



**Special Issue (NSAZ-2022)**

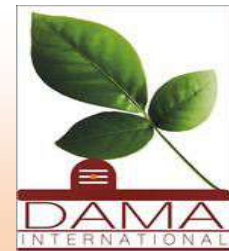


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# **Applied Zoology, Profitable Animal Production, and Health: Current Status and Future Progress (NSAZ-2022)**

**Organized by**

**Department of Zoology and Fishery Science,  
Rajarshi Shahu Mahavidyalaya (Autonomous),  
Latur- 413531, Maharashtra**

**On**

**23<sup>rd</sup> & 24<sup>th</sup> September- 2022**

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## Essential Composition and Analytical Methods of Honey.

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### Abstract

Honey samples for this research were obtained from Gauri Natural Foods in Chakur, Latur, Maharashtra, India. Fresh samples were taken in sterile containers (labelled with numbers, the collecting site, and the collection date) and kept at room temperature until analysis. Wax sticks, dead bees, and comb particles were removed from the samples using cheesecloth prior to examination. This study examined water content, ash content, fibre content, carbohydrate content, energy value, specific gravity, and mineral contents to assess the nutrient content and degree of adulteration of honey samples collected from sources in Gauri Natural Foods, Chakur, Latur district. These metrics were evaluated using AOAC-standard methods. The values of moisture content, ash content, specific gravity, total reducing sugar, sucrose, fructose-glucose ratio, and acidity as formic acid were determined to be 19.0%, 0.1608%, 1.4137, 75.0624%, 0.7791 %, 1.0584%, and 0.0926%, respectively, in honey gathered from a beekeeper. The high carbohydrate, crude fibre, metabolising energy, and low crude fat contents of honey samples, regardless of their source, along with a significant amount of essential mineral components, indicate their nutritive quality and encourage their use in a variety of food products. Fresh and branded honey samples were analysed for their chemical makeup, and their deviations from pure honey gathered from apiary locations revealed the values of the quality factors.

**Keywords:** honey adulteration, ,AOAC

### Introduction

One of the most crucial bee products, it is a sweet, viscous liquid formed from the nectar of plants. Honey was defined as "the sweet compounds made by honeybees from the juice of flowers or from plant discharges, which they collect, transform, and store in honeycombs"[1]. The enzymes in honey ( Glucose oxidase, invertase, amylase, etc.) emerge either from the bees or from the plants that the bees harvested on. They are present in relatively minute quantities, however may possibly have nutritional significance for humans. The enzymes are very susceptible to warmth (above 35°C) and increased storage. Because enzymes are destroyed by heat, a low enzyme content may suggest that honey has been heated. On the market, both labeled and non branded honeys are accessible. There may be major variation in honey brands in terms of nutrition and quality. The majority of individuals are misinformed of the quality of the honey they consume. Due to the growth in concerns related with adulteration and tampering with natural honeys sold on the market, it is essential to conduct quality checks on commercial honey. In order to give valuable information for honey consumers, a study was created to evaluate the quality of commercial honey available in the marketplace and compare it to International Honey Standards. Natural honey is one of the most sought-after items due to its unique nutritional and therapeutic

benefits, which are a result of the many groups of substances it contains. Honey is the natural sweet substance honey, *Apis mellifera*, from the nectar of plants (blooms) or from the secretions of living parts of plants or excretions of plant sucking insects on living parts of plants, which bees collect, transform by combining with specific substances of their own, reserve, dehydrate, store, and allow to ripen and mature in the honey comb. Honey is claimed to be produced by bees in order to provide as a food supply during times of scarcity or severe weather. Natural honey is a viscous solution composed of 80% to 85% carbohydrate (mainly glucose and fructose), 15% to 17% water, 0.1–0.4% protein, 0.2% ash, and trace amounts of amino acids, enzymes, vitamins, and other substances such as phenolic antioxidants. Each of these minor constituents is recognised to have specific nutritional or therapeutic benefits, and their unique combination enables a vast array of products. natural honey applications. The chemical composition and physical qualities of natural honeys vary according to the plant species on which the bees graze, despite the fact that the honey's primary elements are almost identical across all samples.

## Materials and Methods

### Methods for analysing honey include: -

Honey samples were examined according to the AOAC (Association of Analytical Chemists) Method for humidity, carbohydrate content, acidity, sucrose, fructose, ash content, and the Fiehes test. [2]

#### 1. Sample Collection and Preparation:

This investigation's honey samples were obtained from Gauri Natural Foods, Chakur, a local harvester in the Latur district. Fresh samples were gathered in sterile containers (marked with numbers, the collecting location, and the collection date) and kept at ambient temperature until analysis. Wax sticks, dead bees, and comb particles were removed from the samples using cheesecloth prior to analysis.

#### 2. Measurement of relative humidity:

Due to its impact on honey's capacity to stay stable and resist yeast fermentation, moisture is an essential quality criterion for honey shelf life. The maximum moisture content permitted is 20% (w/w). Using readings from a refractometer at 20 °C and the AOAC standard table, the moisture content of honey samples was determined.

#### 3. Acidity Evaluation:

Acidity is defined as the percentage of acidity in a sample as determined by titration with a standard base and quantified as the predominant acid in the sample. 10g of honey was weighed into 75 ml of distilled water, the pH was determined, and the pH of the honey solution was adjusted to 8.3 with NaOH.

1. Acidity = Used NaOH Volume x Honey Weight.

#### 4. Determining Sugar Content:

A and B Fehling solutions, methylene blue solution, sodium chloride solution, and hydrochloric acid. I poured (5.00g) of honey in a beaker, added D. H<sub>2</sub>O, and dissolved it to produce 100 ml. Following the addition of two to three drops of phenolphthelene, NaOH solution was added until the solution turned pink. HCl was added to the solution until its original colour was restored, followed by 200ml of distilled water (honey solution). In a conical flask, 5ml of Honey solution, 5ml of Fehling solution A, and 5ml of Fehling solution B were boiled for 2 minutes. During the process of boiling, add three drops of methylene blue indicator and titrate with honey solution until a brick-red hue is achieved. The employed honey solution volume is provided.



Reducing sugar% = Fehling solution constant 0.051 x total solution volume x 100/ weight of sample solution x titrate volume. Take five grammes of honey solution in a beaker, add d.H<sub>2</sub>O, and raise to a volume of one hundred millilitres with d.H<sub>2</sub>O. Add two to three drops of phenolphthelene followed by NaOH solution until the colour of the solution changes to pink. Then, add HCl to the solution until its original colour is restored, followed by 200 ml (v1) of pure water. Add 5g of citric acid to 50ml (w2) of the aforementioned solution, boil for 10 minutes, and then allow to cool. Then, neutralise it by lowering the sugars, and add enough distilled water to get the whole volume to 200 ml (v2). Combine 5ml of honey solution with 5ml each of fehling solutions A and B, and boil for 2 minutes. Add two to three drops of methylene blue and titrate with honey solution until brick red colour is achieved. Notate the volume of the honey solution.

Sugars Total = Fehling solution constant (0.051) 200200100 / 550 Vol.

**Non-reducing sugars = total sugars minus reducing sugars.**

#### **5.Determining the sugar concentration:**

To determine the sucrose content by inversion, 10 mL of diluted HCl, 50 mL of diluted honey solution, and water were put to a 100 mL volumetric flask. After boiling the solution in a water bath, it was cooled and diluted to the required concentration. The Layne-Enyon method was then applied, and the sucrose concentration was determined using the differential.

#### **6.Determining the concentration of fructose:**

Utilizing the resorcinol reagent method [24], fructose concentration was determined. Before adding 1.0 mL of diluted HCl to a honey sample solution, 1.0 mL of resorcinol reagent was added to the solution and thoroughly mixed. As indicated previously, 0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml standard solutions were also treated with 1.0 ml of the resorcinol reagent and

1.0 ml of diluted HCl. In addition to the regular solution, a blank solution was prepared and handled in the same way. The test solution, the standard, and the blank were then heated in a water bath at 80°C for approximately 10 minutes, the solution was removed from the water bath and cooled by immersion in tap water for five minutes, and the absorbance of the test and standard solutions were read against the blank solution at 520 nm within 30 minutes. The concentration of fructose in the honey samples was then extrapolated from a reference curve based on absorbance.

#### **7. Content: cinder:**

According to (AOAC, 1999) procedures, ash content was evaluated; 5 g of honey was placed in combustion pots, which required preheating to darkness using a gas flame to prevent honey from foaming. The samples were subsequently burned in a burning muffle for five hours at a high temperature (550 °C). Once the collected ash had cooled to room temperature, it was weighed.

#### **8.The Fiehe test:**

It can detect the use of invert sugar to adulterate honey (Acid hydrolyzed sugar). In honey, it identifies the actual existence of HMF (Hydroxymethyl furfural).

Honey has significantly less HMF (approximately 10 mg/kg) than invert sugar. Honey that has been heated or stored for

longer period of time may contain a higher concentration of HMF than honey that has not been heated or stored for as long. 5 millilitres of honey should be placed in a mortar. Add 10ml of solvent ether. With a pestle, thoroughly combine the liquid. Transfer the solution from the mortar's supernatant to a Petri plate. Allow it to dry in the open air. In a test tube, pour 5ml of HCL (Hydrochloric acid). Add 1 g of resorcinol crystals. Shake it vigorously to scatter it. The

Petri plate requires a few drops of resorcinol HCl solution (This should be done once the ether dries out completely). Check the colour of the petri dish.

Test result for Fiehes:

**Positive result:** the presence of a pink or cherry red hue suggests a high HMF content. This means that honey has either inverted sugar or been tampered with.

There is no transformation to a yellowish-pink colour. A negative result indicates that neither ordinary sugar nor invert sugar has been tampered with.

FSSAI test criteria for fiehe: -

The Fiehe test should be negative. If Fiehe's test is positive and the level of hydroxymethylfurfural (HMF) is greater than 80 mg/kg, the fructose:glucose ratio must be greater than or equal to 1.0.

**Conclusion for Fiehes test:**

**Positive result:** a pink or cherry red tint indicates the existence of a high HMF concentration. This indicates that honey has either inverted sugar or has been adulterated with sugar.

**Negative outcome:** -There is no colour change to a yellowish pink hue. A negative result implies that neither regular nor invert sugar has been adulterated.

**FSSAI criterion for fiehe's test: -**

The test for Fiehe should be negative. If Fiehe's test is positive and the hydroxymethylfurfural (HMF) level is greater than 80 milligrammes per kilogramme, the fructose:glucose ratio must be greater than or equal to 1.0.

#### Result and Concluding Remarks :

Sr. No.	Parameter	Results	Standard reference values
1.	Moisture %	19 %	20 % Max.
2.	Acidity (Formic acid %)	0.0926%	0.20 % Max.
3.	Total Reducing Sugar %	75.0624%	70 % Min.
4.	Sucrose %	0.7791 %	5.0 % Max.
5.	Fructose-glucose ratio	1.0584	1.0 Min.
6.	Ash %	0.1608 %	0.50 % Max.
7.	Fiehe's test	Negative	Negative



## Conclusion:

Moisture content is a crucial indicator for certifying honey's quality because it determines honey's shelf life (Bognadov, 2009). The moisture level of honey also affects its viscosity and flavour. Additionally, the yeast count and thus the inclination for fermentation are determined by the moisture content. Thus, below 20% relative humidity, there is negligible fermentation (Bognadov, 1999). AOAC-recommended Layne-Enyon technique was used to estimate reducing sugars. The composition of minerals and trace elements determines the ash. The ash percentage of honey obtained in this study varied from 0.16% to 0.18%, values lower than (0.44-0.58%) recorded from Ado-Ekiti (Oyeyemi et al., 2015) and (0.37-0.54%) reported from North East Nigeria (Oyeyemi et al., 2015). (Buba et al., 2013). The ash content is lower than the average given by the university of Ilorin Apiary (4.16 1.78%) (Ande et al., 2010).

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