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Research Article

Investigation of Antioxidant Activity and Physicochemical Properties of Local and Branded Honeys in Sindh, Pakistan

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Abstract: Honey is the sweetest natural product because it has unique properties, which are collected from dissimilar plant materials by bees. Honey is used for nutritional purposes for human consumption and is also used for medicinal and industrial purposes. Honey is also an important product in international markets. The present study was conducted to analyze the physicochemical and antioxidant activities of different honey samples in Sindh, Pakistan. Fifteen honey samples were analyzed for parameters, including pH, electrical conductivity, moisture, viscosity, ash percentage, density, and antioxidant activity. The average values were as follows: pH, 3.78; electrical conductivity, 2,492 μS/cm; viscosity, 12.34 mPa; moisture, 15.99%; density, 0.78; and antioxidant activity, 19.12 mg/100 g.

Keywords: antioxidant activity, local and branded honey, phyicochemical analysis, Sindh

1. Introduction

Honey is one of the natural sweetest substance produced by honey bees, from nectar of different plants (blossoms) and from the excretions of living parts of the plants.[1] The chemical composition of honey depends single or multi floral types, geographical, botanical origin of the nectars and different environmental conditions. Numerous natural components of honey used as an indicator to determine the floral, botanical and geographical origin of the product. Basically, natural honey is viscous and sticky liquid which content of 80-85% carbohydrate (fructose and glucose), 15-17% water, 0.1-0.5% protein, 0.2% ash and also trace level of minerals, amino acids, vitamins and enzymes as well as some other constituents like phenolic compound and antioxidants also present.[2,3] The honey is used as a different medicinal purposes in the world.[1] The major components of honey are approximately equal in all honey samples, the chemical and physical properties of natural honeys change according to the types of plants and different factors that may affect the properties of the honey.[4,5]

Honey is an oldest well-known as medicines and it was appreciated extremely in the world. Honeybees are possessed enzymes that catalyzed the sucrose into fructose and glucose. Honey in fact, is mainly a combination of these three sugars. The honey is used for several diseases like urinary, respiratory, gastrointestinal, ulcers, skin, wounds, dandruff, eczema, psoriasis diseases.[6,7]

The main objective of the present study to examine the quality of local and branded honey in the study area because no any previous work were reported on the physico-chemical and antioxidant activity of the honey in the Sindh, Pakistan.

1.1 *Review of literature*

There is no work reported on the antioxidant activity and physicochemical on the honey in Sindh but less work was reported in the Pakistan.

Zahoor et al.[8] determined the antibacterial, antifungal and antioxidant activities of honey, collected from Timergara (Dir, Pakistan).

Ahmed et al.[9] analyzed the physiochemical, biochemical, minerals content and antioxidant potential of national and international honeys in Pakistan and results were found between pH 3.38 to 4.28, moisture 10.10 to 19.2 (%), ash 0.04 to 0.22 (g/100 g).

Gulfraz et al.[10] quality assessment of various types of honey of Pakistan and results were found between pH 3.32 to 6.5, moisture 18.0 to 36.8 (%), ash 0.13 to 0.44 (g/100 g).

Farooq Khan et al.[11] physical and spectroscopic characterization of Pakistani honey from Pakistan and results varied from pH 4.2 to 4.3, moisture 16.60 to 16.7 (%), ash 0.30 to 0.38 (g/100 g), density 1.50 to 155 g/cm.

Lanjwani and Channa[12] minerals content in different types of local and branded honey in Sindh, Pakistan and analyzed different minerals like Na, Ca, K, Mg, Fe, Cu, Zn, Mn, Ni, Cr, Cd, Co, Pb.

2. Materials and methods

2.1 *Sample collection and preparation*

The total fifteen (15) honey samples were collected from different parts of Sindh, 8 honey samples were collected from different locations of the study areas and 7 samples were purchased from local market Hyderabad Sindh (Table 1)and analyzed for antioxidant activity, electrical conductivity, pH, ash%, moisture, specific gravity and viscosity. All honey samples were freshly collected in the clean containers and labeled numbers, date of collection and place and samples were kept at normal temperature until analyzed.

Table 1. Name of sampling stations of honey samples

Sample stations	Sampling stations name	Name of branded company
1	Local honey	Collected by local peoples
2	Local honey	Collected by local peoples
3	Local honey	Collected by local peoples
4	Branded honey	Purchase from local market Hyderabad
5	Local honey	Collected by local peoples
6	Local honey	Collected by local peoples
7	Local honey	Collected by local peoples
8	Local honey	Collected by local peoples
9	Local honey	Collected by local peoples
10	Branded honey	Purchase from local market Hyderabad
11	Branded honey	Purchase from local market Hyderabad
12	Branded honey	Purchase from local market Hyderabad
13	Branded honey	Purchase from local market Hyderabad
14	Branded honey	Purchase from local market Hyderabad
15	Branded honey mixed rayal jelly	Purchase from local market Hyderabad

2.2 *Samples analysis*

The pH was examined by (Orion 5 Star) pH meter, kinematic viscosity, specific gravity, moisture content and ash content and antioxidant activity were analyzed using standard analytical methods. The electrical conductivity was analyzed with Orion 115 conductivity meter.[13,14] The Viscosity was analyzed by using ASTM D method[15] the constant temperature was used to change the dilutions of honey, 5 gram honey sample was taken and diluted by Xylene with percentage 50%, then honey sample was put into the viscometer which was already maintained at hot bath at 40 °C and 60 °C, and then viscosity was measured. Moisture content of honey was determined by using methods Association of the Official Analytical Chemists.[16] The 2 g of sample was taken in the china dish, and placed in oven at 80 °C for 2 to 3 hours until constant weight was achieved. The content of moisture was expressed as loss in weight of wet honey samples.

Moisture content was calculated as follows

$$\% \text{ Moisture (w/w)} = \frac{\text{Wt: of wet Sample} - \text{Wt: of dry Sample}}{\text{Wt: of wet Sample}} \times 1{,}100 \qquad (1)$$

Ash content in different honey samples were determined by using method.[17] The 4 g of honey sample was taken in crucible and kept in a muffle furnace for 4 hours at 550 °C. Then crucible was cooled in the desiccators and then weighed. Percentage % of ash content was determined by the resulting inorganic residue. Weight of ash obtained in percentage as follows

$$\% \text{ Ash} = \frac{W_t : \text{of Ash}}{W_t : \text{of wet Sample}} \times 100 \qquad (2)$$

The density of honey samples were analyzed with the help of hydrometer cylinder. The honey sample was transferred to hydrometer cylinder, which was already set at same temperature. When temperature reached at equilibrium position thenthe hydrometer scale was observed and results were noted. The antioxidant activity was determined used method.[18] Taken 0.2 mL of samples in the test tubes and added 2 mL of 0.6M H_2SO_4, and 28 mM sodium phosphate and also 4 mM ammonium molybdate was added in duplicate. After that tubes were capped using aluminum foil and kept in water

bath at 96 °C for 95 minutes than samples were cooled at room temperature. The blank was prepared by using same procedure using deionized water instead of sample and absorbance was carried at 695 nm with the help of Ultraviolet (UV)-visible Spectrophotometer. Standard calibration curve was prepared by using different concentrations (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 ppm) of tocopherol. The statistical analysis like correlation coefficient, principal component and cluster analysis were also determined to interpretive the data using SPSS version 22 software.

3. Results and discussion

The seven parameters of 15 local and branded honey samples were analyzed to check the quality of honey of study area Sindh.

3.1 *Antioxidant property, viscosity and moisture contents in honey samples*

Honey is used subsequently from long period both domestic and medical uses, recently antioxidant activity has come to attention, with increasing demand of antioxidant activity in food, honey is a famous as a source of the antioxidant activity.[19] The oxidative stress results lack of balancing chemical reaction among the production of free radicals and also natural protective effect of our body resulting disruption of genetic structure and cellular damage.[20] The antioxidant activity of the honey are phenols, chyrsin, quercetin, hesperetin and maillard products called melanoidins.

The antioxidant activity of honey was found from 11.09 to 40.08 mg/100 g. The lowest level of antioxidant activity was founded in sample 10, 11.09 mg/100 g and highest was in sample 6, 40.08 mg/100 g (Table 2, Figure 1). It is observed that antioxidant activity of local honey were higher than branded honey which were purchased from local market Hyderabad Sindh.
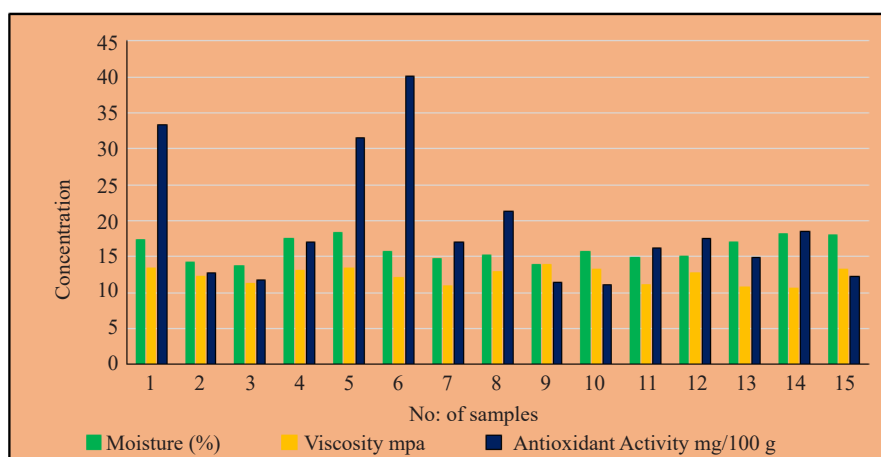

Figure 1. Results of viscosity, moisture and antioxidant activity of honey samples

Viscosity of honey is depend on the moisture%, temperature, existence of crystals and colloidal particles in the honey product. Various studied have been conducted in the viscosity of honey as role of the temperature at the specific moisture%.[21,22] The moisture content in the honey due to the environmental situations and also handling from beekeepers at time of harvest and it may vary in every year. The higher moisture content due to the crystallization in many kinds of the honey.[23] The viscosity content of the honey varied from 10.54 to 13.89 (mpa). The lowest level of viscosity was founded in sample fourteen 10.54 (mpa) and highest was in sample nine 13.89 (mpa). Usually, as rises of temperature, viscosity decrease because there is lower molecular friction and also decrease the hydrodynamic forces. The moisture content in the honey is very important parameter, contributing its stability against granulation and fermentation during storage.[24] The current results of moisture content were between 13.8 to 18.3%. The lowest level of moisture was founded in sample three 13.8% and the highest was in sample five 18.3%. The moisture content of honey varied in different harvest period (Table 2, Figure 1).

3.2 *Physico-chemical properties*

The Electrical Conductivity (EC) is analyzed to check the flow of current from the sample and ions present in the samples. EC is the important physico-chemical determination and for the validation of honeys.[25] The results of electrical conductivity was found from 1,275 to 4,205 µs/cm (Table 2, Figure 2). The highest result was observed in sample 5 and lowest was in sample 3, high value of electrical conductivity in the honey samples may be due to the environmental condition of area from honey samples were collected. The pH is defined as concentration of hydrogen ion, it measures the acidity and alkalinity of the sample.[26]
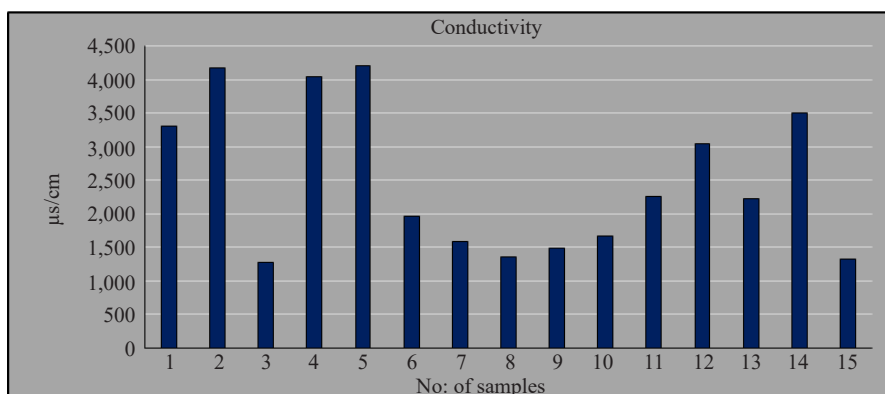
Figure 2. The electrical conductivity results of honey samples

The pH was observed from 3.21 to 4.31 (Figure 3), lowest pH value was found in sample 3 while highest was in sample 2. The pH of all samples were acidic in nature. The Ash content is one of the most important parameter related with geographical and botanical origins of the honey samples. The ash content in honey is normally less and due to the nectar composition of the plants.[27] The ash content of honey samples varied from 0.30 to 0.46 g/100 g. The lowest level of ash was founded in sample six 0.30 g/100 g and the highest was in sample fourteen 0.46 g/100 g. The density is temperature dependent and density of honey is higher than other liquid. The density of honey may also increase with the passage of time, if it is more volatile and less dense than components may lost. After significant loss, the density of some honey will rise. It is described as the mass/volume of a constituent. It is mostly described for samples in the units of g/mL and g/cm^{-1}, and also less in the unit of kg/m^3. The density results of honey samples varied from 0.873 to 0.992 g/cm^{-1}. The lowest level of density was founded in sample fourteen 0.873 g/cm^{-1} and highest was in sample seven 0.992 g/cm^{-1} (Table 2, Figure 3).

Table 2. Results of antioxidant activity and physico-chemical parameters of honey samples

Sampling stations	Cond µs/cm	pH	Ash (g/100 g)	Moisture (%)	Density g/cm^{-1}	Viscosity Mpa	Antioxidant activity mg/100 g
1	3,305	3.82	0.32	17.3	0.851	13.34	33.4
2	4,170	4.31	0.31	14.2	0.793	12.32	12.75
3	1,275	3.21	0.38	13.8	0.843	11.22	11.74
4	4,040	3.81	0.41	17.6	0.791	13.10	17.07
5	4,205	3.76	0.45	18.3	0.754	13.43	31.5
6	1,965	3.62	0.30	15.7	0.799	12.11	40.08
7	1,585	3.45	0.38	14.8	0.892	10.98	17.05
8	1,350	3.28	0.35	15.2	0.797	12.98	21.35
9	1,490	3.87	0.39	13.9	0.722	13.89	11.49
10	1,670	4.01	0.33	15.8	0.711	13.21	11.09
11	2,250	3.92	0.40	14.9	0.786	11.12	16.18
12	3,035	4.10	0.43	15.0	0.776	12.78	17.46
13	2,230	3.56	0.44	17.1	0.689	10.79	14.87
14	3,495	4.23	0.46	18.2	0.683	10.54	18.59
15	1,325	3.79	0.34	18.0	0.821	13.24	12.26
Minimum	1,275	3.21	0.3	13.8	0.683	10.54	11.09
Maximum	4,205	4.31	0.46	18.3	0.892	13.89	40.08
Mean	2,492.67	3.78	0.38	15.98	0.78	12.34	19.12
Standard deviation	1,107.2	0.32	0.05	1.61	0.06	1.12	8.88
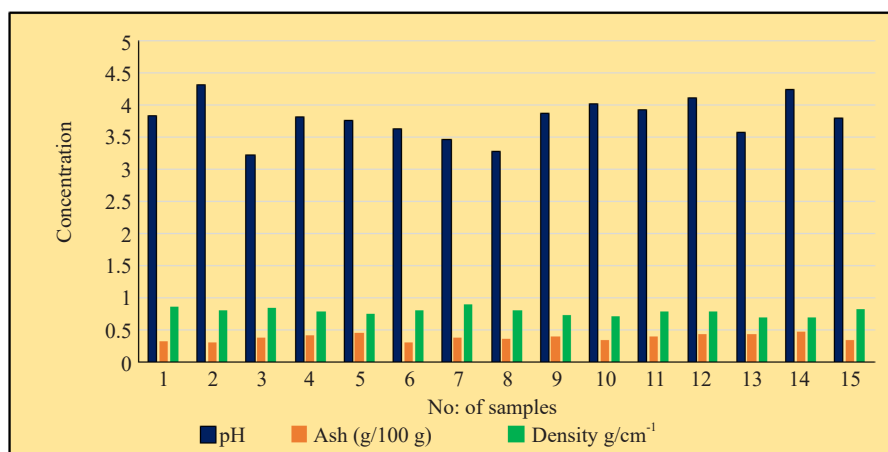
Figure 3. Results of pH, ash and density of honey samples

4. Statistical analysis

4.1 *Coefficient of correlation*

Coefficient of correlation is used to check the degree of similarity between the dissimilar variables.[12] The coefficient of correlation among 7 physicochemical parameters pH, electrical conductivity, ash, moisture, density, viscosity and antioxidant activity were calculated by computer program SPSS Statistics 22 software. The results were based on average value and examine relationship between different variables, one variable exhibits the prediction of other (Table 3). The results indicate that antioxidant activity was less correlated with EC, moisture, density, viscosity and negative correlated with pH and ash. EC was moderate correlated to pH (0.5-0.7), less correlated to ash, moisture, viscosity and antioxidant activity (< 0.5), pH was less correlated with ash, moisture and viscosity and negative correlation to density and antioxidant activity. Ash was less correlated to moisture but negative correlated to others parameters. Moisture and density were less correlated to viscosity and antioxidant activity, viscosity was less correlated to antioxidant activity.

Table 3. Correlation determination of seven parameters of the honey samples

Correlation matrix							
Correlation	Conductivity	pH	Ash	Moisture	Density	Viscosity	Antioxidant
Conductivity	1.000						
pH	0.594	1.000					
Ash	0.296	0.078	1.000				
Moisture	0.434	0.174	0.323	1.000			
Density	-0.178	-0.441	-0.469	-0.268	1.000		
Viscosity	0.094	0.136	-0.319	0.091	0.030	1.000	
Antioxidant	0.282	-0.140	-0.182	0.343	0.196	0.139	1.000

4.2 *Cluster analysis*

Hierarchical cluster analyzed to examine the similarity among the groups.[28,29] The dendrogram grouped all 15 sampling stations into four statistically clusters. Cluster 1 comprises three samples 11. 13 and 6, cluster 2 contains six samples 8, 15, 3, 7, 10, 9 and cluster 3 comprise three samples 2, 5, 4 and cluster 4 contains three samples 1, 14, 12 (Figure 4). It is observed that cluster 4 was highest results of parameters than other clusters, cluster 3 were higher results

of parameters than cluster 2 and cluster 2 were higher results of parameters than cluster 1.
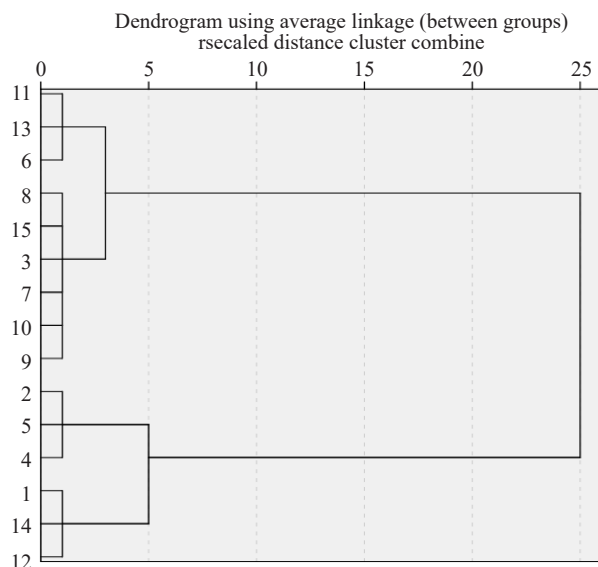
Figure 4. Cluster analysis of honey samples

4.3 PCA

Table 4. Principal Component Analysis (PCA) of the parameters of honey samples

Rotated Component Matrix			
Parameters	Components		
	PCA (1)	PCA (2)	PCA (3)
Conductivity	0.578	0.620	0.026
pH	0.888	0.063	0.181
Ash	0.325	0.173	-0.812
Moisture	0.242	0.769	-0.218
Density	-0.701	0.026	0.391
Viscosity	0.229	0.130	0.749
Antioxidant	-0.320	0.807	0.236
Eigenvalues values	2.314	1.599	1.208
% of Variance	33.052	22.849	17.252
Cumulative %	33.052	55.901	73.153

Principal Components Analysis (PCA) was carried out using SPSS 22 Software. It is a tool which attempts to clarify the variance of the dataset of inter correlated variables with smaller set of the independent variables.[29] PCA extracts eigenvalues from covariance matrix of original variables and is weighted linear combination of the original variables. The rotated components matrix for 7 parameters for the honey samples were shown in (Table 4). It includes loading components for rotated matrix, eigenvalues for every component and cumulative% of variance explained in each component. It shows that 3 principal components account together for 71.153% of the total variance of the dataset. In which the first component is 33.052%, second component 55.901% and third component 71.153% of total variance.

The eigenvalues of three components are greater than 1, and can be used to assess the dominant hydrogeochemical process. The component 1 which is based on loading 33.052% indicated for parameters pH, electrical conductivity, ash, moisture, density, viscosity and antioxidant with negative to positive high loading (negative to 0.888), The component 2 which has 22.849% loading indicated high loading for moisture and antioxidant (0.769, 0.807 respectively). The component 3 which has 17.252% loading with high positive loading for viscosity (0.749), less positive loading for pH, electrical conductivity and antioxidant.

5. Conclusion

The present research work total 15 samples were analyzed for 7 parameters. The pH level was found in acidic nature but found between in the acceptable range. The electrical conductivity was found in higher concentration in half of honey samples. The analyzed honey samples were rich in moisture and viscosity contents and has good antioxidant activity in samples but ash% were founded slightly low. The statistical analysis like correlation coefficient, Principal Component Analysis (PCA) cluster analysis were also determined to interpreting the results. The results indicate that the samples were compare favorably with others samples in various portions of the study area and under the limits of international standards. It is observed that the results of local honey rich in concentration of parameters but founded less concentration in branded honey which were purchased from local market. It is reveal that local honey is more favorable than branded honey but branded honey may be used for nutritional purposes and human consumption.

Conflict of interest

The authors declare no conflicts of interest in this research.

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