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Original article

Comparative study of physio-chemical analysis of fresh and branded honeys from Pakistan

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ABSTRACT

Honey is a nutritious substance produced by bees. Its quality and nutritional value is of great importance for consumers. Keeping this in view physicochemical and minerals determination as quality parameters of fresh floral Pakistani honeys produced by *A. mellifera* and branded honeys was conducted. The results of fresh honey indicated average means of Color as 48.78 mmPfund, pH 4.9, Total acidity 37.14 meq/kg, Moisture content 18.62%, Electrical conductivity 0.23 mS/cm, Ash content 0.49%, HMF content 30.85 mg/kg, Proline 365.84 mg/kg, Diastase activity 34.39(DN) and Invertase activity was 68.61(IN) comparable to honey standards. Natural honey were rich in k+ (408.46 ppm) and Na+ (405 ppm). Although Ca+ was very low. Whereas, Co, Mn and Ba concentrations exceed the 1 ppm. However, Pb, Cr, and Mo were unnoticeable. Similarly, Color, pH, MC, EC, T. Acidity, HMF, Proline, Ash content, Diastase and Invertase activity of branded honey samples average means found were 42.5 mmPfund, 5.05, 20.5%, 0.18 mS/cm, 15.34 meq/kg, 36.5 mg/kg, 181.6 mg/kg, 1.11%, 7.90(DN) and 36.97(IN) respectively. The findings showed that fresh honey samples were good and of consumable quality as per honey standards than branded honey. Higher HMF content and lower enzymatic activity in branded honey sample than the Codex standards revealed its either long or improper storage.

1. Introduction

Honey is defined as "the sweet substance produced by honeybees from the nectar of flowers, which the bees collect, transform and store in honey combs". It is the most important natural substance and categorized by its massive nutritive cost (330 kcal/100 g) and rapid absorption of carbohydrates during consumption (Conti et al., 2007; Khaliq and Swaileh, 2017). Although, the main components of honey are almost identical in all honeys, yet the chemical composition and physical properties of natural honey depends on the floral sources, the processing, storage and climatic conditions (Lazarević et al., 2012; Boussaid et al., 2018; Sakač et al., 2019).

In Pakistan, four species (*Apis florae, Apis dorsata, Apis cerana* and *Apis mellifera*) of honey bees are present (Morse and Calderone, 2000; Qamer et al., 2009). *A. mellifera* is one of them, that improved quantities and Qualities of crops through pollination in the selected study areas and have a key role in honey agro-food chain (Munawar et al., 2009). Honey has become one of the most commercial agricultural products for trade in Pakistan since few years (Waghchoure and Martin, 2009; Adnan et al., 2014; Anjum et al., 2015).

Physicochemical parameters like moisture, acidity, pH, hydroxymethylfurfural (HMF) content, color, sugar composition and specific conductivity of the natural honey are precisely defined, and each characteristic is known to represent quality indicators (Tosi et al., 2008; Ajlouni and Sujirapinyokul, 2010; Khan et al., 2016; Adgaba et al. 2017; Ansari et al., 2018; Boussaid et al., 2018). Honey is also well known natural cheaper source of essential inorganic elements for consumers which are required for body metabolism (Alwaili et al, 2013; Sakač et al., 2019).

The present study was aimed to identifying natural and branded honey varieties quality found collected from different

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areas, local shops and markets of various districts, Punjab Pakistan in terms of their physicochemical properties and mineral contents according to International Honey Standards.

2. Materials and methods

2.1. Honey collection

A total of Sixty-five honey samples (fresh honey n = 50, branded honey n = 15) were collected from shopkeepers and beekeepers from the different areas/ districts such as Chakwal, Sialkot, Lahore, Sahiwal, Narowal, Sheikupura, Nankana Shab and Muree of Punjab Pakistan during the year 2017–2018. Fresh honey samples were stored in sealed plastic jars followed by labeling and dating and kept at the room temperature ±29 °C till completion of analysis. All these honey samples were classified on the basis of their dominant botanical and geographical origin.

2.2. Honey quality tests

Honey water content was detected using a refractometer REF-116, pH by pH-meter ((Bibby Scientific Ltd., UK) with a solution of 75 ml of carbon dioxide-free water in which 10 g of honey was dissolved (AOAC method 1990), EC by Vorwohl (1964) directly with 13% honey solution. Results were expressed in milli Siemens per centimeter (mS/cm). Muffle furnace was used to determine the ash content by burning the samples at 500 °C for 6 h. Acidity was estimated by AOAC Official (1990) and results were expressed as meq/kg. The HMF content in honey were determined by using the actual method of Wrinkler et al. (1955). Honey Color parameters was measured in Minolta Chromameter® CR 410 type instrument as well Lovibond. The L^* parameter (lightness index scale) ranges from 0 (black) to 100 (white). The diastase activity was determined by phadebas tablets as recommendation by International Honey Commission (2009). Siegenthaler (1977) method was used to analyzed Invertase activity. Mineral contents were analyzed using a Varian Spectra Atomic Absorption Spectrophotometer (Model 220, Varian, USA) and emission photometry.

2.3. Statistical analysis

SPSS-2001 software was used to analyze the data. The statistical difference in honey samples were tested with ANOVA at $p < 0.05$

3. Results and discussion

3.1. Physicochemical properties

The physicochemical parameters of the 65 honey samples were analyzed as shown in Tables 1–3. The moisture content of fresh and branded honey samples ranged from 18 to 19.2% and 19.50–21.25% respectively. Despite of different floral sources, water % of all the fresh honey samples were within the international limit (≤21%) than branded honey. The highest moisture content value was found in Bari (19.2) and Qureshi honey (21.25%). Kumar et al. (2018), Al-Ghamdi et al. (2019) and Can et al. (2015) were reported 18.37–22%, 18.50 ± 1.53, and 16.54–20.84% respectively, which seems close to the current findings. Honey moisture content depends on several factors such as degree of maturity reached, yielding season, and ecological factors (Acquarone et al., 2007).

pH is another important parameter during extraction and the conservation of honey. It increases the quality, constancy and shelf life of honey (Terrab et al., 2002). All the examined fresh honeys were acidic except Bairi samples. The average pH values of fresh and branded honey ranged from 4.35 to 7.05 and 4.6 to 5.35. Azonwade et al. (2018) and lokossou et al. (2017) reported pH of 5.08 and 5.00–5.48 respectively, showing almost the same range as found in the present research. However, Laredj and waffa (2017) and Mohammed et al. (2017) recorded acidic pH values (4.17 and 4.20) in Algerian and Saudi honeys lower than present pH. In the same way EC ranges obtained by Bousaid et al. (2018) (0.39–0.89 mS/cm), Lokossou et al. (2017) (0.37–1.43 mS/cm) and Guler et al. (2017) (0.250–0.90 mS/cm) in Tunasian honey, Beninese and Turkish honeys were more or less same as found in fresh (0.11–0.61 mS/cm) and branded (0.17–0.23 mS/cm) honey samples. The resulted variation in EC depends on the different floral origins of honeys.

According to Perez-Arquillué et al. (1994), acidity in honey varies due to floral origins and harvesting seasons. Though acidity of fresh honeys (33–46.5 meq/kg) were within the International limits yet higher as compared to branded (14.16–16.33 meq/kg) samples, El-Haskoury et al. (2018) and Alqarni et al. (2016) reported honey acidity 16.50–59.50 meq/kg and 55.5–145.5 meq/kg which is closely related to the current recorded values of acidity.

Another parameter used for the determination of the botanical origin is Ash content (White, 1978). As compared to branded honey (0.66–2.05%), the fresh honey samples are within the limit (0.4–0.55%) for ash content proposed by the Codex Alimentarius Standards (≤0.6 g/kg). Parviz et al. (2014) and Anhwange et al. (2015) recorded ash content of 0.03–0.52%, 0.6–0.8 and 1.26–1.66 almost in the same range determined in current study.

The determination of color is a use full method for classification of multiflora honeys. The lightness values (L^*) found for the sixty-five honey samples ranged from 27.95 mmPfund (White) to 78 mmPfund (Light amber) and 34.86 mmPfund (Extra light Amber) to 50.50 mmPfund (Light Amber) respectively. Honey color parameter is usually first honey assessment depending upon its ingredients by consumers. Orange, Barseem and Shesham have same values (47) that contain Extra light amber color while Qurshi honey has Light Amber color. Aazza et al. (2018). Boussaid et al. (2018) and Khalafi et al. (2016) found the color range between 71.27 mmPfund, 36.64–51.37 mmPfund and 19–45.6 mmPfund, closely resemble the current research.

HMF is an important parameter used for honey purity and its freshness (Codex Alimentarius, 2001). According to international honey commission, the concentration of HMF should not exceed 40 mg/kg. The measured HMF content in current study ranged from 24.45 to 40.68 mg/kg and 316.86 to 516.26 mg/kg for fresh and branded honeys. Boussaid et al. (2018) and Perviz et al. (2014) reported similar HMF content of 27.43 ± 1.50 mg/kg and 37.31 ± 17.13 mg/kg. In addition, Al-Ghamdi et al. (2019) and Kivrak et al. (2017) determined much lower (3.78 mg/kg and 3.87–4.64 mg/kg) in Saudi and Indian honeys. Storage and floral sources are the major causes of higher HMF (Terrab et al., 2002; Meda et al., 2005).

The estimated Proline content of honey samples range was 287.6–511.1 mg/kg and 103.66–329.66 mg/kg. The proline contents of fresh honey samples were within the codex limit (≥180 mg/kg) and directive 2001/EC (≥180 mg/kg) as compared to branded honey samples. Moloudian et al. (2018), Aazza et al. (2018) and Nayik and Nanda (2015) analyzed the proline content ranged from 240.4 to 848.07 mg/kg, 256.46–924.98 mg/kg and 205.3–551.74 mg/kg respectively, in Iranian and Morocco honey more or less similar to the range of proline content of fresh honeys examined in the current research. The level of proline has been reported to vary depending on the honey flora, but this is more closely associated with the working performance of bees (Manzanares et al., 2014).

The diastase numbers of fresh honey samples in the present research work were 26.97–43.47(DN) with in the international ranges as compared to branded samples 5.95–10.35(DN) respectively. Moloudian et al. (2018) recorded diastase number

Table 1
Physicochemical parameters of fresh honey samples analyzed (mean ± SD).

Parameters	Phulai (*Acacia modesta*) n = 3	Baiker (*Jusdticia adhatoda*) n = 19	Serson (*Brassica comprestris*) n = 8	Bari (*Zizipus jojoba*) n = 2	Orange (*Citrus xsinensis*) n = 3	Barseem (*Trifolium repens*) n = 13	Sheesham (*Dalbergia sisso*) n = 2	P value	Codex*
Color (mm Pfund)	52.67 ± 11.59	27.95 ± 10.16	40.5 ± 23.42	78 ± 24.04	47 ± 6.92	47.38 ± 10.13	48 ± 2.35	0.00	–
pH	4.73 ± 0.47	4.75 ± 0.70	4.35 ± 0.48	7.05 ± 0.21	4.43 ± 0.49	4.95 ± 0.35	4.5 ± 0.92	0.00	–
Acidity (meq/kg)	35.67 ± 1.15	35.65 ± 5.27	34.01 ± 3.99	46.5 ± 2.82	36.63 ± 2.95	38.55 ± 6.18	33 ± 4.8	0.04	≤50 meq/kg
Moisture (%)	18 ± 1	19.07 ± 0.99	18.61 ± 1.27	19.2 ± 1.06	18.83 ± 1.25	18.65 ± 1.12	18 ± 0.43	0.65	≤21%
EC (ms/cm)	0.11 ± 0.04	0.18 ± 0.06	0.19 ± 0.06	0.61 ± 0.04	0.20 ± 0	0.2 ± 0.06	0.17 ± 0.03	0.07	≤0.7 ms/cm
Ash (%)	0.52 ± 0.08	0.50 ± 0.22	0.40 ± 0.12	0.55 ± 0.07	0.43 ± 0.30	0.54 ± 0.26	0.5 ± 0.06	0.88	≤0.6 g/kg
HMF (mg/kg)	33.53 ± 3.46	25.24 ± 4.32	31.67 ± 4.32	24.45 ± 2.82	31.83 ± 3.75	40.68 ± 4.24	28.55 ± 3.6	0.00	≤40 meq/kg
Proline (mg/kg)	308.57 ± 67.56	410.15 ± 35.19	414.17 ± 42.21	511.1 ± 33.79	299.4 ± 8.47	329.95 ± 63.14	287.60 ± 56.1	0.00	≥180 mg/kg
Diastase (DN)	41.47 ± 5.70	29.57 ± 14.52	29.27 ± 7.75	26.97 ± 22.23	43.46 ± 10.46	35.55 ± 12.64	34.5 ± 14.02	0.40	≥8 (DN)
Invertase (IN)	74.33 ± 10.08	61.07 ± 5.04	70.57 ± 4.34	73.6 ± 6.92	81.9 ± 4.83	58.55 ± 8.30	60.3 ± 4.85	0.00	≥50 (IN)

Means in the same column with different letters are significantly different at $p < 0.05$.

Table 2
Comparative Analysis of Macro and Micro Elements Based on Botanical (floral) Origin.

Parameters	Phulai (*Acacia modesta*) n = 3	Baiker (*Jusdticia adhatoda*) n = 19	Serson (*Brassica comprestris*) n = 8	Bairi (*Zizipus jojoba*) n = 2	Orange (*Citrus xsinensis*) n = 3	Barseem (*Trifolium repens*) n = 13	Sheesham (*Dalbergia sisso*) n = 2	P value
Potassium (ppm)	465.66 ± 108.56	398.68 ± 252.83	454.22 ± 269.26	166.5 ± 23.33	361.4 ± 254.08	370.81 ± 235.60	295.42 ± 115.2	0.80
Sodium (ppm)	335 ± 361.13	445.22 ± 345.40	445.77 ± 38.28	399.5 ± 47.37	579.6 ± 317.96	422.81 ± 362.94	211.6 ± 165.7	0.11
Calcium (ppm)	0.73 ± 0.23	0.57 ± 0.26	0.50 ± 0.30	0.69 ± 0.03	0.65 ± 0.23	0.62 ± 0.20	0.69 ± 0.12	0.77
Cobalt (ppm)	2.47 ± 0.63	3.68 ± 1.90	4.43 ± 0.56	4.54 ± 0.80	4.37 ± 0.50	4.39 ± 0.57	2.60 ± 0.65	0.43
Manganese (ppm)	3.11 ± 0.16	2.37 ± 0.91	2.75 ± 0.9	1.75 ± 0.18	2.22 ± 0.83	2.80 ± 0.14	1.05 ± 0.16	0.55
Barium (ppm)	4 ± 0.16	1.57 ± 0.93	3.88 ± 0.12	1.1 ± 0.2	3.8 ± 2.16	2.36 ± 0.25	3.24 ± 0.9	0.55
Lead (ppm)	0.10 ± 0.08	0.07 ± 0.02	0.04 ± 0.08	0.19 ± 0.06	0.09 ± 0.01	0.17 ± 0.09	0.07 ± 0.02	0.54
Chromium (ppm)	0.03 ± 0.02	0.25 ± 0.41	0.1 ± 0.03	0.023 ± 0.01	0.26 ± 0.1	0.11 ± 0.01	0.10 ± 0.07	0.50
Molybdenum (ppm)	0.05 ± 0.02	0.3 ± 0.61	0.09 ± 0.03	0.26 ± 0.24	0.03 ± 0.01	0.2 ± 0.06	0.07 ± 0.02	0.60

Means in the same column with different letters are significantly different at $p < 0.05$.

Table 3
Physicochemical parameters of Branded honey samples analyzed (mean ± SD).

Parameters	Quershi Honey n = 2	Marhaba honey n = 2	Sulman honey n = 3	Saudi honey n = 3	Young honey n = 2	Alshifa honey n = 3	P values	Codex*
Color (mm Pfund)	50.50 ± 21.92	44 ± 4.24	34.86 ± 20.21	41.22 ± 15.95	44.50 ± 31.81	40 ± 22.5	0.93	–
pH	5.00 ± 0.84	5.35 ± 0.21	5.23 ± 0.15	4.86 ± 0.40	5.30 ± 0.70	4.6 ± 0.20	0.39	–
Acidity (meq/kg)	16 ± 2.12	15.50 ± 0.70	16.33 ± 1.60	15.33 ± 3.81	14.75 ± 0.35	14.16 ± 1.60	0.87	≤50 meq/kg
Moisture (%)	21.25 ± 0.35	19.50 ± 0.00	20.33 ± 0.76	20.58 ± 1.66	20.62 ± 0.53	21 ± 1.73	0.74	≤21%
EC (ms/cm)	0.17 ± 0.02	0.19 ± 0.03	0.23 ± 0.10	0.12 ± 0.02	0.21 ± 0.04	0.18 ± 0.03	0.38	≥0.7 ms/cm
Ash (%)	1.05 ± 0.21	0.85 ± 0.07	0.99 ± 0.10	2.06 ± 0.65	1.05 ± 0.35	0.66 ± 0.11	0.01	≤0.6 g/kg
HMF (mg/kg)	389.18 ± 296.95	316.86 ± 205.54	319.63 ± 268.43	331.26 ± 263.38	318.78 ± 214.42	516.26 ± 273.27	0.92	≤40 meq/kg
Proline (mg/kg)	161.48 ± 49.83	195.19 ± 19.60	103.66 ± 50.59	329.66 ± 425.02	140.17 ± 49.99	159.93 ± 70.89	0.82	≥180 mg/kg
Diastase (DN)	9.45 ± 1.48	5.95 ± 1.20	8.61 ± 1.99	6.9 ± 0.55	10.35 ± 1.48	6.15 ± 0.72	0.02	≥3 (DN)
Invertase (IN)	6.8 ± 0.84	8.21 ± 11.00	3.10 ± 0.48	5.7 ± 0.95	8.35 ± 1.20	9.66 ± 0.45	0.39	≥10 (IN)

Means in the same column with different letters are significantly different at $p < 0.05$.

17.75–28.68(DN) which are almost like that of current work. Diafat et al. (2017) analyzed diastase 129.49–43.67 (DN) was above the range of present study.

Invertase is a natural honey enzyme which is commonly used for determinant of freshness. In current study the invertase activity ranges from 58.55 to 81.9 (IN) and 3.10–9.66(IN). All the fresh honey samples showed a significant result as compared branded samples. Boussaid et al. (2018), Parvanov et al. (2012) and Lichtenberg-Kraag (2012) reported invertase number 46.25–184.68 (IN), 1.47–15.2(IN), 23.91–0.02(IN) and 86.95–33.76(IN) and the same range as found in the present research. Over all the Invertase activity indicate storage and processing condition.

3.2. Mineral composition

The mineral composition of the fresh fifty honey samples were also analyzed as shown in Table 2. In general, the most abundant macro elements found in the honey samples were Potassium and Sodium ranging from 166.5 to 642 and 211 to 579.6 ppm respectively. Boussaid et al. (2018) showed the sodium range from

497.54 to 362.55 ppm and 251.34 to 521.22 ppm almost same as found in present research. Ca+ level of all the fresh honey samples was below the international limit (200–2300 ppm). The micro minerals such as Cobalt, Manganese and Barium were found in normal range, while Chromium was found less according to standard range (>1 ppm) respectively. Khaliq and Swaileh (2017) analyzed potassium and sodium concentration range between 183.86 ppm and 104.66 ppm which is lower values as compared present research. Mineral results showed that fresh honey is rich in nutritive elements and free for toxic metals.

4. Conclusion

The results of this study indicated that the physiochemical characteristics of fresh honey samples were within recommended limits of international standards then branded samples. Evidence showed that the freshness and purity of fresh honey was due to dominant flora. The trace amount of heavy metal like Pb, Cr and Mo in all fresh honey samples showed the clean environment, while the richness in other essential metals represented the high nutritional values of Pakistani honey.

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