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ESTIMATION OF HMF (HYDROXYMETHYLFURFURAL) CONTENT IN PROCESSED HONEY – A COMPARATIVE ANALYSIS USING HPLC AND WINKLER METHODS

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ABSTRACT

Honey processing is a controversial aspect of Ayurveda owing to predicted toxicity associated with heating process. Processed honey is strongly recommended to achieve specific outcomes. HMF (*Hydroxymethylfurfural* increases after heating process and said to be associated with carcinogencity. HPLC method is emphasized as accurate technique to measure HMF in Honey. Processed honey was analysed to evaluate HMF content by two different methods (conventionally followed Winkler method and HPLC analysis). Triphala Kashaya samskarita honey showed very high HMF content in HPLC analysis compared to the sample analyzed with Winkler method. Phenolic content of the processed honey is thought to be the reason behind increased value. Winkler method seems to be ideal for the honey samples having more phenolic content.

KEYWORDS: Hydroxymethylfurfural, HPLC, Winkler method, Processed Honey

INTRODUCTION

Honey is a natural substance produced by bees using nectar obtained from flowers. Honeybees travel from flower to flower and collect nectar which is converted to honey by enzymatic action of their saliva. Invertase in bee saliva converts sucrose in the nectar to glucose and fructose which is the major constituent of honey. Enzymatic action of Glucose oxidase oxidizes glucose to gluconolactone and further reducing this to Hydrogen peroxide.[1] Honey also contains a number of Phenolic acids and flavonoids which contribute to its antioxidant properties. In Ayurveda, Honey is termed as Pushpasava[2] and has mentioned 8 types based on the types of honeybee collecting the nectar from flowers. Though heating honey is contraindicated in Ayurveda as it is said to transform honey in to Visha, contradictory remarks are also found in some books saying honey must not be used unless heated. It is even mentioned as to process honey with different Kashaya kalpana to achieve specific outcomes.[3] Though satisfactory answer has

not been given for honey becoming toxic after heating, it is often linked with HMF (Hydroxymethylfurfural), which is claimed to be carcinogenic. HMF is a compound formed by the triple dehydration of fructose, obtained by the isomerization of glucose. It is a white- yellow solid substance which is highly soluble in Water and organic solvents.[3] HMF is naturally absent in fresh foods and is formed over storage or by drying or cooking and is best observed in Caramelized sugar [5]. Although HMF is used as a food additive, there are ambiguities regarding its safety as it was thought to have cytotoxic and mutagenic effects. Recent studies have also revealed some beneficial effects. The concentration of HMF rises on processing and on ageing.[6] Daily consumable limits can be upto 30-40g per day but this might increase up to 350mg on consumption of certain beverages.[7]

The concentration of HMF can be determined by different methods. High Performance Liquid Chromatography (HPLC) is said to be very accurate and efficient methods among them.[8] HPLC consists of a solid- liquid phase extraction followed by a solid phase extraction using silica as adsorbent, coupled with mass spectrometry analysis. The other method of HMF detection and also a conventionally adopted is spectrophotometric method designed by Winkler [9] in which p-toluidine solution is added to the honey and compared with the sample containing p-toluidine and barburitic acid. The solutions are checked for absorbance at 550nm.[10]

Hence the study was undertaken to determine HMF concentration of unprocessed, water processed and Triphala Kashaya processed honey by HPLC and Winkler methods.

MATERIALS AND METHODS

Honey was procured form Honey National Society, Marthandam, Nagercoil dist., Tamilnadu and aged for one year in a food grade plastic container, under room temperature protected from excessive moisture and light. This was then used for analysis.

Preparation of sample:

Three samples were taken for the study:

I. One year aged honey in unprocessed form

II. One year aged honey processed with water: Eight parts of honey was mixed with one part of potable water and heated over low flame till the volume reduced to the quantity of honey taken. [11]

III. One year aged honey processed with Triphala Kashaya: Fruits of Haritaki, Amalaki and Vibhitaki were procured and pound to a coarse powder and mixed in equal proportions to make Triphala churna. Triphala kashaya was prepared as per Kashaya paka vidhi of Sharngadhara Samhita.[12] One year aged honey and Triphala kashaya were taken in equal proportions and mixed to form a homogenous solution. It was then heated over low flame till paka lakshana was obtained. [13]

HMF determination:

High performance liquid chromatography method: [14]

Five grams of sample was diluted up to 50mL with distilled water and filtered through a 0.45µm filter and injected into a HPLC equipped with a Diode Array Detector immediately. All the solvents used were of HPLC grade. The HPLC column was a Merck Lichrospher, RP- 28, 5µm, 125x4mm, fitted with a guard cartridge packed with the same stationary phase. The HPLC operated under the following conditions: isocratic

mobile phase of 90% water, 1% acetic acid and 10% methanol. It was set at a flow rate of 0.7ml/min with an injection volume of 20µL. The chromatograms were monitored at a wavelength of 285nm within the working range of 220-660nm. HMF was identified by splitting the peak in honey with a standard HMF and by comparing the HMF spectrum with that of the samples.

Spectrophotometric method of Winkler: [15]

Before performing the actual Winkler's test, it is essential to perform the FIehe's test to detect the presence of HMF. To perform this, 1g of resublimed Resorcinol was dissolved in 100mL hydrochloric acid. Five gram of the honey sample was taken in a mortar and pestled with 10mL of ether. This procedure was repeated twice and the extract was then decanted to a porcelain dish. The extract was allowed to dry at room temperature and then added with a large drop of freshly prepared Resorcinol solution. An instant cherry red color appeared by the reaction, indicating the presence of HMF in the sample.

To quantitatively determine the HMF content in the sample, Winkler's test was then performed.

Preparation of p-toluidine solution: Analytical grade 10g of p-toluidine was dissolved in 50mL isopropanol by warming slightly on a water bath. This was transferred to a 100mL graduated flask and added with 10mL glacial acetic acid. It was then cooled and made up to volume using isopropanol. This solution was kept unused in the dark for about 24 hours. Preparation of Barbituric acid solution*:* 500mg of barbituric acid was taken and transferred to a 100mL graduated flask using 70mL water and placed in a hot water bath until it dissolved and the it was added with more water to make up for the volume.

Ten grams of honey was dissolved in 20mL of oxygen free distilled water and transferred to a volumetric flask. 2mL of the solution and 0.5mL of p-toluidine solution were put in two different test tubes. To one tube, 1mL of distilled water was added and to the other 1mL of barbituric acid solution was added without any delay. The HMF in the honey reacted with the p-toluidine and barbituric acid to change color. The absorbance of this solution was determined at 550nm using a VARIAN mod. Cary 1E UV-visible. The quantitative value of HMF was determined by using both the external standard method and by using the proposed formula for the method.

RESULTS

Honey aged for a year was brown in colour (Fig 1) and turned bit darker after processed with water (Fig 2). Triphala Kashaya samskarita Madhu was dark brown in colour with thick consistency (Fig 3).

Though there was no change in the odor much after processing with water, astringent sour odor was observed in Triphala Kashaya samskarita honey.

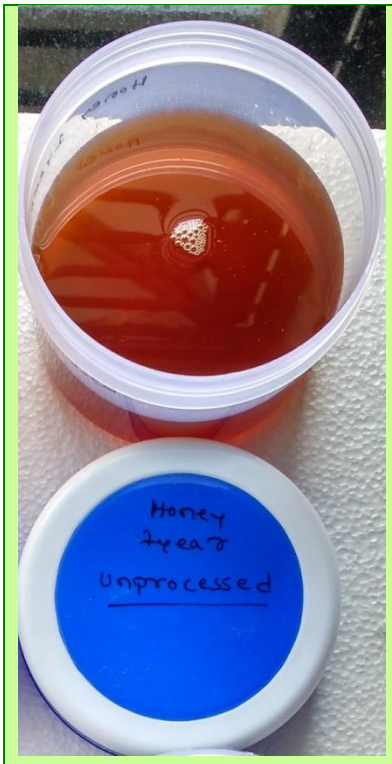

Fig 1(Unprocessed Honey)

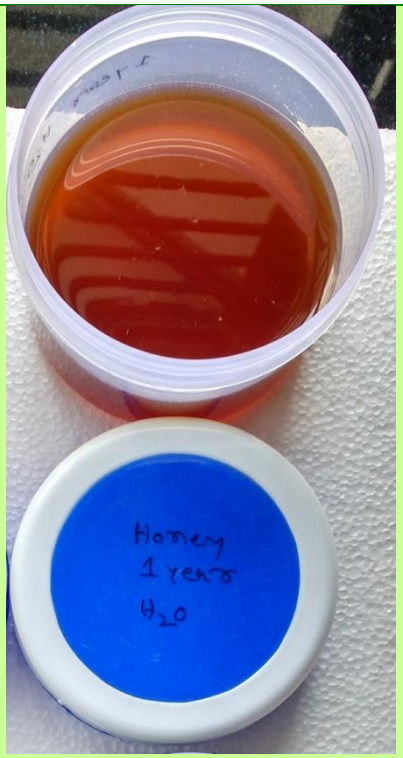

Fig 2 (Processed with water)

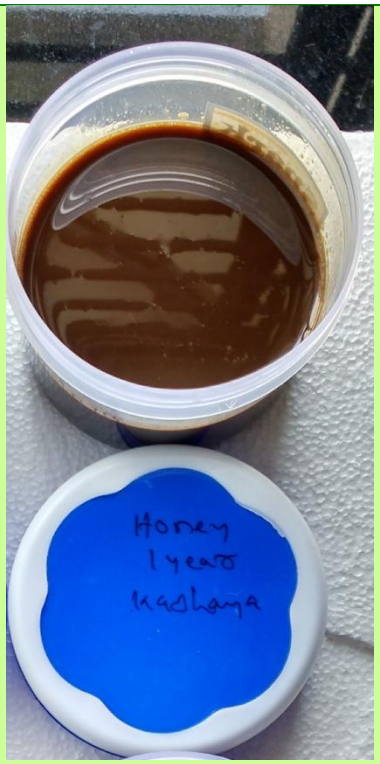

Fig 3 (Processed with Triphala Kashaya)

The results of the HMF values estimated by both HPLC and Winkler methods are reported in Table 1.

Sl. No.	Sample	HPLC Method (mg/kg)	Winkler's method (mg/kg)
1.	One year aged honey-unprocessed	6.573	9.2
2.	One year aged honey processed with water	66.82	68.7
3.	One year aged honey processed with Triphala kashaya	558.159	292.4

Table 1

From the above data, it is observed that the results significantly vary between the two methods after processing with Triphala Kashaya. However, the same honey samples were used for both the analytical methods, gradient increase in values is found between samples in the Winkler's method but there is a sharp rise in the value of HMF in the Triphala Kashaya processed sample analyzed By HPLC method, which is actually double

the amount observed in Winkler method.

The HMF values of the aged honey are still within limits of international standards in unprocessed honey and Water Processed Honey.

DISCUSSION

Honey is basically a food product and consumed almost every day. It also carries medicinal value and often used as weight reducing agent.[16] Practice of using honey with hot water is found worldwide, which is not advocated in classical Ayurvedic books.[17] Though no satisfactory answer is available for toxicity after heating of honey in ancient literature, modern science throws some light on possible changes happen during storage and processing of sugar containing stuffs including honey. HMF is said to be carcinogenic but effect of it is not established in human beings. Previous studies carried out on experimental animals as well as on human beings using processed honey did not show any harmful effects when used for one mandala kala (48 days).[18][19][20][21]

When honey is heated, it results in increased production of HMF and other substances as a result of Maillard Reaction. These values cannot be applied for all samples of honey as the production of HMF and other reaction products of Maillard reaction are significantly influenced by the sugar and amino acid composition of the honey, in addition to its pH value, heating and storage conditions. It is also observed that some phenolic acids accelerate or inhibit the formation of HMF based on the composition of honey.[22]

Phenolic acids are one of the major components present in honey. Due to the interference of phenolic acids, the quantity of HMF cannot be fixed for the wide range of honeys available in the market. Determination of Phenolic acids using HPLC method is challenging. Phenolic acids do not separate easily giving erroneous results. Hence they need to be determined using a suitable non polar solvent system with Gradient method and C18 reverse phase columns for their separation. HMF is said to interfere with Phenolic estimation in HPLC method at the wavelength of 280nm.[23] Hence in the HPLC method, the results may not be accurate due to the improper separation of phenolic acids, resulting in a combined peak. Results of previous studies have showed steep increase in Phenolic content after processing of honey.[24] owing to the release of bound phenolics after heating process[25] This might have resulted in more interference of phenolics in HMF estimation by HPLC method.

In the Winkler's method, the sample is added with barbituric acid and p-toluidine solutions which react only with HMF in the sample resulting in a color change. Spectroscopically this is observed at 550nm and the values are calculated. As the reagents only react with HMF in the sample and not with any other compounds in the sample, phenolics might not have interfered during spectroscopic analysis suggesting reliability of Winkler method in HMF analysis of processed honey with phenolic rich drugs.

CONCLUSION

Winkler method holds good with processed honey samples compared to HPLC in estimation of HMF owing to phenolic interference. Much accurate and versatile tool is needed to prevent usage of harmful chemicals during analysis as it happens in Winker method.

REFERENCES

1. The chemistry of Honey | Bee Culture [internet]. Bee culture, 2018 [cited 24 December2021]. Available from: http:// www.beeculture.com/the-chemistry-ofhoney

2.Narahari pandita, Tripathi Rajanighantu. (5st edn). Varanasi: Chowkham ba Krishnadas Academy; 2010.

3. Sharma P V et al. Kaiyadeva Nighantu of Kai yadeva, 2nd Edition. Varanasi: Chowkambha orientalia; 2009. p 42.

4. Shrotri, A. Catalytic Conversion of Structural Carbohydrates and Lignin to Chemicals. In: Song, C (ed.) Advances in Catalysis. : Academic Press; 2017. p. 59-123.

5. Wikipedia. Wikipedia. [Online]. Available from: https://en.m.wikipedia.org/wiki/Special:Hist ory/Hydroxymethylfurfural [Accessed 9 April 2022].

6. National Center for Biotechnology Information (2022). PubChem Compound Summary for CID 237332, 5-Hydroxymethylfurfural. Retrieved March 22, 2022 from https://pubchem.ncbi.nlm.nih.gov/comp ound/5-Hydroxymethylfurfural.

7. Wikipedia. Wikipedia. [Online]. Available from: https://en.m.wikipedia.org/wiki/ Special:History/Hydroxymethylfurfural [Accessed 9 April 2022]

8. Zappalà, M, Fallico, B, Arena, E, Verzera, A. Methods for the determination of HMF in honey: a comparison. Food Control. 2005; 16(3): 273-277.

9. Apiary industry sectional committee. Bureau of Indian Standards. (2 ed.). New Delhi: 1994.

10. Martysiak-zurowska*, D, Borowicz, A. A Comparison of Spectrophotometric Winkler Method and HPLC Technique for Determination of 5-Hydroxymethyl furfural in Natural Honey. Chemia Analityczna. 2009 ; 54(5): 939-947

11. Sharma P V et al. Kaiyadeva Nighantu of Kaiyadeva. 2nd edn. Varanasi: Chowkambha orientalia; 2009. Shloka no : 210. p 42

12. Srikantamurthy K R Trans. Sharangadhara Samhita of Sharagadhara with English translation: Madhyama khanda. 1st Edn. Varanasi: Chowkamba orientalia; 2009. p 335

13. Sharma P V et al. Kaiyadeva Nighantu of Kaiyadeva. 2nd edn. Varanasi: Chowkambha orientalia; 2009. p 43

14. Mannino, S, Schleining, G. Practical tasks for food quality assurance. : ; 2001.

15. Apiary industry sectional committee. Bureau of Indian Standards. (2 ed.) New Delhi: ; 1994.

16. Sadeghi, F, Salehi, S, Khohanmoo, A, Akhlaghi, M. Effect of Natural Honey on Glycemic Control and Anthropometric Measures of Patients With Type 2 Diabetes: A Randomized Controlled Crossover Trial. International Journal of Preventive Medicine. 2019;10(1): 3.

17. Acharya Agnivesha YT, editor. 1st Chapter, commentary for 6th Shloka, Charaka Samhita with Ayurveda Deepika commentary by Chakrapanidatta. 5th ed. Varanasi: Chaukhambha Sanskrit Sansthana; 2001. Agnivesha, Sutra sthana; p. 4.

18. Annapoorani, A., Anilakumar, K. R., Khanum, F., Murthy, N. A., & Bawa, A. S. (2010). Studies on the physicochemical characteristics of heated honey, honey mixed with ghee and their food consumption pattern by rats. Ayu, 31(2), 141–146. https://doi.org/10.4103/0974-8520.72363

19. Satish Pai, Ravi Nargis, Manula S N. Role of Samyoga and Samskara on

Antihyperlipidaemic / Anti-obesity activity of Honey An Experimental Study. J. res. tradit. med. (2015), [cited April 05, 2022]; 1(1): 16-22.
20. Sowmya Hariharapura Yogendra, Satish Pai, Manjula Santhepete Nanjundayya. Evaluation of Anti - Hyperglycaemic activity of Madhu (Honey) in High fat diet induced diabetes - An Experimental study. J. res. tradit. med. (2017), [cited April 05, 2022]; 3(5): 129-136.
21. Pai, S, Shivappa, C.B. Anti-obesity and Anti-hyperlipidemic activity of Processed Honey - A Randomised, Open labeled, Controlled Clinical Study . J res tradit med . 2018;4(2): 40-48.
22. Abrantes, T, Moura-nunes, N, Perrone, D. Gallic acid mitigates 5-hydroxymethylfurfural formation while enhancing or preserving browning and antioxidant activity development in glucose / arginine and sucrose/arginine Maillard Model Systems. Molecules. 2022;27(848): .
23. Chen, P. X., Tang, Y., Zhang, B., Liu, R., Marcone, M. F., Li, X., & Tsao, R. (2014). 5-hydroxymethyl-2-furfural and derivatives formed during acid hydrolysis of conjugated and bound phenolics in plant foods and the effects on phenolic content and antioxidant capacity. Journal of agricultural and food chemistry, 62(20), 4754–4761. https://doi.org/10.1021/jf500518r
24. Liaudanskas, M, Zymonė, K, Viškelis, J, Klevinskas, A, Janulis, V. Determination of the Phenolic Composition and Antioxidant Activity of Pear Extracts. Journal of Chemistry. 2017;2017(1): 9.
25. Niwa Y, Kanoh T, Kasama T, Neigishi M. Activation of antioxidant activity in natural medicinal products by heating, brewing and lipophilization. A new drug delivery system. Drugs Exp. Clin. Res. 1988;14:361-372

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