

A Comparative Study of Carbohydrates in Unifloral Honeys of East-Godavari, A.P India

K. Hemalatha¹, P. Satyanarayana²

^{1,2}Department of Chemistry, Ideal College of Arts and Sciences, Kakinada-533003, India

Abstract: Honey is an extraordinary and unique sweetening agent synthesized by the honey bees by collecting different nectars from different kinds of flowers. It is a natural product containing both food and medicinal values without causing any adverse effects. Out of all nutrients of the food carbohydrates are the most important nutrient which gives instant energy (power house in terms of energy). These carbohydrates are taking a very crucial role in metabolic activities (biological activities of the body) carried out in the body. They also responsible for the texture of the honey. Thus we have studied and investigated the comparative amounts of carbohydrates. The amounts of carbohydrates are calculated as reducing, non-reducing, and dextrin's. The reducing and non-reducing sugars are given by F/G ratios in each kind of uni-floral honeys of East-Godavari, A.P India. For the above study the samples are collected from different areas in different seasons for A.P. This information can give an idea about the selection of floral honey and the requirement basing on their health condition (diabetes). Origin of honey decided by the pollen analysis of the honey.

Keywords: Meleto Palionology, Frequency classes, Pollen morpho types, Chromoproteins, Metal activators, Unifloral, Multifloral.

I. Introduction

Floral honey is primarily a mixture of fructose (levulose), glucose (dextrose) and sucrose and an ill-defined material (donor 1977) known as dextrin₀. Following these general 3 different honeys for fructose, glucose and sucrose. Honey is a colloidal suspension of nearly 300 different chemicals which gives the food and medicinal values and texture to the honey. And can be preserved very long time without adding any preservatives. Our ancestors know that honey as food, later on they come to know their medicinal values too. Hence honey is an excellent natural sweetening agent.

It is well established by studies carried out in different countries over many years that honey is a saturated solution of mainly three sugars fructose, glucose and sucrose in the proportion of 35:45:0.5 respectively. This proportion allows a maximum quantity of sugars to remain in solution and prevents microbial spoilage and packs a maximum quantity of energy per unit quantity of solution. On average a gram of honey gives 3Cal of energy. The high nutritional values of honey are because of high fructose and glucose content of nectar (EST. et al., 1997). These sugars completely utilized by organisms in their metabolism

The nectar is aqueous sugar containing secretion of floral glands called nectaries. Sugars are mainly present in nectars, but their relative proportion is highly variable. However, they are consistent for certain plant families (Maurizio 1965, Percival 1961, baker n baker 1983). Bahadur .et al, 1986 classified the nectar types into 6 combinations. SGF, SG SF, GF, G&S. Out of 100 floral species Bahadur et al 1986 analyzed nectars for their constituent sugars, 56 of SGF and 31 of SG. Thus these two types have been found that they are dominating over other combinations. Subbareddy 1998 analyzed the nectars of 42 Indian plant species and found that 28 species have glucose dominance, and the other 14 species are rich in sucrose. Thus, in many Indian nectars fructose content is either low or absent.

In general honeys have more fructose than glucose because of the existence of the invertase enzyme secreted by honey bees from their body. And also presence of excess fructose than glucose may be caused by transglucosidase's from honey bees. The variety of minor oligo saccharides that are appearing in honey are nothing but the sugars under gone changes due to the X-gluco sidase originating in honey bees (Siddique and furgula 1970). Honey dextrin's are mixtures of 11 disaccharides (Siddique: Low & sporns 1988). 11 trisaccharides identified by white & hobon 1959. Designating particular species as an adagate source of nectar is based on pollen analysis of honey samples.

II. Pollen Analysis

Honey samples were procured from different areas of East Godavari district, Andhra Pradesh, India in different seasons [2]. 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)[9]. The pollen morphotypes were identified with the help of reference slides mentioned in the Central Bee Research Institute (CBRI, Pune) palynarium.

The pollen types recovered and identified were placed under four frequency classes as mentioned below. The three E.G samples were investigated for their origin by using pollen analysis of honey is known as Meletto palinalysis [3,11].

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

The honey sample was treated as Unifloral if the prepared slide contains a predominant pollen morphotypes. If several morphotypes are represented, the honey sample was termed as Multifloral [4]. Basing on the above information honey samples were identified out of three, two are Unifloral and one is as Multifloral.

List of Unifloral honeys of East Godavari district, Andhra Pradesh – India investigated

EGH 1	Psidium Guajava (Guava)
EGH 2	Syzygium cumini
EGH 3	Cajanus Cajan (Arhar)

TABLE I. Frequency classes and frequencies of Pollen morphotypes in the Unifloral honey samples of the present study

Honey sample	*Frequency class	Pollen morphotype	Frequency (%)
EGH-1	P	Psidium Guajava	72
	S	Borassus flabellifer	21
	I	Cocos nucifera	5
	M	Ageratum conyzoides	2
EGH-2	P	Syzygium cumini	58
	S	Flacourtia indica	29
	I	Borassus flabellifer	13
	M	-Nil-	0
EGH-3	P	Cajanus Cajan	64
	S	Sorghum vulgare	20
	I	Fabaceae	14
	M	Cyanotis sp.	2

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



Fig 1 . Psidium Guajava , Syzygium cumini, Cajanus Cajan

III. Methods

1. Determination of reducing (glucose and fructose) and non-reducing (sucrose) sugars (AOAC.1975):

The reducing sugar value was determined by the volumetric method of Lane and Eyon (1923)[5,6]. The method involves the reaction of sugars with an alkaline CuSO₄ solution to form red copper oxide.

Fehling solution A and B were prepared according to the procedure. Standardization of Fehling solution was done as follows. Accurately 0.95 g of sucrose was weighted and dissolved in about 10-15 ml water. Concentrated HCl of 2 ml was added and kept aside for 24 h. It was then neutralized with NaOH solution and made to 100 ml volume.

Preliminary titration : Equal volumes of (5ml) each of Fehling A and B solutions were pipetted into a set of 250 ml conical flasks and 10 ml of distilled water was added and mixed. About 15 ml of sucrose solution from above was added from a burette having an off- set tip and the mixer was allowed to boil for 2 min on a hot plate. While still boiling, 3-5 drops of 1% methylene blue solution was added and the titration was continued until the blue colour was completely decolorized to brick - red end point. The titer value was noted down.

Final titration: Sucrose solution (less than 1ml of the titer obtained in the preliminary titration) was added to the mixer of Fehling A and B (5ml each) and titrated as described earlier. Titration was performed in

triplicate and a volume of invert sugar required to reduce the Fehling solution was noted down and the following way.

$$\frac{\text{Fehling Factor (in g)}}{\text{(for invert sugars)}} = \frac{\text{Titer value} \times 10}{100} \quad (1)$$

Preparation of honey solution: About 15 g of honey was accurately weighed and dissolved in 30 ml of distilled water and to the resulting solution 2 ml alumina cream was added and filtered. The filtrate was made up to 100 ml. Titration was performed as per the method described in standardization of Fehling solution [7]. The average of triplicate titer values was noted down and the percentage of reducing sugars (as invert sugar) was calculated as mentioned below.

$$\frac{\text{Reducing sugars \%}}{\text{(for invert sugars)}} = \frac{\text{Fehling factor} \times \text{dilution} \times 100}{\text{Wt. of sample} \times \text{titer value}} \quad (2)$$

2. Determination of total reducing sugars (AOAC 1975):

From the diluted honey solution (2:13), an aliquot (20ml) was transferred to a 100ml volumetric flask, concentrated HCl (5ml) was added and kept in a hot water bath maintained at 65°C for 15 min. It was kept aside for 24hr and neutralized with NaOH solution and the total reducing sugar content was determined by titration against Fehling solution as described earlier.

3. Determination of sucrose:

Sucrose was calculated using the formula as shown below.

$$\text{Sucrose \%} = \frac{\text{Total reducing}}{\text{after inversion \%}} - \frac{\text{Reducing sugars}}{\text{Before inverting \%}} \times 0.95 \quad (3)$$

as invert sugar as invert sugar

4. Determination of glucose (IS: 4941-1974):

Hypiodite method : From the diluted honey solution, an aliquot (20 ml) taken in an iodine flask and 40 ml of 0.1 N Iodine and 25 ml of 0.1N NaOH solution were added and mixed well. It was stoppered with 10ml of H₂SO₄ (1:1) and titrated quickly against standard sodium thiosulphate solution using soluble starch solution (1% as indicator). A reagent blank was also simultaneously carried out.

$$\text{Glucose \%} = \frac{\text{Titer value (blank)} - \text{Titer Value (sample)} \times \text{dilution } 0.009004 \times 100}{\text{Wt. of sample}} \quad (4)$$

5. Determination of Fructose:Glucose ratio (IS: 484 -1968):

The fructose: glucose ratio was determined by computation as indicated below:

$$\text{Fructose \%} = (\text{Total reducing \%} - \text{glucose \%}) \quad \text{as invert sugar}$$

$$\text{Fructose : Glucose} = \frac{\text{Fructose \%}}{\text{Glucose \%}} \quad (5)$$

6. Determination of dextrin (Kirk- Wood et al. 1960):

Accurately 5g honey was weighed into a beaker and dissolved in 5ml of distilled water. The solution was acidified with 0.5ml of concentrated HCl and 50ml of absolute ethanol was added drop-wise from a burette, with continuous and vigorous stirring. The precipitate was allowed to settle overnight. A further 10 of absolute ethanol was added to verify completeness of precipitation and the precipitate was then separated on a No. 42 filter paper and washed with five 20 ml portions absolute ethanol. The filter paper was dried to a constant weight in a vacuum oven at 40°C in vacuum of 29 inches of mercury. The filter paper was then washed with three 20 ml portions of boiling distilled water and dried to constant weight as before, the difference in weight was taken as the amount of dextrin in the sample.

$$\% \text{ Dextrin} = \frac{\text{Weight of dextrin} \times 100}{\text{Weight of sample}} \quad (6)$$

IV. Results

The differences between individual unifloral honey types are mainly responsible because of sugars, flavoring agents, acids, minerals and pigments. The coloring of the honey is largely due to the plant resource and climatic conditions. The largest portion of the dry matter, nearly 85% in honey consists of carbohydrates or

sugars. In above percent, nearly 70% is because of fructose and glucose [8,10]. They are building blocks for more complex sugars.

From the results of carbohydrates of uni- floral honey samples of East Godavari A.P . India under present study, all 3 EGH samples are showing the same order of TDS & TDS - TRS values are as follows. TRS values before and after inversion are given in the table below. Since TDS indicate the total dissolved solids of the sample, their relative proportion are in decreasing order, which are shown in the table.

TDS is mainly due to the sugars or Brix. There is no much difference was found in three EGH samples, i.e., *Cajanus*, *Psidium* and *Syzygium*.

According to this data *Syzygium* is more sweeter than other two floral honeys. It can be explained as follows: As the comparative amounts of sugars in three EGH honey samples the amounts of fructose and Glucose is more and sucrose amount is less this can be explained as follows.

The main sugars of nectar are glucose, fructose and sucrose. Sucrose (non reducing sugar) undergoes biochemical transformation by invertase (enzyme) giving fructose and glucose. The enzyme invertase is added by honey bee from its body. Conversion of sucrose into two different monosaccharide's making the *Syzygium* rich in fructose & glucose and low amount of sucrose content.

Table II Comparative results of EGH samples

Parameters	Order according to their percentages
TDS	<i>Cajanus</i> > <i>Syzygium</i> > <i>Psidium</i>
TDS-TRS	<i>Cajanus</i> > <i>Syzygium</i> > <i>Psidium</i>
Fructose	<i>Syzygium</i> > <i>Psidium</i> > <i>Cajanus</i> .
Glucose	<i>Syzygium</i> > <i>Psidium</i> > <i>Cajanus</i>
Sucrose	<i>Cajanus</i> > <i>Psidium</i> > <i>Syzygium</i>
Dextrin	<i>Psidium</i> > <i>Cajanus</i> > <i>Syzygium</i>
F/G %	<i>Psidium</i> > <i>Syzygium</i> > <i>Cajanus</i>

Dextrin another carbohydrate present in honey and gives gel character to honey. Gel nature of cesium is more and sizing is less. F/G ratio of above three honeys is approximately 1, which is prone hence these three samples of honey are less prone to granulation or crystallization. Fructose to glucose ratio (F/G) is the standard quality measurement for honey. It should be one (1). If it exceeds more than one indicates glucose dominants and prone to granulation. Hence total reducing power (TRS) depends upon carbohydrates before and after inverting. Since TRS estimated using common Fehling factor and the various saccharides differ in their copper reducing power aside diarity may occur. Above values are clearly indicating the quality standards of honeys under present study.

V. Conclusion

Syzygium honey stood in first when compared with other unifloral honeys. *Syzygium* honey is unique in all aspects, and can be preferred over other honeys. As the dextrin value is more gel nature is more, it is high in *Psidium* honey and predominant values of fructose proving that *Syzygium* honey is much sweeter over *Cajanus* and *Psidium* honeys.

Identification of pure honey: Pure honey can be identified by its gel nature and unique smell it does not adhere to the paper. Different branded honeys are available in the market. But the collection of unifloral honeys are possible with beekeepers, color and flavor of the honey also helps the consumer to get the honey which he wants.

References

- [1] Wakhle, Z. D.M.1998, Beekeeping technology production, Characteristics uses of honey and other products, pp.150-186..In: R.C Mishra(Ed), Perspectives in Indian Apiculture. Agro Botanica. Bikaner, India.
- [2] Wakhle, D.M.Desai, D.B.1983, Enzymes in Indian honey pp.703-710: In: Proc. 2nd Int. Conf. Apic Trop. Climates, IARI, New Delhi.
- [3] Weston.R.J. and Brocklebank.L.K.1999. The Oligosaccharide composition of some New Zealand honeys, Food chemistry,64: 33-37
- [4] White, Jr.J.W.1975.Pysical properties of honey, pp.207-239; In: Crane(Ed) honey: A comprehensive Survey, Heinemann, and London.
- [5] Kirkwood, K.C., Midchell, T.J.and Smith, D.1960. Examination of the occurrence of honey dew honey. Analyst 85: 412-416.
- [6] Giri.K.V.1938. Chemical composition and enzyme content of honey. Madras Agric., J., 26:68-72.
- [7] Herich, K. (Ed) 1990.Official methods of analysis, 15th Edition, AOAC. Inc., Arlington, VA, USA.
- [8] Echigo, T.1970. Determination of sugar in nectar and honey the mutarotation coefficient of glucose, by gas- liquid chromatography. Bull. Fac. Agric. Tamagawa Univ.10: 3-13.
- [9] Chaturvedi, M.1998. Indian honeys: an evolution of qualitative analysis, pp., 196-208. In: Nectar biology: structure, function and utilization (Ed) Bir Bahadur, Dattson, Nagpur India.
- [10] Crane .E .1975 .In: E.Cran (Ed) Honey: A comprehensive survey. pp. 3-76 Heinemann, London.
- [11] Donner, I.W. 1977. The sugars honey - A review J. Sci.Food Agric, 28: 443-446.