al. (2017) reported that the variation in free acidity among different honeys can be explained by blossom origin, the presence of different organic acids or some inorganic ions, geographical origin or harvest season.

3.2.5. Diastase activity. The diastase activity of *Acacia* honeys was found to be in the range of 3 DN in sample Ac57 to 30 DN in Ac80 with an average of 12 DN on Gothe's scale. Out of 80 *Acacia* honeys 15 of them showed diastase activity > 8. Three of them (Ac12, Ac89 and Ac50) were from comb extracted honeys. The range of diastase activity in *Robinia* honeys was between 5 DN in Rb7 and 12 DN in Rb10. The average was 8 DN. Because *Robinia* honey has a low natural enzyme content (White, 1992), values of less than 8 DN are accepted in CAC standards. The diastase activity in ten samples had > 8 DN and four samples viz, Rb6, Rb7, Rb13 and Rb14 showed < 8. The range of diastase activity was wider in *Acacia* samples compared to *Robinia* (Table 1 and Table 2). Diastase (α -amylase) is one of the most important enzymes in honey added by the bees during the conversion of nectar into honey. It converts starch to short-chain sugars. Therefore no starch is found in honey. The diastase content varies according to the floral source, long storage periods and exposure to high temperatures (White and Bryant, 1996).

Heating honey denatures the enzyme, therefore the legislation has set a minimum level (< 8 DN) for diastase activity (Codex Alimentarius Commission, 2001). It is undesirable to have either very low or very high diastase activity in honey (Tolon, 1999). The latter may occur due to the formation of acid and may result in fermentation (Yardibi and Gumus, 2010). The current study showed a wide range of diastase activity in *Acacia* honey samples from 3.32 to 30.13 DN. This observation is common in the fresh, unheated honeys as reported by White (1994) and indicates their variable geographical and biological origins (White and Bryant, 1996).

Our results illustrated that the diastase activity in 81% *Acacia* honeys was > 8 DN, whereas only 19% of the samples shown < 8 DN. This was not caused by the aging, adulteration or heating of these samples since they were collected fresh from the beekeepers in the season and stored in glass bottles with tight caps in a freezer. Besides, heating honey is not usually practiced by the Omani beekeepers. Out of these 15 samples, 3 of them (Ac12, Ac30 and Ac50) were from comb honeys, which show natural low diastase activity (White, 1994). The low diastase activity might be due to the high temperature environment within the bee colonies. This explanation also gathers support from German and American investigators who observed that honeys from warmer regions and comb honeys could have low diastase activity (White, 1994).

Studies from Saudi Arabia, Algeria and Yemen also documented similar low diastase activity (< 8) in honey samples (Al-Khalifa and Al-Arif, 1999; Al-Zoreky et al., 2001; Chefrour et al., 2009) respectively. White (1992) explained the reason of low diastase which occurs naturally in some honeys. Since it is agreed that the enzymes are added by the honey bee during the collection and the ripening of the nectar to make it a thick consistency, also it is known that some nectars are naturally much thicker to start with than others, therefore some nectar types require less manipulation by the bees in the hive to attain that thick consistency. This results in the addition of lower levels of diastase and invertase and other materials by the bee. In the United States diastase activity is mainly controlled to ensure low diastase values because much of the honey supply is used in bakeries for mixing with starch-containing food ingredients and a high diastase number could cause poor bread texture (White, 1992). A low diastase activity range (5–12 DN) was found in *Robinia* honey samples, compared with the *Acacia* honey samples. This was within the range (3.1 - 20.4 DN) of European *Robinia* honeys found in the database of the IHC (Persano Oddo and Piro, 2004).

The low diastase activity (< 8) is accepted by EUC and CAS because *Robinia* honey is classified as a low diastase containing honey (Persano Oddo and Piro, 2004). White (1992) advised against using diastase activity as a basis for measuring honey quality because of its uncertainty due to its extreme variation in the level of its starting point. The diastase activities in honey vary in wide limits depending on botanical origin of honey. The enzyme has no nutritional basis as a dietary requirement; therefore, diastase measurement should not be used as a regulatory standard for honey quality. This recommendation is respectfully made to Saudi Arabian and other Gulf authorities dealing with honey imports.

3.2.6. Invertase activity. *Acacia* honeys were in the range of 39.95 U/kg in sample Ac49 to 312.37 U/kg in sample Ac24 with an average of 141.07 U/kg (Table 1). The range of invertase activity in *Robinia* honey was between 3.26 U/kg in sample Rb7 to 55.26 U/kg in sample Rb4 with an average of 17.53 (Table 2). Significant correlation was found ($r = 0.688$, $P < 0.05$) between diastase and invertase activities in *Acacia* honey samples (Figure 8 and Table 3).

Figure 8. The correlation between diastase and the invertase activities of 80 *Acacia* honey samples

However, there was weak correlation ($r = 0.229$, $P < 0.05$) (Table 4) for these parameters in *Robinia* honey samples. Invertase (α -glucosidase) is the enzyme added to the nectar by the bee that converts the sucrose of the nectar to glucose and fructose, a vital step in the ripening of nectar to honey. Invertase is more susceptible to temperature than diastase. The activity of this enzyme decreases depending on the temperature and time of honey processing (White, 1992). Similar to diastase, it is hard to interpret the results of invertase activity in terms of freshness, since the initial value is unknown (Dinkov and Vashin, 2001). There were

significantly ($P < 0.005$) variable activities of this enzyme in the studied *Acacia* honey samples (40–312 U/kg). Al-Khalifa and Al-Arif (1999) recorded the range of invertase activity 26.7 to 76.7 U/kg for three *Acacia* honey samples from Saudi Arabia.

Invertase activity ranged (3.26–55.26 U/kg) in *Robinia* samples which is within the permitted range (3.4 – 107.5 U/kg) of European *Robinia* honeys found in the database of IHC (Persano Oddo and Piro, 2004). It was observed that the range of invertase activity of *Acacia* samples was larger than the *Robinia* samples. In addition, both ranges of *Acacia* and *Robinia* were wide. This variation among honey samples is due to a number of factors such as enzymatic activities, concentration and composition of the nectar, amount of sucrose in honey, and even the age of honeybees. Therefore, honeys from rich nectar sources such as *Robinia* often contain low natural enzyme activities (White, 1994). All honeys with high enzyme content are produced in summer when brood rearing is less intensive and foragers are predominant. Therefore the nectar collection time (the physiological state of the colony) and consumption of pollen by bees also have influence on the enzyme activities (Huang and Otis, 1989).

3.2.7. Correlations amongst the quality control criteria. The correlation matrix (Table 3 and Table 4) showed a significant correlation between the quality control criteria. This study verified uniform pattern of the results for all the parameters studied. For example, *Acacia* honey sample Ac49 was collected from a commercial apiary that fed sugar to the bees for a long time that was reflected in its quality. The results of this sample showed the lowest values of pH, EC, free acidity and invertase activity (4.11, 0.54 mS/cm, 29.46 meq/kg and 39.95 U/kg, respectively) in comparison to the rest of the samples. The EC of *Acacia* honey samples was the only criterion found to correlate significantly and positively with pH, moisture content, free acidity, diastase and invertase activities. This may indicate the influence of these parameters in raising the EC of this honey. The significant and positive correlation between free acidity and EC of *Acacia* honey may indicate the richness of this type of honey in minerals and organic acids. All of these point to its rich nutritional and medicinal value.

Many investigators such as Persano Oddo et al. (1999) and Serrano et al. (2007) have reported a highly significant and positive correlation between diastase and invertase activities; at the same time demonstrating the correlation between the two enzymes, showing that honeys with a low content of invertase also have a low content in diastase, and vice versa. This correlation maybe useful in excluding honeys in evaluation of adulteration. In the current study, the correlation between diastase and invertase in *Acacia* honey was found to be significant ($P < 0.05$) with the coefficient of correlation ($r = 0.688$), which was similar to that reported by Horn and Boehm (2004) ($r = 0.700$) but lower than those obtained by Persano Oddo et al. (1999) ($r = 0.835$). The low correlation coefficient between both enzymes found in tested *Robinia* honeys ($r = 0.2$) was similar to the reports of Vit and Pulcini (1996) and Bentabol Manzanares et al. (2017). The low correlation may be because of the low content of diastase in these types of honeys probably due to the high nectar flow that does not allow bees to adequately process it (White, 1994). Data in this study are in agreement with the reports of (Persano Oddo et al., 1999) that these enzymes may not provide useful information about the freshness of honey because they are variable from one type of honey to another.

4. Conclusion

This study was undertaken to elucidate the true *Acacia* honey from white *Acacia* (*Robinia*) honey. It is highly recommended to label them with specific names, which can be used by both scientific and public communications. We suggest the name “*Acacia*” for *Acacia* spp. honeys and “*Robinia*” for *Robinia pseudacacia* honey. This suggestion is put forward to honey regulators such as the CAC, IHC, and EUC directive relating to honey.

The quality control criteria showed that Omani *Acacia* honey possesses relatively higher values of physicochemical parameters, which mostly meet the major permitted specifications. The EC of this honey was found to correlate significantly with pH, moisture content, free acidity, diastase and invertase activities. This may point out the influence of these parameters in raising the EC value indicating the richness of *Acacia* honey with mineral and organic acid contents, which may increase its nutritional and medicinal value. It was found that most of the *Robinia* honey samples purchased from the local markets in Oman met the national and international standards. This may satisfy the consumer when going for this product safety although its quality is lower than that of Omani *Acacia* honey.

Some criteria of *Acacia* honey such as EC and free acidity were not within the range of the currently available standards. This shows that Oman needs to build its own honey database and standards for the regional honeys. The values in the current standards are not in accordance with those obtained from honey from the Arabian Peninsula, which, were formulated based on European and imported honeys. Originally, they were promulgated during the 1960s.

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