# Elemental Analysis of Multi Floral Honeys of E.G.Dist., Andhra Pradesh, India.

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*Abstract* -— **People are more health conscious nowadays, hence they are giving preference to the natural ones rather than synthetic products. So in order to fulfill the requirement of the present generation, we have chosen honey, which is having both medicinal and food values with zero adverse effects. Nearly 300 different chemicals are giving this valuable, unique quality of the honey, which is synthesized by the honey bees by collecting nectar from different kinds of flowers which is useful to the mankind and other organisms. We investigated honey sample and decided that the sample belongs to multifloral basing on the different frequency classes and frequencies of pollen morphocytes. The biological systems of living organisms require minerals for their biological activities, hence this paper deals with elemental composition of some multifloral honeys and their ash contents at different places. It also describes the importance of essential (micro to macro) and non essential elements (whose functions are unknown).**

**Keywords —** ***Meleto Palionology, Frequency classes, Pollen morpho types, Chromoproteins, Metal activators, Multifloral.***

## I. INTRODUCTION

The Honey is an excellent unique sweetening agent which contains both medicinal and food values and can be preserved for a long time without adding any preservative. This unique and excellent property gained by honey is from three different chemicals which are in colloidal suspension in nature. The properties gained by honey are its ingredients which are about 300 different chemicals out of which 30 micro to macro elements have been observed[1]. And these elements play a vital role in the biological reactions of living organisms. Even though their quantities are less, their importance is very essentially or high. We have investigated the elements (mineral contents) of the honey by recognizing the origin of honey. Origin of honey again investigated by pollen analysis using different pollen morphotypes.

We have randomly collected various floral honeys of East Godavari Dist., investigated and identified their maximum elemental composition.

## II. ELEMENTS AND ITS ROLE IN BIOLOGICAL REACTIONS

Geenrally human body requires 13 metal, out of which we have detected 9 micro elements in all EGH samples of honeys. Fe, Cu, Mn, Zn and Mg play very important role in biochemical reactions. These metals directly links with proteins and known as metalproteins or chromoproteins. These elements present in enzymes and acts as metal activators and helps in group transfer, redox and hydrolysis reactions of the organisms[1]. And the rest of the metals have their own significance in biochemical reactions of the organism. The human body requires Na, K, Mg and Ca are in macro levels and Zn, Cu, are in micro levels. Fe is in between micro and macro levels.

## III. MINERAL CONTENT OF THE HONEY

The present study determined ash content, electrical conductivity and 3 individual elements like K, Na, Mg, Mn, Cu, Fe, Zn and Ni of different honeys. The related data are presented, where the K / Na ratio is also included. The values of ash content are expressed as percent of honey weight, and those of individual elements are in ppm[9]. The ash content of honey is generally small ranging from 0.02 to slightly over 1% in the US honey ( white nd Doner 1980)[5], and from 0.16 to 0.21 % in Indian honeys( Wakhle 1998). The calcian honeys are reported to have a higher ash content 0.408 (Rodriguez et.al 1994). Wild honeys possesses more ash content than apiary honeys in India ( Dhadke et.al 1970). In a general belief dark coloured honeys contains more minerasl than light coloured honey (Kalimi and Sohonie 1964, Dhadke et.al.1970). The minerals contains both cations and anions.

## IV. POLLEN ANALYSIS OF SAMPLES

Honeybees collect nectar from flowers to make honey by concentrating the nectar and adding enzymes. Analysis of the pollen in honey (melliso-palynology) can be used to determine its origin. Honey samples were procured from different areas of East Godavari district, Andhra Pradesh, India in different seasons[2][8]. The samples were subjected to qualitative and quantitative pollen analysis following the methodology recommended by the International Commission for Bee Botany (ICBB) (Louveaux et al 1978)[6]. The pollen morphotypes were identified with the help of reference slides mentioned in the Central Bee Research Institute (CBRI, Pune) palynarium.

## V. METHODS

The pollen types recovered and identified were placed under four frequency classes as mentioned bellow. The three E.G.H samples were investigated for their origin by using pollen analysis of honey is known as Meleto palionalysis[3],[7].

- Predominant pollen type: More than 45% of the total pollen grains counted.
- Secondary pollen type: Between 16 and 45% of the total pollen grains counted.
- Important minor pollen type: Between 3 and 15% of the total pollen grains counted.
- Minor pollen type: Less than 3% of the pollen grains counted.

Fig 1a. Trifolium alexandrinum

Fig 1b. Helianthus annuus

Fig 1c. Syzygium cumini

Fig 1d. Carthamus tinctorius

The honey sample was treated as Unifloral if the prepared slide contains a predominant pollen morphotype. If several morphotypes are represented, the honey sample was termed as Multifloral [4].The plants and flowers of EGH1 is shown in Fig 1a-1d .

**TABLE I**. FREQUENCY CLASSES AND FREQUENCIES OF POLLEN MORPHOTYPES IN THE MULTIFLORAL HONEY SAMPLES OF THE PRESENT STUDY

| Honey type | Frequency class | Pollen morphotype | Frequency (%) |
|---|---|---|---|
| EGH1 | P | Trifolium alexandrinum | 58 |
| | S | Helianthus annuus | 37 |
| | I | Syzygium cumini | 14 |
| | M | Carthamus tinctorius | 1 |
| EGH2 | P | Limonia acidissima | 67 |
| | S | Delonix regia | 21 |
| | I | Syzygium cumini | 12 |
| | M | -Nil- | 0 |
| EGH3 | P | Brassica Juncea | 54 |
| | S | Litchi chinensis | 41 |
| | I | Poaceae | 5 |
| | M | -Nil- | 0 |

P= Predominant S= Secondary I= Important minor M= Minor

## VI. DETERMINATION OF TOTAL ASH (AOAC 1975):

About 25g of the well homogenated honey sample was placed in an ashing vessel of known weight. The materials in the vessel was carbonized under the hood initially on a low heat to prevent spattering. Then, the silica crucible was transferred to a muffle furnace maintained at $550^0$C and incarcerated for about 6hrs. The dish was taken out, cooled to room temperature and the ash was wetted with minimum amount of water, followed by the addition of a few drops of concentrated $HNO_3$.The contents were dried on low heat and again turned into ash at $550^0$C, till carbon free ash was obtained. The crucible was cooled down to $100^0$C and transferred to a desiccator for further cooling to room temperature and later weighed. (The ash was reserved for the estimation of mineral matter).

$$\%\ of\ Ash = \frac{Weight\ of\ Ash}{Weight\ of\ sample} \times 10 \qquad (1)$$

### *A. Preparation of sample solution suitable for mineral component (determination of ash):*

1) The ash obtained was dissolved in 10ml concentrated HCl, boiled and evaporated to near dryness on a hot plate.

2) The residue was redissolved in 10ml HCl of 2N normality by boiling gently and filtered through the fast ash less filter (Whatman 41) into 50ml volumetric flask. The residue and paper were washed thoroughly with water collecting all the filtrates into the same volumetric flask. The solution was made with 50ml with water mixed well.

Fig 2a. Limonia acidissima

Fig 2b. Delonix regia

3) The concentration of metals in ash solutions under consideration was measured directly or diluted with 0.5N HCl to obtain solutions within the range of standards.

*B. Detailed procedure for each metal as follows :*

i. *Determination of Calcium :* The determination was done by complexometric titration .

Reagents :

- Ethylene diamine tetra acetic acid salt (0.1ml).
- Murexid indicator ( ammonium purpurate ) .
- Sodium hydroxide solution ( 2N ) .

ii. *Determination of sodium and potassium:* Flame photometer was used for this purpose.

Reagents:

- Stock solution - 1000 ppm (1 ml = 1 mg ).
- Stock potassium solution -1000 ppm (1 ml = 1mg )

Procedure:

The sample was aspirated after waiting for a few minutes the readings of sodium and potassium were noted .

$$Sodium(mg/l) = \frac{Reading \times ppm\ of\ Na\ std.}{100} \quad (2)$$

$$Potassium(mg/l) = \frac{Reading \times ppm\ of\ K\ std.}{100} \quad (3)$$

iii. *Determination of Mg, Cu, Zn, Ni, Fe and Mn :* The ash samples of Mg, Cu, Zn, Ni, Fe and Mn were aspirated in the calibrated Atomic absorption spectrophotometer (A.A.S) and the concentrations of the elements in ppm were recorded. The parameters used for the elements are as follows.

**TABLE II**. Parameters used for the elements

| Element | Lamp Current (mA) | Fuel | Support | Wave length (nm) | Slit width (nm) | Reagent (1:1 and 1 ml stock solution) |
|---|---|---|---|---|---|---|
| **Mg** | 4 | Acetylene | Air | 285.5 | 0.1 | $HNO_3$ in Dist. Water |
| **Cu** | 4 | Acetylene | Air | 324.7 | 0.5 | $HNO_3$ in Dist. Water |
| **Zn** | 5 | Acetylene | Air | 213.9 | 1.0 | $HNO_3$ in Dist. Water |
| **Ni** | 4 | Acetylene | Air | 232.0 | 0.2 | $HNO_3$ in Dist. Water |
| **Fe** | 5 | Acetylene | Air | 248.3 | 0.2 | Hcl in Dist. Water |
| **Mn** | 5 | Acetylene | Air | 279.9 | 0.2 | $HNO_3$ in Dist.Water |

Fig 3a. Brassica Juncea

Fig 3b. Litchi chinensis

Fig 3c. Poaceae

Procedure :

The ash sample was aspirated in the calibrated A.A.S and the concentration of each element was noted in ppm .

$$Content\ of\ element\ (in\ ppm) = \frac{C \times D \times 50}{W} \qquad (4)$$

Where C = Concentration of metal (in µg/ml) read from instrument , D = Dilution factor , if the original ash solution (of 50 ml ) was distilled further , W = Weight ( in g ) of the sample taken for ashing .

## VII. Results

Among the three multifloral honey types of East Godavari district, EGH 1 had the lowest percentge (0.04%) of ash content. The individual elements Fe, Ni, Zn, Mn were comparatively rich in EGH 3( figure shown in Fig 3a-3c) . The lowest values of Fe, Ni, Mn, Na, K were realized with EGH 2 honey , and Zn in EGH2( figure shown in Fig 1c, Fig. 2a-2b).

The difference could be attributed to the difference in the mineral content in the soil of the different areas. Since plants obtain minerals in soil, aeration between the mineral content of honeys and of soil may be expected. The values of ash content obtained in recent study are comparable with similar data reported earlier for Indian honeys. The values of the East Godavari District honey are either close to or exceed the values of ash content reported for honeys in other countries like USA (White and Doner 1980) and (Whits et al. 1962 ). The Calcian honeys also appear to exceed the ash content values of Indian honeys.

Majority of honey types content of K greatly exceeded the other elements. The Molise workers found a positive correlation between pH and ash content of honeys and attributed the same to the high cationic content, particularly K that influences the Stalinized fraction of the acids. A similar positive correlation exists between ash content and pH of the honey types of the present study as seen above.

*Recognition:* Honey of multifloral can be recognized by its tart taste . It doesn't adhere to the paper. Multifloral honeys are synthesized by honey bee, by collecting nectar from different flowers.hence one cannot recognise its origin. It is always better to choose dark coloured honey for general use. For any medical usage preferably choose unifloral honey.

## VIII. Conclusion

Elemental analysis of some multifloral honeys of East Godavari Dist. Of A.P, India. We investigated three multifloral honeys for the elemental composition. All multifloral honeys contain all elements i.e, Na, K, Ni, Fe, Mg, Zn, Cu, Mn and Ca. However, some multifloral honeys are rich with some elements (Cu, Zn, Mn are trace elements).As the individual element ratio is less in EGH1, the ash content is also low. The major morphotype influences this citeria. Trifolium alexandrinum is dominating 58% over Helianthis annuus, which is 37%. Comparing with other the syzygium is very less. If we go through the importance in human biological system Ni functions are not completely established. However, it comes to know that Ni has an important key role in the human biology. Fe is the most important macro element of the life. K activity is most important in biological systems like Na, Zn meta improves mental ability. Na other elements present in this honey are very good electrolyte and who maintains body fluid osmotic pressure.

Hence we can recommend each floral honey as an additional food supplement accordingly instead of taking other food supplements. Because honey is a very fine mixture of different foods (components) which are essential for the human body and having no adverse results if we consume. Most of the honeys contain their flower color and smell. Different branded honeys are available in the market. They are pure, but we cannot say its origin.

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