



# Study of the Hydroxymethylfurfural and Sugar Profile of *Apis mellifera* Honey in Madhya Pradesh, India

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## Authors' contributions

This work was carried out in collaboration among all authors. Authors SS and ASY designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors ASY and SPST managed the analyses of the study. Author SS managed the literature searches. All authors read and approved the final manuscript.

## Article Information

DOI: 10.9734/IJECC/2023/v13i92356

## Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/102975>

Original Research Article

Received: 08/05/2023

Accepted: 10/07/2023

Published: 17/07/2023

## ABSTRACT

**Aims:** The present study was undertaken to determine the Hydroxymethylfurfural and sugar profile of *Apis mellifera* honey.

**Place and Duration of Study:** Honey samples were collected from beekeepers of eight selected villages of Guna and Morena region of Madhya Pradesh during two season 2021/22 - 2022/23

**Methodology:** The study covers the sugar characterization in term of reducing sugar, sucrose, glucose, fructose, fructose/glucose ratio and Hydroxymethylfurfural content. Collected honey samples were investigated in lab and data analysis by two factor without replication.

**Results:** The result recorded different parameters Hydroxymethylfurfural, (9.77-15.73 and 9.50-15.91 mg/kg) total reducing sugars (70.08-73.53 and 69.16-73.83 %), sucrose (1.70-3.43 and 1.77-

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3.39%), glucose (31.60-35.60 and 31.20-35.30), fructose (36.47- 39.94 and 36.73-.39.69) and fructose glucose ratio (1.03-1.22 and 1.04-1.22) respectively, during 2021-22 and 2022-23. All the parameters showed significant variation in honey among the selected locations.

**Conclusion:** According to the study, all of the honey samples met the allowed limit as required by Indian standards and were of acceptable quality.

**Keywords:** Honey; hydroxymethylfurfural; sugar; fructose; glucose; *Apis mellifera*.

## ABBREVIATIONS

*HMF* : Hydroxymethylfurfural,

*F/G ratio*: Fructose-Glucose ratio

*TRS* : Total Reducing Sugar

*FSSAI* : Food Safety and Standard Authority of India

## 1. INTRODUCTION

Honey is a sweet, viscous substance supersaturated sugar solution, principally contains mainly fructose and glucose with traces of maltose and sucrose [1,2]. Total reducing sugar found in monosaccharides form which include fructose and glucose and primary disaccharide is sucrose, which is composed of one glucose molecule and one fructose molecule. Sucrose is present in small quantities in honey and contributes to its overall sugar composition.

These simple sugars provide the sweet taste and energy content of honey and ratio of glucose to fructose is mainly responsible for the crystalline nature of honey. The sugars in honey are easily digestible and provide a quick source of energy, making it an ideal choice for athletes or individuals needing an energy boost. The specific combination of these small components, each of which is known to have unique nutritional or therapeutic characteristics, explains the wide range of uses for natural honeys [3]. Although the majority of the sugar in each sample of honey is essentially the same, the precise chemical makeup of natural honey varies depending on the types of flora, and nectar collected by worker bee [4-6]. Additionally, varying agroclimatic conditions and vegetation types are significant elements that might impact the composition of honey.

Its unique flavor profile and perceived health benefits make it an attractive option. It adds flavor and sweetness to dishes, beverages, and baked goods. It can also be used as a natural preservative in certain food preparations. The chemical properties of honey, one of the major

components hydroxymethylfurfural, is a quality indicator and measures honey's freshness [7,8]. Hydroxymethylfurfural (HMF) is a natural compound which absent or present in low quantity in fresh honey when it heated in order to facilitate processing but elevated levels of HMF may indicate overheating or extended storage, which can degrade the quality of honey or adulterated with invert syrup [9].

The sugar and HMF analysis of honey is important to the honey industry, since these elements are closely linked to storage quality, granulation, flavour so this study based on analyzing sugar and HMF of local honey samples collected from beekeepers of different villages.

## 2. MATERIALS AND METHODS

Eight honey samples were collected from *A. mellifera* beekeepers of adjoining villages of Guna and Morena district of Madhya Pradesh during 2021-22 and 2022-23. The honey samples collected from village name V1-Sumaoli, V2-Mungavali, V3-Sahadpura (Morena district) and V4-Padampura, V5-Barkhedahat, V6-Khumbraj, V7- Ramnagar and V8-Pahadiya (Guna districts). Each village's honey samples were gathered, placed in pristine glass bottles, and sealed tightly. Honey samples were analyzed using the facilities of the Soil Science lab, Department of Soil Science, College of Agriculture, Gwalior. The study covers the sugar characterization *i.e.* reducing sugar, sucrose, glucose, fructose, fructose/glucose ratio and hydroxymethylfurfural, of honey.

The reducing sugars content of the honey samples was estimated by the reduction of Soxhlet's modification of Fehling's solution by titration with copper sulphate at boiling point (60°C) against a solution of reducing sugars in honey using methylene blue as an internal indicator. The fructose, glucose and sucrose content of the honey samples was determined according to the procedure of Lane and Eynon [10] and AOAC [11,12]. Titration was done following a similar procedure

as for the determination of reducing sugars. Fructose content divided by glucose content for obtained fructose- glucose ratio. The hydroxymethylfurfural (HMF) content of the honey samples was determined spectrophotometrically according to White and Doner [13] and Bogdanov [14].

## 2.1 Statistical Analysis

The data from eight locations of honey samples obtained were analyzed by two factors without replication the analysis of variance using Microsoft excel. significant at 5% levels were determined using F-table.

## 3. RESULTS AND DISCUSSION

The results and basic statistics obtained for hydroxymethylfurfural and various sugar parameters of the eighth honey samples collected from different villages of Guna and Morena district are summarized in Table (1&2) and Figs. (1&2). Analysis of variance has shown that differs significantly for the entire honey sample.

### 3.1 Hydroxymethylfurfural (HMF)

HMF concentration is a quality indicator that has been linked to the freshness of honey. This is a by-product of fructose degradation that develops during heating or storage. Its presence is therefore regarded as the primary sign of honey degradation. The high value of HMF suggests processing that was overheated, storage that was too long, or adulteration with invert sugars. The current findings of HMF content recorded the lowest (V2, 9.77 and 9.50 mg/kg) in Mungavali honey which was at par with Sumaoli honey (V1, 9.83 and 9.98 mg/kg), The highest HMF (V3, 15.73 and 15.91 mg/kg) was found in a honey sample of Sahadpura during 2021-22 and 2022-23 respectively. The result varied significantly among the location and all the sample under the range of (40 mg/kg) Codex Standards and (80 mg/kg) FSSAI [15] regulations which show all honey sample are good in quality. The result was an agreement with Nayik and Nanda [16] and Painkra et al [17].

### 3.2 Total Reducing Sugar (TRS)

The reducing sugar values of entire samples were significantly different and sample collected from Pahadiya honey recorded the highest (V8, 73.53 and 73.83 %) reducing sugar followed by Ramnagar (V7, 73.27 and 73.09%) while the lowest reducing sugar was reported in

Barkhedahat (V5, 70.08 and 69.16%) during 2021-22 and 2022-23 respectively. These values were in agreement with Berhe et al [18] and Charathi [19]. According to FSSAI [15] the result of TRS percentage found in the acceptable minimum standard ( $\leq 65\%$ ) honey.

### 3.3 Sucrose

The results of the sucrose analysis showed a significant difference among the sample and Sahadpura honey sample was found highest sucrose (V3, 3.43 and 3.39%) while lowest sucrose (V2, 1.70 and 1.77 %) was found in the Mungavali honey during 2021-22 and 2022-23 respectively. The amount of sucrose varies depending on the honey's nectar compound's origin and maturation stage. Our findings showed approximately similarity with the results of Charathi [19], and Painkra et al [17]. The final result is within the 5% range set by FSSAI [15]. This indicates honey in the collected area was fully ripe with good-quality.

### 3.4 Fructose

The data of fructose content was recorded significantly higher in Pahadiya (V8, 39.94%) honey and the lowest content (V1, 36.47%) was found in Sumaoli honey during 2021-22. However, Ramnagar sample had maximum (V7, 39.69%) fructose content and minimum in Mungavali (V2, 36.73%) among the honey sample during 2022-23. Similar fructose values were also described by Berhe et al [18] and Painkra et al [17]. Fructose content contributed to sweetness in honey and the result ranged between 27.2 to 44.3% set by the Indian standard [20].

### 3.5 Glucose

The investigation on glucose percentage of the honey samples found significant difference among the study area and Mungavali honey has maximum (V2, 35.60 and 35.30%) glucose content and minimum glucose content found in Barkhedahat (V5, 31.60 and 31.20%) honey sample during 2021-22 and 2022-23 respectively. These values were in agreement with Buba et al [21], and Berhe et al [18]. The result was accepted in the range of 22 to 40.7% set by the Indian standard [20].

### 3.6 Fructose-Glucose Ratio

Fructose to glucose ratio measured the quality of honey for crystallization. The ratio of fructose to

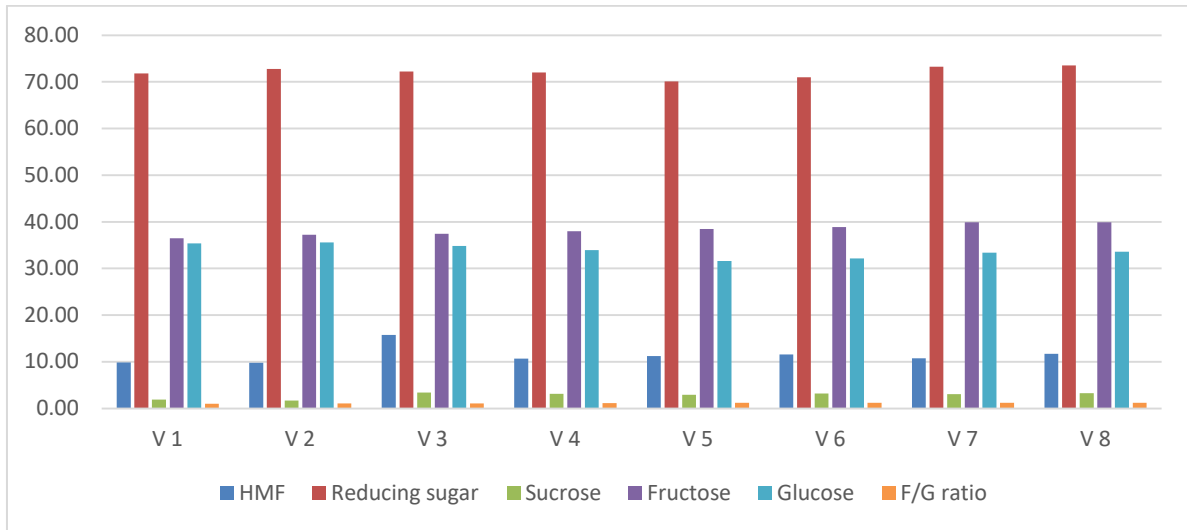


Fig. 1. The graphical depiction of HMF and sugar parameters of honey collected from the study area during 2021-22

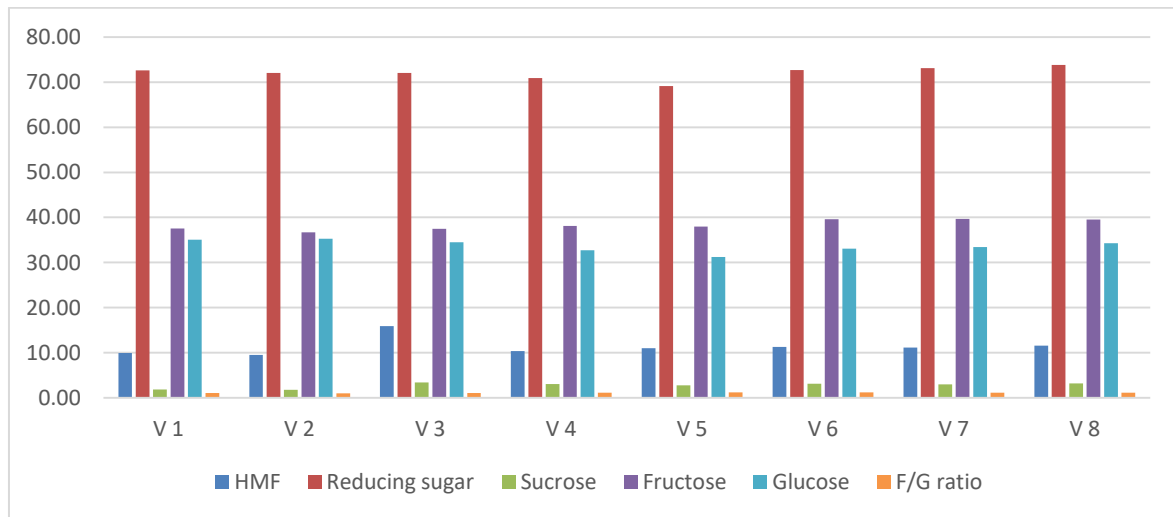


Fig. 2. The graphical depiction of HMF and sugar parameters of honey collected from the study area during 2022-23

Table 1. Hydroxymethyl furfural and sugar profile of honey collected from different locations in study area during 2021-22

Locations	HMF (mg/kg)	Reducing sugar (%)	Sucrose (%)	Fructose (%)	Glucose (%)	F/G ratio
V 1	9.83	71.82	1.87	36.47	35.35	1.03
V 2	9.77	72.80	1.70	37.20	35.60	1.05
V 3	15.73	72.24	3.43	37.42	34.83	1.07
V 4	10.70	71.99	3.16	38.02	33.97	1.12
V 5	11.23	70.08	2.90	38.48	31.60	1.22
V 6	11.57	71.01	3.23	38.88	32.13	1.21
V 7	10.77	73.27	3.07	39.90	33.37	1.20
V 8	11.70	73.53	3.26	39.94	33.59	1.19
S.Em±	0.74	0.66	0.27	0.65	0.57	0.03
CD At 5%	2.23	2.00	0.80	1.98	1.73	0.09

Significance level at 5% (HMF- Hydroxymethyl furfural, F/G ratio- Fructose-Glucose ratio)

**Table 2. Hydroxymethyl furfural and sugar profile of honey collected from different locations in study area during 2022-23**

Locations	HMF (mg/kg)	Reducing sugar (%)	Sucrose (%)	Fructose (%)	Glucose (%)	F/G ratio
V 1	9.98	72.61	1.90	37.53	35.07	1.07
V 2	9.50	72.03	1.77	36.73	35.30	1.04
V 3	15.91	72.00	3.39	37.48	34.53	1.09
V 4	10.38	70.90	3.05	38.15	32.75	1.17
V 5	11.05	69.16	2.78	37.96	31.20	1.22
V 6	11.33	72.64	3.13	39.58	33.07	1.20
V 7	11.14	73.09	3.01	39.69	33.40	1.19
V 8	11.55	73.83	3.19	39.55	34.28	1.15
S.Em±	0.52	0.66	0.17	0.39	0.47	0.02
CD At 5%	1.57	2.01	0.52	1.17	1.42	0.05

Significance level at 5% (HMF- Hydroxymethyl furfural, F/G ratio- Fructose-Glucose ratio)

glucose is less than one, which makes honey sensitive to the crystallization ingredient of monohydrate glucose crystals, which, vary in quantity, shape, size, and quality depending on the composition of the honey and the storage conditions. The data presented on the (F/G) ratio maximum (V5, 1.22) was found in the case of the samples collected from Barkhedahat and the lowest (V1, 1.03) F/G ratio reported in Sumaoli honey during 2021-22. However, Barkhedahat reported a maximum (V5, 1.22) fructose/glucose ratio while the lowest (V2, 1.04) was found in Mungavali honey during 2022-23. There was a significant difference in the values obtained from the study area. Buba et al [21], and Berhe et al [18] support the finding. The result falls within 0.95 to 1.5. are in agreement with FSSAI [15] regulation.

#### 4. CONCLUSION

The study concludes that Hydroxymethyl furfural content in the current honey sample was found low value which indicates honey sample freshness. The sucrose content noted in all honey samples below Indian standard shows that honey was fully ripe. Fructose content high than the glucose and fructose glucose ratio show less crystallized nature of honey. The current findings indicate sugar and HMF analysis of all collected honey samples showed variations in composition because of differences in agro-climatic and botanical origin and all were of good quality honey for commercialization compared to national standard.

This study would be helpful to understand local honey properties and very important towards the commercialization of regional honey.

#### ACKNOWLEDGEMENTS

The author expresses sincere thanks to the Department of Entomology and Krishi Vigyan Kendra Morena for proper guidance and encouragement during the research work.

#### COMPETING INTERESTS

The authors have declared that no competing interests exist.

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