PHYSICO-CHEMICAL AND BIOCHEMICAL CHARACTERIZATION OF HONEY FROM LYCHEE (LITCHI CHINENSIS) AND INDIAN MUSTARD (BRASSICA JUNCEA) FLOWERS FROM WEST BENGAL STATE IN INDIA

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RESEARCH ARTICLE

Abstract

Honey is a natural product of animal origin very important in human diet from many points of view, defined as "the sweet substance produced by honeybees from the nectar of blossoms or from secretions on living plants, which the bees collect, transform and store in honeycombs". It plays an important part in our nutrition, and it is well-known for its positive effects on health.

Litchi Chinensis (or Lychee) is an evergreen tree form the South-Eastern regions of Asia, well known throughout history for its sweet fruit. The fruit features a red coloured harder inedible outer shell, followed by the fruit pulp and the seed.

Brassica Juncea (or Brown Mustard, Indian Mustard, Chinese Mustard) is a species of mustard plant widely used in South-Eastern and African Cuisine, where its leaves are cooked, or its oil gets cold pressed and extracted for frying purposes.

Both plants are commonly found in West Bengal State, in North-Eastern India, thus making their honey rather easily obtainable. The aim of the following study was to analyse certain Physico-chemical and Biochemical parameters from these types of honey from the forementioned region.

Keywords: Honey, Lychee Honey, Mustard Honey, Indian Honey Analysis
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INTRODUCTION

Honey is defined as "the sweet substance produced by honeybees from the nectar of blossoms or from secretions on living plants, which the bees collect, transform and store in honeycombs" (Codex Alimentarius Commission, 2002). Naturally, honey has been traditionally recognized as a valuable source of energy. It has also been recognized for its antimicrobial characteristics, possessed excellent antioxidant activity and is such a natural product with several therapeutic properties. It is a concentrated aqueous solution of invert sugar, that contains a mixture of other carbohydrates, amino and organic acids, minerals, aromatic substances, pigments, waxes and pollen grains to make it complex (Al-Quassemi and Robinson, 2003).

Litchi chinensis belongs to the Sapindaceous family and is well-known in the Indian traditional system for its traditional uses. The parts of the plant used are leaves, flowers, fruits, seed, pulp, and pericarp. All parts of the plant are rich sources of phytochemicals––epicatechin; procyanidin A2 and procyanidin B2; leucocyanidin; cyanidin glycoside, malvidin glycoside and saponins; butylated hydroxytoluene; isolariciresinol; kaempferol; rutin; and stigmasterol.

Ayurveda and the naturopathic system of medicine (indigenous to India) clearly state the use of medicinal plants for treating various disorders. India has a rich knowledge of phytotherapy from ayurveda and hundreds of potent drugs are yet to be evaluated scientifically. Keeping this in view, the lychee is one of the potential plants that has edible fruits and the other parts of which also have potent traditional application, but it has not been studied much (Kilari and Putta, 2016). The component of interest for honey production and implicitly for our research is the flower. Lychee flowers are small and inconspicuous, greenish in colour, and are borne in loose terminal clusters sometimes up to 30cm long (Britannica, 2024).

Mustard (*Brassica juncea*) is a cruciferous vegetable used as a food spice and folk medicine worldwide. Mustard contains numerous phytochemicals such as: vitamins, minerals, dietary fiber, chlorophylls, glucosinolates (and their degradation products), polyphenols and volatile components (allyl-isothiocyanate, 3-butyl isothiocyanate, etc.) Tian, and Deng (2020).

Mustard honey, a monofloral honey derived from mustard flower is considered a great source of nutritional and medicinal values. The honey is traditionally used as ethnomedicine in different parts of the world to cure many health problems (Billah et al, 2019).

The aim of the present study was the physical-chemical and biochemical characterization of two types of honey from India: Lytchee and Mustard honey. I discovered these assortments during my participation in the Erasmus placement, carried out at the University of Calcutta in 2023.

MATERIALS AND METHODS

As mentioned before, in the region of the Sundarbans, in the district of West Bengal, it is common to see Lychee tree orchards as well as Indian mustard plantations. Therefore, in the pollinating season, the honey production from these specific plants increases.

In the study, 2 samples of honey were analysed, one of lytchee and one of mustard.

To obtain the most accurate values, we worked with 3 samples from each type of honey.

For both types of honey, an organoleptic examination was carried out, certain physico-chemical and biochemical parameters.

The Physico-chemical and biochemical analysis conducted were the following: colour determination, water, total soluble substances (TSS), ash an HMF content, the total polyphenols (TPC) and antioxidant activity (AA).

For the organoleptic analysis, we looked into the general aspect of the honeys: degree of impurity (d.o.i.), consistency, colour, taste, smell.

Physical-chemical parameters, like: colour, water, pH, acidity, ash, were analysed according to the Romanian Standard Analysis Methods (National Standard, 2009) and Harmonised methods of the IHC (Bogdanov, 2009), or with specific methods.

- Colour determination: ABS 450nm/ and Pfund scale
- Water and Total soluble substances (TSS): Brix index - refractometric determination
- Total acidity - titrimetric method
- HMF determination - WHITE method (spectrophotometric determination)
- Ash content - calcination at 525°C
- Total Polyphenol (TPC) - Folin-Ciocalteu method
- Total Antioxidant (AA) - FRAP method

Colour Analysis

To help us further expand our information in the colour differences, we conducted a colour intensity analysis. The net absorbance ABS 450, was defined as the difference between spectrophotometric absorbance at 450nm and 720nm. Determinations were made with a Shimadzu UV Mini 1240 spectrophotometer (Beretta, 2005).

The colour of honey was then determined by spectrophotometric measurement of the absorbance of a 50% honey solution (w/v) at 635 nm. The honeys were classified according to the Pfund scale after conversion of the absorbance values using the formula:

mm Pfund = -38.70 + 371.39 × Abs 635 where mm Pfund is the intensity of honey colour in the Pfund scale and Abs is the absorption of honey solution (White, 1984).

Ash Content Determination

The ash content was determined using calcination in a calcinating oven, by gradually heating the samples from 90°C all the way to 525°C. The process was stopped when only white ash remained in the porcelain crucible.

After cooling, the crucibles were weighed, and the ash percentage was calculated for both types of honey.

Water and Total Soluble Substances – BRIX

The total sugar content corresponds to the concentration in the water-soluble substance, expressed in BRIX degrees, because in honey sugar is the main water-soluble substance, the rest of the compounds being quantitatively insignificant. The desired results were obtained using a digital refractometer (KRUS, AR 2008 model, Germany).

Total acidity

Total acidity was determined by titration method (Bogdanov, 2009).

Hydroxymethylfurfural (HMF) Content determination

HMF content was determined by spectrophotometric method (White, 1979). Each of the honey samples was divided into 2 clarified aliquots; water was added to one of the aliquots and absorption was read at λ=284 and 336 nm. This was compared to a second solution in which this absorption was eliminated by the addition of sodium bisulphate. Results were expressed in milligrams of HMF per kilogram of honey.

HMF (mg/kg) = $(A_{284} - A_{336}) \times 149.7 \times 5/m$, where A_{284} = absorbance reading at 284nm,

A_{336} = absorbance reading at 336nm,

m = sample mass (g),

5 = nominal mass of the sample and 149.7 = (126/16830) x (1000/10) x (1000/5).

Total polyphenolic content (TPC) and antioxidant activity (AA)

The total polyphenolic content (TPC) of the samples was determined by using the Folin-Ciocâlteu colorimetric method developed by Singleton and Rossi (1965). The method relies on the transfer of electrons in alkaline medium from phenolic compounds to form a blue chromophore constituted by a phosphotungstic complex where the maximum absorption depends on the concentration of phenolic compounds. For the calibration curve, gallic acid solution were used as the standard.

The results of the spectrophotometric reading are expressed in mg GAE 100mg/kg honey.

The total antioxidant activity were determined using FRAP method (Berzie and Stain, 1996). This method is based on the ability of substances to reduce ferric to ferrous ions, which in the presence of TPTZ at an acidic pH, form a blue colored complex. The absorbance was read at a wavelength of 593 nm. For calibration curve, Trolox solution were used.

Results are further expressed as FRAP values for a 10% honey solution, equal with the µmol Trolox equivalent.

RESULTS AND DISCUSSIONS

Organoleptic analysis

The results of the organoleptic analysis conducted on the two types of honey are presented in the table 1.

Table 1
Organoleptic results of Lychee and Indian Mustard Honey

Analyzed parameters	Lychee honey	Indian mustard honey
General Aspect	homogenous without foam or bubbles	homogenous small air bubbles on the surface
Colour	light colour, golden hue	darker colour, brownish-yellow
Taste and smell	sweet taste, subtle floral smell	sweet taste, specific sharp smell
Consistency	fluid, homogenou, without signs of sugar crystals	fluid, homogenou, without signs of crystals
d.o.i.	without any impurities	without any impurities

Therefore, based on the results of the organoleptic tests carried on the two honey samples, we can see the differences to be mainly in the colour field, as well as the smell, which goes from a floral one in lychee honey to a sharper, almost pungent scent in the mustard honey.

Colour

After performing the calculations, it was determined that the colours of the two honey samples on the Pfund scale were defined as “White” for the Lychee honey and "Light Amber" for the Indian Mustard honey.

Fig 1. Pfund colour scale – graphic representation

Table 2
Colour intensity (ABS450n) and Pfund Colour intensity results

	Lychee Honey	Mustard Honey
Abs 450nm	0.223	0.41
Abs 720nm	0.002	0.01
Abs 450n	0.221	0.4
Colour	white	Extra light amber
Abs 635nm	0.11	0.201
Pfund	2.15	35.95
Colour Pfund	white	Extra light amber

Ash Content

The values obtained for the ash content were within the limits provided by the FSSAI (Food Safety and Standards Authority of India) specifications, but also fall within the values allowed for ash according to European regulations (0.6%) (Bogdanov, 2009, Council Directive 2001/110/EC of 20 December 2001).

Table 3
Honey ash percentage together with FSSAI maximum permitted value

	Lychee honey	Mustard honey	FSSAI max. value
Ash %	0.040	0.047	0.050

Water and Total Soluble Substances – BRIX

As per the values, the obtained water percentages were both above the EU regulatory standards which allow a water percentage of up to 20% in regular honeys (European

Commission, 2014). However, according to the Indian regulatory standards, which include a calculation correction according to the collecting temperature of the honey as well as the temperature of the lab environment when analyzing the honey samples, the two values which can be observed in the table below are safe for consumers and, therefore, keep the product on the market (FSSAI, 04B.003:2023).

For the Total Soluble Substance percentage, the same refractometer was used, and the value ranged from 73.8% to 77.0%.

Total acidity values respect the limits established for honey in International Regulatory Standards and in FSSAI.

Values for water, TSS and total acidity can be found in table 4.

Table 4

Honey water and TSS and total acidity percentage together with RO/FSSAI maximum permitted value

	Water %	TSS Brix %	Total acidity °T/kg
Lychee	24.8	73.7	27.6
Indian mustard	21.6	77.0	14.7
RO/ FSSAI	20 (25.2*)	-	50

Hydroxymethylfurfural (HMF) Content

The determined values represented the content of HMF expressed in mg/kg and are presented in the table 5.

Table 5

HMF content together with FSSAI maximum permitted value

	Lychee honey	**Mustard honey**	**FSSAI standard**
HMF (mg/kg)	77.67	11.76	80.0

The FSSAI standard allows for a HMF content in honeys from warm and humid areas up to double the European Commission's value (40 mgHMF/kg honey), therefore making these honeys fit within the regulatory standards of the Indian market.

Total polyphenolic content (TPC) and antioxidant activity (AA)

The results of the spectrophotometric determination of TPC, was expressed in mg GAE/kg honey.

The calibration curve for TPC was represented in Fig.1 and for AA, in Fig.2

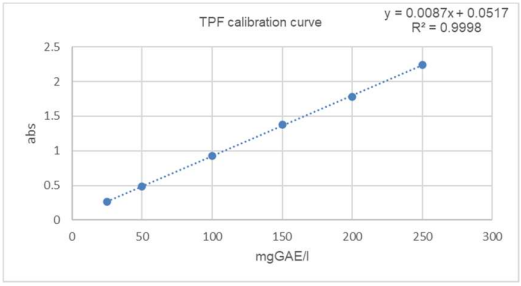

Fig.1. Total Polyphenolic content calibration curve

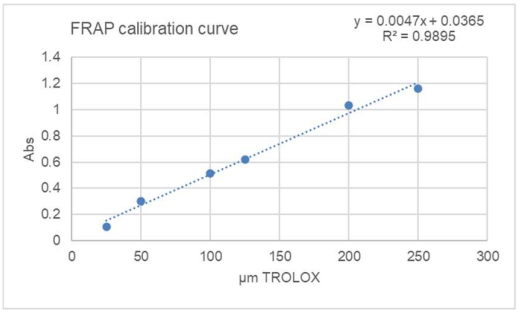

Fig.2. Total antioxidant activity FRAP -calibration curve

Both the Antioxidant Activity results and the TPC results were included in the table 6.

TPC of Mustrad honey was almost 4 times higher than TPC for Lychee honey.

Total AA was 2 time higher in mustard honey in comparison with Lytchee honey.

Table 6

Results regarding TPC and AA values

Sample	**TPC (mg GAE/kg)**	**AA (µmol Trolox equivalent)**
Lychee honey	2.26	70.32
Mustard honey	8.57	155.422

CONCLUSIONS

The two samples of honey we analysed are honeys with a specific aroma, strong flavours and a higher fluidity, the latter one thanks to the higher average temperature in the areas of cultivation in India.

The values obtained for the physical-chemical parameters fit within the European regulatory standards except HMF and water content, which have a different limit in the Indian market.

Comparing the 2 types of honey, comparing the 2 types of honey, it can be observed that the Mustard Honey has a lower water content, with a higher TPC and AA.

The composition of the honeys varies depending on the floral source, seasonal and environmental factors.

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