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Original Article

# Physiochemical Analyses of Four Mono-Floral Honeys Collected from Different Parts of the Tamil Nadu, India

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## ABSTRACT

**Background:** Honey is a key, recognized and commonly utilized food product for cosmetic, medicinal, and nutrient purposes. Recent years mono-floral honeys are gaining good market values as well as got good appreciation among the consumers. **Objectives:** In the present study, we are aiming to study the physicohemical properties of five selected honey samples namely i) Acacia (Honey 1), ii) Commercial honey (Honey 2), iii) Litchi (Honey 3), iv) Naval (Honey 4) and v) Neem (Honey 5). **Materials and Methods:** The physiochemical analysis includes specific gravity, moisture and Total Solid (TS) content, refractive index, pH, acidity, viscosity, ash content, sugar profile, Total Dissolved Solid (TDS) and Electrical Conductivity (EC), optical density, fiehe's test, Hydroxymethylfurfural (HMF) content, total phenolic content, diastase activity, proline content, Iron (Fe) and Calcium (Ca) content. **Results:** The physiochemical analysis results showed that all the five honey samples comply well with the Bureau of Indian Standards (BIS). And moreover, the present investigation the naval mono-floral honey (honey sample 4) has showed better physiochemical properties, when compared with other tested honey samples. **Conclusion**: The present finding of physiochemical analysis revealed the best quality and as well as purity of five selected Indian honey samples which satisfies the standards of natural honey.

**Keywords:** Acacia honey, Hydroxymethylfurfural (HMF), Mono-floral honey, Naval honey, Neem honey, Physiochemical properties.

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## INTRODUCTION

Honey is a natural food product mainly produced by honey bees. Honey has been broadly classified as i) blossom honey (flower nectar) and ii) honeydew honey (plant-sucking insect secretions or plant sap). Blossom honey again sub-divided as i) mono floral honey (single flower) and ii) multi floral honey (mix of wild flowers). In mono floral honey is otherwise known as uni-floral honey, where bees forage mainly from single plant source and hence the honey was named after plant name for instance sunflower honey (Naik *et al*., 2020).

India is well known its rich flora and fauna, being one of the globe mega diverse countries. It also stands 7$^{th}$ huge honey producer and exporter in the globe (Nidhi *et al*., 2025). In India various types of honeys are reported which includes i) Acacia / Babhul honey (*Acacia nilotica*), ii) Neem honey (*Azadirachta indica*), iii) Cotton / Kaapus honey (*Gossypium herbaceum*), iv) Litchi honey (*Litchi chinensis*), v) Eucalyptus honey (*Eucalyptus globulus*), vi) Drumstick / Moringa honey (*Moringa oleifera*), vii) Jambhul / Jamun honey (*Syzygium cumini*), viii) Coriander /Kothmir honey (*Coriandrum sativum*), ix) *Aegle marmelos* honey, x) *Terminalia arjuna* honey, xi) *Schleichera oleosa* honey, xii) Asian bee honey (*Apis cerana*), xiii) Rock bee honey (*Apis dorsata*), xiv) Little bee honey (*Apis florea*), xv) European bee honey (*Apis mellifera*), xvi) Stingless bee honey (*Lepidotrigona arcifera*), xvii) Dammar / Stingless bee honey (*Tetragonula* species), xviii) Tulsi honey (*Ocimum sanctum*), xix) Ajwain honey (*Trachyspermum ammi*), xx) Mustard honey (*Brassica juncea*), xxi) Hirda honey (*Terminalia chebula),* xxii) Mango honey (*Mangifera indica*), xxiii) Sunflower honey (*Helianthus annuus*), xxiv) Jujube honey (*Ziziphus mauritiana*), xxv) Neela kurinji / Kurinji honey (*Strobilanthes kunthianus*), xxvi) Field mustard / Rapeseed honey (*Brassica campestris*), xxvii) *Eucalyptus lanceolatus* honey, xxviii) Egyptian Clover / Berseem Clover honey (*Trifolium alexandrinum*), xxix) Pongamia / Karanj honey (*Pongamia pinnata*), xxx) Coconut palm honey (*Cocos nucifera*), xxxi) Brown Salwood honey (*Acacia mangium*), xxxii) *Terminalia bellirica* honey, xxxiii) *Areca catechu* honey, xxxiv) *Mimosa pudica* honey, xxxv) Khejri tree / Desert honey (*Prosopis cineraria*), xxxvi) Sesame honey (*Sesamum indicum*), xxxvii) Sulai / Suli honey (*Plectranthus rugosus*), xxxviii) Hungarian acacia (*Robinia pseudoacacia*),

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xxxix) Buckwheat honey (*Fagopyrum esculentum*), xxxx) Apple honey (*Malus domestica*), xxxxi) Cherry honey (*Prunus avium*), xxxxii) Saffron honey (*Crocus sativus*), xxxxiii) Horse Chestnut (*Aesculus hippocastanum*), xxxxiv) Mistu / Thumbai honey (*Leucas aspera*), xxxxv) Karavi honey (*Strobilanthes callosa*), xxxxvi) Rosewood / Sheesham honey (*Dalbergia sissoo*), xxxxv) Alfalfa honey (*Medicago sativa*), xxxxvi) Sweet orange (*Citrus sinensis*) honey, xxxxvii) Peach (*Prunus persica*) honey, xxxxviii) Agastache (*Agastache rugosa*) honey.

In Indian Ayurvedic system of Medicine, honey has been used as a vehicle for enhancing the absorption of various herbal drugs. Similarly, honey may be applied singly (or) in blend with other compounds/substances (Tarfe and Deshpande, 2018). Moreover, honey has been applied both by orally and topically. Furthermore, honey is generally utilized as home remedy to manage severe exacerbations (in allergic conditions) and pathogens (Tarfe and Deshpande, 2018). Apart from these, honey has been reported to possess various pharmacological activities, which includes i) anti-bacterial, ii) anti- cancer, iii) anti-diabetic, iv) anti-hypertension, v) anti-inflammatory, vi) anti-microbial, vii) anti-nociceptive, viii) anti-oxidant, ix) anti-ulcer, x) anti-viral, xi) hepato-protective, xii) immune-stimulatory, xiii) nematicidal and xiv) wound healing (Narayanaswamy *et al.*, 2015; Anand *et al.*, 2019; Rathod *et al.*, 2019; Nidhi *et al.*, 2025).

The physicochemical analysis of honey is simple and effective tool, which is useful to assess the honey quality as well as authenticity nature (Ma *et al.*, 2019). The physicochemical properties of honey have been widely reported globally (Marghitas *et al.*, 2010; Mohamed *et al.*, 2018; Ma *et al.*, 2019; Vijayakumar *et al.*, 2020; Goswamy *et al.*, 2023; Joshi *et al.*, 2024; Nidhi *et al.*, 2025; Zhang *et al.*, 2025). Thus, the current investigation was aimed to study the physiochemical properties of four mono-floral honeys like Acacia, Litchi, Naval, Neem and one commercial (multi-floral) honey.

## MATERIALS AND METHODS

### Chemicals

Chemicals and solvents of analytical grade were purchased from SD Fine Chemicals, Mumbai, Maharashtra State, India.

### Collection of honey samples

The four mono-floral honeys used in this study were obtained directly from beekeepers' farms (located in Virudhunagar, Tirunelveli and Thoothukudi districts) and one commercial honey (one multi-floral honey which was purchased from a supermarket). Subsequently, five distinct honey samples namely i) Acacia (Honey 1), ii) Commercial honey (Honey 2), iii) Litchi (Honey 3), iv) Naval (Honey 4) and v) Neem (Honey 5) were chosen for the present physicochemical analysis.

### Specific gravity

The specific gravity of five selected honey samples was analysed using pycnometer at 25°C (Kavapurayil *et al.*, 2014).

### Moisture and total solid content

The moisture and Total Solid (TS) content of five selected honey samples was determined using Sartorius MA35 Infrared moisture analyser (Sleagun and Popa, 2012).

### Refractive index

The refractive index of five selected honey samples was calculated using Labman Abbe's refractometer (Kavapurayil *et al.*, 2014).

### pH

The pH of five selected honey samples was determined using pH meter- Eutech, PC700 multi-parameter (Goswamy *et al.*, 2023).

### Acidity

The acidity of five selected honey samples was calculated by titrating prepared honey solution against sodium hydroxide and phenolphthalein (Kavapurayil *et al.*, 2014).

### Viscosity

The viscosity of five selected honey samples was recorded using spindle 4 Brookfield viscometer.

### Ash content

The ash content was determined by subjecting honey samples at 600°C in muffle furnace (Kavapurayil *et al.*, 2014).

### Sugar profile

The sugar profile consists of Total Reducing Sugar (TRS), Total Sucrose Content (TSC) and Fructose: Glucose (F: G) ratio of five selected honey samples were determined by titration method (Kavapurayil *et al.*, 2014).

### Total dissolved solid and Electrical conductivity

The Total Dissolved Solid (TDS) and Electrical Conductivity (EC) of five selected honey samples were recorded using Eutech CON 700 digitals conductivities meters (Goswamy *et al.*, 2023).

### Optical density

The Optical Density (OD) of five selected honey samples was recorded at 660 nm.

### Fiehe's test

The Fiehe's test was carried out for the five selected honey samples using the freshly prepared resorcinol to the ether extract honey samples (Kavapurayil *et al.*, 2014).

### Hydroxymethylfurfural (HMF) content

The Hydroxymethylfurfural (HMF) content of five selected honey samples was determined using carrez (1 and 2) reagents by spectrophotometric method (Mohammad *et al.*, 2023).

### Total phenolic content

The total phenolic content of five selected honey samples was measured at 765 nm using spectrophotometric method (Kavapurayil *et al.*, 2014).

### Diastase activity

The diastase activity of five selected honey samples was measured at 620 nm using spectrophotometric method (Goswamy *et al.*, 2023).

### Proline content

The proline content of five selected honey samples was measured at 520 nm using ninhydrin as reagent by spectrophotometric method (Czipa *et al.*, 2012).

### Iron (Fe) and Calcium (Ca) content

Iron (Fe) and Calcium (Ca) content of five selected honey samples were measured at 248 and 422 nm using Atomic Absorption Spectrometric method (Marghitas *et al.*, 2010).

### Ethics statement

The current *in vitro* study does not require any ethical approval, as it does not involve any animal or human subjects.

### Statistical analysis

For each analysis, triplicates ($n$=3) were performed and standard deviation was carried out using Microsoft Excel 2019 (Microsoft Corporation, USA).

## RESULTS

In the current investigation, Table 1 represents the specific gravity, moisture content, Total Solid (TS) content, Refractive Index (RI) and pH of five selected honey samples.

Similarly, in the present study Table 2 represents acidity, viscosity, ash content, Total Reducing Sugar Content (TRS), Total Sucrose Content (TSC) and fructose: glucose ratio.

In the current study, Table 3 represents the Total Dissolved Solids (TDS), Electrical Conductivity (EC), Optical Density (OD), fiehe's test and Hydroxymethylfurfural (HMF) content.

Similarly, in the present investigation Table 4 represents total phenolic content, diastase activity, proline content, iron (Fe) and Calcium (Ca) content.

## DISCUSSION

The physicochemical analysis could give valuable knowledge about the composition and properties of honeys and moreover physicochemical analysis are also potential to determine the quality and authenticity of the honeys (Ma *et al.*, 2019). Furthermore, specific gravity of honey is also important in knowing quality and purity of the honey (Terrab *et al.*, 2002). In the current investigation, specific gravity of five selected honey ranges from 1.31±0.02 to 1.35±0.02 (as shown in the Table 1). The present finding has shown good correlation with earlier reports (Kavapurayil *et al.*, 2014, Vijayakumar *et al.*, 2020). The moisture content of five selected honey ranges from 15.84±0.16 to 18.53±0.14% w/w (as shown in the Table 1). The current result has shown good agreement with previous report, where moisture content of pure honey has ranged from 14 to 18% (Kavapurayil *et al.*, 2014). The total solid content of five selected honey ranges from 81.44±0.14 to 84.15±0.12% w/w (as shown in the Table 1). This finding was on par with previous report, where total solid content of honey has ranged from 74.40 to 83.20% (Vijayakumar *et al.*, 2020). The pH of five selected honey ranges from 3.58±0.34 to 4.25±0.41. The present finding has shown good correlation with earlier report (Vijayakumar *et al.*, 2020).

Acidity serves as one of key indicating factor for determining the quality of honey (Prica *et al.*, 2014). In the present investigation, acidity of five selected honey ranges from 0.05±0.02 to 0.25±0.05% w/w (as shown in the Table 2). The current result was good agreement with previous report (Kavapurayil *et al.*, 2014). Ash content demonstrates the direct assess of inorganic residues present in the honey (Kavapurayil *et al.*, 2014). In the current study, ash content of five selected honey ranges from 0.14±0.02 to 0.21±0.02% w/w (as shown in the Table 2). This finding was on par with previous report (Kavapurayil *et al.*, 2014). The Fructose: Glucose (F: G) ratio of honey has been reported to influence the flavour of honey, as the fructose is known to sweeter than glucose and sucrose (Julika *et al.*, 2020). In the current investigation, Fructose: Glucose (F: G) ratio of five selected honey ranges from 1.30±0.20 to 1.44±0.13 (as shown in the Table 2). The current finding has shown good correlation with earlier reports (Marghitas *et al.*, 2010, Zae *et al.*, 2020).

Recently, the total amount of organic and inorganic contents present in the honey is determined by the total dissolved solids (Raweh *et al.*, 2026). In the present study, Total Dissolved Solids (TDS) content of five selected honey ranges from 134.49±0.34 to 187.56±0.24 ppm (as shown in the Table 3). The present result was on par with previous report (Shukla and Kumar, 2020). The electrical conductivity of honey is more associated to the concentration of organic acid, minerals and proteins. And moreover, it has been utilized for distinguish honeys of different botanical source (Živkov-Baloš *et al.*, 2018). In the current investigation, electrical conductivity of five selected honey ranges from 0.35±0.02 to 0.56±0.03 mS/cm (as shown in the Table 3).

The current finding has shown good correlation with earlier report (Živkov-Baloš *et al.*, 2018). The Hydroxymethylfurfural (HMF) content analysis has been reported as main quality marker of honey deterioration (loss of freshness), heating (thermal treatment) and adulteration (Althaiban, 2024). In the present study, HMF content of five selected honey ranges from 32.45±0.43 to 78.19±0.24 mg/kg (as shown in the Table 3). The present result has demonstrated good agreement with previous report (Vijayakumar *et al.*, 2020).

The phenolic compounds have been reported as key components, which responsible for anti-oxidant activity of honey (Sagdic *et al.*, 2013). In the current investigation, total phenolic content of five selected honey ranges from 7.26±0.17 to 15.40±0.17 mg of Gallic acid equivalents per 100 g (as shown in the Table 4). The current finding was on par with earlier report (Marghitas *et al.*, 2010, Sagdic *et al.*, 2013). The determination of diastase activity of honey is utilized as one of key indicator of honey quality (Alhejely *et al.*, 2025). In the present investigation, diastase activity of five selected honey ranges from 10.53±0.34 to 19.59±0.25 Diastase number (as shown in the Table 4). The present result has shown good correlation with previous report (Goswamy *et al.*, 2023). The proline content of honey serves as one of criterion for assessing the quality of honey (Truzzi *et al.*, 2014). In the current study, proline content of five selected honey ranges from 388.17±0.55 to 563.63±0.69 mg/kg (as shown in the Table 4). The current result has demonstrated good agreement with earlier report (Czipa *et al.*, 2012). Minerals are one of the minor components of honey, which serves as significant role in estimating quality of honey (Tlak Gajger *et al.*, 2024). In the current investigation, iron (Fe) content of five selected honey ranges from 12.12±0.17 to 18.49±0.17 mg/kg (as shown in the Table 4). The current finding has demonstrated good correlation with previous report (Melaku and Tefera, 2022). Similarly in the present study, Calcium (Ca) content of five selected honey ranges from 34.42±0.19 to 58.28±0.17 mg/kg (as shown in the Table 4).

**Table 1: Physicochemical (specific gravity, moisture content, Total Solid (TS) content, Refractive Index (RI) and pH) analysis of five selected honey samples.**

| Honey samples | Specific gravity | Moisture content (% w/w) | Total Solid (TS) content (% w/w) | Refractive index (RI) | pH |
|---|---|---|---|---|---|
| Honey 1 | 1.32±0.03 | 18.53±0.14 | 81.44±0.14 | 1.4905±0.00 | 3.58±0.34 |
| Honey 2 | 1.33±0.06 | 16.83±0.15 | 83.12±0.14 | 1.4946±0.00 | 4.25±0.41 |
| Honey 3 | 1.32±0.04 | 16.45±0.19 | 83.56±0.18 | 1.4956±0.00 | 4.05±0.21 |
| Honey 4 | 1.31±0.02 | 18.11±0.14 | 81.95±0.15 | 1.4915±0.00 | 4.22±0.17 |
| Honey 5 | 1.35±0.02 | 15.84±0.16 | 84.15±0.12 | 1.4971±0.00 | 4.15±0.24 |

**Table 2: Physicochemical (acidity, viscosity, ash content, total reducing sugar content, total sucrose content and glucose: fructose ratio) analysis of five selected honey samples.**

| Honey samples | Acidity (% w/w) | Viscosity in centipoise (cP) | Ash content (% w/w) | Total Reducing sugar content (% w/w) | Total Sucrose content (% w/w) | Fructose: Glucose (F: G) ratio |
|---|---|---|---|---|---|---|
| Honey 1 | 0.06±0.02 | 7018±0.45 | 0.15±0.01 | 70.88±0.88 | 4.49±0.36 | 1.41±0.14 |
| Honey 2 | 0.25±0.05 | 7012±0.37 | 0.21±0.02 | 69.20±0.78 | 4.18±0.25 | 1.33±0.11 |
| Honey 3 | 0.05±0.02 | 5883±0.16 | 0.16±0.02 | 69.64±0.61 | 4.20±0.42 | 1.30±0.20 |
| Honey 4 | 0.06±0.03 | 5369±0.38 | 0.14±0.02 | 69.65±0.28 | 4.52±0.52 | 1.38±0.05 |
| Honey 5 | 0.05±0.02 | 5368±0.53 | 0.12±0.02 | 68.91±0.91 | 4.24±0.28 | 1.44±0.13 |

**Table 3: Physicochemical (total dissolved solids, electrical conductivity, optical density, fiehe's test and hydroxymethylfurfural content) analysis of five selected honey samples.**

| Honey samples | Total Dissolved Solids (in ppm[a]) | Electrical Conductivity (in mS/cm) | OD[b] at 660 nm | Fiehe's test | HMF[c] content (in mg/kg[d]) |
|---|---|---|---|---|---|
| Honey 1 | 187.56±0.24 | 0.35±0.02 | 0.23±0.02 | Negative | 50.59±0.41 |
| Honey 2 | 137.57±0.39 | 0.56±0.03 | 0.27±0.02 | Negative | 78.19±0.24 |
| Honey 3 | 134.49±0.34 | 0.45±0.02 | 0.31±0.02 | Negative | 55.30±0.39 |
| Honey 4 | 177.76±0.32 | 0.36±0.02 | 0.28±0.02 | Negative | 32.45±0.43 |
| Honey 5 | 135.49±0.23 | 0.44±0.03 | 0.45±0.02 | Negative | 50.54±0.25 |

Note: ppm[a]- Parts Per Million, OD[b]- optical density, HMF[c]- hydroxymethylfurfural, mg/kg[d]- milligram per kilogram.

**Table 4: Physicochemical (total phenolic content, diastase activity, proline content, iron and calcium content) analysis of five selected honey samples.**

| Honey samples | Total Phenolic content (mg GAE[a]/100 g) | Diastase activity (in DN[b]) | Proline content (in mg/kg[c]) | Iron (Fe) content (in mg/kg[c]) | Calcium (Ca) content (in mg/kg[c]) |
|---|---|---|---|---|---|
| Honey 1 | 10.13±0.16 | 19.59±0.25 | 471.27±0.59 | 12.12±0.17 | 58.28±0.17 |
| Honey 2 | 13.28±0.13 | 15.53±0.34 | 388.17±0.55 | 18.49±0.17 | 55.02±0.19 |
| Honey 3 | 15.40±0.17 | 13.43±0.43 | 416.77±0.99 | 14.70±0.16 | 36.25±0.18 |
| Honey 4 | 9.73±0.12 | 10.53±0.34 | 563.63±0.69 | 13.41±0.15 | 52.83±0.16 |
| Honey 5 | 7.26±0.17 | 12.62±0.18 | 481.73±0.51 | 13.12±0.18 | 34.42±0.19 |

Note: mg GAE[a]/100 g- milligram of Gallic Acid Equivalents per 100 grams, DN[b]- Diastase number, mg/kg[c]- milligram per kilogram.

The present finding was on par with earlier report (Kacaniova *et al.*, 2009).

## CONCLUSION

In the present investigation, all the honey samples have exhibited pH values (3.58 to 4.25) in acidic range. Interestingly, in the present investigation honey sample 4 (Naval honey) has shown least specific gravity of 1.31, diastase activity of 10.53 mg/kg and Hydroxymethylfurfural (HMF) content of 32.45 mg/kg. On other hand honey sample 4 (Naval honey) has exhibited highest proline content of 563.63 mg/kg. Thus these physicochemical properties of honey sample 4 (Naval honey), has suggested good quality nature of naval honey (honey sample 4). And furthermore, this unique quality paves way for biomedical applications of naval honey.

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## ABBREVIATIONS

**BIS:** Bureau of Indian Standards; **Ca:** Calcium; **cP:** Centipoise; **DN:** Diastase Number; **EC:** Electrical Conductivity; **Fe:** Iron; **F: G:** Fructose:Glucose Ratio; **GAE:** Gallic Acid Equivalents; **HMF:** Hydroxymethylfurfural; **OD:** Optical Density; **ppm:** Parts Per Million; **RI:** Refractive Index; **TDS:** Total Dissolved Solids; **TRS:** Total Reducing Sugars; **TS:** Total Solids; **TSC:** Total Sucrose Content.

## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHOR CONTRIBUTIONS

Conceptualization: **S.K., R.N.;** Methodology, **S.K., R.N.;** Software, **S.K., R.N.;** Formal analysis, **S.K., S.B.S., B.D., R.N.;** Investigation, **S.K., S.B.S., B.D., R.N.;** Resources, **S.K., R.N.;** Data curation, **S.K., S.B.S., B.D., R.N.;** Visualization, **S.K., S.B.S., B.D., R.N.;** supervision, **R.N.;** Project administration, **S.K., R.N.;** Writing-original draft preparation, **S.K., S.B.S., B.D., R.N.;** Writing-review and editing, **S.K., S.B.S., B.D., R.N.;** All authors have read and agreed to the published version of the manuscript.

## SUMMARY

In the current study, physiochemical analysis results showed that all the five honey samples (Acacia (Honey 1), Commercial honey (Honey 2), Litchi (Honey 3), Naval (Honey 4) and Neem (Honey 5)) comply well with the Bureau of Indian Standards (BIS). Moreover, honey sample 4 (Naval honey) has shown least specific gravity of 1.31, diastase activity of 10.53 mg/kg and Hydroxymethylfurfural (HMF) content of 32.45 mg/kg respectively.

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