

Analysis of sugar composition in honey samples using HPLC separation and RI detection

M.P Sathianarayanan*, Karishma Hemani and Shraddha Gaonkar

The Bombay Textile Research Association, L B S Marg, Ghatkopar (W), Mumbai 400086

Abstract

Honey, a complex natural foodstuff is used for various purposes without any processing. Honey has been used in medicine as well as raw food since ancient times. Essentially, it is a blend of sugars, especially fructose and glucose. Honey which is available in the commercial market may not be pure and could be adulterated with sugar. The objective of the study is to determine the major sugar composition in honey and to assess its freshness. A total of twenty five samples were collected from local markets and analyzed for the content of glucose and fructose. Total sugar content in terms of glucose and fructose was in the range of 51 to 69%. The ratio of fructose to glucose in these honey samples was in the range of 1.0 to 1.3 which is also an important parameter to assess the purity of honey. Out of twenty five samples, only one sample has fructose to glucose ratio below 1.0 indicating lesser chances for honey to crystallize on storage. A convention for the assurance of two important monosaccharide sugars (fructose and glucose) in honey is set up within the current study by utilizing ordinary stage amino column (NH₂) partition liquid chromatography using a Refractive Index detector.

Keywords:

Fructose, Glucose, Honey, HPLC, RI detector, Sugar

Citation

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1.0 Introduction:

Honey is a natural sweet substance produced by honey bees from the flower nectar, which the bees collect and transform by, combining with specific substances of their own, deposit, dehydrate, store and leave in the honeycomb to ripen and mature. [1] It is a complex natural foodstuff. Honey has been in use in medicine as well as raw food since ancient times. Essentially, it is a blend of sugars, especially fructose and glucose. Additionally, very small amounts of several compounds such as enzymatic and non-enzymatic antioxidants, glucose, ascorbic acid, flavonoids, phenolic compound amino acids etc. are also present in honey [2]. Honey is primarily composed of the simple sugars glucose and fructose – known as monosaccharides and a further 17% to 20% of water. Honey also contains other types of sugars such as sucrose (which is a disaccharide composed of fructose and glucose linked together through α -1–4 linkage). The chemical structure of fructose and glucose is presented in various fundamental textbooks of Chemistry and Biochemistry, therefore, this isn't critical to mention in this article. Usually, fructose is slightly sweeter than sucrose and glucose is less sweet. The sweetness of mono-floral honey – honey made from a single flower source – is dependent on

the ratio of fructose to glucose that results from the bee's processing the nectar of the homomono-specific flower. Carbohydrates are one of the most important components in many food items and they may be either present in an isolated form or associated form to other macromolecules.

Honey has simple carbohydrates and is important for everyday life biological functions such as providing energy for running vital roles of the living body. The majority of the natural honey contains 6 or 12 carbon atoms in their molecules. Honey is semi solid gel like soluble in water and generally has a sweet taste [3]. The concentration of these compounds is determined by numerous elements like natural conditions during the assortment of the crude material, sorts of blooms, assortment of honey bees, climatic conditions, etc [4].

2. Materials and analytical methods

2.1. Material

Twenty five honey samples were collected from different cities of Maharashtra.

Acetonitrile (HPLC grade) was purchased from Supelco. Standard glucose and fructose were purchased from Sigma Aldrich. Agilent 1200 series HPLC equipped with RID

*Corresponding author,
E-mail: ecolab@btraindia.com

detector was used for the analysis. HPLC grade water collected from the TKA water purifier was used for sample preparation.

2.2 Analytical method

Standard glucose and fructose solutions were prepared individually and injected into HPLC to identify the peaks and system suitability. Mobile phase and flow rate were optimized in such a way as to get well resolved glucose and fructose peaks. Optimized HPLC test parameters are given in Table 1.

A known weight of honey sample (0.25g) was dissolved in 25 ml of mobile phase (75/25 acetonitrile/water) and sonicated for 10 min. The solution was filtered through a 0.25 µm nylon membrane to remove the suspended solid particles. The filtered solution was filled in 2 ml autosampler vials and analyzed by HPLC as per the test conditions given in Table 1. A mixture of standard glucose and fructose (1% in mobile phase) was prepared and analyzed under identical conditions for quantification of glucose and fructose in honey samples.

Table 1. HPLC Method Parameters

Column	ZORBAX NH2 [4.6mm X 250mm X 5µm].
Detector	Refractive Index
Mobile phase	Acetonitrile/Water 75/25
Flow	1ml/min
Column temperature	30°C
RID temperature	40°C
Injection Volume	20µl
Analysis time	20 minutes

3. Result and Discussion

Twenty five honey samples collected from various parts of Maharashtra were analysed by HPLC for the content of glucose and fructose. The determination of different sugar contents was employed to distinguish pure honey from adulterated ones. One of the major characteristics of honey is that fructose is present in higher amounts than glucose. Pure honey has approximately 40% fructose and 30% glucose.[4] However, this can vary depending on the source, storage time, temperature and other factors. Glucose, Fructose, Fructose/Glucose ratio and Sugar content in various honey samples tested by HPLC are given in Table 2.

Fig-1 is the HPLC chromatogram of one honey sample and Fig- 2 is the HPLC chromatogram of a mixture of fructose and glucose standard. It can be seen that in both sample and standard, fructose and glucose peaks match with respect to retention time, which indicates that all honey samples contain fructose and glucose. Both fructose and glucose peaks are well resolved with a base to base with a retention time of 7.6 min and 9.0 min respectively. Fructose and

Glucose content in honey is calculated as per the given formula.

$$\text{Fructose / Glucose (\%)} = \frac{\text{Asp X Cst X V}}{\text{Ast X W}}$$

Where Asp is the peak area of fructose/glucose in the honey sample

- Ast is the peak area of fructose/glucose in standard
- Cst is the % concentration of fructose/glucose standard
- W is the weight of the honey sample in g
- V is the volume of dilution in ml.

Table 2. Major sugar components in honey samples determined by HPLC

Sr. No.	Sample mark	Glucose (%)	Fructose (%)	F/G ratio	Sugar content (%)
1	H1	29.93	36.32	1:21	66.2
2	H1a	29.07	36.03	1:24	65.1
3	H1b	30.83	37.67	1:22	68.5
4	H1C	28.63	35.23	1:23	63.8
5	H1d	30.30	36.41	1:20	66.7
6	H2	32.02	35.28	1:10	67.3
7	H2a	30.05	32.06	1:07	62.1
8	H2b	32.69	34.97	1:07	67.6
9	H2c	32.38	34.06	1:05	66.4
10	H2d	30.93	31.98	1:03	62.9
11	H3	31.07	38.18	1:23	69.2
12	H3a	29.98	37.48	1:25	67.4
13	H3b	28.95	36.44	1:26	65.3
14	H3c	28.80	35.80	1:24	64.6
15	H3d	27.87	34.83	1:25	62.7
16	H4	30.09	36.47	1:21	66.5
17	H4a	30.57	37.08	1:21	67.6
18	H4b	30.07	36.26	1:21	66.3
19	H4c	30.83	36.88	1:20	67.7
20	H4d	29.42	35.47	1:21	64.8
21	N1	27.12	33.77	1:25	60.8
22	N2	29.74	34.81	1:17	64.5
23	N3	22.85	31.91	1:40	54.7
24	N4	23.33	28.20	1:21	51.5
25	N5	32.78	31.38	0.96	64.1

Table 3, Table-4& Table-5 are the specifications of honey laid by GSO standard, IS 4941 and ICH standard respectively for reference.

Table 3. Specifications of honey as per GSO standard

Honey composition	Specification
Total reducing sugar	Not less than 60%
Fructose	27–44.3%
Glucose	22–40.7%
Sucrose	Not more than 5%
Fructose/Glucose ratio	Not less than 1%

Table 4. Specification of honey as per IS 4941 standard

Sugar content	Special grade	A grade	Standard grade
Total sugar (min) %	70	65	65
Sucrose (Max)%	5	5	5
Fructose/Glucose ratio (min)	1	1	1

Table 5. Specification of honey as per international honey commission ICH standard

Total sugar (min) %	60
Fructose %	27-44.3
Glucose %	22-40.7
Fructose/Glucose ratio (min)	0.95

Apart from glucose and fructose, sucrose is also present in low amounts ideally not more than 5% except honey from a few specific plants [5]. The total sugar content in twenty five honey samples was in the range of 51.5–69.2 g/100 g of honey. Highest sugar content was 69.2 % in sample H3 and the lowest was 51.5 % in sample N4. Out of twenty five honey samples, only one sample (N5) is having fructose/glucose ratio below 1.0. The ratio of fructose to glucose is also an important parameter for honey. The more glucose a honey has, the faster it tends to crystallize. In honey, the ratio of fructose to glucose ideally should range from 1.0 to 1.35. A fructose to glucose ratio below 1.0 leads to faster honey crystallization, whereas crystallization becomes slower when this ratio is more than 1.0 [6,7]. In the present study, except for one sample all twentyfour honey samples fructose /glucose ratio was in the range of 1.07 to 1.40. The amount of fructose in all samples except sample N5 was found to be higher than the amount of glucose, which is a characteristic of natural honey. The honey sample H3 was found to contain the highest amount of fructose (38.18%) and the lowest 28.2% in sample N4. Sample N5 glucose content was higher than fructose.

If we compare the test results of honey samples with various specifications, Table-3, Table-4 & Table 5, out of twenty five samples only fourteen samples comply with IS 4971 in terms of sugar content (65% min) for A grade/standard grade honey. Twenty four samples comply with fructose to glucose ratio (1 min). As per GSO standard, all twenty five samples comply with glucose and fructose content and twenty three samples comply with a minimum sugar content of 60%. Only one sample (N5) has to have fructose to glucose ratio of less than 1.0 which is noncompliance with GSO and IS specifications. Honey samples N3 & N4's total sugar content is less than 60% which is non compliance with all three specifications.

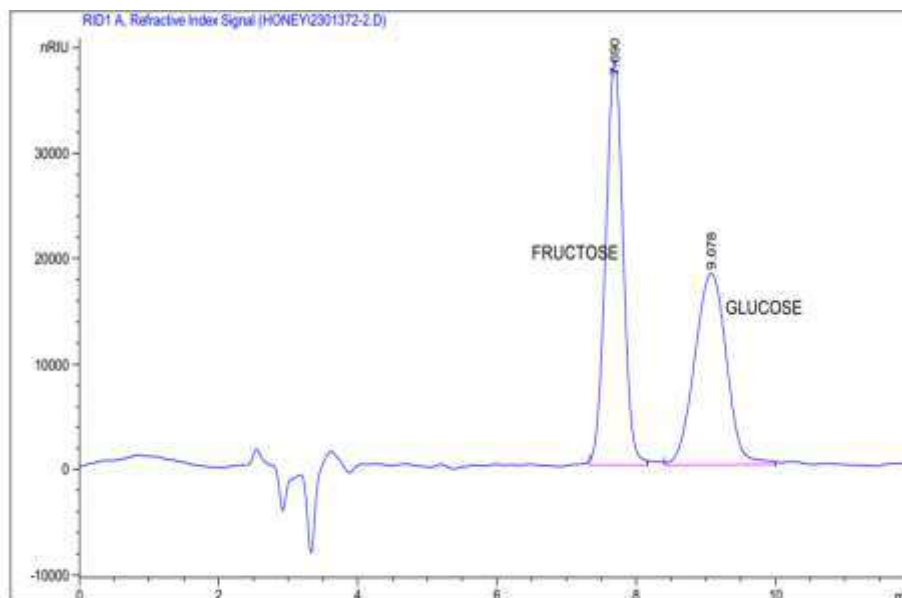


Figure 1. HPLC chromatogram of honey sample

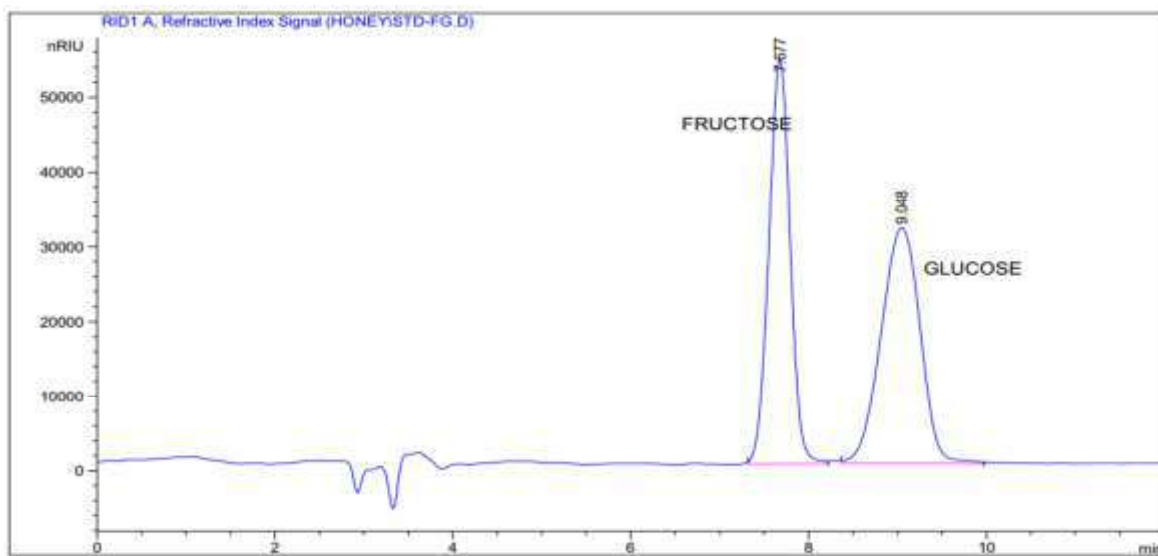


Figure 2. HPLC chromatogram of fructose and glucose standard

4. Conclusion

All the honey samples except two contained more than 60 % sugar as the sum of glucose and fructose, which is a requirement for all specifications. The ratio of fructose and glucose was also typical of honey. The more glucose a honey has, the faster it tends to crystallize. Natural honey normally contains high concentrations of fructose than glucose and the same is observed in most of the honey samples except two samples. This test method can be used to distinguish between

natural honey and adulterated honey. The data demonstrated the effective chromatographic separation of fructose and glucose using the NH₂ column. Since glucose and fructose have specific refractive index, both have shown good responses to RI detectors. The developed methodology demonstrates the effective determination of fructose and glucose concentrations in honey.

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