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Determination of hydroxy methyl furfural concentration in honey using ultra violate-visible spectrometry in West Gojjam Zone of Amhara Region, Ethiopia

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Abstract

This paper aimed to determine the concentration of hydroxyl methyl furfural (HMF) using UV-visible spectroscopy to assess the quality of honey. The honey samples were collected from three honeys productive temperature zones: temperate, sub-tropical and tropical. Following the procedure of white method, the concentration of HMF of temperate, sub-tropical and tropical zone honey are found to be 11.18 ± 0.052mg/kg, 24.95± 0.119mg/kg, and 56.94±0.366mg/kg respectively. There is statistically significance differences between the groups in HMF concentration at 95% confidence level ($p<0.05$). All the samples are found to have HMF value less than the maximum concentration of HMF in honey set by standard controlling international organizations, which shows good quality of the honey in the study areas.

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Introduction

Honey is the natural sweet substance produced by honey bees from the nectar of plants which bees collect and transform by combining with specific secretions of their own and deposit, store and leave in the honey comb to ripen and mature (Gebiru *et al*., 2015). Honey is considered as healthy and wholesome food with curative properties as it contains plenty of nutrients and plays effective antimicrobial effects against many bacteria (Buba *et al*., 2013).

Honey consists mainly of three sugars: Fructose, Glucose and Sucrose together with water with about 1% made up of the pollen grains, amino acids and other minerals (Tadesse *et al*., 2014). Composition of honey is tightly associated to its botanical source and also to the geographical area from where it originated, because soil and weather determine melliferous flora (Kaskoniene *et al*., 2010). Its properties and composition also depend on bee species, season, mode of storage, and even harvest technology and conditions (Everaldo *et al*., 2017). However, honeys produced from different floral sources may have distinctly different aromas and tastes due to differences in volatile composition which in turn is dependent on the extraction methods and also on the botanical and geographical origins (Gebreegziabhere *et al*., 2013).

Nowadays the quality of honey is determined by the concentration of hydroxyl methyl furfural (HMF). HMF occurs naturally in most honeys and increases rapidly with heat treatment of honey (Moralles *et al*., 2009). Therefore, it can be used as an indicator of heating and storage time at elevated temperatures. Good quality honey has lower amount of HMF (Kooh, 2009). Even though HMF is not a harmful substance, many countries restrict the maximum allowable amount of HMF in honey (Tadesse *et al*., 2014; Shapla *et al*., 2018; Nicole *et al*., 2010).

In West Gojjam Zone, Ethioipa, particularly at Dembecha Woreda honey is harvested abundantly in all corridors of the Woreda but the quality and purity of the honey has been evaluated ordinarily by tasting and observing color of the honey. This paper was intended to measure concentration of HMF in honey for the indication of quality of honey at Dembecha Woreda in West Gojjam, Ethiopia.

Materials and methods

Sample collection techniques

The study area has three temperature zones namely Temperate (Dega), Sub-tropical (Woyna Dega) and Tropical (Kola). Sample representatives were collected from each temperature zone. Samples were collected directly from the bee keepers**.** Each honey sample was assigned a code that was used throughout the experimental trail.

Apparatus

Plastic bottles were used to handle the honey samples. Beakers and volumetric flasks were used to handle chemical and reagents during solution preparations. Pipette were used to suck solutions while Pipette tips were t ovoid contamination of samples and reagents. Filter paper was used to filter residuals and cuvettes of 10 mm path length were used to handle the final prepared samples solutions during recording of absorption spectra with UV-Vis spectrophotometer (Jenway 6705) at maximum absorbance at wave length of 284nm.

Reagents and chemicals

Carrez solution I (Potassium Ferro cyanide ($K_4Fe(CN)_6{\cdot}3H_2O$)) and Carrez solution II (zinc acetate ($Zn(CH_3CO_2)_2{\cdot}2H_2O$) were used to precipitate protein from honey while deionized water was used to prepare solutions and to washing different materials which are set in to the experiment. Sodium bisulfite ($NaHSO_3$) solution was used to make a reference solution. HMF standard was used to make spiked solution.

Sample preparation method

Experimental method in this work followed the method established in University of Malaya Honey Research Lab, Department of Molecular Medicine (Machado De-Melo *et al*., 2017). This method was used to determine the concentration of HMF in honey. Three solutions were prepared simultaneously. The firs solution is a 100ml of Carrez solution I was made by dissolving 15g of Potassium Ferro Cyanide ($K_4Fe(CN)_6{\cdot}3H_2O$) and the second

solution is a 100ml of Carrez solution II was also made by dissolving 30g of Zinc Acetate $Zn(CH_3CO_2)_2{\cdot}2H_2O$) in water and make up to a volume of 100ml. The e third solution is a 100ml of 0.2% Sodium Bisulphite solution made by dissolving 0.20g of solid hydrogen sulfite, $NaHSO_3$, in water.

From each honey sample 5.0g was completely dissolved in 25.0ml of distilled water. The mixed solution was then transferred into a 50ml volumetric flask and 0.5ml of Carrez solution I and Carrez solution II were again well mixed by vortex. This mixture was then filtered using a filter paper. From the total mixed solution in the volumetric flask, the first 10ml of filtered solution was rejected while the remaining solution after filtration was collected. A volume of 1.0ml of the filtrated solution was pipetted into two separate test tubes with the volume of 1.0ml each. Oneml of water was added in one of the test tube and mixed well. This solution is named as sample solution. Also 1.0ml of 0.2% sodium Bisulphite solution was added to the second test tube and mixed well. This solution is named as reference solution.

Validation methods

Method validation is used to confirm the analytical procedure employed for specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of the result. In order to validate the analytical method, the following method validation parameters such as recovery test, instrument detection limit, method detection limit, limit of quantification, precision and accuracy and linearity studies were carried out.

Limit of detection (LOD)

The limit of detection (LOD) is the lowest concentration of the measurement that can be detected at a specified level of confidence. In this work, LOD was obtained from triplicate measurement of the blank solution as:

LOD = Standard deviation of blank×3 ..... (1)

Limit of quantification (LOQ)

Limit of Quantification is the lowest concentration of the measurement that can be measured with an acceptable uncertainty. Similar to limit of detection, LOQ was obtained from triplicate measurement of the blank solution as:

LOQ= Standard deviation of blank×10 ..... (2)

Recovery test

Recoveries were determined by spiking the sample with the known concentration of HMF by preparing on the same procedure of the sample in triplicate and measured its value for recovery and accuracy test. The method can be an accurate if the recovery test can be in the range 80% _ 120% (Ololo and Goroya, 2018). The recovery test in this work was determined as

$$\%RT = \frac{M.\,value\ of\ spiked - m.\,value\ of\ un\ spiked}{amount\ of\ added\ to\ spike} \times 100 \ldots (3)$$

Precision

The precision of the experiment was cheeked by triplicate measurement of the sample and determines the percentage relative standard deviation (RSD).

$$\%RSD = \frac{\text{standard deviation}}{mean\ value} \times 100 \ldots\ldots\ldots\ldots (4)$$

Statistical data analysis methods

All analyses were performed in triplicates and data was presented as mean and standard deviation. Differences between individual/group of honey samples were analyzed using analysis of variance one way ANOVA, SPSS Statistics (IBM) corporation version 20.

Results

Calibration Curve

To determine the concentration of HMF out of the quantity taken in the measurement, the known amount of concentration of the HMF standard solution were used. These known concentration and absorbance of the reading enables to plot the calibration graph displayed in Fig. 1.

Table 1. Absorbance of the HMF standard solution.

Concentration (mg/kg)	6	12	18	24	30
Absorbance	0.132	0.206	0.251	0.319	0.335

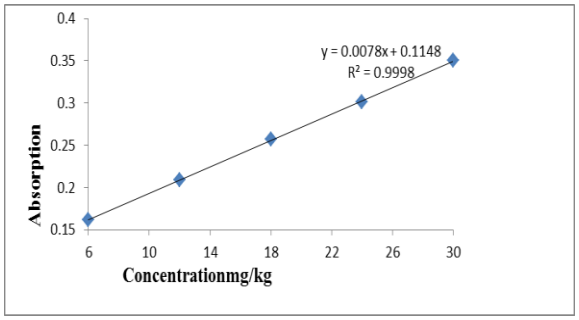

Fig. 1. Absorbance versus the concentration of the HMF standard solution.

The calibration curve produced correlation relation coefficient (R^2) of 0.9998. This value indicates that absorbance and concentration are directly proportional so Beer Lambert's law is valid to determine the concentration of HMF in the sample.

Method Validations Result

Results of methods of validations and spectral lines used in this experiments are displayed in Table 2.

Table 2. Spectral lines and method validation results in this work.

Wave length (nm)	LOD (mg/kg)	LOQ (mg/kg)	Recovery test (%)	Precision (%)
284	0.00458	0.1528	88.63-109.85	0.528

The triplicate measurement of the blank solution gives the value of LOD and LOQ as 0.00458mg/kg and 0.01528mg/kg by applying equations (1) and (2), respectively. Accuracy of the experiment was cheeked by spiking the stoke solution with the sample as it described in the methodology part. The recovery test ranges from 88.63% to 109.85%. This result shows that the method was accurate because the recovery test falls in the accepted range 80% - 120% (Ololo and Goroya, 2018). Measurement of the experiment in this work gives% RSD of 0.528. This value of%RSD shows the measurements were precise, because RSD value falls in the accepted range of less than 15% (Rehman *et al.*, 2008).

Concentration HMF

The mean HMF concentration in the honey samples collected from the three different temperature zones were determined and displayed in Table 3. Maximum of 57.547 ± 0.909 and minimum of 11.051± 0.513 was obtained.

Table 3. Mean HMF concentration of honey in this work (n = 3).

Sample	Mean Absorbance at 284nm	Concentration of the samples (mg/kg)
A	0.201	11.051± 0.513
B	0.311	25.154 ± 1.049
C	0.564	57.547 ± 0.909

A, B and C are Honey samples of Anjenie, Yemehel, and Enewond

Discussion

The most commonly monitored parameters for the determination of honey quality and freshness is the level of HMF concentration of the honey (Bogdnov and Martine, 2002). Naturally, in fresh honey HMF is found in a very small amount from 0.06 - 0.2mg/kg (Ghazal *et al.*, 2008). As can be seen from Table 3, the HMF level of this work is 11.051 ± 0.0513mg/kg, 25.154±1.049 and 27.547 ±0. 099mg/kg for honey samples which were collected from temperate, sub-tropical and tropical, respectively. One way ANOVA analysis shows that there is statistical significance difference between the group at 95% confidence level $p<0.05$.

Except the valued obtained in tropical area, HMF concentrations are less than 40mg/kg which is the maximum limit of HMF for none tropical areas as declared by EU council directive (2001), the international honey quality regulatory commission (Bogdnov *et al.*, 1999). The Honey which were collected from the tropical area has an HMF concentration of 57.547 ± 0.909mg/kg. This value is also fall in the range of the accepted values of HMF level for tropical areas. The EU council directives 2001, Korean food code, International honey quality regulatory commission, National food regulatory institution, limits the maximum concentration of HMF in honey for tropical climatic zone to be less than 80mg/kg.

Nuru (1999) observed that Ethiopian honey contains mean HMF concentration of 32.4mg/kg while Jose *et al.* (2009) analyzed honey samples and found its HMF content ranged from 0.9 to 22.8mg/ kg. Similarly, Tadesse and Gebremedihn (2014) reported that the HMF concentration of their honey sample ranges from 8.32mg/kg to 45.26mg/kg. Results of current work are in good agreement with these previous findings. Table 4 shows short summery and comparisons of this work's result to the maximum limits of standards of HMF and the result of other researchers.

Table 4. HMF concentration of this work with maximum limits of standards.

Results	Current work	Nuru, (1999)	Gebiru *et al.* (2015)	Tadesse (2014)	G/Egzeabher, (2013)	Daniel (2018)	Ethiopian standard	EU standard	IHC standard
HMF mg/kg	11.18-56.94	32.4	14.55-15.13	8.32-45.26	11.18	30.1-64.5	80	40&80	40&80

Conclusion

In this paper different honey samples collected from different temperature zones were evaluated for their quality by the assessment of their HMF concentration by using UV visible spectroscopy. The HMF concentration is found to be in the range of value of 11.1776 ± 0.0511.051± 0.513mg/kg to 56.9359 ± 0. 57.547 ± 0.909/kg with lower side corresponding the temperate and the upper values corresponding to tropical temperature zones. The honeys which are produced in colder area have low concentration of HMF. Moreover, it can be said that all the honey samples analyzed in this work are in a good quality as their values found in the acceptable range for the tested parameter of the international and national standards.

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