

A Relative Study on the Chemical Composition Among the Pure and Branded Honey Types Collected from Diverse Sources of Tamilnadu, India

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Abstract: Determination of standard criteria of food products is extremely important, since their consumption, quality and validity depend on it. The composition and properties of honey differ with the floral and honeydew sources utilized by honey bees, besides regional and climatic conditions. Honey gets its sweetness from the monosaccharide, fructose and glucose. The existing study was carried to probe the chemical composition of fresh and treated honey samples collected from different sources. A total of five samples was collected from different places. In this study diverse parameters were estimated to identify the physio-chemical difference between the raw and processed honey. The typical methodology was used to determine the chemical composition of honey samples. The outcomes obtained from this study govern that the raw honey and processed honey samples diverse with the standards. All the pure honey samples chemical parameters were within the Codex standard. The branded honey samples to some extent deviated with the parameters like ash content, moisture, HMF and sucrose content. Apart from these both of the pure and branded honey samples are rich with diastase activity and proline content records that the honey samples are not adulterated and their nutritional properties are enriched. The results ascertained the quality of the honey, which will create a worthy profit in the global market and its therapeutic value.

Key words: Honey • Treated honey • Fresh honey and codex alimentarius standard

INTRODUCTION

Natural honey is the resulting product of nectar and sweet deposits gathered from various floral sources, modified and stored in the honey combs by the honey bees. Honey is basically a sticky and viscous cocktail of carbohydrates (especially glucose and fructose), water, protein, ash and trace quantities of amino acids, different vitamins, enzymes, flavonoids and phenolic contents [1-5]. These trace contents are known to have distinctive nutritional and therapeutic values and hence have varied applications for enhancing the human health and improving immunity. Various factors such as botanical, geographical and environmental conditions influence the biochemical composition of natural honey [6-8]. Natural honey is a very good source of antioxidant due to the presence of phenolic components and enzymes which promote the removal of oxygen [9 and 10]. It has an antagonistic action against various diseases causing pathogens because of its physical property of osmosis and production of hydrogen peroxide [11, 12].

Honey is hygroscopic in nature and has low heat conductivity and surface tension [13-15]. It is highly concentrated sugar solution having very less water content. Presence of high sugar contents determines the viscosity of honey [16]. pH of honey determines the acidity and the nature of the floral source [17]. It ranges from 3.4 to 6.1 whereas the average pH is 3.9. Diastase activity is dominant in honey, where it takes part in the conversion of starch to maltose [18]. Ash content determines the chemical composition and the nectar source and the content present in the honey. It measures the mineral content present in honey. Fructose decomposes in acidic condition to give out Hydroxy Methyl Furfural which is one of the main components predominating in honey. Presence of HMF contents measures the degree of deterioration of honey.

Honey could be in unprocessed (raw) form or in processed form. Due to variation in constituents of natural honey with a comparison of processed honey, various tests were conducted. These tests were important for establishing the quality parameters of the honey.

The present study was intended to analyze the biochemical properties of unprocessed natural (raw) honey and processed honey samples and compared their results. Although various biochemical studies on honey were done, but there is a lack of information on the comparison of the raw and processed honeys.

MATERIALS AND METHODS

Sampling of Honey Samples: For the present study five honey samples were chosen among them the samples A1 and A2 was directly collected from the beekeepers in the pure form (raw honey, unprocessed). The honey samples PH1, PH2 and PH3 were collected from the local market and they were branded. The raw, unprocessed honey samples were triple filtered using muslin cloth and stored in glass containers to retain its naturalness and the processed, commercial honey samples were stored at room temperature ($25\pm 2^\circ\text{C}$). Further, all the honey samples were tested for their chemical composition.

Details of the Honey Samples Collected from Different Sources

Chemical Characterization of Honey Samples

pH Value: pH values were determined using a pH meter [19].

Ash content (%) w/w: To determine the ash content, 5g of each honey sample was placed in a china dish and subjected it into a muffle furnace at 660°C and incinerated, i.e., until it turned into ash and the values were tabulated. The test was carried out in triplicates, mean and standard error were recorded.

Refractive Index: The determination of the refractive index of the selected honey samples was prepared by using digital refractometer. Calibration of the instrument was done before carrying out the experiment. The test was carried out in triplicates, mean and standard deviation were noted.

Moisture content (%) w/w: Moisture content is the major parameter of honey sample, which will yield the amount of adulteration present in the samples easily. Experimentally moisture content is measured in wet base and dry base. Moisture content is measured for the storability and agglomeration. The moisture content percentage values corresponding to the corrected refractive index values were calculated using Wedmore's tables [19].

Hydroxymethylfurfural (HMF) Content ($\text{Mg}\cdot\text{kg}^{-1}$):

The HMF was determined using [20]. 5g of honey sample was dissolved in 25 ml of deionised water. 0.5 ml of Carrez Solution I (150 mg/ml potassium ferrocyanide and 0.5 ml of Carrez Solution II (300 mg/ml zinc acetate) were added to the each sample and mixed well. The sample was brought to a final volume of 50 ml with deionised water using a drop of alcohol to suppress surface foam. The sample was filtered and the first 10 ml of filtrate was discarded. 5 ml of the remaining filtrate was transferred into each of the two test tubes. A reference sample was prepared by adding 5 ml of 0.20% sodium bisulphite to one test tube with the filtrate. A test sample was prepared by adding 5 ml of deionised water to the other test tube with the filtrate. Both samples were vortexed later the absorbance of the test sample was measured against the reference sample at 284 nm and 336 nm. The absorbance of a clarified aqueous honey solution was measured against a reference solution of the same honey in which the 284 nm chromophore of HMF was destroyed by bisulphite. The test was carried out in triplicates, mean and standard error were noted.

Diastase Activity: Schade Method was used to determine the diastase activity of the selected honey samples. It is calculated as DN (Diastase Number). DN expresses the units of diastase activity. One unit is defined as the amount of the enzyme that converts 0.01g of starch to the prescribed end point in 1 hour at 40°C under the test conditions. It was evaluated calorimetrically at 600 NM. The test was carried out in triplicates, mean and standard error were recorded.

Proline Content ($\text{mg}\cdot\text{kg}^{-1}$): Proline content was determined by using 5 g of each honey sample into a clean, dry beaker and dissolved it in 50 ml of deionized distilled water, quantitatively transferring it into a 100 ml volumetric flask. Water was added to make up the volume and was shaken well. 0.5 ml of the sample was pipetted out into a test tube, 0.5 ml of water (blank test) into another test tube and 0.5 ml of proline standard solution in the third test tube. 1ml of formic acid and 1ml of Ninhydrin solution were added to each test tube. The tubes were capped carefully and vigorously shaken for about 15 minutes. The test tubes were placed in a boiling water bath for 15 minutes, immersing the tubes below the level of the solution. Test tubes were then transferred to a water bath at 70°C for 10 minutes. 5ml of the 2-propanol-water-solution was added to each test tube later capped immediately. After cooling down the

absorbance was determined at 510nm after 45 minutes. The test was carried out in triplicates, mean and standard error were recorded.

Proline in mg.Kg⁻¹ honey at one decimal place is calculated according to the following equation:

$$\text{Proline (mg.Kg}^{-1}\text{)} = (E_s / E_a) \times (E_1/E_2) \times 80$$

Where

E_s = Absorbance of the sample solution

E_a = Absorbance of the proline standard solution (average of two readings),

E₁ = mg proline taken for the standard solution

E₂ = Weight of honey in grams.

80 = Dilution factor

Reducing Sugars (%): Glucose content of honey samples was determined by enzymatic oxidation with glucose oxidase reagent (Randox Laboratories Ltd., UK). 5 ml of each honey solution was taken and 40 ml of iodine and 25 ml of sodium hydroxide solution was added to it, the flask was sealed tightly and kept in the dark for 2 hrs, 12 ml sulphuric acid, tirate against sodium thiosulphate and conduct blank using 50 ml of water. The test was carried out in triplicates, mean and standard error were recorded.

$$\text{Glucose \%} = (B-S) * 0.009005X 100/ 2$$

$$\text{Fructose \%} = \text{TRS- Glucose \%} / 0.925$$

Sucrose Content (%): Estimation of the reducing sugar was carried out using the Layne-Emyon method as described in AOAC. 5 ml of Fehling solution A and 5 ml of Fehling solution B were taken and 0.26g of honey solution were made. Honey solution was added from the burette to 1 ml methylene indicator to complete the titration. The colour change was observed from blue to red. The test was carried out in triplicates, mean and standard error were recorded.

$$\text{Sucrose \%} = \text{TRS (after) - TRS (before)} \times 0.95$$

Adulteration Confirmation Test

Fiehe's Test: Fiehe's test is a qualitative test based on the detection of the Hydroxymethylfurfural that results from the dehydration of fructose, obtained by the acidic hydrolysis of sucrose. The furfural reacts with the resorcinol, forming a colour. If the colour formed is red, then it is confirmed as positive [21]. If there is no colour, then the test confirms negative.

Aniline Chloride Test: 5 gm of honey sample was placed in a porcelain basin and 2.5 ml of freshly prepared aniline chloride reagent was added and stirred well. If there are any commercial invert sugars, then within one minute the orange red colour turns down to dark red, hence it is confirmed that the honey sample is adulterated with commercial invert sugars.

Statistical Analysis: The result was investigated statistically, the entire assay was carried out in triplicate and the data was represented as mean and standard deviations. One way analysis of Variance (ANOVA) followed by least difference (LSD) was compared with the data. Differences between means at the 95% (p ≤ 0.05) level were considered statistically significant.

RESULTS

Chemical Composition of the Pure and Branded Honey Samples: The results show that the pH of the honey samples collected was acidic in nature which reveals that it has enhanced gluconic acid, which benefits the digestion of glucose content easily. The pure honey sample AH1 had a maximum pH of 4.56±0.14 and the branded honey with 4.09±0.14 (table 1 and graph 1).

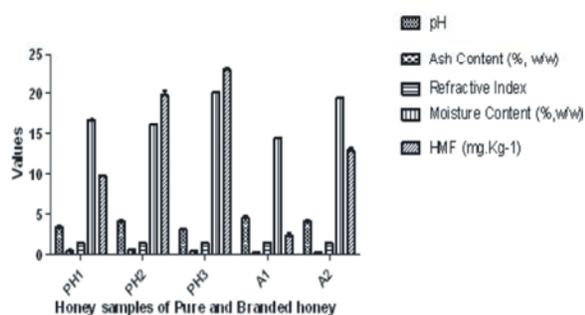
The ash content present in the selected honey samples was from 0.24±0.01 to 0.58±0.13% with an average value 0.39±0.13. The highest value of ash content was recorded for the sample PH1 with 0.58±0.13% for branded honey and for pure honey it was 0.27±0.02. According to the European Honey Commission [22] the permissible limit of ash content in honey is 0.60% (table 1 and graph 1).

The moisture content (%) w/w: The moisture content in honey mainly depends on the botanical origin and also several other factors like ripening of the honey, processing technique, blossom, availability of nectar source and air moisture. The results of the moisture content of the selected honey samples were below 21% and ranged from 14.4±0.02 to 20.12±0.02%. The highest was recorded in the sample PH3 and lowest in AH1 (table 1 and graph 1). The maximum value allowed by the Codex Alimentarius standard is 20%. The moisture content determines the freshness and fermentation free honey [23].

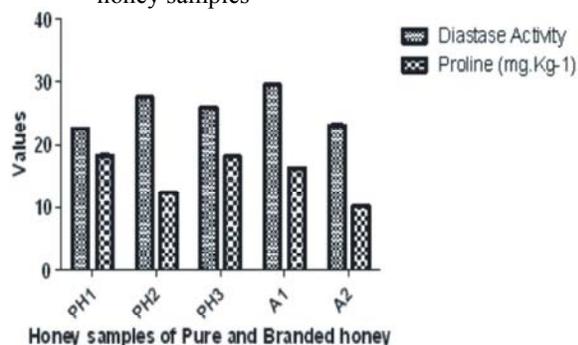
Refractive Index: The value of the water content in honey is estimated by the refractive index with the use of a standard table. The refractive index of the honey samples ranged from 1.4860 to 1.5007 (table 1 and graph 1).

Table 1: Chemical composition of the pure and branded honey samples

Sample No	pH	Ash Content (%) w/w	Refractive Index	Moisture Content (%) w/w	HMF (mg.Kg ⁻¹)
PH1	3.43±0.06	0.45±0.13	1.4946	16.8±0.05	9.73±0.12
PH2	4.09±0.14	0.58±0.07	1.4961	16.24±0.04	19.86±0.53
PH3	3.14±0.12	0.43±0.01	1.4860	20.12±0.02	22.88±0.22
A1	4.56±0.14	0.27±0.02	1.5007	14.42±0.02	02.41±0.31
A2	4.13±0.05	0.24±0.02	1.4875	19.51±0.03	02.86±0.33



Graph 1: Chemical composition of the pure and branded honey samples



Graph 2: Diastase activity and Proline content in honey samples

Hydroxymethylfurfural (Mg.kg⁻¹): The freshness of the honey sample is mainly determined by the hydroxymethylfurfural. The HMF is absent in the freshly stored honey by the bees, but later it increases as the product gets older. The process is done by the simple sugars like fructose due to the action of the acids, thus it indicates the storage of honey in poor conditions [24]. Storage condition, pH, time of heating, temperature and the floral source of the honey is influenced by the levels of HMF [25]. The HMF of the selected honey samples ranged from 2.88±0.22 to 22.41±0.31 mg.Kg⁻¹ (table 1 and graph 1). The branded honey had the highest HMF content ie, 22.41±0.31 mg.Kg⁻¹ than the pure honey.

Diastase Activity: The freshness, improper storage and heat induced defect of honey is mainly indicated by the diastase activity [26]. α amylase the enzyme naturally

Table 2: Diastase activity, Proline and sugars present in pure and branded honey samples

Sample No.	Diastase activity	Proline mg.Kg ⁻¹	Reducing Sugar (%)	Sucrose (%)
PH1	22.63±0.05	18.25±0.14	21.25	5.01
PH2	27.54±0.11	12.30±0.05	35.68	5.43
PH3	26.01±0.03	18.11±0.04	23.65	4.61
A1	29.53±0.05	16.31±0.01	24.67	2.30
A2	23.16±0.20	10.14±0.05	22.23	1.16

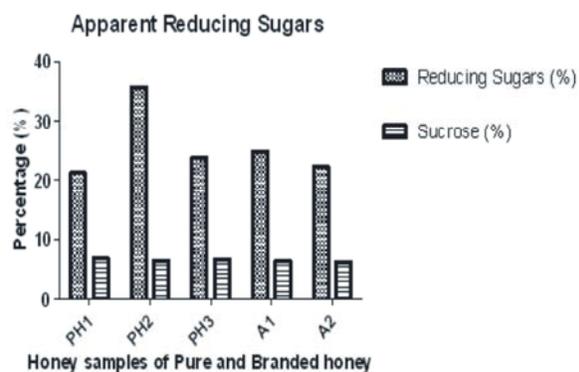
present in honey, which is weakened and destroyed by heat [27]. The mean average value of the diastase activity of honey samples ranged from 22.63±0.05 to 29.53±0.05 (table 2 and graph 2). The values obtained were accorded the Codex standard. Thus it proves that the samples were fresh and of good quality.

Proline Content (mg.Kg⁻¹): Proline content in honey is an indication of adulteration and determines the quality of the honey. It is defined as the colour developed with Ninhydrin compared with the standard proline expressed in mg.Kg⁻¹. The mean average value of the proline content in the selected honey samples ranged from 10.14±0.5 to 18.25±0.14 (table 2 and graph 2). The highest value was recorded in PH1 and PH3 honey sample.

Sugar Content (%): In honey the sugars are mainly composed of glucose, fructose and sucrose. The other constituents present in honey are about 12% [28]. The disaccharides present in honey are maltose, isomaltose, trisaccharides and tetrasaccharides [29]. The sugars in honey were determined by a modification of the Lane and Eynon procedure. The reducing sugar percentage for the selected honey samples had a mean average of 21.25 to 35.68% and the sucrose content was from 1.16% to 5.43%(table 2 and graph 3). The results revealed that the sucrose content was less compared to that of the reducing sugar. The analysis of the sugar content with higher values for glucose (29.49% and 37.45%), fructose (41.52% and 47.53%) and lower values for sucrose (between 0 and 2.68%) [30].

Table 3: Detection of Adulteration in pure and branded honey samples

Sample No	Pure and Branded Honey	
	Fiehe's Test	Aniline Chloride Test
PH1	No Colour	Orange Red Colour
PH2	No Colour	Orange Red Colour
PH3	No Colour	Orange Red Colour
A1	No Colour	Orange Red Colour
A4	No Colour	Orange Red Colour



Graph 3: Apparent reducing sugars and sucrose content in the selected honey samples

Fiehe's and Aniline Chloride Test: The adulteration detection was done by Fiehe's and aniline chloride test. Both the tests confirmed that the samples did not turn into either red or dark red in colour. For Fiehe's test there was no colour formed and for the aniline chloride test the honey solution was orange red in colour (table 3).

DISCUSSION

The need for determining the chemical composition of the honey reveals its physical and chemical characteristics about the honey of that region. The essentiality of the chemical composition is for its storage capacity and the market value [31].

pH: The pH values of the selected honey samples ranged from 3.14 to 4.16. According to the research reports published earlier the standard range for pH of the honey should be between 3.2 to 4.5. The studies pertaining to India honey also recorded with the same pH values. The honey samples from Czech Republic [32] and in Moroccan honey were also similar [33].

Ash Content (%) w/w: The ash content of the pure and branded honey samples varied from 0.24 ± 0.02 to 0.58 ± 0.07 (%)w/w. Ash content is one of the criteria that is associated with the floral and geographical origins of the

honey [34]. It determines the mineral content in honey, where the composition of the mineral content is less in honey, but it provides the nutritional value and also the colour of the honey [35]. Usually the content of ash in honey is meager and the ash content in the selected honey samples were in the suitable range. The mineral content of Spain honey was from 0.06% to 1.34%. The aroma, flavour, medicinal value and other qualities of the honey mainly depends upon the mineral content [36].

Moisture content (%) w/w: The preservation of the honey mainly depends upon the moisture content. The average value of the moisture content is 20% and if it exceeds then the honey starts fermentation. The moisture content of the selected honey samples was below the prescribed range and the sample PH1, 2 and AH1 had lower moisture content. The similar studies were done on *Apis cerana* and *Apis mellifera* honey reported by different authors was similar [37]. The Taiwan honey samples the values were 20-24 [38]. There are also reports showing lower values of moisture content in honey and one of that was reported in American honey 16.72 [27]. The lower values of moisture content may be due to various factors like time of extraction, the process of ripening, climatic conditions, more humid and storage conditions [39].

Refractive Index: The measure of the ratio of the velocity of light in free space is the refractive index of honey. Refractive index increases due to the presence of sugars like laevulose and dextrose besides minerals and amino acids in honey samples [40], lesser refractive index indicate the higher moisture content and higher refractive index indicates the lesser moisture content [41]. Refractive index of *A. cerana* honey was 2.2305 and *A. mellifera* honey had a value of 2.235 [42]. The selected honey sample refractive index ranged from 1.4860 to 1.5007. Similar results were reported in Venezuelan honeys with refractive index 1.499 [40].

Hydroxymethylfurfural (mg.Kg⁻¹): Usually the HMF in fresh honey is less compared to that of the old honey. This condition is mainly due to the simple sugars like fructose in an acid environment [24]. The results of the HMF analysis became apparent that the pure honey samples had lesser values compared to that of the branded honey. The HMF results of the raw and processed honey samples collected from *Apis* species ranged from 13.mg.Kg⁻¹ to 74mg. Kg⁻¹ and the processed

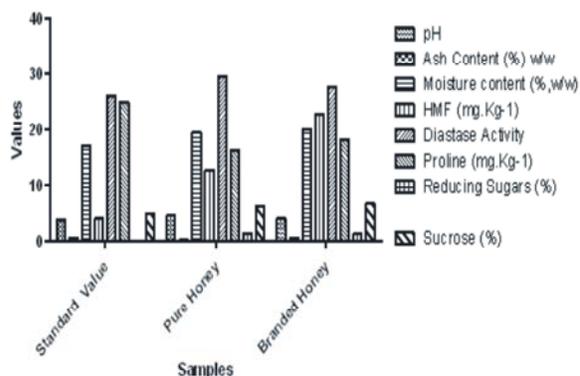
honey samples had higher value [43] A maximum HMF value can be 40 mg.Kg⁻¹ but the value changed for Eucalyptus honey with a minimum of 2.30 mg.Kg⁻¹ and a maximum of 38.20 mg.Kg⁻¹ [44].

Diastase activity: The honey quality can be determined by the diastase activity parameter to show whether the honey has been heated extensively during the processing. The selected honey samples resulted that they were within the limit range of Codex Alimentarius Standard. Similar results were reported for *Apis* species ranging from 21.667 to 28.2 mg.Kg⁻¹ [43].

Proline Content (mg.Kg⁻¹): Adulteration of honey can be detected by the amino acid proline present in honey. It determines the genuineness and ripening of the honey. The proline in honey originates from the secretions of the honeybee [45]. The proline contented from different honeybee species varied [46, 47]. The proline content was higher in *Helianthus annuus* honey when compared with *Eucalyptus lanceolatus* [48]. The proline content recorded in the present study accepted the Codex Alimentarius Standard.

Sugars (%): Reducing sugars, mainly Glucose and fructose are the important components of honey [49]. The honey samples analysed resulted that the reducing sugars ranged from 21.25 to 35.68. The sucrose content of the selected honey samples was in the permissible limit with below 5%. The Romanian lime honey sample analysed had 42.49% of combined glucose and fructose content [50]. 5% Sucrose is the maximum prescribed limit as per the Codex standard [51]. If the sucrose content is higher than 5%, then it indicates that the honeybees are over fed with sugar syrup, early harvesting or any adulteration [52, 53]. The sucrose (%) content in the seven honey sample analysed ranged from 0.4 to 8.8 %. The seventh sample had high sucrose content and all the other were below 5% [9].

Adulteration Test: The Fiehes test and the aniline chloride test performed to detect if there are any adulterants in the selected pure and branded honey samples was negative. This preliminary test reveals that the samples are not adulterated. Similar tests were done for the honey samples analysed for any adulterants were negative for Brazilian honeys [21].



Graph 4: Differences between the pure and branded honey samples compared with the standard values

CONCLUSION

All the five honey samples which were fresh and branded honey analysed for their chemical features and their differences between the pure honey collected from the apiary sites and the branded honey samples from the local market revealed the values of the quality parameters. All the pure honey sample chemical parameters were within the Codex standard. The branded honey samples slightly deviated with the parameters like ash content, moisture, HMF and sucrose content (graph 4). Apart from these both of the pure and branded honey samples are rich with diastase activity and proline content records that the honey samples are not adulterated and their nutritional properties are enriched. The results ascertained the quality of the honey, which will create a worthy profit in the global market and its therapeutic value.

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