Analysis of Apiculture Honey Samples and Determination of its Vitamins Content

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Abstract

Two honey samples, apiculture honey (Ha) and Zee honey (Hz) were collected from Meiktila Township, Mandalay Region. The hydroxymethylfurfural (HMF) contents were determined by White spectrophotometric Method. Total reducing sugar and non-reducing sugar of honey samples were determined by the Lane and Eynon method. Mathematical investigation of using efficient discrimination function equation reveal the samples were floral or honeydew. Qualitative determination of vitamin B_2 was carried out by using UV lamp. Vitamin B_6 and vitamin C were also determined by UV absorption spectroscopy and titration method. By comparing the resultants factors to those of literature values, consumers have many chances of choosing the best quality honey for curing age related diseases and defy ageing process.

Key words: hydroxymethylfurfural (HMF), reducing sugar, vitamins

Introduction

Honey

Honey is the most important primary product of beekeeping both from a quantitative and an economic point of view. It was also the first bee product used by human beings in ancient times (Crane, 1980). Bees collect nectar, pollen and water each day to take back to the hive so that future generation can live. The raw nectar comes from flowers. They mix this with secretions from their glands, thereby transforming it and after it is deposited in the comb, it ripens into honey. Nectar secretions consist of the production of a sugar solution secreted from special organ of the plants known as nectaries. These organs are located in various parts of the flower or even or the stem, leaf, node, or bract of the plant, they are known as extra-flora nectarines when they occur elsewhere than in the flower (Lovell and Grout, 1973).

Figure1. Apiculture honey (Ha)

Figure 2. Zee honey (Hz)

Properties of Honey

Honey's hygroscopic properties also make it an ideal ingredient in a lot of cosmetics as it helps skin hydrated and fresh and prevent drying. Thus, some people call honey a natural "humectant" as it attracts and retains moisture. The effective antimicrobial agent in honey prohibits the growth of certain bacteria. It contains an enzyme that produces hydrogen peroxide which it believes to be the main reason for the antimicrobial activity of honey. Be pleasantly surprised by the amazing honey properties, antimicrobial, antioxidant, and hygroscopic which all make honey a popular food as well as a medicine.

Floral honey

Nectar secretion consists primarily of the production of a sugar solution secreted from special organs of the plant known as nectaries. These organs are located in various parts of the flower or even on the stem, leaf, node, or bract of the plant; they are known as extra-floral nectarines when they occur elsewhere than in the flower (Lovell and Grout, 1973).

Honeydew honey

The most important sources of honeydew are trees, and of these, conifers (which produce no nectar) give the highest yields. Droplets of honeydew fall on to the plant surface, and are collected by other insects, especially bees, wasps and ants. The characteristic composition of honeydew is somewhat different from that of nectar, because honeydew contains enzymes derived from the gut and saliva of the plant-sucking insect (Crane, 1990).

Materials and Methods

Collection of Honey Samples

Honey samples were collected from Meiktila Township, Mandalay Region. Pann-Nham Honey (Ha) and Zee Honey (Hz) were the products of apiculture from local beekeeper.

Determination of Hydroxymethylfurfural (HMF) Content

5 g of honey sample was accurately weighed and put into a beaker. The sample was dissolved in 25 mL

of deionized water and transferred into a volumetric flask (50 mL). Then, Carrez solution I (0.5 mL) was added and mixed. And then, 0.5 mL of Carrez solution II was added, mixed and made up to the mark with deionized water. A drop of ethanol was added to suppress foam. This solution was filtration and rejection the first 10mL of the filtrate. The filtrate 5 mL was pipetted into each of two 2 test tubes. 5 mL of deionized water was added to one of the test tubes and mixed well. This solution was sample solution. The second test tube, 0.2 % of sodium bisulphate solution (5 mL) was added and mixed well. This solution was reference solution. The absorbance of the sample solution was determined against the reference solution at 284 nm and 360 nm in 10 mm quartz cells within one hour. If the absorbance at 284 nm exceeds a value of about 0.6, dilute the sample solution with deionized water and the reference solution with sodium bisulphate solution to the same extent in order to obtain a sample absorbance low enough for accuracy (White, 1979).

Determination of Apparent Reducing Sugars

A mixture of 5 mL of each Fehling's solution A and Fehling's B was added into 150 mL of conical flask. A solution mixture was prepared by using 16 mL deionized water, little antibumping agent, followed by about 5 mL diluted honey solution. The cold mixture was heated to boil over a stove and maintained moderate ebullition for 2 minutes. 0.2 % of aqueous methylene blue solution (1 mL) was added whilst still boiling and completed the titration within the total boiling time of 3 minutes by repeated small addition of diluted honey solution used (X mL), tilled the color of the indicator was disappeared. The total volume of added reactant at the completion of the redox titration must be 35 mL.

The amount of added water necessary was calculated to bring the total volume of the reactants at the completion of the titration to 35 mL by subtracting the preliminary titration (X mL) from 25 mL. 5 mL of Fehling's solution A was pipetted into a 150 mL conical flask and added 5 mL Fehling solution B. (25-XmL) deionized water was added and little antibumping agent was added from a burette, all but 5 mL of the diluted honey solution volume determined in the preliminary titration. The cold mixture was heated to boil over a hot plate and maintained moderate ebullition for 2 minutes. 1 mL of 0.2 % methylene blue solution was added until boiling and completed the titration within the total boiling time of 3 minutes by repeated small addition of diluted honey solution until the indicator is decolorized. Noted the total volume (Y mL) (Lane and Eynon, 1923).

Determination of Apparent Sucrose

The honey solution (50 mL) was placed in a conical flask, together with 25 mL deionized water. This solution was heated to 65℃Cover a boiling water bath. Then the flask was removed from the water bath and added 10 mL of hydrochloric acid solution. The mixture was allowed to cool naturally for 15 minutes and then neutralized with sodium hydroxide solution (5 M) using litmus paper as indicator, cooled again, and the volume was adjusted to 100 mL (diluted honey solution).

Classification of Honey Sample

Depending on the source from which bees obtained the material for honey, it is classed either as floral or honeydew (Loyrish, 1974). Bennetl and Franklin (1954) distinguished honeydew from floral honey by a linear discriminant function which was evolved from the experimental results being subjected to discriminatory analysis. The numerical value of that function for a given sample can be classified the honey sample as floral or honeydew in origin. Mathematical investigation showed that an efficient discriminant function is given by the following equation:

$$X = -8.3x_1 - 12,3x_2 + 1.4x_3$$

X = discriminant function

x_1 = pH

x_2 = ash percent

x_3 = apparent reducing sugar percent

If the value of X is greater than 73.1, the honey is classified as floral, whereas if the value is less than 73.1 the honey is classified as honeydew.

Determination of Vitamin B_2 (Riboflavin) Content

1 mL of honey sample was taken and dissolved with 20% sodium hydroxide solution until alkaline to litmus paper (neutral) and then under the UV lamp. If B_2 was present, this solution shows the green florescence (British Pharmacopoeia, 1993).

Determination of Vitamin B_6 (Pyridoxine) Content

About (5.2 g) of honey sample was put into a beaker (50 mL) and then transferred to separation funnel (250 mL). 20 % sodium hydroxide solution was added until alkaline to litmus paper (neutral). Then, it was extracted with three times of 30 mL chloroform. Combine chloroform solution was washed with 10 mL of distilled water. The combine chloroform solution was evaporated to dryness on water bath and obtained residue. This residue was dissolved with 25 mL of 0.1 M hydrochloric acid in volumetric flask (25 mL). This solution was measured the ultraviolet absorption spectrum at 291 nm (Clarke, 1978).

Determination of Vitamin C (Ascorbic Acid) Content

Honey sample (6.0 g) was put into a beaker (50 mL) and dissolved with 10 mL of distilled water. Then the solution was transferred to 250 mL of iodine flask and added 25 mL of 0.01 M iodine solution. Next, the stopper was closed and cooled in the fridge for 30 minutes. Then dilute sulphuric acid (10 mL) was added immediately and washed the stopper with small portion of distilled water. It was titrated

available iodine with 0.01 M sodium thiosulphate solution, using 1 mL of 0.5% starch solution as indicator and then indicator was changed from blue to colorless. Blank titration was the same procedure without sample (Griebel and Hess, 1940 and British Pharmacopoeia, 1993).

Results and Discussion

Honey Sample

Two honey samples were collected, apiculture honey (Ha) and Zee honey (Hz) from Meiktila Township, Mandalay Region. These samples were extracted by traditional local method and resultant honey was known as pressed honey. Local method was easier to extract then other methods but which was more weight of waste than others. The inducement to fermentation was greater with the local method (where the fingers were used for pressing) was employed for extraction of honey. Therefore, more tedious and greater precautions were taken when using the local method of extraction.

Hydroxymethylfurfural (HMF) Content

The hydroxymethylfurfural (HMF) Content of honey samples were determined by White Spectrophotometric Method (White, 1979). HMF content indicates the honey freshness and overheating. The standard maximum value is 40 mg/kg. The HMF content of honey samples Ha and Hz were found to be 5.016 and 6.843 mg/kg. The HMF contents of these samples lie under the allowable maximum range.

Apparent Reducing Sugars and Apparent Sucrose

Apparent reducing sugar and apparent sucrose were determined by using modification of the Lane and Eynon method. Any given samples of honey contain reducing sugar (glucose and fructose) and non-reducing sugar (sucrose). The amount of glucose, fructose and sucrose may be determined by hydrolysis of honey before titration with Fehling's solution. The difference in these two determinations will give the sucrose content. The literature values of reducing sugar is not less than 65% and sucrose is not more than 5%.The observed values for honey samples were showed in Table 1.

Table 1. Apparent reducing sugar and apparent sucrose content of honey samples

Sample	Reducing Sugar (%)	Apparent reducing sugar (%)	Apparent sucrose (%)
Ha (apiculture honey)	82.36	78.54	3.22
Hz (zee Honey)	89.43	84.27	3.68

Classification of Floral and Honeydew Honey

Qualitative tests such as color, physical state or flavor and most of the qualitative tests described are not suitable to differentiate the two types of honey with certainty and more satisfactory methods are needed. The percentages of reducing sugar, ash and pH values are also important indicators of whether the honey is floral or honeydew. Mathematical investigation showed that an efficient discriminant function (X) is given by the equation of

$$X = -8.3x_1 - 12.3x_2 + 1.4x_3.$$

If the value of X is greater than 73.1, the honey is classified as floral, whereas if the value is less than 73.1, the honey is classified as honeydew. The X value of Ha is 78.27 and so it is floral. The X value of Hz is 69.43 and it is regarded as it is honeydew.

Table 2. Classification of honey samples on the basic discriminant function

Sample	Discriminant Function(X)	Type of Honey
Ha (apiculture honey)	78.27	Floral
Hz (zee Honey)	69.43	Honeydew

Vitamins Content of Honey Samples

Water soluble vitamins are Thiamine (B_1), Riboflavin (B_2), Niacin (B_3), Pantothenic acid (B_5), Pyridoxine (B_6). Biotin, cyanocobalamin (B_{12}), folic acid and ascorbic acid (C). Fat soluble vitamins are vitamin (A, D, E and K).In this paper, qualitative determination of vitamin B_2 (riboflavin) was carried out by using the UV lamp. Under the UV lamp, green fluorescence was observed in the presence of B_2.B_6 was determined by UV absorption spectroscopy and vitamin C was determined by titration method. All honey samples showed green fluorescence color indicating the presence of B_2 (riboflavin). Vitamin B_6 (pyridoxine) contents of honey samples were 0.0006 mg (Ha) and 0.0007mg (Hz)/ 100g respectively. Vitamin C contents of honey samples were 0.19mg/ 100g for Ha and 0.15mg/100g for Hz.

Table 3. Vitamins (B_6 and C) content in different honey samples

Sample	B_6 (mg/100g)	C (mg/100g)
Ha (apiculture honey)	0.0006	0.19
Hz (zee Honey)	0.0007	0.15

Conclusion

Honey samples were extracted by the local method. In fresh honey, there is no hydroxymethylfurfural (HMF), but it increased as long storage, depend upon pH and storage temperature. It is used as an indicator of honey freshness and heat damage. HME is highly neurotoxic and therefore it is very important to reduce its content. When honey is stored above 20 °C the HMF content will increase. HMF content greater than 40 mg/kg is not allowed to be marketed. The major sugar constituents such as apparent reducing sugar and apparent sucrose were quantitatively determined by the Lane and Eynon's method. Total reducing sugar of honey samples Ha and Hz were found to be 82.36 and

89.43 % and non-reducing sugar obtained by acid hydrolysis was found to be 3.22 and 3.68 %. According to discriminant function, apiculture honey was classified as floral and zee honey was regarded as honeydew. Several of the essential vitamins were present in honey, but some have significant figures. Vitamin B_2 was present in all honey samples. Vitamin B_6 and C were determined by UV absorption spectroscopy and titration method. According to the literature, vitamin C content is the largest among the vitamins. In this paper, vitamin B_6 content of the Ha and Hz were found to be 0.0006 mg/100g and 0.0007 mg/100g respectively. Vitamin C contents were 0.19 mg/100g and 0.15 mg/100g for Ha and Hz honey samples.

Since honey samples are rich in sugar, mineral elements, enzymes, antioxidant and vitamins, that may be used in the formulation of high nutritional drinks and foods and medicine for the young and the old.

Acknowledgements

We would like to express our deepest thanks to Dr Ba Han, Rector, Meiktila University and Dr Tin Tun Aung, Pro-Rector, Meiktila University, for their kind permission and research facilities to carry out this paper.

References

Bennetl, C.A. and Franklin, (1954) *Statistical Analysis in Chemistry and the Chemical Industry*, Chapman Hall Ltd., London

"British Pharmacopoeia" (1993) "Volume-1", Printed in United Kingdon, London, pp.553-579.

Clarke, E.G.C. (1978) *Isolation and Identification of Drugs*, The Pharmaceutical Press, London

Crane, E. (1980) *A Book of Honey*, Oxford University Press, Oxford

Griebel, J. and C.F. Hess, (1940) *Iodine Absorption of Honey and Artificial Honey*, Z Unters. Lebensm

J. W. Jr. (1979) "Spectrophotometric Method for Hydroxyl-methyl-furfural in Honey", *J.Ass.Off.Anal.Chem*, Italy, vol.62, pp.509-514.

Lane, J.H. and L. Eynon, (1923)"Determination of Reducing Sugars by Means of Fehling's Solution with Methylene Blue as Internal Indicator", *J.Soc.Chem. Ind.Trans*. Denmark,pp.32-36.

Lovell, H.B. and R.A. Grout, (1973) *The Hive and the Honey Bee :Sources of Nectar and Pollen*, Dandant & Sons, Hamiltion, Illinois

Loyrish, N. (1974) *Bees and People*, Translated from the Russian by Kozlova, G.A, MIR Publishers, Moscow

White, J.W., M.L. Reilhof, and I. Kushnir, (1961) "Composite of Honey: The effect of storage on Carbohydrate Acidity and Diastase Content", *J. Food Sci*., vol.26, pp.63-71.