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Comparative fourier transform infra red (FTIR) analysis of commercial honey and enriched honey of *Apis cerana indica* (Fabricius)

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Abstract
Honey the most explored apiproduct from medicinal as well economic point of view worldwide. It has been widely adopted as food and medicine by both ancient and modern generations. Honey is a viscous, sweet and palatable liquid palatable with high nutritional value and array of health benefits. The colour of honey is characteristics of its floral source due to minerals and other minor components. Raman infrared spectra provide complementary intonation about the vibrations of molecules and in consequence about the functional groups that constitute the product analysed the enriched coloured honey.by FT - Raman spectroscopy and chemometrics. Fourier transforms infrared spectroscopy (FTIR) reveals to phytochemical profiles containing overlapping singles from a wide array of the compounds. This study was carried out to identify the reactive functional groups present in enriched coloured honey.

Keywords: fourier transform, infra-red, *Apis cerana indica*

Introduction
Honey the natural sweet liquid produced by honey bees from the nectar of blossoms or from the secretion of living parts of plants which the honey bees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature (Alimentarius, 1989) [1] is an important food for human beings. Crane (1983) [9] was of the view that the relationship between bees and Homo sapiens existed for a long time. Crane *et al*., (1984) [10] differentiated a wide variety of honey with different taste and colours depending on their botanical origin. Caillavy (2008) [7] suggested that the colour of honey is largely a reflection of plants from which the nectar was sourced and also reported that honey colour is an important consideration as it determines the value of the product and the products final destination. These preferences are largely social and cultural as honey colour does not reflect any intrinsic value of the product, Wongsiri *et al.*, (2000) [23] suggested that different honey sources can differ in terms of their taste, smell and colour.
The colour of honey is characteristics of its floral source due to minerals and other minor components. Exposure to heat and storage time may affect the colour of honey (National Honey Board, 1995) [17]. Honey is classified into seven colour categories Water white, extra white, white, extra light, amber, light amber and dark amber by USDA (National Honey Board, 1995) [17]. White (1975a) and Crane (1980) [22, 9] stated that honey colour is frequently given in millimetres on a p fund scale (an optical density reading generally used in international honey trade). Gheldof *et al.*, (2002) [11]. Bertoncelj *et al.*, (2007) [5]. Vela *et al.*, (2007) [21], Zegarac *et al*., (2009) and Marghitas *et al.*, (2009) [24, 14] reported that the darker the honey, the higher its phenolic content and its antioxidative power. Honey inhibits the growth of micro-organisms and fungi. The antibacterial effect of honey mostly against gram positive bacteria is well documented by Bogdanov (1997) and Molan (1992, 1997) [6, 15]. Kwakman *et al.*, (2010) [13] characterized an antibacterial honey protein as defensin - 1 which originate in royal jelly. Honey also has antiviral and anti-parasitic activity. It was reported that honey has been shown to inhibit *invitro* the *Rubella* virus (Zeina *et al.*, 1996) and *Herpes* virus (Alwaili, 2004) [25, 3] and three species *of the Leishmania* parasite (Zeina *et al.*, 1997). 2004) and three species *of the Leishmania* parasite (Zeina *et al.*, 1997) [26].

Materials and Methods
Experimental hives
Six experimental hives were placed in the Annamalai university campus - Chidambaram. The hives were properly cared for to ensure that the hives contained sufficiently large populations of honey bees as well as sufficient amount of brood and pollen.

Coloured honey
The experiment was carried out during February, when nectar producing flowers did not blossom, thus minimizing the interference of floral nectar with the experiment. The colouring agent (dye) was mixed with diluted honey (honey and water in the ratio 1:1) and placed in shallow vessels in the super chamber. The honey

bees fed on this honey and started depositing it on the combs present in the super chamber. Feeding was continued till the combs were sealed with the artificially coloured honey. The coloured honey was subjected to FTIR tests.

Enriched Honey

Bees in a colony were fed daily with 50 ml of honey solution with 50% of carrot extract, 5 ml of egg albumen and 1 ml of vitamin E (Tocopherol) solution. The honey bee converted this mixed enriched feed into natural enriched coloured honey and stored in their comb. After extracting the enriched honey, the FTIR studies were carried out.

FTIR (Fourier Transform Infra Red Analysis)

For FTIR analysis, the sample were presented in the form of KBr discs. The honey were first dried and measured quantities of 0.1 g of the extract was ground with an equal amount of KBr and the mixture was compressed into a transparent wafer or disc. The grinding was done under an infrared lamp to avoid condensation of atmospheric moisture.

The frequency of the spectra was set between 4000 - 5000 per cm wave number and the vibration spectrum was recorded as graphical chart. FTIR analysis was done using Shimadzu analyzer, Japan, Resolution: 4500, Sample gain: 1.0, Minor velocity: 0.6329, and Detector: DTGS – KBr. The infrared spectra were interpreted based on the reference library of Silverstein e/<3/. (1981).

Spectrophotometry

Absorbance spectrum

The extent to which a sample absorbs light depends upon the wave length of light. In analyzing honey samples the absorbance spectrum of the sample was first determined and then the wave length of maximum absorbance was found out.

Fig 1: Worker bees-cells filled with red coloured honey

Fig 2: Final instar larvae in their cells

Fig 3: Cells filled with enriched coloured honey

Table 1: FTIR measurement of commercial honey

SI. No	Peak	Intensity	Base(H)	Base(L)	Area
1.	373.34	11.992	381.92	358.77	18.047
2.	394.56	17.407	405.06	381.92	17.2
3.	526.8	16.842	536.23		55.913
4.	562.3	16.536	574.81	536.23	29.824
5.	593.24	16.143	611.45	574.81	28.707
6.	634.67	16.281	692.47	611.45	60.323
7.	779.34	21.275	802.41	756.12	28.006
8.	819.85	25.427	850.64	802.41	25.432
9.	868.07	31.57	883.43	850.64	15.66
10.	921.15	33.776	949.01	906.57	18.459
11.	1061.03	6.378	1070.53	1039.67	35.95
12.	1258.7	26.93	1292.35	1207.48	46.176
13.	1349.36	25.899	1381.08	1292.35	49.966
14.	1423.87	24.099	1446.66	1381.08	38.808
15.	1641.62	23.453		1550.82	101.823
16.	2938.76	23.937	2997.48	2389.88	228.623
17.	3374.83	2.074	3421.83	3383.26	64.384

Table 2: FTIR measurement of enriched honey

Sl. No	Peak	Intensity	Base (H)	Base(L)	Area
1	369.34	15.253	385.78	358.77	20.207
2	528.73	20.994	536.23	466.79	45.284
3	571.3	20.57	572.88	536.23	24.879
4	592.24	20.1 58	61 1.45	572.88	26.603
5	621.81	20. 1	688.61	61 1.45	5 1.115
6	781.34	25.1 53	800.49	754.19	25.242
7	827.85	29.236	848.7 1	800.49	23.1 9
8	868.07	34.907	885.36	848.71	1 6.016
9	928.15	36.445	949.01	906.57	1 7.286
10	1051.03	8.002	1097.53	949.01	120.07
11	1257.77	29.603	1290.42	1205.55	42.91 5
12	1349.36	28.407	1359.86	1290.42	36.009
13	1481.87	26,8 1	1 525.74	1 38 1.08	66.314
14	1651.69	26.909	1 828.58	1 550.82	97.566
15	2941.76	27.664	2997.48	2391 8 1	2 1 0.832
16	3368.68	2.754	3381.33	2999.41	385 235
17	3397.83	2.742	3421.83	3383.26	59.652

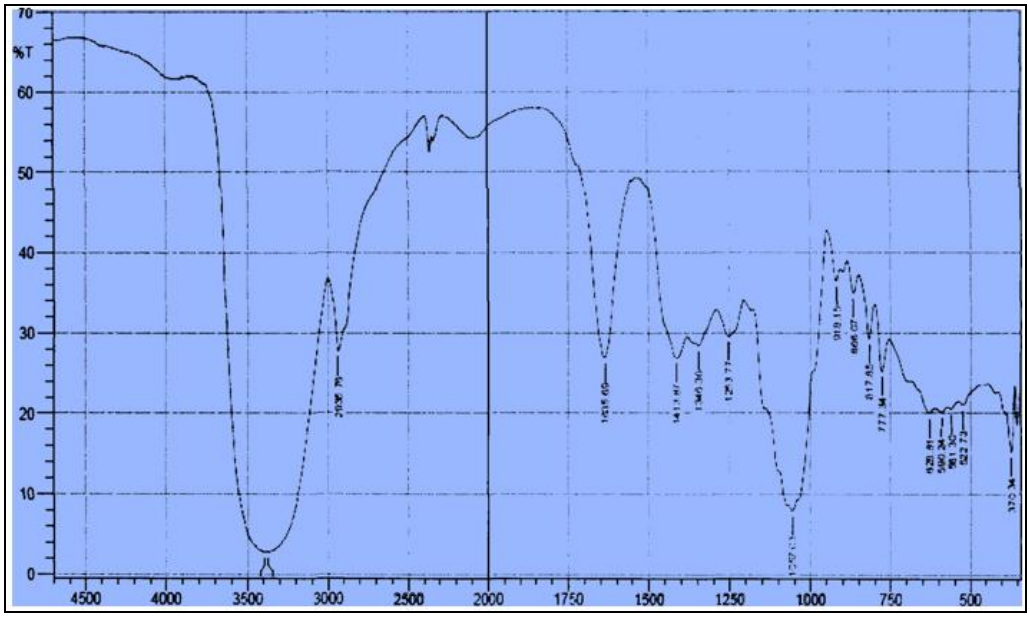

Fig 4: FTIR analysis of commercial honey

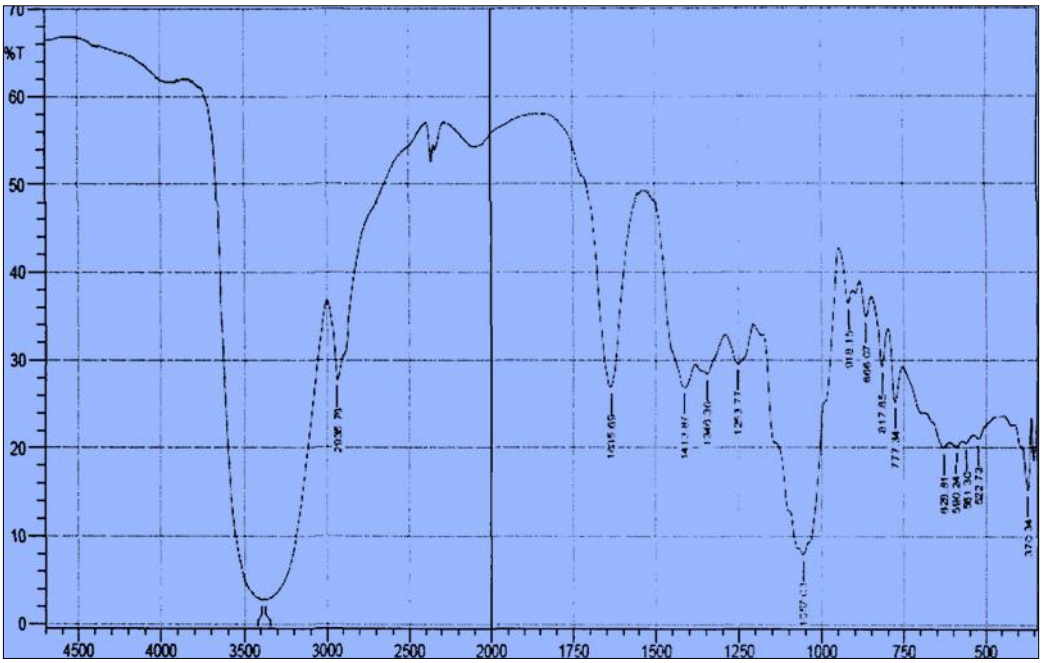

Fig 5: FTIR analysis of enriched honey

Result and Discussion

It is possible to artificially enrich the aesthetic and nutritional quality of natural honey to enhance its marketability. Alissandrakis *et al.*, (2010) [2] investigated the bioconversion of nectar components through the mediation of honey bees. Colour is an important characteristic upon which honey is classified by honey producers, packers and users. An estimated 75 percent of industrial users of honey include colour designations in their specifications (NHB and AB, 1990) [18]. The colour of the honey is characteristic of its floral source due to minerals and other minor components.

The dye was mixed with diluted honey (50 percent) and a supplementary feed was produced. The supplementary feed containing dilute honey and colouring agent was fed to the honeybees (*Apis cerana indica*). Dark red colour could be seen in the crop of honeybees that consumed the artificial coloured feed. The honey bees act as bioreactors and convert the artificial feed into coloured honey. The department of Agriculture and co-operation has laid down standards of honey under the Grading and Marketing rules of Agmark, which lays down the grades designation of honey as Special, Grade A and Standard to indicate the quality of honey for the purpose of certification. Honey is attractive to the consumers for several reasons. In addition to its sweet taste, flavour is one of its most significant attributes. Consumer preferences related to flavour depend mainly on country or regional customs. Dark honey dew honeys of more prenounced flavor and taste are highly priced in central regions of Central Europe, while they are less valued than floral honeys in North America (Siddiqui, 1970).

The IR frequencies of enriched honey ranges from 370.34 to 3394.83 and similar range is observed in the control honey. In addition to these ranges, the medium band frequency of 3371 is observed in the enriched honey (Figures 1and 2). This frequency suggested the presence of amine group with N - H stretching vibration, indicating the presence of protein in the enriched honey.Pierna *et al.*, (2011) [20] analysed the Corsican honey by FT - Raman spectroscopy and chemometrics and reported that the FT - Raman spectrum of honey has a large band in the vicinity of 3,234 cm^{-1}. characteristic of O -H group stretching vibration and several sharp peaks in the 200 - 1500cm' region characteristic of several chemical groups. Paradkar and Irudayaraj, (2002) [19] and Batsoulis, (2005) [4] reported Raman spectrum of honey is considered as a combination of absorption due to different compounds and the major compounds are carbohydrates.

Conclusion

The advantage of enriched coloured honey is that the honey bees are used as bioreactors and this type of beekeeping can be practiced even in sterile areas where honey flow is minimum or absent. Food is supplied to the honey bees along with additive supplements and the bees need not depend on flowers in the surroundings. So, the honey bees are sustained and in the meantime there is storage of honey which could be extracted. Through this method, we can produce different coloured and enriched honey. The disadvantage is that additional economic inputs are required. The bees are fed with diluted honey and not sugar syrup. Hence the enriched honey has to be sold at a very high price to compensate for the inputs made.

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