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Review Article

# A comparative review on sweet honey and bitter honey

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**ABSTRACT**

Honey is a sugary secretion deposited in the honey comb by the bee, *Apis dorsata* and possibly other species of *Apis*, e.g. *Apis indica, Apis florae,* etc. usually contain a variety of nutritional and mineral substances. Honey is an ingredient in most of the Ayurvedic preparations due to its therapeutic use in the treatment of cancer, nervous disorder, and muscular weakness and to treat several infections. There are different types of honey available, in that, bitter honey has been shown that it contains bioactive compounds. Antinutritional qualitative and quantitative analysis of bitter honey shows the presence of flavonoids, saponin glycosides whereas steroids were detected only in sweet honey. Bitter honey obtained from strawberry tree has showed interesting antioxidant antiradical activities and protective effect against thermal cholesterol degradation. It also induces the growth inhibition of human cancer cells. The quality of honey is governed by FSSAI, AGMARK and various pharmacopoeias available for honey. But there is no standard monograph available for bitter honey also the demand for this natural product is increasing globally. So the review work is proposed for the further research in developing the standard monograph and pharmacognostical study for bitter honey.

**Keywords:** Bitter honey; Sweet honey; Strawberry tree honey; Comparative study.

## INTRODUCTION

Honey bees are the most fascinating and useful creatures to mankind (Reema Saha et al., 2008). The process involved in the transformation of nectar into honey is regurgitation and evaporation (Ishan Ullah Khan et al., 2014). Honey contains mainly carbohydrates and also other minor substances like organic acids, amino acids, proteins, vitamins and minerals. The major sugar present in all the types of honey is fructose. The characteristic flavour of honey depends on the volatile substances present in it (Abdel Moneim E.Suleiman et al., 2013). Strawberry tree honey is a unifloral honey which has a distinct fragrance and bitter after taste and is locally known as "bitter honey" which is a product of Sardinia. It is amber coloured when liquid and beige- brown when crystallised; the smell is intense the taste is slightly sweet initially and decidedly bitter and astringent later There are insufficient data on its phytochemical composition or biological properties (Pablo A Ulloa et al., 2015).

## TYPES OF HONEY

**Types of honey concerning origin** (Nicola Bradbear., 2009).

**Blossom honey**: Honey is obtained mainly from the nectar of flowers.

**Honey dew honey:** Honey produced by bees after they collect honeydew secretions of insects which pierce plant cells, ingest plant sap and then secrete it again. The colour of honey dew honey varies from very light brown or greenish to almost black.

**Monofloral honey**: The bees have been foraging mainly on one type of plant.

**Multifloral honey**: This type of honey is also known as polyfloral honey which has several botanical sources, none of which is predominant.

**Types of honey concerning processing**

**Comb honey** is pieces of honeycomb which is produced by the bees and no processing has been done to separate honey from the beeswax, where both the beeswax comb and honey is edible.

**Strained honey** is obtained when the honey is separated from the beeswax by straining. The colour of the honey is amber when liquid and beige- brown when crystallised. The smell is intense and the taste is slightly bitter and astringent later.

**Chunk honey** is a jar of liquid honey containing a piece of comb honey. The liquid honey should be very light and clear, and will not granulate over a long period. Honeys from *Acacia and Robinia pseudo acacia* are commonly used.

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**Extracted honey** is obtained by centrifuging the honeycombs.

**Pressed honey** is extracted by pressing honeycombs with or without applying moderate heat.

**Crystallised or granulated honey** is strained honey which is crystallised.

**Creamed honey** is strained honey which has been seeded to start crystallisation and then stirred to prepare a honey of uniform and soft consistency**.**

The energy value of honey along with its major and minor constituents is represented in table 1, 2 and 3 respectively.

The Qualitative phytochemical composition, Nutritional composition and mineral composition of sweet and bitter honey are represented in tables 4, 5, and 6 respectively (Adeniyi et al., 2016, Adeniyi et al., 2014).

**Physicochemical properties of honey**

**Colour**: The colour in liquid honey varies from clear and colorless to dark amber or black (Tahereh Eteraf-Oskouei et al., 2012). Pfund classifier is used to measure the color intensity of honey. Honey samples were transferred into a cuvette with a 10mm light path length until it is approximately half full and is then inserted into a colour photometer. Colour grade is expressed in millimetre by comparing with glycerol standard (S.A.EI Sohaimy et al., 2015).

**Aroma/ Flavour:** The aroma of honey depends on its volatile fraction composition, which is influenced by the floral origin and nectar composition. GC-MS is usually used for aroma profile determination (Luis F. Cuevas –Glory et al., 2007).

**Sweetness:** The sweetness of honey depends mainly on the monosaccharide fructose and glucose. Chemical tests can be performed for the qualitative determination of carbohydrates (Kushwaha et al., 2010).

**Weight per millilitre:** it is the weight of liquid present in gram of 1ml of liquid at 25℃.The weight per millilitre of honey sample can be determined by dividing its weight in air in gram which fills the pycnometer by the capacity of the pycnometer in ml at the same temperature (The Indian Pharmacopoeia 1996).

**Viscosity**: Honey viscosity plays a significant role in the crystallization process (Stawomir bakier. 2009). Viscosity of honey samples are measured using an Ostwald viscometer. The viscometer is calibrated using double distilled water and 20% sucrose solution and viscosities of the samples were measured at 15,25,35,45 and 60℃ (O.O.James et al., 2009).

**$P^H$:** The $P^H$ of the honey samples was determined using a digital $P^H$ meter. Weigh about 10 g of honey and add 75 ml of distilled water to it. The $P^H$ meter is initially calibrated with buffers at $p^H$ 4 and 10 before sample measurement (Agbagwa et al, 2011, M.O. Adenekan et al., 2010, Muhammad Shahanawaz et al., 2013, Shobam et al., 2017).

**Moisture content:** The natural moisture of honey in the comb is that which remains after the nectar has ripened. Moisture content of honey depends on the factors involved in ripening like weather conditions and original moisture of the nectar (Kashinath, 2006). Moisture content of honey can be determined by drying 2g of sample at 70℃ to constant weight in hot air oven (Fatimah Buba et al., 2013, R.A. Lawal et al., 2009).

**Ash content:** The honey samples about 5-10g weighed in a crucible is placed in a muffle furnace at 600℃ for 4 hours .The ash content is determined by comparing both the weight of the samples obtained before and after measurement (Mahmood Ahmed et al., 2016, O.M. Atrooz et al., 2008).

**Sugar content:** Qualitative and quantitative esatimation of saccharide in honey is performed by using HPLC or GC in order to determine honey adulteration (Helena Rybak – Chmielewska. 2007). HPLC with RI detector and analytical stainless steel column in polar aminopropylsilane is used (Amina Chakir et al., 2011). The chromatographic mobile phase consists of a mixture of water: acetonitrile (25:75), whose flow rate was kept constant at 1 ml per minute (P. Mondragon- Cortez et al., 2013).

**Total acidity:** The acidity in honey is due to the presence of the organic acids like tartaric, citric, oxalic, acetic acid obtained either from nectar or bees secretions. The acidity of honey adulterated with sugar syrup is low when compared to that adulterated with inverted sugar (Desissa Yadata., 2014). The acidity of honey was determined by volumetric method .Twenty five millilitres of each sample was titrated against 0.1N NaOH using 0.25 ml phenolphthalein as indicator (Nadežda Prica et al., 2014).

**Proline content:** Proline is the main amino acid of honey obtained from the honey bee and is a criterion of honey ripeness (Stefan BOGDANOV et al., 2004). Proline comes mainly from the salivary secretions of honey bee during the conversion of nectar into honey (K.J.Cherian et al., 2011).Weigh about 2.5 g honey and transfer it to a 50 ml volumetric flask and make up the volume.. Pipette 0.5 ml into 3 test tubes, and then add 0.25 ml of acetic acid and 1.00 ml of ninhydrine solution. Shake well, boil in a water bath for15 minutes. Cool for 5 minutes and then pipette out 5ml of aqueous isopropanol into each .Mix well and read the absorbance at 520nm and read blank using water (Kenneth Helrich, AOAC 1990).

**Pollen Analysis:** Pollen analysis determines the botanical, geographical and ecological origin of honey (Aina, D. Oet al., 2015). Weigh about 10 grams of

**Table 1: Energy value of honey**

| | |
|---|---|
| Total Energy value of Honey | 3,040 kcal per kg |
| Sweetness of Honey | High |
| Sugar content of honey | 80% |
| Minerals, protein, enzymes | Very little |

**Table 2: The Major constituents of honey (99%)**

| Constituents | Percentage | Mean (%) |
|---|---|---|
| Water | 13.4-26.6 | 17 |
| Fructose | 21.7-53.9 | 39.3 |
| Glucose | 20.4-44.4 | 32.9 |
| Sucrose | 0.0-7.6 | 2.3 |
| Other sugars | 0.1-16.0 | 8.5 |

**Table 3: Minor constituents of honey (1%)**

| Constituents | Percent of 1% |
|---|---|
| Acids (gluconic) | 0.17-1.17 |
| Minerals | 0.002-1.03 |
| Nitrogen (protein) | 0.00-0.13 |
| Enzymes | >0.1% |
| Aroma | >0.1% |
| Others (HMF,etc) | >0.1% |

**Table 4: Qualitative Phytochemical composition of Sweet and Bitter honey**

| Phytochemicals | Sweet honey | Bitter honey |
|---|---|---|
| Tannis | + | ++ |
| Resins | - | - |
| Saponin | +++ | +++ |
| Flavonoid | + | + |
| Steroid | + | - |
| Terpenes | + | ++ |
| Alkaloid | + | + |
| Balsam | ++ | + |
| Carbohydrate | + | + |

Key: - ++= relatively presence, += presence, -=absence.

**Table 5: Nutritional Composition of Bitter and Sweet Honey**

| Sample | Moisture (%) | Ash (%) | Fat (%) | Protein (%) | Crude fibre (%) | Carbohydrate (%) |
|---|---|---|---|---|---|---|
| Bitter Honey | 19.93±0.10 | 1.18±0.15 | 0.33±0.03 | 0.74±0.04 | 2.03±0.08 | 77.86±0.84 |
| Sweet Honey | 20.14±0.04 | 1.73±0.74 | 0.23±0.02 | 0.69±0.03 | 1.25±0.08 | 76.44±0.64 |

**Table 6: Mineral Composition of Bitter Honey and Sweet Honey (mg/kg)**

| Sample | Sodium | potassium | Calcium | Iron | Magnesium | Phosphorus | Energy |
|---|---|---|---|---|---|---|---|
| **Bitter Honey** | 2.80±0.00 | 7.50±0.00 | 3.90±0.21 | 1.53±0.00 | 0.22±0.00 | 2.62±0.07 | 329.12±1.82 |
| **Sweet Honey** | 3.10±0.00 | 9.50±0.01 | 4.30±0.15 | 1.25±0.00 | 0.10±0.00 | 3.45±0.02 | 333.64±0.35 |

honey and dissolve in 20 ml of warm water (40℃) centrifuge for 10 min at 2500 rpm/min. Decant the supernatant solution and collect the sediments into a conical tube and add acetolysis mixture containing acetic anh ydride : conc. sulphuric acid in the ratio 9:1 V/V for about 30 min at room temperature. After acetolysis the sediments were rinsed with distilled water and centrifuged for 5 min at 2500 rpm/min, and preserved for study. The types of pollen present in the sample were identified by comparing with that of reference slides of pollen (Xiao-Yan Song et al., 2012). The honey is considered to be monofloral when there is one dominant pollen and if none is dominant, it is of mixed floral (K.A. Hamid et al., 2015).

**Hydroxy methyl furfural content**. HMF is an indicative of overheating and poor storage of honey. The European Union and the Codex Alimentarius Commission established that the concentration of HMF present in honey should not exceed 40 or 80 mg /kg respectively. The two methods used for HMF

determination are after White (1979) and after Winkler (1955).

10 ml of the filtrate. Transfer 5 ml in two test tubes, to one tube add 5 ml of distilled water (sample solution);

**Table 7: Therapeutic properties of honey**

| Sl.No | Therapeutic properties | Attributed factors | Actions | References |
|---|---|---|---|---|
| 1 | Antimicrobial | Hydrogen peroxide. | Bactericidal/Bacteriostatic. | Araya Geber eyes-us Wasihun 2016 |
| | | High osmolarity, hydrogen peroxide and non H2O2 components. | Bactericidal. | Jose M.Alvarezsuarez et al.,2014 |
| | | H2O2. | Bacteriostatic | R A Cooper et al., 1999 |
| | | Kynurenic acid, glucose oxidase, catalase. | Bactericidal. | M Asaduzz-aman et al.,2015 |
| 2 | Anticancer (antitumor) | Flavanoids | Cytotoxicity towards tumor cells and antitumor activity in experimental animals. | Ahmed Hegazi, et.al.,2014 |
| | | Flavanoids | Stimulation of tumor necrosis factor (TNF-α), inhibition of cell proliferation inducation of apoptosis, cell cycle arrest. | Pongasthon premratana chai, et al.,2014 |
| | | Phenolic com-pounds | Anti leukemic activity. | Murtala .B Abubakar 2012 |
| | | Polyphenols | Apoptosis Induction. | Sarfraz Ahmed et al., (2013). |
| 3 | Antioxidant | Phenolic compounds, Enzymatic and non enzymatic antioxidants | Prevents the formation of free radicals. | Sumitramaurya et.al.,2014 |
| | | Phenolic compounds flavonoids | Radical scavenging | Norul Liza A-Rahaman et al.,2013 |
| | | Enzymatic and non enzymatic antioxidants | Radical scavenging | Eleazu,C.O et.al.,2013 |
| 4 | Antiulcer | Flavonoids | Gastroprotective activity , reducing the intensity of ulcer sores. | Nabila Al- Jaber 2013 |
| 5 | Antihyperli pid-emic | Niacin | Reduces VLDL and LDL through decreasing fatty acid mobilisation from adipose tissue triglyceride stores , inhibiting hepatocyte diacyl glycerol acyl transferase and triglyceride synthesis. | E.A.Alagwu,et al., 2011 |

**Table 8: Therapeutic Properties of Bitter Honey**

| Sl.No | Therapeutic property | Attributed factors | Actions | References |
|---|---|---|---|---|
| 1 | Antioxidant and antiradical | Homogentisic acid | Radical scavenging activity | AngelaAtzer et al., 2011 |
| 2 | Growth Inhibition of Human Colon Cancer Cells | Phenolic compound, flavonoid | Cytotoxicity | Sadia Afrin et al., 2017 |

**Spectrophotometric method (White)**: Weigh about 5g of honey and dissolve in 25 ml of water transfer it to a 50 ml volumetric flask and add 0.5 ml of carrez solution I followed by 0.5 ml of Carrez II , make up to 50 ml with water. Filter the solution through paper reject the first

to the second add 5 ml of sodium bisulphite solution 0.2% (reference solution). The absorbance of the solutions was determined at 284 and 336 nm.

**Table 9: FSSAI standards of honey**

| Parameters | limits |
|---|---|
| (a) Specific gravity at 27°C | Not less than 1.35 |
| (b) Moisture | Not more than 25 per cent by mass |
| (c) Total reducing sugars | Not less than 65 per cent by mass |
| (c-i) for Carbia colossa and Honey dew | Not less than 60 per cent by mass |
| (d) Sucrose | Not more than to 5.0 per cent by mass |
| (d-i)for Carbia colossa and Honey dew | Not more than 10 per cent by mass |
| (e) Fructose-glucose ratio | Not less than 0.95 |
| (f) Ash | Not more than 0.5 percent by mass |
| (g) Acidity (Expressed as formic acid) | Not more than 0.2 per cent by mass |
| (h) Fiehe's test | Negative |
| (i) Hydroxy methyl furfural(HMF), | Not more than 80 |

If Fiehe's test is positive and hydroxy methyl furfural (HMF) content is more than 80 milligram/kilogram, then fructose: glucose ratio should be 1.0 or more.

**Table 10: Agmark Standards of Honey: Criteria for grade designation**

| Sl.no | Parameters | Grade designation | | Standard |
|---|---|---|---|---|
| | | Special | Grade-A | |
| 1 | Specific gravity at 27 Degree Centigrade, (Minimum) | 1.4 | 1.37 | 1.35 |
| 2 | Moisture, percentage by mass, ( Maximum) | 20 | 22 | 25 |
| 3 | Total reducing sugars, percentage by mass, (Minimum) | 70 | 65 | 65 |
| 4 | Carvia- callosa and Honeydew honey, percentage by mass, (Minimum) | 60 | 60 | 60 |
| | Sucrose, percentage by mass, (Maximum) | 5 | 5 | 5 |
| | Carvia-callosa& Honeydew honey, (Maximum) | 10 | 10 | 10 |
| 5 | Fructose and Glucose ratio (F/G Ratio) (Minimum) | 1 | 0.95 | 0.95 |
| 6 | Ash, percentage by mass, for honey (Maximum) | 0.5 | 0.5 | 0.5 |
| | Ash, percentage by mass for Honey dew honey or a mixture of honey dew honey and blossom honey, (Maximum) | 0.5 | 0.5 | 0.5 |
| 7 | Acidity expressed as formic acid percentage(by mass), (Maximum),or | 0.2 | 0.2 | 0.2 |
| | Free Acidity Milli equivalents acid/1000g, (Maximum) | 40 | 40 | 40 |
| 8 | Fiehe's Test | | Negative | |
| 9 | Aniline chloride test | | Negative | |
| 10 | Total count of pollen and plant elements/g (i). Apiary honey (Maximum) (extracted honey) | 50,000 | 50,000 | 50,000 |
| | (ii). Squeezed honey (Minimum) | | | |
| 11 | Hydroxy Methyl Furfural (H.M.F.) mg/kg. (Maximum) | 80 | 80 | 80 |
| 12 | Optical Density at 660 nm. Percentage (Maximum) | 0.3 | 0.3 | 0.3 |
| 13 | Diastase activity. in schade units, (Minimum) | 3 | 3 | 3 |
| 14 | Water insoluble matters percentage (Maximum), | 0.5 | 0.5 | 0.5 |
| | For Pressed honey Other than Pressed honey | 0.1 | 0.1 | 0.1 |

If the pollen count is higher than 50000/gm. Then honey may be categorized as Squeezed honey.

**Spectrophotometric method (Winkler):** Weigh about 10 g of honey and dissolve in 20 ml water and transfer it to a 50ml of volumetric flask. 2 ml of solution and 5.0 ml of p-toluidine solution were transferred in test tubes, to one tube add 1 ml of distilled water (reference solution) and to the second add 1 ml of 0.5% barbituric acid solution (sample solution). The absorbance of the solutions was determined at 550nm (M. Zappala et al., 2005).

**Fiehe's test:** Fiehe's test is a qualitative test based on the detection of the hydroxyl methyl fufural that results from the dehydration of glucose, 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 .If there is no colour then the test confirms negative (S.Umarani et al., 2015).

**Therapeutic properties of honey**

The therapeutic properties of sweet honey and bitter honey are represented in tables 7 and 8 respectively.

**STANDARDS OF HONEY**

The quality of honey is governed by Food Standards and Safety Authority of India (FSSAI), AGMARK , Bureau Of Indian Standards (BIS), Indian pharmacopoeia (I.P) and are shown in tables 9,10 11 and 12 respectively.

varieties. *World Journal of Pharmaceutical Research*,

**Table 11: BIS Standards (Bureau of Indian standards, 1994) requirements for extracted honey**

| Sl .No | Characteristic | Special grade | A Grade | Standard grade |
|---|---|---|---|---|
| 1 | Specific gravity at $27^0$c, min | 1.37 | 1.37 | 1.37 |
| 2 | Moisture, percent by mass, max | 20 | 22 | 25 |
| 3 | Total reducing sugar, percent by mass, min | 70 | 65 | 65 |
| 4 | Sucrose, percent by mass, max | 5.0 | 5.0 | 5.0 |
| 5 | Fructose-glucose ratio[1)],min | 1.00 | 1.00 | 1.00 |
| 6 | Ash, percent by mass, max | 0.5 | 0.5 | 0.5 |
| 7 | Acidity (expressed as formic acid), percent by mass, max | 0.2 | 0.2 | 0.2 |
| 8 | Fiehe`s test | Negative | Negative | Negative |
| 9 | Hydroxymethylfurfural (HMF), mg/kg, max | 80 | 80 | 80 |
| 10 | Total count of pollens and plant elements/g of honey, max | 50000 | 50000 | 50000 |
| 11 | Optical density, at 660 nm, percent, max | 0.3 | 0.3 | 0.3 |

If Fiehe`s test is positive, carry out the determination of hydroxymethyl furfural (HMF) content. If it is more than 80mg per kg, then fructose glucose ratio should be more than 1.00.

**Table 12: The Indian Pharmacopoeia standards of Honey (IP 1966)**

| Sl.No | Parameter | Limits |
|---|---|---|
| 1 | Weight per ml | Should not be less than 0.36g |
| 2 | Acid value | Not exceeding 0.5ml N NaOH in 10g |
| 3 | Azo dye | Absence essential |
| 4 | Specific rotation | +0.3 to +0.5 |
| 5 | Chlorides | 18.75 parts per million |
| 6 | Sulphates | 25 parts per million |
| 7 | Invert sugar | Fiche test should be negative |
| 8 | External dye,Starch, Dextrin | Absent |
| 9 | Ash content | Not to exceed 0.5% |

## CONCLUSION

An extensive survey of literature on honey revealed that the traditional use of honey has a long history for various diseases and exhaustive research work has been done on honey, but little work has been done on bitter honey. FSSAI, AGMARK, BIS and Pharmacopoeial standards are available for honey, but not for bitter honey. So the further research work is proposed to develop a standard monograph for bitter honey.

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