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FTIR Characterization and Physico-chemical Studies of Honey Samples in Kanpur Nagar

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Abstract

Honey is a valuable food entity that gains a lot of attention due to its potential benefits for health and its uses to sweeten foods. It contains about 60% invert sugar and appreciable amounts of maltose and sucrose. In this study, the Physico-chemical characteristics of pure and adulterated honey samples were investigated. The grade and limitation of adulteration of the honey samples also were checked. The moisture, ash and pH were carried out. The Fourier Transform Infrared Spectroscopy (FTIR) of different samples were analyzed. The result of FTIR spectroscopy provides reliable results.

Keywords: Honey, Moisture, Total ash, pH.

Introduction

Honey is a natural sweet substance obtained from honeycombs. It is a unique sweetening substance that can be used by humans without processing.[1] Honey is used in health described in traditional medicine and as an alternative treatment for clinical conditions and is also used in brewing honey wine.[2] The major component of honey is fructose and glucose with a lesser amount of water. The composition of honey varies with the floral and honeydew sources utilized by honey bees as well as climate conditions.[3] The chief sources of microorganisms in honey are the nectar of flowers and the honeybee yeasts have been shown to come from the nectar and from the intestinal content of the bee, bacteria also come from the later source. Honey has been found to contain lysozyme, an enzyme with a bacteriostatic as well as a lytic effect on most gram-positive bacteria. The use of antibiotics, such as neomycin[4] and streptomycin[5] is widespread in beekeeping, and these antibiotics have been found in the honey obtained from treated larvae and bees. The gluconobacter and lactobacillus are the two main groups of bacteria present during the maturation of nectar to honey.[6] Honey is extracted from the comb, strained, and marked. Honey contains larger quantities of fructose[7] (about 38%) than glucose (31%) and Sucrose[8] constitutes about 2% of the total sugar[9] content. Honey has the capacity to retain water, and hence, cakes, candies, etc., made with honey remain moist for a longer period than those made with other sweetening agents[10] determined by the Physico-chemical parameter i.e., refractive index,

specific gravity, water-insoluble solids, and total soluble solid, etc. The physical properties such as conductance, surface tension, and pH determine by using digital instruments.[11] Honey is essentially a concentrated aqueous solution of invert sugars, but it also contains a very complex mixture of other saccharides, proteins, enzymes, amino acids, organic acids, polyphenols, and carotenoid-like substances, vitamins, and minerals.[12] Dietary frauds in particular adulteration are practices in constant progress.[13] If any coloring matter other than prescribed in respect of the amounts is not within the prescribed limits of variability present in substances.[14,15] The colored inverted sugar syrups are used to adulterate honey.[16,17,20] One of the methods that provide information on the total chemical composition of any sample is infrared spectroscopy.[18,19] So, the aim of this study was to confirm the pure and adulterated honey samples to be collected from the local market of Kanpur Nagar by physicochemical and IR spectroscopy methods.

Materials and Methods

Sample Collection

Three samples of honeys produced in Kanpur Nagar were collected from beekeepers. The samples were stored in refrigerator in airtight plastic containers until analysis.

Physico-Chemical Analysis

Determination of moisture content

The sample material was taken in a flat bottom dish and kept for 12 hours in an oven at 100 -110 °C and weighed. The loss in weight was a measure of moisture content.

Moisture content = M_1- M_2 / M_1-M_0

Where

M_0 - Weight of the dish

M_1 - Weight of the fresh sample +dish

M_2 - Weight of the dried sample +dish

Determination of ash content

Ashes were obtained in a muffle furnace at 550 °C for 5-6 hours to obtain constant weight.

The percentage of Ash was calculated:

Ash (%) = (Weight of crucible + Ash) – (Weight of empty crucible) X 100 / sample weight

Determination of pH

The pH of honey is determined by using a digital

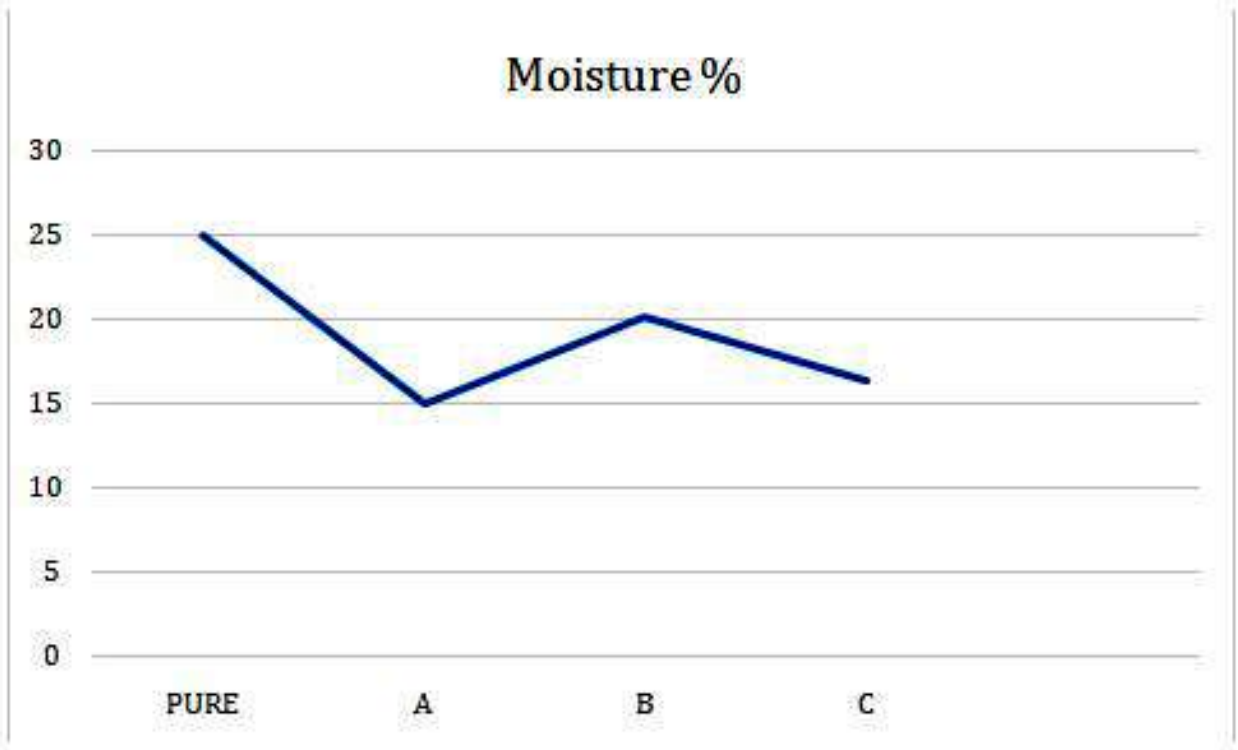

Fig. 1: Moisture in various honey samples

Fig. 2: Ash content in various honey samples

pH meter. The pH meter was calibrated with buffers at pH 4,7 and 10. The sample was taken in a beaker dissolved in distilled water by using a Magnetic stirrer. Electrode inserted in the beaker containing the sample and recorded the pH.

Determination of Adulteration

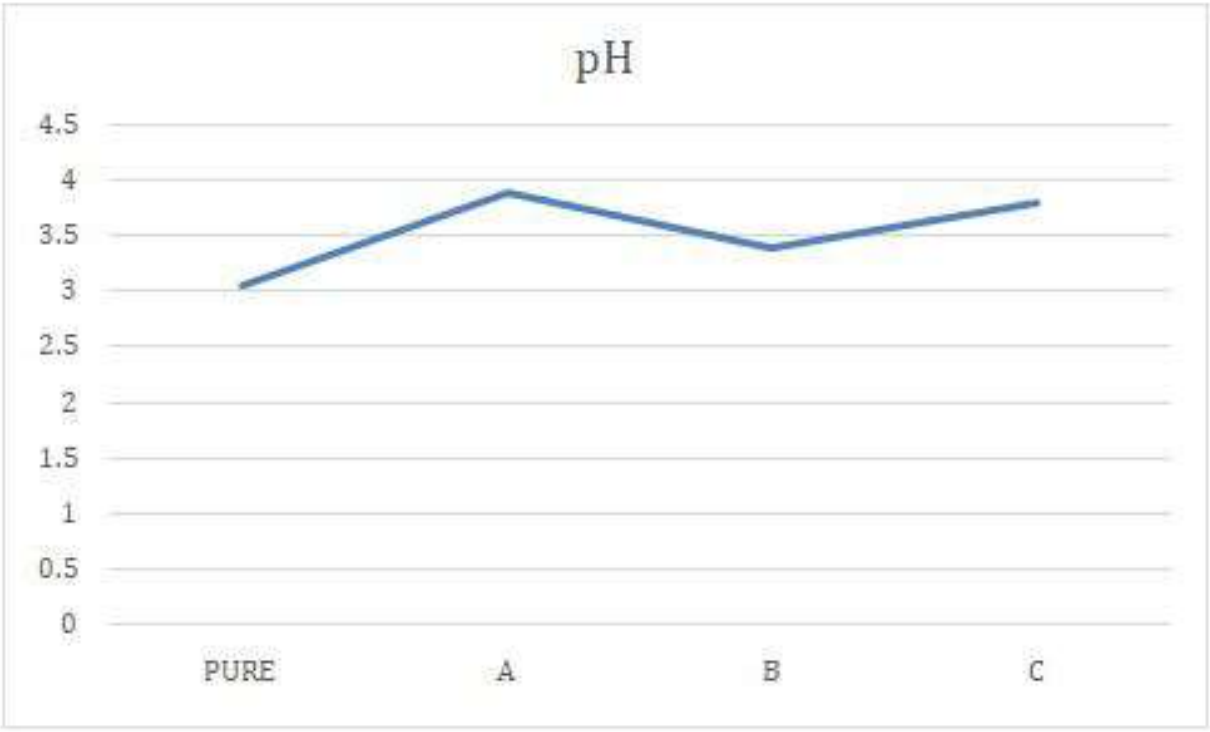

Fig. 3: Data of the pH in various honey samples

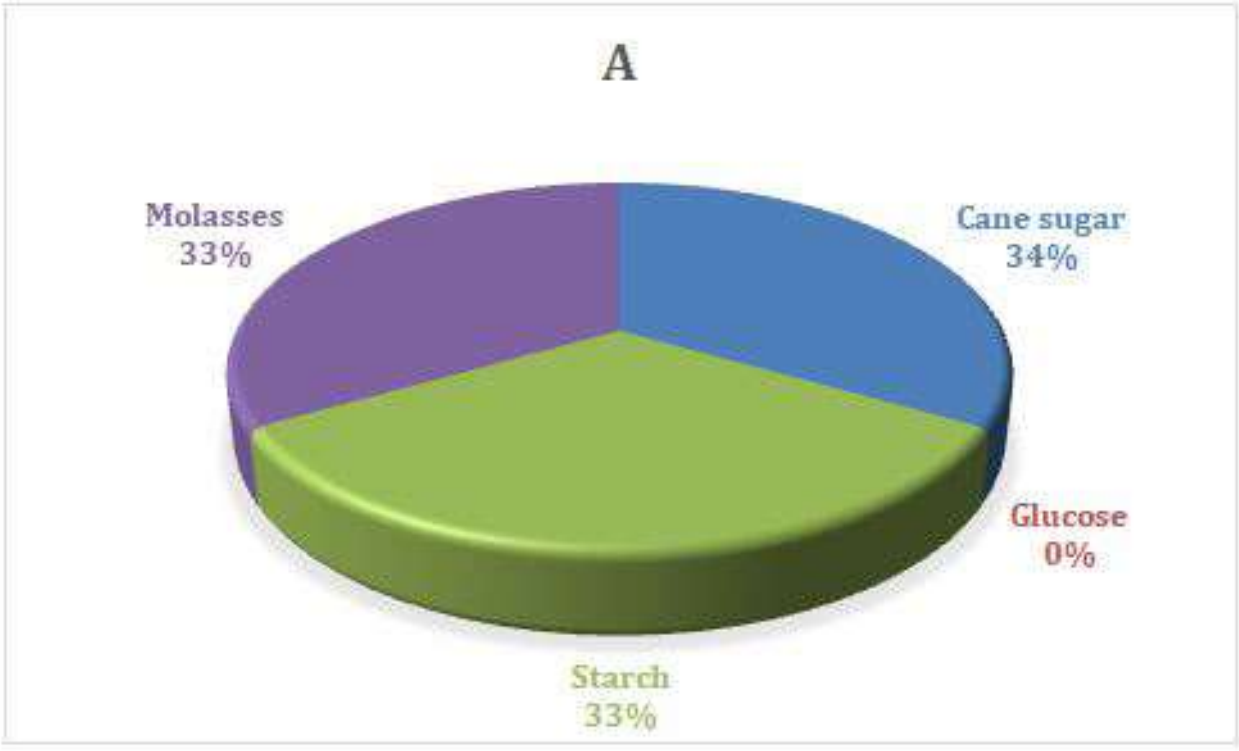

Fig 4: Adulteration in Honey samples

FTIR Spectra

IR spectra of honey samples were recorded using the Fourier transform infrared spectrometer (FTIR Spectrometer). The spectral range from 1200 to 700 cm-1 (Maximum absorbance established at 1026 cm-1) was subjected to further statistical analysis since the adulteration in honey has been identified in this particular spectral region.

Results and Discussion

The moisture content of honey samples had a value of 15%, 20.11%, and 16.42%. The moisture levels are one of the most crucial parameters of honey which affects the optical density, refractive index, surface tension, and viscosity. Honey is an excellent hygroscopic product and tends to absorb atmospheric moisture and thus readily increase its moisture levels. In BIS the maximum content of moisture is 25%.

The Ash content in Fig 2, sample A has a high amount of Ash value, Sample B has a little high

Table 1: Infrared Band Assignments for pure honey and compare with other honey samples collected from local market of Kanpur Nagar

Functional Group	Pure Honey Frequency (cm-1)	Sample A Frequency (cm-1)	Sample B Frequency (cm-1)	Sample C Frequency (cm-1)
O-H (H-bonded)	3276.79	3521.42	3518.92	3521.81
C-H stretching	2948.35	2929.28	2929.16	2928.32
O-H (Water) bonding	1638.19	-	-	-
-CH2 bending (Strong)	1426.05	1419.86	1426.12	1421.02
-CH2 bending (Medium)	1362.05	1346.20	1362.68	1361.66
C-C, O, -CH2, C-OH bending	1254.15	1255.66	1259.19	1260.14
C-O Stretching	1032.05	1034.24	1057.47	1057.64
Extra peak	-	3095.92	3099.01	-
Extra peak	-	2343.51	-	2343.72

but Sample C has a low according to the Bureau of Indian standard (BIS). Bureau of Indian standard (BIS) maximum percentage of ash is reported as 0.5%.

Data of pH of samples shown in Fig. 3. pH content of honey varied a 3.9, 3.4, and 3.8. Bureau of Indian standard (BIS) reported pH in the range of 3.05 – 4.50. According to data pH range is not more than the reported value.

FTIR Spectrum Analysis

In this study, FTIR was used to compare honey samples based on their spectral difference in the range 4000- 650 m-1.[11] The spectrum of honey samples is shown in Fig. 5, 6, and 7. Table-1 represents the bond assignment along with the corresponding vibration and compares it with pure honey.

The O-H stretching vibration band in carboxylic acids is very broad and occurs in the field of 3300-2500 cm-1.[12] The pure honey samples obtained the O-H peak at 3276.79 cm-1 but adulterated honey samples 1, 2 and 3 show a peak at 3521.42, 3518.92, and 3521.81, respectively. Fig. 5,6,7 and Table:1 show a band at 2929 cm-1 was observed which according to Anions et.al.[13] also corresponds to C-H stretching of carboxylic acids and NH3 stretching band of free amino acids. However, the band under 1440-1395 cm-1 cannot be distinguished from C-H bending bends which also occur in the same frequency region. The O-H water bonding peak is approx. 1638 cm-1 did not appear in sample1, 2, and 3. The vibration with the maximum bandwidth of about 1255 cm-1 1259 cm-1 and 1260 cm-1 are characteristic and bending vibrations of C-C, C-CH2, and C-OH grouping. The band range from 1250 to 1140 cm-1 is in turn vibration characteristic for the stretching vibration of the C-H in carbohydrates or stretching vibration of CO in carbohydrates. The vibration with the maximum at approximately 1100 and 1080 cm-1 constitutes a band that can originate in the vibration of C-O stretching but samples A, B, and C showed these peaks at 1034 cm-1, 1057 cm-1, and 1057 cm-1. Some extra peaks i.e., 3095 cm-1 and 3099 cm-1 showed in samples A and B but not shown in sample C. Similarly, the peak 2343 cm-1 appeared in samples A and C but not appeared in samples B. The extra peaks which showed in Table-1 were due to adulteration.

Conclusion

Honey is a low-cost natural product that can be used for different purposes. Now, commercially honey is used in various industries for product formation and this trend is increasing day by day as industrialists are finding honey to be a cheap

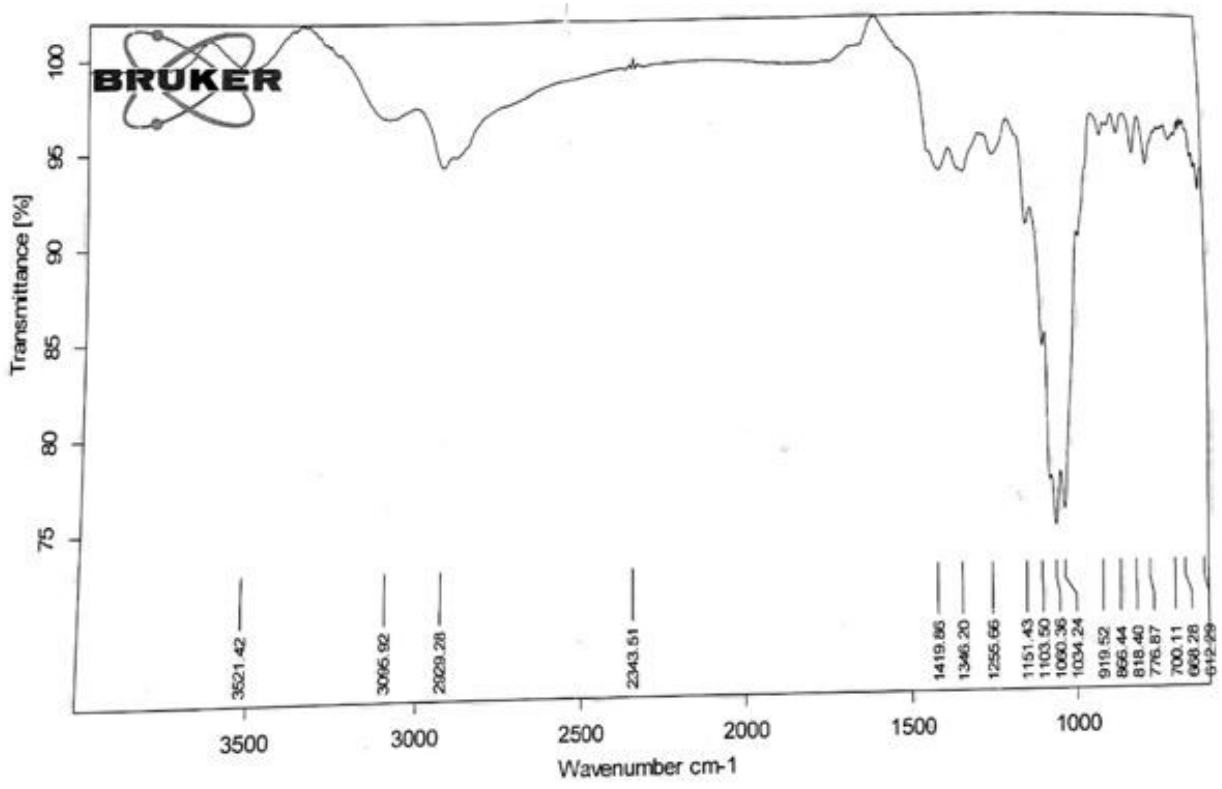

Fig. 5: FTIR Spectra of sample A. **Source:** https://www.bruker.com/en.html

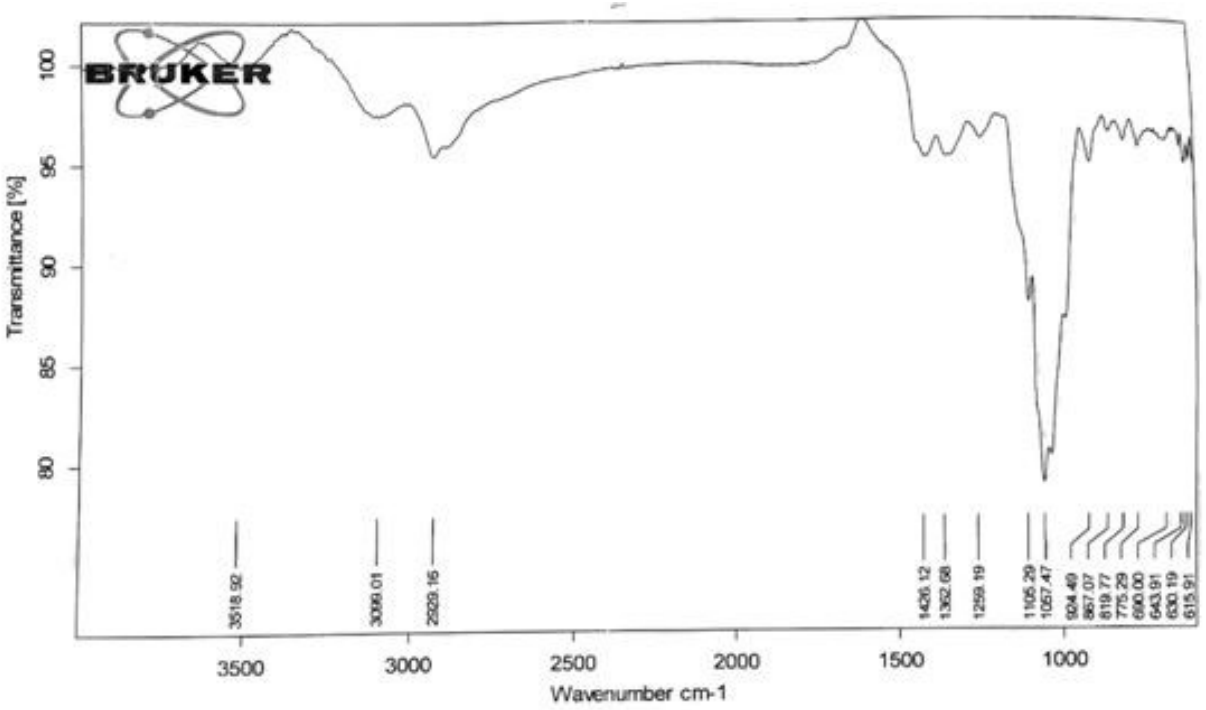

Fig. 6: FTIR Spectra of sample B. **Source:** https://www.bruker.com/en.html

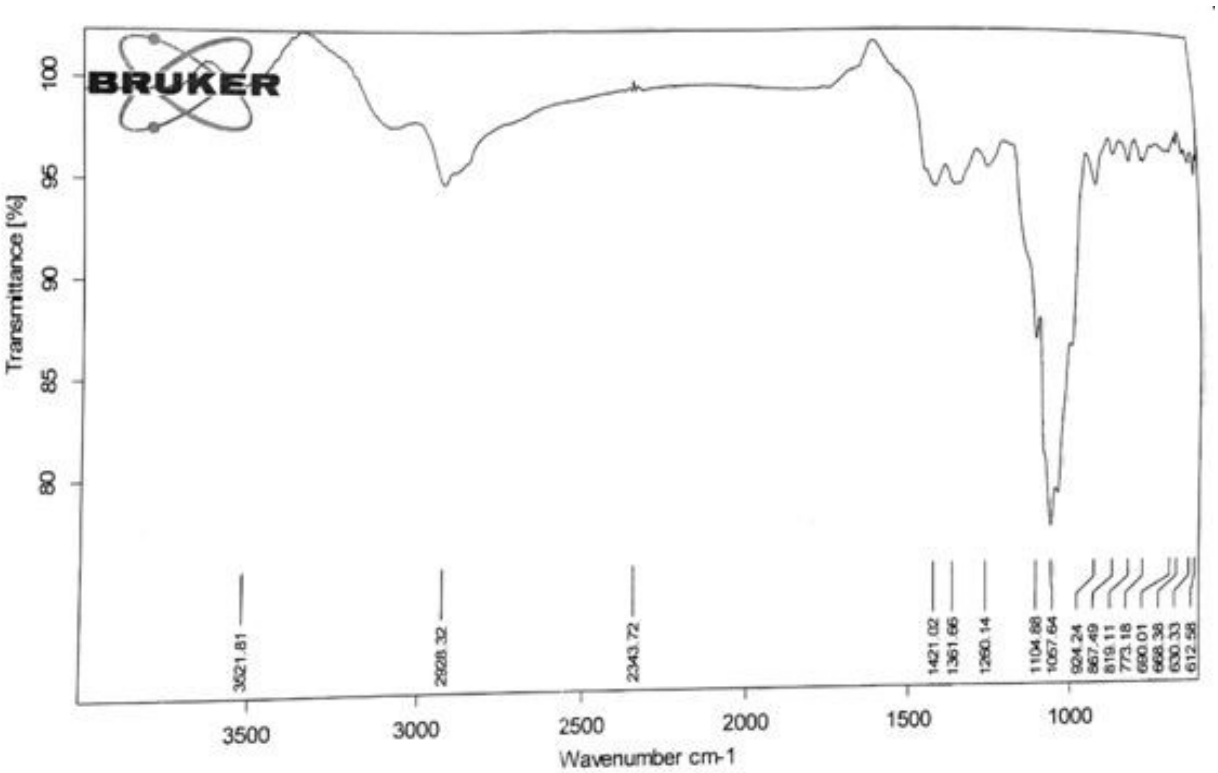

Fig. 7: FTIR Spectra of sample C. **Source:** https://www.bruker.com/en.html

source of sweetening agent without any side effects as in the case of synthetic sweeteners. Due to variation in botanical origin honey differs in appearance, sensory perception, and composition. According to obtained data from studied literature, it is to some extent obvious that nearly the majority of physiochemical properties of honey depend on floral sources. The honey samples collected from Kanpur Nagar were not found to have good quality physicochemical characteristics and adulterants

also found in some samples also might have been due to unhygienic handling during processing and storage. The results of this study showed that IR spectroscopy provides equally reliable results, but also represents a rapid and cheap analytical tool in comparison to commonly used standard analytical methods. Therefore, it is expected that IR spectroscopy will contribute to the authentication and quality control of honey samples.

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