IJBPAS, June, 2024, 13(6): 2714-2723

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

CHEMICAL CONSTITUENTS OF *NAVA* VS *PURANA MADHU*

HASSAN GP[1*] AND KAVITA MB[2]

1: Assistant Professor, Dept of PG studies in Swasthavritta, JSS Ayurveda Medical College, Mysuru

2: Professor & HOD, Dept of PG studies in Swasthavritta, SDM College of Ayurveda and Hospital, Hassan

***Corresponding Author: Dr. Pooja Hassan G: E Mail: drpoojaunnikrishnan17@gmail.com**

Received 15th April 2023; Revised 8th June 2023; Accepted 10th Sept. 2023; Available online 1st June 2024

https://doi.org/10.31032/IJBPAS/2024/13.6.8077

ABSTRACT

Introduction: The literature related to Ayurveda mentions that newly collected honey makes the body fat and as the honey gets older, it attains the ability to scrape off the deposited fat/lipid underneath the skin and in blood vessels. **Objectives:** The analysis was conducted to study the change of chemical constituents of honey in two different samples based on its time after collection. **Material and methods:** The samples of honey were purchased from bee keeper and market and stored for a year to make them old. Just before study, the newly collected honey samples were also purchased from both. All four samples were tested for total protein, total lipid, total carbohydrate, ash content, moisture content, total solids, energy value, HMF level, reducing sugars, total phenols and total flavonoids. The study was conducted at Central Food Technological Research Institute (CFTRI), Mysore. **Results and Conclusion:** In comparison, there was not much difference in proximate analysis of the Nava vs Purana in both the honey samples. HMF and total phenols were more in Purana Madhu samples. Bee-keeper's sample had higher amounts of protein, ash and moisture than the market samples; low levels of total carbohydrates, reducing sugars and high energy. The higher amount of HMF, phenols and flavonoids in it indicate its potency in anti-oxidant activity. The Purana Madhu bought from the bee keeper had the highest moisture (25.5%). The storage at ambient temperature for one year increased total phenols, flavonoids and HMF concentration while it reduced the concentration of reducing sugars. The honey stored for a year exhibited its ability to be a potent diet in oxidative stress related disorders.

Keywords: Ayurveda, Flavonoids, HMF, Madhu, Poly Phenols, Proximate analysis, Reducing sugars

INTRODUCTION:

Ayurveda lists honey among the daily consumable foods **[1]**. It classifies honey mainly into two types based on the time after collection viz., new (Nava) and old (Purana) **[2]**. The honey is called old only after completion of one year of it's collection **[3]**. The newer honey adds on weight to the body **[4]**. As the honey gets older, it attains the ability to scrape off the deposited fat/lipid underneath the skin and in blood vessels **[5]**. In this study, we hypothesize to study the change of chemical constituents of honey based on its time after collection in two different samples.

MATERIALS AND METHODS:

The samples of honey were collected directly from beekeeper and also local market in Hassan district of Karnataka state in India. Honey thus procured was stored separately in dark room at room temperature for one year to make old honey from market (OHM) and beekeeper (OHB). In parallel, new sample from market (NHM) and beekeeper (NHB) were procured just before the study was started. The study was conducted at Central Food Technological Research Institute (CFTRI), Mysore. All the four samples were tested for their chemical constituents viz., proximate analysis (total protein, total lipid, total carbohydrate, ash content, moisture content, total solids, energy value, HMF level, reducing sugars, total phenols and total flavonoids.

A. Proximate composition [6]

1. Moisture:

Clean porcelain crucibles were oven dried at 105^0C. The crucibles were later cooled and weighed. Approximately 10 gram of the sample was weighed into the crucibles. The whole crucible with the sample was weighed again [initial weight]. The crucibles with the sample were oven dried at 105^0C. For every one hour the crucible was cooled and re-weighed. The procedure was repeated in intervals for every one hour till a constant value is obtained [final weight]. The moisture content present in the sample was calculated using the following formula and expressedas percentage per 100 gm.

$$\text{Moisture} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Weight of the sample taken}} \times 100$$

2. Proteins [7, 8]:

Percentage of protein content in the sample was assessed using a protein analyzer. About 50 grams of moisture free sample was covered in aluminium foils, covered well and kept in respective slots of protein analyzer and analyzed against a known protein standard. The percentage of

protein content present in the sample was calculated by N/P (nitrogen/ protein) method.

3. Lipid analysis:

Lipid extraction was done using Folch method **[9]**. Five grams of each sample was added with 10 ml acetone methanol mixture (70:30) and vertexed well. This is allowed to stand for one hour and solvent was filtered through whatman filter paper 41 to pre-weighed test tube (t1). This process was repeated till clear solvent was seen. Solvent was completely evaporated through nitrogen gas and the test tube is re-weighed (t2). Total lipid content was calculated using the formula,

$$\text{Total lipid} = \frac{t2 - t1}{SW} \times 100$$

[SW = sample weight]

4. Ash:

10 grams of the sample was taken in pre-weighed crucibles [W1]. This was charred on a mantle to jet black till it becomes smokeless. The crucibles were further ignited in muffle furnace at 500^0 C, until the samples turned grayish white [for around 3 to 4 hours after temperature reaches 500 ^{0}C]. The weight of the crucible with the ash was measured [W2]. The total ash content present in the sample was calculated by using the following formula and expressed in percentage per 100 grams.

$$\text{Total Ash} = \frac{(\text{Wt of the crucible+ash}) - \text{Wt of the crucible}}{\text{Weight of the sample taken}} \times 100$$

5. Carbohydrate:

Total carbohydrate was calculated by using the formula; value was expressed in percentage per 100gm.

Carbohydrate = 100-[Moisture + Fat + Protein + Ash + Fiber]

6. Energy value:

Total energy value of the samples was calculated by using the formula and expressed as kcl per 100gm.

Energy value = [4×Protein%] + [9×Lipid%] + [4×Carbohydrate%]

7. Total solids:

Total solids present in the sample was calculated using the following formula and was expressed in percentage per 100 grams.

Total solids = 100 – Moisture

B. Other chemical analysis

1. HMF (Hydroxy Methyl Furfural):

Four grams of each sample was taken in a test tube (sample 5 was taken in 250µg and 500µg) and 10ml of distilled water was added and mixed well. To this 5ml of Oxalic

acid was added and kept in boiling water bath for 1hour and cooled. After cooling, 5ml of TCA (Trichloroacetic acid) was added for protein precipitation. After 5 minutes it is centrifuged for 10minutes with 4000 rpm. 4ml of the supernatant was taken in another test tube and added with 1 ml of

2. Reducing sugars:

Stock solution was prepared by taking 100µl of raw sample, to which 10 dilutions were done. From this dilution again 100µl was taken and 100 dilutions were done. From the stock solution, 2ml of each sample was taken to which 1ml of DNS reagent was added and kept for incubation in boiling water bath for 1 hour. After cooling 3ml of distilled water was added to it and absorbance was read at 550nm. Glucose was used to calculate the standard curve (200, 400, 600, 800 and 1000µg/ml, r^2=0.996). The results were expressed as mg/ml.

3. Total phenols / Phenolics:

Phenolic compounds were detected by spectrophotometric Folin-Ciocalteu method **[10]**.50ml of each sample were taken and 50 dilutions were done. From this 50µl and 100µl of sample was taken to which 2.95 ml of distilled water was added. To this mixture 1 ml of FC reagent and 1 ml of Na_2CO_3 was added and incubated for 20 minutes in dark. The absorbance was read at 760nm. Gallic acid was used to calculate the standard curve (5, 10, 15, 20 and 25µg/mL, r^2=0.999). The results were reported as the mean ± standard deviations and were expressed as mg of Gallic acid equivalents per ml.

TBA (Thiobarbituric acid). This mixture is incubated in 40^0C for 40 minutes and the absorbance was read at 443nm.

Total HMF was calculated using the formula,

(OD443 – 0.055) × 28.4 = µ mol total HMF per 100g of dry matter of sample.

4. Total flavonoid content:

The total flavonoid contents were determined using aluminium chloride cocorimetric method **[11]**. One gram of each sample was taken and 5ml of methanol was added, mixed well. This mixture was centrifuged for 10 minutes and supernatant methanol phase of 2ml was taken as stock solution. To this 2ml, 3ml of 5% $AlCl_3$ was added and incubated for 30 minutes in dark and absorbance was read at 437nm against methanol blank. Quercetin was used to draw standard curve in concentration range of 100mg to 1200mg/ml. The results were reported as the mean ± standard deviations and were expressed as mg of Quercitin equivalents per 100g.

OBSERVATIONS:

Effect of sampling on taste: The taste, one of the sensory characteristic, of honey brought from bee keepers (OHB) was sweet during collection and turned sour after one year of storage. The souring may be due to the Kala Samskara given to honey. This is supported various researches related to effect of moisture content on honey. The excessive moisture content causes fermentation in honey on storage by the action of osmotolerant yeasts, which results in the formation of ethyl alcohol and carbon dioxide. The alcohol can be further oxidized to acetic acid and water, which leads to a sour taste **[11, 12, 13]**.

RESULTS:

Table 1: Results of Proximate analysis of samples (per 100 grams)

S. No.	Parameters	Groups			
		OHB	NHB	OHM	NHM
1.	Protein (%)	2.5	1.5	ND	ND
2.	Lipid (%)	ND	ND	ND	ND
3.	Carbohydrates (%)	71.5	71.5	79	78.5
4.	Moisture content (%)	25.5	22	15.5	14
5.	Ash (%)	1	2	0.5	0.5
6.	Total solids (%)	75	75	79.5	79
7.	Energy value (kcal)	296	291	316	314

NOTE: OHB (Old honey from beekeeper), NHB (New Honey from Bee keeper), OHM (Old Honey Market), NHM(New Honey from Market, ND = Not Detected

Table 2: Results of bio-chemical tests

S. No.	Name of the experiment	OHB	NHB	OHM	NHM
1.	Reducing sugars (gm/100ml)	48.40	56.44	69.06	69.93
2.	Total phenols (mg/100ml)	140.53	86.4	266.13	67.2
3.	Total flavonoids (mg/100gm)	19.95	11.5	5.85	8.15
4.	HMF (mg/kg)	77.72	60.40	41.17	34.63

NOTE: OHB (Old honey from beekeeper), NHB (New Honey from Bee keeper), OHM (Old Honey Market), NHM(New Honey from Market), MPT (Madhupaka with Triphala rasa) and MPJ (Madhupaka with Jala)

DISCUSSION:

Proximate Analysis

Proximate analysis is very important to know the beneficial effect of honey before and after processing. The proximate components such as water content (moisture), total protein, total fat, total carbohydrates, total ash and energy value of old and new honey samples from beekeeper and market were assessed and presented in **Table 1**.

Protein: Protein was found to be higher in OHB (2.5 %) compared to NHB (1.5%) however no protein was found in the two market samples OHM and NHM. The amount of protein content varies based on source of honey. Protein content was found to be below the detectable limit in market samples. This may be due to microfiltration of honey to remove the pollens (as they may cause allergic reactions when consumed) which are the main source of

proteins in honey. The other marketing reason is to avoid it's tracing to the floral source from which honey is formed **[14, 15]**. Presence of protein in OHB and NHB hints that they are not subjected to any processing to remove the pollens and difference in their protein content (2.5 and 1.5 %) is probably due to pollens from different floral sources.

Total Lipids:

The study revealed no lipids in all the four samples analyzed. Many of the studies done on proximate principles and nutritional values also revealed that the honey has either no or negligible amount of lipids **[16, 17]**.

Total carbohydrate:

The highest carbohydrate content was found to be higher market samples with negligible difference viz., OHM (79%) and NHM (78.9%) compared to bee keeper's samples viz., both NHB and OHB with 71.5% of carbohydrates. The values obtained from all samples are abovethe minimum value (60 gm /100 gm) recommended by Council Directive of the European Union **[18]**.

Moisture content:

The moisture content was found to be higher in OHB (25.5%) compared to NHB (22%), OHM (15.5%) and NHM (14%). Based on storage time in both bee keeper's and market sample, the older honey had higher moisture content than the newer sample. Ideal moisture content of honey is not more than 20%[18]. A study by Martinez *et al* revealed temperature and length of storage

increases the humidity of honey. Moisture content of honey stored at ambient temperature will be higher than that was refrigerated **[19]**.

Ash:

Ash content was found to be higher in NHB (2%) compared to OHB (1%), OHM (0.5%) and NHM (0.5%).

Total solids:

Total dissolved solids were found to be slightly higher in market samples i.e., 79.5% and 79% in OHM and NHM respectively when compared to samples from bee keeper i.e., 75% in both OHBand NHB. Total solids or Total Dissolved Solids (TDS) is a measure of all the inorganic and organic substances present in honey either in molecular or ionized form **[20]**. The result indicates that the beekeepers samples had lesser organic and inorganic substances in them when compared to market samples.

Energy value:

Total energy value was found to be highest in market samples OHM (316 KCal) and NHM (314 KCal) when compared to OHB (296 KCal) and NHB (291 KCal). The slight increase in energy value in old honey in both market sample and that from bee keeper are

probably due to change insugar component of old honey on storage. da Silva *et al* **[21]** analyzed both stabilized and non- stabilized honey stored for 24 weeks. Sucrose concentration in non-stabilized honey decreased 79% in honey stored at room temperature (20^0C). Fructose content increased 7% and the glucose content increased by 8.8%.

Biochemical analysis:

The results of bio-chemical constituents are summarized in **Table 2**.

Reducing sugars:

The reducing sugars were highest in NHM (69.93 gm/100ml) followed by OHM (69.06 gm/100ml). It was least in OHB (48.40 gm/100ml) followed by NHB (56.44 gm/100ml).

Total phenols:

The total phenols were highest in OHM (266.13gm/100ml) followed by OHB (140.53 gm/100ml). It was least in NHM (67.2gm/100ml) followed by NHB (86.4 gm/100ml). This difference in total phenolic content can be attributed to the difference in botanical sources and geographical regions from which honey was collected in market sample to bee keeper's sample **[22]**.

Total flavonoids:

The total phenols were highest in OHB (19.95 gm/100ml) followed by NHB (11.5 gm/100ml). It was least in OHM (5.85 gm/100ml) followed by NHM (8.15 gm/100ml).

HMF:

HMF content was found to be highest in OHB (77.72 mg/kg) compared to NHB (60.40 mg/kg), OHM (41.17 mg/kg) and NHM (34.63 mg/kg) respectively. The standard limit for HMF in unprocessed honey is 40 mg/kg **[23, 24]** Increased HMF in OHB and NHB might be due to hot climate in place of procurement and storage (OHB). Fallico *et al*. (2004) **[25]** reported that the amount of HMF after processing should not be more than 40 mg/kg, and not more than 80 mg/kgin honey procured from regions with high temperature. Tosun *et al*. (2003) **[26]** in their study reported that, HMF formation increases on storage and this causes change in colour from light to dark which is also evident in colour assessment of this study. HMF is naturally present in honey, which is produced by the action of acidity in honey on reducing sugars and sucrose usually at room temperature. So, exposure to HMF cannot be completely prevented **[27]**. Moreover, as per the statement of bee-keeper honey was exposed to intense sun rays for two days to prevent crystallization of it. This processing given by exposing to sun light further might have enhanced the HMF content in OHB and NHB. Abraham *et al*. (2011) **[28]**

in an animal experiment reported that, there were no ill effects observed at a daily dose of HMF in the range of 80 – 100 mg/kg. Tamanna *et al*. (2015) **[29]** reported that, HMF and other Millard reaction products in low amounts acts mainly as anti-oxidants.

CONCLUSION:

There is no much difference in proximate analysis of the two varieties of honey viz., new vs old. HMF and total phenols are more in old honey samples. Bee-keeper's sample has highest amount of protein, ash and moisture than the market samples. It has low levels of total carbohydrates, reducing sugars and high energy. It also has higher amount of HMF and anti-oxidant activity.

REFERENCES:

[1] Acharya J. Charaka Samhita by Agnivesha. 1st ed. Varanasi: Chaukhambha Orientalia;2011.p.38

[2] Bhavamisra. Madhu varga/25. In: Misra B, Vaisya R, ed. by. Bhavaprakasha. 1st ed. Varanasi: Chaukhambha Sanskrit Bhavan; 2013. p. 791.

[3] Bhavamisra. Madhu varga/26. In: Misra B, Vaisya R, ed. by. Bhavaprakasha. 1st ed. Varanasi: Chaukhambha Sanskrit Bhavan; 2013. p. 791.

[4] Sushruta. Dravadravya Vidhi/140. In: Acharya J, Acharya N, ed. by. Sushruta Samhita.7th ed. Varanasi: Chaukhambha Orientalia; 2002. p. 207.

[5] Sushruta. Dravadravya Vidhi/141. In: Acharya J, Acharya N, ed. by. Sushruta Samhita.7th ed. Varanasi: Chaukhambha Orientalia; 2002. p. 207.

[6] A.O.A.C., 1990. Official Methods of Analysis. Association of Official Analytical Chemists, The Association: Arlington, VA, Vol. II, 15th ed. Sec.985.29.

[7] Kjeldahl J. Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern. Fresenius' Zeitschrift für analytische Chemie. 1883;22(1):366-382.

[8] Hanne K. Mæhre, Lars Dalheim, Guro K. Edvinsen, Edel O. Elvevoll, Ida-JohanneJensen. Protein Determination—Method Matters. Foods [Internet]. 2018 [cited 1 January 2019];7(1):5. Available from: https://www.mdpi.com/2304-8158/7/1/5

[9] Folch J, Ascoli I, Lees M, Meath JA, Lebaron FN. Preparation of Lipide Extracts From Brain Tissue*. The Journal of Biological Chemistry [Internet]. 1951 [cited 3 March 2019]; 191: 833-841. Available from: http://www.jbc.org/content/191/2/833.long

[10] Blainski A, Lopes G, de Mello J. Application and Analysis of the Folin Ciocalteu Methodfor the Determination

of the Total Phenolic Content from Limonium Brasiliense L. Molecules. 2013;18(6):6852-6865.

[11] Bag GC, Grihanjali Devi P, Bhaigyabati T. Assessment of Total Flavonoid Content and Antioxidant Activity of Methanolic Rhizome Extract of Three Hedychium Species of Manipur Valley. Int J Pharm Sci Rev Res [Internet]. 2015 [cited 14 April 2019];30(1):154-159. http://globalresearchonline.net/journalcontents/v30-1/28.pdf

[12] Chirife J, Zamora MC, Motto A. The correlation between water activity and% moisturein honey: fundamental aspects and application to Argentine honeys. J Food Eng. 2006;72(3):287–292. doi: 10.1016/j.jfoodeng.2004.12.009.

[13] Moniruzzaman M, Sulaiman SA, Khalil MI, Gan SH. Evaluation of physicochemical and antioxidant properties of sourwood and other Malaysian honeys: a comparison with manuka honey. Chemistry Central Journal. 2013 Aug 12;7(1):138.

[14] Bauer L, Kohlich A, Hirschwehr R, Siemanna U, Ebner H, Scheiner O, Kraft D, Ebner C.Food allergy to honey: Pollen or bee products?: Characterization of allergenic proteins in honey by means of immunoblotting. Journal of allergy and clinical immunology. 1996Jan 31;97(1):65-73.

[15] Honey without Pollen | Bee Informed Partnership [Internet]. Beeinformed.org. 2018[cited 1 January 2018]. Available from: https://beeinformed.org/2011/11/14/honey- without-pollen

[16] Ajibola A, Chamunorwa J, Erlwanger K. Nutraceutical values of natural honey and its contribution to human health and wealth. Nutrition & Metabolism. 2012;9(1):61.

[17] McCance RA, Widdowson EM. The composition of foods. 6th ed. Cambridge, England: Food Standards Agency; 2010.

[18] Council Directive 2001/110/EC of 20 December 2001 relating to honey. Official Journal of the European Communities [Internet]. 2002 [cited 2 January 2019];9-35. Available from: https://www.wipo.int/edocs/lexdocs/laws/en/eu/eu159en.pdf

[19] Martínez R, Schvezov N, Brumovsky L, Román A. Influence of temperature and packaging type on quality parameters and antimicrobial properties during Yateí honey storage. Food Science and Technology [Internet]. 2017 [cited 18 February 2019];38(suppl 1):196-202. http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0101-20612017005051103

[20] Ahmed M, Imtiaz Shafiq M, Khaleeq A,

Huma R, Abdul Qadir M, Khalid A *et al*. Physiochemical, Biochemical, Minerals Content Analysis, and Antioxidant Potential of National and International Honeys in Pakistan. Journal of Chemistry [Internet]. 2016 [cited 3 February 2019; 2016: 1-10. https://www.hindawi.com/journals/jchem/2016/8072305/

[21] da Silva P, Gauche C, Gonzaga L, Costa A, Fett R. Honey: Chemical composition, stability and authenticity. Food Chemistry [Internet]. 2016 [cited 3 February 2019];196:309-323. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26593496

[22] Bertoncelj J, Dobersek U, Jamnik M, Golob T. Evaluation of the phenolic content,antioxidant activity and colour of Slovenian honey. Food Chem. 2007;105:822–828. doi: 10.1016/j.foodchem.2007.01.060.

[23] Bogdanov S, Lüllmann C, Martin P, von der Ohe W, Russmann H, Vorwohl G *et al*. Honey quality and international regulatory standards: review by the International Honey Commission. Bee World. 1999;80(2):61-69.

[24] Bogdanov S, Martin P. Honey Authenticity: A Review [Internet]. http://www.bee- hexagon.net. 2002 [cited 8 June 2019]. Available from: http://www.bee-hexagon.net/files/file/fileE/Honey/AuthenticityRevue_Internet.pdf

[25] Fallico B, Zappala M, Arena E, Verzera A. Effects of conditioning on HMF content in unifloral honeys. Food Chemistry. 2004 Apr 30;85(2):305-13.

[26] Tosun I, Sule Ustun N. Nonenzymic browning during storage of white hard grape pekmez (Zile pekmezi). Food Chemistry. 2003;80(4):441-443.

[27] Stadler RH, Lineback DR, editors. Process-induced food toxicants: occurrence, formation, mitigation, and health risks. John Wiley & Sons; 2008 Dec 9.

[28] Abraham K, Gürtler R, Berg K, Heinemeyer G, Lampen A, Appel K. Toxicology and risk assessment of 5-Hydroxymethylfurfural in food. Molecular Nutrition & Food Research. 2011;55(5):667-678.

[29] Tamanna N, Mahmood N. Food Processing and Maillard Reaction Products: Effect on Human Health and Nutrition. International Journal of Food Science. 2015;2015:1-6.