Minerals content in different types of local and branded honey in Sindh, Pakistan

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ABSTRACT
The 15 honey samples were collected from different areas of Sindh, 8 samples were collected from local honey and 7 samples were purchase from local market Hyderabad Sindh. The 13 minerals were analyzed like potassium (K), magnesium (Mg), sodium (Na), calcium (Ca), manganese (Mn), iron (Fe), cadmium (Cd), nickel (Ni), copper (Cu), lead (Pb), chromium (Cr), zinc (Zn) and cobalt (Co). The results of the major and trace elements in honey samples were found in the range from Na 77.5–200 mg/kg, K 225–439 mg/kg, Ca 46.1–98.1 mg/kg, Mg 31.3–73.8 mg/kg and trace elements Fe 2.98–16.2 mg/kg, Zn 1.11–4.1 mg/kg, Co 0.01–0.23 mg/kg, Cu 0.08–0.33 mg/kg, Mn 0.12–0.95 mg/kg, Cr 0.012–0.10 mg/kg, Ni 0.06–0.33 mg/kg, Pb 0.01–0.14 mg/kg, Cd 0.01–0.38 mg/kg. The results of K was found higher while the chromium was found lowest value in Sindh honeys. The statistical analysis correlation determination, principal components analysis and cluster analysis determined to evaluate the data.

1. Introduction
Honey is the sweetest natural food, honeybees collected this sweetest food from nectar of flowers and plants. The honeybees gather this sweet substance and collect it with exact materials of their store, own, leave in comb of honey to mature and ripen (White and Doner, 1980). Honey is mostly monosaccharides glucose and fructose but it also comprises pigments waxes, minerals, aromatic substances, amino acids proline and pollen grains (Bogdanov et al., 1999; Qiu et al., 1999). Though, the presence of other components like vitamins, enzymes and other components in honey was described by different researchers (Coco et al., 1996).

Honey is a supersaturated sweet natural viscous liquid, around 200 substances have been reported in honey natural liquid but composition of honey especially its secondary metabolites, quality of the honey can be changed by external factors, seasonal and environmental factors, storage, processing and handling (Gheldof et al., 2002; Khalil et al., 2012; Saxena et al., 2010). The major substances of honey are sugars (glucose 31%, fructose 38%), vitamins (ascorbic acid), moisture (10–20%), minerals (potassium, sodium, calcium, magnesium, phosphorus, organic acids (glucronic acid and acetic acid).

The different amount of major and trace minerals present in honey which is depend on the arrangement of the soil, many types of the minerals, floral plants are entered into the plants by the roots and reached to nectar and then finally reached into the honey (Anklam, 1998). Other factors, environmental pollution, beekeeping practices, honey processing contribute the content of minerals in the honey (Pohl, 2009). The contents of mineral also depends on the geographical area, climatic conditions and floral sources, iron and copper also have anti-oxidant or reducing properties.

Trace minerals are useful if present in low concentrations, but higher levels of tarce minerals contribute to harmful effect in the humans. The toxicity of minerals may be occurs due to the incapability of minerlas to be metabolized competely by human body, leading to gathering in animal or tissues of human without competely destroyed or inactivated (Ajibola et al., 2012). Trace elements caused health problems include respiratory disorders, headaches, metabolic abnormalities, vomiting and nausea. For instance, lead (Pb) may cause damage to human nervous system, brain, kidney and red blood cells. According to Agency for Toxic Substances and Disease Registry, trace elements dangerous include Pb, Cd and As, arsenic poisoning is relatively less common in the honey because of lower use of honey, but Pb and Cd contamination is commonly reported (Bogdanov, 2006).

Honey contains minerals, the amount of their contents varies depend on the geographical and botanical origins of honey. The reported some research publication about minerals coentns in honey, which include...
major (Ca, Mg, Na, K, Cl, P, S) and trace minerals (Zn, Al, Mn, Pb, Cd, Cu, Ti, Co, Nb, Bi, Be, Pt, V, Pd, U, Fe, Te, Mo, Hf, Sn, La, Sm, I, Tb, Dy, Th, Nd, Pr, Lu, Yb, Gd, Er, Ho, Ce, Cr, B, As, Br, Cd, Se, Hg and Sr (Tuzen and Soylak, 2005; Devillers et al., 2002; Birge and Price, 2001; Nanda et al., 2003).

The main objectives of the current study was to examine the major and trace minerals as well as to compare the quality of the various types of local and branded honey from Sindh Pakistan.

2. Materials and methods

2.1. Honey samples collection

In Sindh no any scientific apiculture form available, honeybee keep own place in plants and flowers of the local areas. The 15 honey samples were in different areas in Sindh Pakistan, eight local honey samples were collected from different areas and difference sources with the help of peoples of those areas of Sindh (Table 1, Fig. 1). The honey samples were collected in 250 ml plastic bottles correctly labeled with numbers, names and date of collection. The unwanted substance such as dead bees, wax sticks and also combs particles were also removed before analyzing these trace minerals, local honey samples were collected in countryside areas of Sindh and seven samples were purchase different varities of imported honey from local market hyderabad Sindh. The analysis of samples were carried out within two months and all honey samples were stored at 4°C.

2.2. Reagents and chemicals

All chemicals and reagent used in current study were of analytical grade. The nitric acid (HNO₃) hydrogen peroxide (H₂O₂) (Merck, Germany) were used for digestion the honey samples for minerals analysis. The stock standard solutions (1000 ppm) for the minerals like (Mg, Na, Ca, K, Fe, Cr, Ni, Cd, Co, Pb, Cu, Zn, Mn) were also used for the preparation of the calibration standards for instrumental analysis. The deionized water and double distilled was used throughout for the preparation of reagents, stock solutons, dilution, washing and rinsing apparatus for analysis.

2.3. Acid digestion of honey for mineral contents

The five gram of honey sample was taken in to the 100 ml beaker and added 9 ml of pure HNO₃ and 3 ml of H₂O₂ acids (Merck, Germany) and kept sample in hot plate for digestion. After digestion the samples were analyzed...
removed from hot plate and then were cooled and then honey samples were transferred into the volumetric flask (25 ml) and made final volume up to mark with the deionized water. The prepared honey samples were kept in the refrigerator. The samples were determined by the flame atomic absorption spectrophotometer (FAAS) (Qadir et al., 2015; Pohl et al., 2017).

2.4. Instruments conditions using for metals analysis

The elements Na, K, Ca, Mg, Cr, Co, Ni, Mn, Zn, Pb, Fe, Cu and Cd were examined using (Perkin Elmer AA-800, Singapore) flame atomic absorption spectrophotometer (FAAS) at mentioned conditions by manufacturer. The minerals examination was measured in triplicate \( n = 3 \) with delay time 4 s and integration time 4 s (Lanjwani et al., 2019; Pohl., et al., 2017). Instrument (FAAS) was controlled by the Win Lab software with computer (Table 2).

2.5. Statistical analysis

The statistical examination minimum, maximum mean and standard deviation value of the results data were analysed by Microsoft of office excel 2013. The coefficient of correlation \( r \), principal component analysis (PCA) and hierarchical cluster (CA) analysis among metals ions were calculated using computer program (SPSS 22 Inc., Chicago, IL, USA) (Khuhawar et al., 2018).

3. Results and discussion

There are many minerals contents in honey but we analyzed most abundant minerals such as major minerals such as Na, Ca, K, Mg (Table 3), trace minerals Zn, Fe, Cr, Co, Mn, Ni, Cd, Pb and Cu (Table 4). The results of potassium was found highest from all other elements. It is may be due to the higher levels of potassium present in the tissues of plant, presence of minerals in honey good for the humans.

### Table 3

| Sample Id | Na mg/kg | K mg/kg | Ca mg/kg | Mg mg/kg |
|-----------|----------|---------|----------|----------|
| 1         | 180      | 422     | 80.2     | 67.4     |
| 2         | 192      | 435     | 85.24    | 69.7     |
| 3         | 102      | 240     | 66.1     | 54.3     |
| 4         | 200      | 439     | 98.1     | 69.7     |
| 5         | 177      | 428     | 79.2     | 73.8     |
| 6         | 143      | 288     | 75       | 70.4     |
| 7         | 148      | 305     | 78.9     | 68.5     |
| 8         | 136      | 278     | 72.7     | 61.6     |
| 9         | 98.4     | 238     | 48.2     | 34.8     |
| 10        | 81.1     | 228     | 46.1     | 31.3     |
| 11        | 86.8     | 295     | 81.7     | 55.6     |
| 12        | 88.6     | 271     | 75.3     | 68.7     |
| 13        | 95.2     | 285     | 77.4     | 63.4     |
| 14        | 100      | 295     | 83.1     | 70.3     |
| 15        | 77.5     | 225     | 67.2     | 51.1     |

| Min-Max   | 77.5-200 | 225-439 | 46.1-98.1 | 31.7-71.8 |

| Mean (St.d) | 127.04 (43.48) | 311.47 (78.77) | 74.39 (13.38) | 60.86 (13.19) |

### Table 4

| Sample Id | Fe mg/kg | Zn mg/kg | Co mg/kg | Cu mg/kg | Mn mg/kg | Cr mg/kg | Ni mg/kg | Pb mg/kg | Cd mg/kg |
|-----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| 1         | 10.6     | 2.14     | 0.018    | 0.22     | 0.75     | 0.019    | 0.13     | 0.028    | 0.38     |
| 2         | 14.33    | 3.64     | 0.019    | 0.28     | 0.95     | 0.034    | 0.25     | 0.015    | 0.18     |
| 3         | 16.2     | 2.89     | 0.012    | 0.24     | 0.34     | 0.012    | 0.31     | 0.012    | 0.065    |
| 4         | 12.2     | 3.68     | 0.032    | 0.33     | 0.45     | 0.032    | 0.21     | 0.023    | 0.056    |
| 5         | 11.4     | 4.10     | 0.032    | 0.32     | 0.78     | 0.014    | 0.22     | 0.14     | 0.087    |
| 6         | 9.81     | 3.65     | 0.23     | 0.21     | 0.92     | 0.076    | 0.18     | 0.06     | 0.012    |
| 7         | 10.2     | 2.91     | 0.098    | 0.19     | 0.54     | 0.083    | 0.33     | 0.08     | 0.023    |
| 8         | 13.8     | 2.34     | 0.043    | 0.20     | 0.12     | 0.101    | 0.28     | 0.034    | 0.011    |
| 9         | 6.21     | 2.21     | 0.087    | 0.11     | 0.18     | 0.023    | 0.08     | 0.014    | 0.034    |
| 10        | 5.17     | 2.12     | 0.011    | 0.13     | 0.34     | 0.021    | 0.07     | 0.12     | 0.101    |
| 11        | 3.42     | 1.98     | 0.12     | 0.10     | 0.24     | 0.054    | 0.10     | 0.13     | 0.034    |
| 12        | 7.92     | 1.43     | 0.092    | 0.15     | 0.32     | 0.026    | 0.16     | 0.14     | 0.043    |
| 13        | 8.23     | 2.18     | 0.054    | 0.09     | 0.35     | 0.076    | 0.14     | 0.091    | 0.054    |
| 14        | 3.13     | 1.21     | 0.023    | 0.08     | 0.40     | 0.065    | 0.09     | 0.075    | 0.11     |
| 15        | 2.98     | 1.11     | 0.027    | 0.12     | 0.20     | 0.087    | 0.06     | 0.087    | 0.014    |

| Min-Max   | 2.98-16.2 | 1.11-4.1 | 0.01-0.23 | 0.08-0.33 | 0.12-0.95 | 0.01-0.10 | 0.06-0.33 | 0.01-0.14 | 0.01-0.38 |

| Mean (St.d) | 9.04 (4.2) | 2.51 (0.9) | 0.06 (0.05) | 0.18 (0.07) | 0.46 (0.27) | 0.05 (0.03) | 0.17 (0.09) | 0.07 (0.05) | 0.08 (0.09) |
mg/kg, lowest value of Ca was found in sample 10 (branded Hashami honey) and highest found in sample 4 (Mango Tree). The Mg is also most abundant mineral and results was found from 31.3-73.8 mg/kg, the highest concentration was found in sample 5 and lowest found in sample 10. The K, Na, Ca and Mg found about more than 90% of total weigh of tested minerals. The results of major minerals K, Na, Ca and Mg were found higher concentration in local honey as compared to branded honey. The color of local honey samples was dark and viscous and color of branded honey was light and also less viscous.

The trace minerals also abundant in honey, it found in trace level as compare to major minerals The trace minerals also analysed in Sindh honey and found range from Fe 2.98–16.2 mg/kg, the higher concentration of Fe was found in sample 3 (Tooth brush plant) and lower concentration was in sample 15 (Islamic honey mixed with rayal jelly), Fe is essential for human metabolism, red blood cell and other functions. The zinc was found ranged between 1.11–4.1 mg/kg, lower level was found in sample 15 (Islamic honey mixed with rayal jelly) and higher level was found in sample 5 (Musk Willow tree honey), Zn is also essential for human, hair, skin and other functions. The cobalt was found range from 0.011-0.23 mg/kg, higher level of Co was found in sample 6 (Mango tree honey) and lower level was found in sample 10 (Hashami honey), Co is not essential for human, and no have any effect on human health at appropriate level. The concentration of Cu was found from 0.08-0.33 mg/kg, the lower concentration was found in sample 14 (Islamic honey) and higher concentration was found in sample 4 (Mango tree honey 1), Cu has no toxic effect on human health at the suitable limit. The Mn was found ranged from 0.12-0.95 mg/kg, the lowest results of Mn found in sample 5 (Guava tree honey) and higher was found in sample 2 (Acacia tree honey), Mn found lower levels as compared to the Fe and Zn. The Cr trace metal content in the honeys was found from range from 0.012-0.101 mg/kg, the lowest results of Cr found in sample 3 (Tooth brush plant) and higher was found in sample 8 (Guava tree honey), Cr found lower levels as compare to all other minerals Fe, Zn, Cu, Co, Ni, Cd and Pb. The Ni value found between 5.72 0.06 mg/kg, higher results of Cd found in sample 1 (Neem tree Honey) and lower was found in sample 8 (Guava tree honey). No any sample results of Pb and Cd was exceeded maximum suggested level (Pb 1.0 and Cd 1.0 mg/kg) respectively by European Commission (Byrne, 2000). The Cr was found less in all other minerals and K was found higher to all other minerals.

A review of literature showed that no work was reported minerals on the honey in Sindh but in less work was reported in the Pakistan, quality analysis and contamination in coastal line of Pakistan. The literature review showed that results of major elements were correlated to present study. Ahmed et al., 2016; Bibi et al., 2008; Farooq Khan and Maqbool 2008; Gulfraz et al., 2010) were reported the minerals contents in honey samples in Pakistan. The reported results were found K, Na and Mg, 340–1506.4, 71–856, 51–1035.5 and 18–72.02 mg/kg respectively, the results of major minerals were slightly higher than present values. The trace minerals also present slightly less than previous study, it may be due to the climate behaviour of study area. The K comprises 53% of the whole mineral composition in the honey samples. This is may be due to the higher levels of K present in the plant tissues. Nutritionally, existence of these minerals makes honey an excellent food for the humans, particularly for the children.

3.2. Pie chart

The pie chart draws for major and trace minerals of honey samples with the help of (Microsoft excel 2013) (Fig. 2). A diagram is used to identify the % of parameters. The pie chart showed major element like K, Na, Ca and Mg contain 98% of total minerals contents and trace minerals like Fe, Zn, Cu, Pb, Cd, Mn, Co, Ni and Cr only 2% contents. The K contain higher 53%, Na 22%, Ca 13%, Mg 10%.

3.3. Coefficient of correlation (r)

The coefficient of correlation (r) between 13 minerals (K, Ca, Na, Mg, Cu, Mn, Zn, Fe, Cd, Ni, Pb, Co and Cr) were calculated by using computer program SPSS Statistics (version 22) software (Table 5). Results of parameters are based on mean values and determine relationship among two variables (Khuhawar et al., 2018). The one variable shows the prediction of the other variable. If variable is (<5) it means which is less correlated, (0.5–7) moderate correlated, (>0.7) good correlated. The result shows that Na was good correlated with K, Zn, Cu and Mn (>0.7), Moderate correlated with Ca, Mg, Fe and Ni (0.5–0.7) and less (<0.5) and negative (-) correlated with other parameters. K was good correlated with Ca (>0.879), Moderate correlated with Mg, Zn Cu, Ni, Mn and Cd. The Ca was good correlated with Mg (>0.7), but less correlated with other parameters. The Fe was good correlated with Zn, Cu, Ni (>0.9) and less and
negative correlated for other. The Zn was good correlated with Na (0.766) and Fe (0.702). But other parameters less and negative correlated to each other. The correlation results showed that major parameters were good and moderate correlated to each other.

3.4. Hierarchical cluster analysis

The cluster analysis (CA) was accomplished by SPSS version 22 software to identify the similarity between different groups. The level of the similarity in which explanations are joint together may be used to create a dendrogram. The dendrogram clustered all fifteen honey samples into 4 statistically clusters. The cluster 1 comprises four samples, cluster 2 contains four samples and cluster 3 also comprises four samples, cluster 4 contains three samples (Fig. 3). Cluster 1 is based on samples 1, 2, 4 and 5 (local honey samples). Cluster 2 contains four samples 3, 9, 10 and 15 (branded samples except sample 3). The cluster 3 is also based on four samples 11, 12, 13 and 14 (all branded samples). The cluster 4 comprises three samples 6, 7 and 8 (local honey samples). The cluster 1

| Parameters | Na  | K   | Ca  | Mg  | Fe  | Zn  | Co  | Cu  | Mn  | Cr  | Ni  | Pb  | Cd  |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Na         | 1.000 |    |     |     |     |     |     |     |     |     |     |     |     |
| K          | 0.912 | 1.000 |     |     |     |     |     |     |     |     |     |     |     |
| Ca         | 0.617 | 0.725 | 1.000 |     |     |     |     |     |     |     |     |     |     |
| Mg         | 0.623 | 0.665 | 0.879 | 1.000 |     |     |     |     |     |     |     |     |     |
| Fe         | 0.656 | 0.281 | 0.398 | 1.000 |     |     |     |     |     |     |     |     |     |
| Zn         | 0.766 | 0.323 | 0.702 | 1.000 |     |     |     |     |     |     |     |     |     |
| Co         | -0.087 | 0.023 | 0.139 | -0.151 | 0.164 | 1.000 |     |     |     |     |     |     |     |
| Cu         | 0.850 | 0.470 | 0.452 | 0.826 | 0.783 | -0.132 | 1.000 |     |     |     |     |     |     |
| Mn         | 0.714 | 0.406 | 0.593 | 0.401 | 0.672 | 0.198 | 0.548 | 1.000 |     |     |     |     |     |
| Cr         | -0.187 | 0.160 | 0.174 | -0.214 | -0.254 | 0.332 | -0.309 | -0.228 | 1.000 |     |     |     |     |
| Ni         | 0.523 | 0.351 | 0.481 | 0.858 | 0.615 | -0.003 | 0.664 | 0.288 | 0.069 | 1.000 |     |     |     |
| Pb         | -0.414 | -0.056 | 0.010 | -0.541 | -0.257 | 0.165 | -0.484 | -0.118 | 0.040 | -0.327 | 1.000 |     |     |
| Cd         | 0.440 | 0.177 | 0.189 | 0.174 | 0.018 | -0.425 | 0.244 | 0.481 | -0.461 | -0.108 | -0.258 | 1.000 |
and 4 contain only natural honey samples, which showed these samples similar to each other and cluster 2 and 3 contains branded samples except sample 3, which correlated to each other. It is observed that cluster 4 showed higher results of parameters as compare to other cluster. Marchini et al. (2007) described the cluster analysis of different honey samples in Brazil and used cluster analysis for the determination of the data.

### 3.5. Scree plot

Scree plot of eigenvalues versus components number drawn with the help of SPSS 22 statistical software (Fig. 4). The scree plot the first component showed higher eigenvalues and component number 13 showed lowest eigenvalues. The eigenvalues decrease in order first component to last 13 component.

### 3.6. Principal component analysis (PCA)

PCA was measured by the help SPSS 22 version Statistical software. PCA is very powerful device that describes variance of the big dataset which inter linked variables with lesser set of the independent variables (Simeonov et al., 2003). The PCA determine eigenvalues from covariance matrix of the original variables, weighted of linear grouping of original variables. Rotated components matrix for 15 honey samples parameters of Sindh, are shown in (Table 6). It is include loading PCA for the rotated matrix, eigenvalues for the each component, cumulative percent and variance percent described by every component. It indicates that PCA account contains together for 84.526% of total variance of dataset. The first component eigenvalues 6.070 and variance% is 46.694, second component eigenvalues 2.032, cumulative 62.325%, variance 15.631%, third component eigenvalues 1.748, cumulative 75.775%, variance 13.450% 2.153% and fourth component eigenvalues 1.138, cumulative 84.526%, variance 8.751% of total variance and eigenvalues of first components were higher than others. PCA may be used to evaluate the dominant geochemical process. Marchini et al. (2007) described the Principal components analysis of different honey samples in Brazil and PCA used for the determination of the data.

#### Table 6

| Parameter | 1     | 2     | 3     | 4     |
|-----------|-------|-------|-------|-------|
| Fe        | 0.924 | 0.191 | 0.133 | -0.065|
| Ni        | 0.850 | 0.252 | -0.185| 0.107 |
| Cu        | 0.798 | 0.377 | -0.327| -0.026|
| Zn        | 0.706 | 0.288 | 0.400 | 0.408 |
| Pb        | -0.658| 0.059 | -0.019| 0.376 |
| Ca        | 0.146 | 0.930 | -0.117| -0.042|
| Mg        | 0.199 | 0.922 | -0.074| 0.125 |
| K         | 0.289 | 0.767 | -0.490| -0.136|
| Na        | 0.578 | 0.650 | 0.393 | -0.031|
| Cr        | -0.103| 0.198 | -0.833| 0.181 |
| Cd        | -0.059| 0.331 | 0.642 | -0.536|
| Mn        | 0.247 | 0.571 | 0.588 | 0.313 |
| Co        | -0.084| 0.046 | 0.153 | 0.872 |
| Eigenvalues| 6.070 | 2.032 | 1.748 | 1.138 |
| % of Variance| 46.694| 15.631| 13.450| 8.751 |
| Cumulative %| 46.694| 62.325| 75.775| 84.526 |
4. Conclusion

In this study major and trace minerals contents of honey samples from selected areas of Sindh, has been investigated. These major and trace metals were analyzed and measured by flame atomic absorption spectrophotometer. The results of minerals of this study indicated that potassium was highest level followed by sodium, calcium and magnesium in all honey samples. When comparing the present study values with other reported values in Pakistan and as well as other country almost they are comparable. The obtained results of the present study are in good agreement with reported for honey in Pakistan as well as other country. According to present research the local honey was darker and more viscos than branded company honey, branded honey was lighter and less viscos, the darker honey were richer in mineral contents as compare to lighter honey. Therefore the present work showed that local honey rich in mineral contents as compared to branded honey which was purchase from local market hyderabad Sindh. The obtained results of mineral contents of honey in order of decreasing K > Na > Ca > Mg > Fe > Zn > Mn > Cu > Ni > Cd > Pb > Co > Cr. Therefore obtained results of minerals (K, Ca, Na, Mg, Fe, Mn, Cu, Zn, Co, Cr, Ni, Pb and Cd) in present study indicated that the honey produced in selected areas of Sindh were suitable for the human health. The statistical analysis correlation determination, principal components analysis and cluster analysis determined to evaluate the data. The PCA showed and correlation coefficient showed that major element have higher positive loading to each other.

Declarations

Author contribution statement

Muhammad Farooque Lanjwani: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data. Fayyaz Ahmed Channa: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data. Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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