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Physicochemical Analysis of Honey in the Kenyan Retail Market

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Abstract

Retail honey has always been subjected to some form of processing during honey production thus its crucial to assess its quality periodically so as to ascertain whether it satisfies the minimum set requirements. This study intended to determine the quality of honey in the Kenyan retail market through physicochemical analysis and comparing them with the set standards by the Kenya Bureau of Standards (KEBS) and International Honey Commission (IHC). Nineteen (19) honey samples both locally produced and imported were obtained from the market; Samples obtained were analyzed following harmonized methods of the International Honey Commission. Even though there was great variation in electrical conductivity, acidity, diastase activity, and HMF Values, results obtained indicated that most of the honey samples met the minimum set requirements. Diastase activity and HMF proved to be the only possible criteria to discriminate between samples which satisfied and those that did not satisfy the minimum set standards.

Keywords: Honey, quality, physicochemical analysis

1. Introduction

There are numerous bee products, but the best known primary products of beekeeping are honey and beeswax. Others include: pollen, propolis, royal jelly, bee venom, queens, bees and their larvae (Krell, 1996). Honey is produced by honeybees from nectars extracted from the nectaries of flowers (Adebyi *et al*, 2004) or from the secretion of living parts of plants or from the excretion of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honey combs to ripen and mature (White and Landis, 1980). Honey is composed primarily of the sugars: glucose and fructose, its third greatest component is water. It also contains in small quantities numerous other types of sugars, as well as acids, proteins and minerals (White *et al*, 1962).

Honey has been claimed to have therapeutical properties in the treatment of digestive, respiratory, cardiac and rheumatic disorders (Pereira *et al*., 1998). Since ancient times honey, has been used for its medicinal properties in many cultures. Clinical tests done on the effectiveness of the use of honey in the treatment of human disorders have yielded positive results (Jeffrey and Echazarreta 1996).

The reason for testing honey for quality control purposes is to verify the authenticity of the product and to reveal the possible presence of artificial components or adulterants, as well as to address processing and market needs (Krell 1996). This not only requires determination of the moisture and mineral content (ash), but also other physicochemical parameters such as pH, the levels of Hydroxymethylfurfural (HMF), acidity, diastase activity and reducing sugars (Bogdanov et al. 1999). The moisture content is the only composition criteria, which as a part of the Honey Standard has to be fulfilled in world honey trade. This quality criterion determines the capability of honey to remain stable and to resist spoilage by yeast fermentation. The higher the moisture content, the higher the probability that honey will ferment upon storage.

A maximum value of 19 g/100g is suggested by the International Honey Commission (IHC), (Bogdanov *et al*., 1999). Harvesting of honey with high water content leads to spoilage by fermentation resulting to a product with an off taste, high levels of yeast, glycerol, butandiol and ethanol (Russman, 1998).

2.0 Experimental

2.1 Sampling

Before sampling, a survey was carried out to establish the various brands of honey sold at various stores in Nairobi's retail outlets. Systematic random sampling was employed to determine the sampling points. A list of brands present in the various stores was prepared and coded with numerical numbers. A list of the retail stores where each of these samples was present was made and coded with alphabetical letters. To establish which store to

sample from, each alphabetical letter representing each store was put down on piece of paper, which were then folded and placed in a jar and jumbled. A random pick-from-the-hat method was used, and the store whose letter was picked was sampled.

Nineteen different brands of honey samples were obtained from the local Kenyan market (from supermarkets) between March and April 2012, all within Nairobi and its environs.

2.2 Sample collection transportation and storage

250 g of the selected sample brands were purchased from the selected store and transported to the laboratory in Jomo Kenyatta University of Agriculture and Technology whereby they were stored at room temperature in a well secured cool dark drawer while awaiting subsequent analysis. The processing and expiry dates were noted.

2.3Physical Chemical Analysis

The physicochemical properties for each of the parameters were determined according to the Harmonized Methods of the International Honey Commission (Bogdanov 1999). Parameters which were determined are Moisture Content,Electrical Conductivity, pH, free acidity,HMF, Apparent reducing Sugars and diastase activity. Below is a brief description of the procedures followed.

2.3.1Moisture Content

10.0 g of honey were placed in an air tight flask, closed and placed in a water bath at 50 °C until all the sugar crystals were dissolved. The honey solution was cooled to room temperature and homogenized. the surface of the prism of a Bellingham + Stanley RFM 330 refractometer was covered evenly with the sample and after 2 minutes the refractive index was read noting the temperature. Refractive index obtained at temperatures below or above 20 ^{0}C were adjusted by adding 0.00023 per ^{0}C for temperatures above 20 ^{o} C and subtracting 0.00023 per ^{o} C for temperatures below 20 ^{0} C (Bogdanov, 2002). The percentage moisture content was determined by using the correlation table of percentage moisture content with the adjusted refractive index

2.3.2 Electrical conductivity

20.0 g of honey were dissolved in distilled water transferred to a 100 ml volumetric flask and made up to volume with distilled water. 40 ml of this solution was poured into a beaker and placed in thermostated water bath at 20 °C. Electrical conductivity measurements were obtained with a low range conductivity meter (JenWay TDS meter 4076) with a cell constant of 1.03. The conductivity cell was thereafter immersed in the sample solution and the conductance in mS read after temperature equilibrium had been reached.

Electrical conductivity was calculated using the formula:

$$SH = K \cdot G$$

Where:

SH = electrical conductivity of the honey solution in mS.cm^{-1}

K = cell constant in cm^{-1}

G = conductance in mS

The results were expressed to the nearest 0.01 mS.cm^{-1}

2.3.3 Mineral/Ash Content.

The mineral/ash content was determined indirectly by relating the results obtained from the electrical conductivity measurements using the formulae shown below.

$$A = (c - 0.14)/1.74$$

Where: A = ash content in g/100 g of honey.

C = electrical conductivity in milli Siemens cm^{-1}

2.3.4 pH and free acidity

10.0 g of ahomogenized sample wwere dissolved in 75 ml of carbon dioxide-free water stirred with a magnetic stirrer and the pH determined by immersion of the pH electrodes in the solution (pH readings were reported to two decimal places). In determination of free acidity, the sample solution was titrated with 0.1M NaOH to pH 8.30 (using a digital pH meter) ensuring a steady pH reading was obtained within 120 sec of starting the titration. The readings were recorded to the nearest 0.2ml.

Free acidity was calculated using the expression below and the results were expressed to one decimal point:

Free acidity (milliequivalents) = ml of 0.1M NaOH x 10.

2.3.5 Hydroxymethylfurfural

This was determined according to the method after White (Bogdanov, 2002). 5.0 g of honey were accurately weighed into beaker and dissolved in approximately 25 ml of water and quantitatively transferred into a 50 ml volumetric flask. 0.5 ml of Carrez solution I was added into the sample and mixed followed by further addition of 0.5 ml of Carrez solution II, mixing and finally topping it up to the mark with water.The prepared sample solution was filtered through Whattman filter paper # 42. The first 10 ml of the filtrate were rejected. 5.0 ml of the filtrate was transferred in to each of the 2 test tubes ,5.0 ml of water was added into one of the test tubes and mixed well (the sample solution). 5.0 ml of 0.2% sodium bisulphite was added into the second test tube and mixed well (the reference solution).The absorbance of the sample solution was determined against the reference solution at 284 and 336 nm in 10 mm quartz cells within one hour. The determinations were done in triplicates.The HMF values were calculated using the formula shown below.

HMF in mg/kg = $(A_{284} - A_{336})$ x 149.7 x 5 x D/W

Where:-A_{284} = absorbance at 284 nm

A_{336} = absorbance at 336 nm

149.7= Constant

D = dilution factor, in case dilution was necessary

W = Weight in g of the honey sample

The results were expressed in mg/kg to the nearest 1 decimal place.

2.3.6 Total Reducing Sugars

Total reducing sugars were determined through the Lane-Eynon Method (Bogdanov, 2002). This involved three procedures.

a.) Standardisation of Fehling's solution 1

5ml of Fehling solution 1 and 2 were pipetted into a conical flask; 19ml of Standard invert sugar solution was added from a burette. The mixture was boiled on a tripod using a Bunsen flame for 4 minutes, 3 drops of methylene blue indicator added, and the titration carried to completion while still on the Bunsen flame. The factor of Fehling solution (f) was calculated using the formulae shown below

Factor of Fehling's solution (f) = ml of standard invert sugar required/20.36

b.) Preliminary titration

5ml of Fehling's solution 1 and 2 were pipetted into a conical flask; 15 ml of sample solution (prepared by dissolving 1.0 g of honey in water and diluted to 250ml in a volumetric flask) was added from a burette and mixed well. The mixture was boiled on a tripod using a Bunsen flame for 4 minutes, 3 drops of methylene blue indicator added, and the titration carried to completion while still on the flame.

c.) Final titration

5ml of Fehling's solution 1 and 2 were pipetted into a conical flask, 1 ml less of sample solution volume determined from the preliminary titration was added and boiled for 2 minutes, 4 drops of methylene blue indicator were added and without removing the flame, the titration was carried on until when the blue colour disappeared. The titrations were done in triplicates and for all the samples. The formula shown below was used to calculate the total reducing sugars

$$Total\ Reducing\ Sugars = a \times f \times {}^{v}/_{b} \times {}^{1}/_{1000} \times {}^{1}/_{s} \times 100$$

Where: a = Lane –Eynon factor as mg of reducing sugar corresponding to the millimetres of sample sugar solution required from the table

b = ml of sample sugar solution required

f = factor of Fehling's solution 1

s = weight of sample taken in grams

v = volume of sample sugar solution prepared.

2.3.7 Diastase activity

Diastase activity was determined using the method after Schade (Bogdanov, 2002). This method involved several procedures.

a) Determination of starch dry weight:

2.0 g of air dry soluble starch were accurately weighed and spread in a thin layer over the bottom of a weighing bottle (diameter 5 cm, height 3 cm) with a lid. The starch was dried in an oven for 90 minutes at 130 °C. The closed weighing bottle was allowed to cool for about 1 hour in a desiccator and re-weigh.

b) Preparation of starch solution:

2.0 g of anhydrous starch was accurately weighed into a 250 ml conical flask; 90 ml of water were added and mixed thoroughly by swirling. The suspension was rapidly boiled while constantly swirling the flask for 3 minutes. Immediately the hot solution was transferred to a 100 ml volumetric flask and cooled down rapidly to room temperature in running tap water. The solution was topped up to the mark with water and mixed thoroughly.

c) Sample solution preparation

10.0 g of honey were accurately weighed into a beaker and dissolved completely in approximately 15 ml of water and 5 ml of acetate buffer without heating. The solution was quantitatively transferred into a 50 ml volumetric flask containing 3 ml of sodium chloride solution and volume adjusted to the mark with water.

d) Calibration of the Starch Solution / Adjustment of Blue Value

This procedure was carried out to determine the amount of water that had to be added to the reaction mixture so that the absorbance range of the iodine starch solution is between 0.745 and 0.770.

20, 21, 22, 23, 24 and 25 ml of water were pipetted into 6 suitable glass test tubes and 5 ml of dilute iodine solution added. Starting with the first test tube, 0.5 ml of a mixture containing 10 ml of water and 5 ml of starch solution (prepared in part b) was added and mixed well by agitating and immediately reading the absorbance at 660 nm against a water blank in a 1 cm cell. The same procedure was repeated with the other test tubes, to obtain an absorbance in the range 0.770 to 0.745. The amount of water determined in this way was the standard dilution for every determination carried out with the starch solution.

e) Determination in the honey sample solution

10 ml of honey solution was quantitatively transferred into a 50 ml flask and placed in a 40°C water bath. A second flask containing about 10 ml of starch solution was also placed into the same water bath. After 15 minutes, 5 ml starch solution was pipetted into the honey solution mixed and the timer started. At periodic intervals, for the first time after 5 minutes, 0.5 ml aliquots were removed and added rapidly to the 5 ml of diluted iodine solution. The amount of water as determined in "Calibration of the starch solution" was added, mixed well and immediately the absorbance of each separate solution read at 660 nm against a water blank in a 1 cm cell. Two more aliquots were withdrawn at the 10^{th} and 15^{th} minute interval and the same procedure repeated.

A calibration curve of absorbance versus the corresponding reaction times in minutes was plotted and the time (tx) at which the absorbance was 0.235 determined.

The formulae shown below was used to determine the Diastase Number (DN)

$$DN = 60\,minutes\,{}^{60\,minutes}/_{tx} \times {}^{0.10}/_{0.01} \times {}^{10}/_{20} = {}^{300}/_{tx}$$

Where

tx = reaction time in minutes when the absorbance from the calibration is 0.235

2.3.8 Specific Rotation

12.0 g honey were accurately weighed anddissolved in distilled water, 10 ml of Carrez I solution was added and mixed thoroughly for 30 seconds. 10 ml of Carrez II solution was later added and mixed again for 30 seconds and made up to volume in a 100 ml volumetric flask using distilled water. After 24 hours the solution was filtered. A clean 20 cm polarimeter tube was rinsed with the solution and filled with the solution, the tube was placed in an ATAGO POLAX-2L Polarimeter and the angular rotation (α) read at 20^0C. The formula shown below was used to calculate the specific angular rotation.

$$Specific\ Angular\ rotation\ [\alpha]_D^{20} = \frac{\alpha \times 100}{l \times P}$$

Where α = angular rotation found,

l = length in decimeters of the polarimeter tube

p = grams of dry matter taken.

All the determinations were carried out in triplicate and the means and standard deviations were calculated using MS Excel software.

3.0 Results and Discusion

Several physiochemical quality Parameters employed in routine honey quality control purposes were analyzed in nineteen retail honey samples. They are: pH, Moisture content, electrical conductivity, free acidity, HMF, Diastase, specific rotation and total reducing sugars as shown in table 1

3.1 pH

From the results obtained the pH of samples was in the range of 3.82±0.006to 4.43±0.006, these were within the acceptable range of 3.4-6.1 for pure honey. The low pH of the honey samples showed that they are acidic and this indicated their ability to inhibit the presence and growth of microorganisms (National Honey Board, 2006)

3.2 Moisture Content

The moisture content varied from 16.87±0.12 to 19.13±0.06, one sample had moisture content below 13%, and its refractive index was 1.5007 and thus could not be determined from the table. The Maximum water content for honey according to Kenya Bureau of Standards is 20%, moisture content is used to assess the likelihood of honey to ferment; honey with high water content is more likely to ferment (Bogdanov, S. *et al*, 1999)

3.3 Optical Rotation

Honey can be classified as to either blossom/nectar honey or honeydew honey depending on where bees forage. Blossom/nectar honey is honey which comes from nectars of plants while honeydew honey comes mainly from excretions of plant sucking insects on the living parts of plants or secretions of living plants (Bogdanov. *et al* 1999). Optical rotation has been used to distinguish between these two types of honey, blossom honey have negative values while honeydew honey has positive values (Persano, 1999; Battaglini and Bosi 1973). Fifteen honey Samples had negative values of optical rotation ranging from -0.5 to -10.0 indicating that these honeys were blossom/nectar honey while four of the honey samples had positive values ranging from 1.5-6.5 indicating that they were honeydew honey.

3.4 Electrical Conductivity

From the results obtained (table 1) there was great variation in the electrical conductivity ranging from 0.08±0.006 mS/cm to 0.78±0.003 mS/cm, this confirmed that the honeys obtained were blossom and or mixture/blends of blossom and Honeydew honey. Blossom honeys, mixtures of blossom and honeydew honey honeys should have less than 0.8 mS/cm while pure honeydew honey and chestnut honeys should have more than 0.8 mS/cm (Bogdanov.*et al*, 1999)

3.5 Free Acidity

Free acidity has been used as a quality criterion for assessing whether fermentation has taken place; honey fermentation causes an increase in acidity. The maximum acceptable levels of free acidity as per the codex alimentarius standard are 40 miliequivalents /kg. Only three honey samples had values much greater than the recommended limit (sample 1, 3, 6 with values of 56.67±1.15, 43.67±1.53and 60.33±1.15 respectively).For honey whose free acidity was well within the recommended limit, the range was 10.00±1.00 to 36.67±0.58 the great variation in free acidity in the samples could be attributed to the existence of great natural variation in the acidity of honey as established by Horn, 1992.

3.6 HMF and Diastase Activity

The most commonly monitored parameters for determining honey freshness include HMF Levels and diastase activity (Oddo *et al* 1999; Bogdanov and Martin 2002).

From the results obtained four samples had HMF values way beyond the recommended limit in the codex Alimentarius of 40mg/kg i.e. sample 8,11,13,19 with values 85.36±0.151, 95.47±1.369, 108.38±0.041 and 123.44±0.462 respectively. HMF values for the fifteen honey samples which were within the recommended limit

ranged from 2.68±0.373 to 22.14±0.041.

In fresh honeys there is practically no hydroxymethylfurfural (HMF), but it increases upon storage, depending on the pH of honey and on the storage temperature (Bogdanov.*et al*, 1999). The high levels in the four honey samples could be as a result of heating during processing or increase in temperature during storage or while being transported .All the honey analyzed had not stayed more than three years on the shelf.

The Diastase activity ranged from 4.53±1.04 to 11.23±0.44 Schade units. 43% of the samples had diastase activity higher than 8 Schade units ranging from 8.08±0.77 to 11.23±0.44 while the remaining 57% had diastase activity in the range of 4.53±1.04 to 7.82±0.41.

In interpretation of diastase activity results it is critical to do so hand in hand with HMF values, In general honey should have diastase activity not less than 8 but honey with low natural enzyme content and an HMF content of not more than 15 mg/kg should have diastase activity not less than 3 Goethe or Schade units (Bogdanov and Martin 2002). 37 % out of the 57% of the sample which had diastase values lower than 8 had HMF values lower than 15mg/kg this is a good indicator of their low natural enzyme content, absence of heating during processing and storage. The four honey samples whose HMF values were beyond the acceptable limit (sample 8,11,13,19 had diastase values of 7.31±2.06, 6.53±0.79, 6.39±0.99 and 6.39±0.99 respectively). This indicates that the honey had no low natural enzyme activity and had been subjected to some form of heating at high temperature during processing or storage under high temperatures.

3.7 Total Reducing Sugars

The total reducing sugars ranged from 54.82±0.29 to72.76±0.62 g/100g of honey, the general standard for a minimum content of the sum of fructose and glucose of 60 g/100 g for all blossom honeys and 45 g/100 g for all honeydew honey honeys (Bogdanov.*et al*, 1999). Three honey samples had less than 60 g/100 g, of which only one honey sample was honeydew honey showed no anomaly while the remaining two with 54.82±0.29 and 59.55±0.33 had optical rotation values of -1 and -0.5 respectively this values are below what was expected but due to their low magnitude in rotating plane polarized light in a laevorotatory direction they could have been blends of both blossom and honeydew honey. This can be further proved from the electrical conductivity measurements obtained and discussed earlier.

4.0 Conclusion

In conclusion, this study obtained physicochemical data for several honey samples obtained from Kenya's retail market. Diastase activity and HMF were the only possible criteria to establish whether honey meet the minimum set standards, 21% of the honey samples analyzed did not meet the set standards based on diastase and HMF measurements.

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Table 1. A compilation of results of 19 honey samples obtained from Kenya's retail market

Sample	Moisture Content (%)	Electrical Conductivity in mS/cm	pH	Free Acidity in miliequivalent/ kg	HMF in mg/kg	Diastase in Schade units	Total Reducing Sugars in g/100g	Specific Angular Rotation
1	17.83±0.06	0.74±0.006	4.06±0.00	56.67±1.15	22.14±0.04	8.91±0.63	72.76±0.62	-3
2	18.30±0.10	0.32±1.02	3.85±0.006	33.33±1.15	20.09±0.07	5.55±0.46	61.98±0.66	-4.5
3	19.03±0.06	0.30±0.001	3.82±0.006	43.67±1.53	7.98±0.04	7.13±0.63	62.61±0.60	-0.5
4	17.73±0.06	0.26±0.001	3.88±0.000	28.00±2.000	3.58±0.04	6.41±0.39	60.99±0.17	1.5
5	17.73±0.06	0.34±0.002	4.19±0.006	36.00±1.00	17.42±0.04	9.26±0.09	54.82±0.29	-1
6	19.13±0.06	0.78±0.003	4.41±.000	60.33±1.15	16.23±0.07	10.29±2.68	59.55±0.33	-0.5
7	17.07±0.06	0.42±0.002	4.43±0.006	35.00±1.00	11.94±0.04	8.69±0.14	64.29±0.20	-1.5
8	16.57±0.06	0.25±0.006	4.00±0.006	14.00±0.00	85.36±0.15	7.31±2.06	66.82±0.56	2
9	17.03±0.06	0.32±0.005	4.03±0.006	36.67±0.58	20.78±0.11	8.08±0.77	58.99±0.31	-1
10	17.57±0.06	0.42±0.008	4.24±0.06	19.33±0.58	2.68±0.37	4.53±1.04	62.66±0.39	-4
11	17.83±0.12	0.08±0.006	3.97±0.000	10.00±1.00	95.47±1.36	6.53±0.79	65.82±041	-2.5
12	16.87±0.12	0.31±0.004	4.15±0.000	23.33±0.58	19.37±0.08	8.37±2.34	65.20±0.40	-3.5
13	16.87±0.06	0.09±0.005	4.24±0.006	17.00±1.00	108.38±0.04	6.39±0.99	59.19±044	3.5
14	17.27±0.12	0.25±0.005	3.72±0.000	34.00±1.00	5.16±0.04	6.18±0.14	63.79±0.53	-1.5
15	below 13	0.23±0.005	3.98±0.000	25.67±0.58	13.06±0.04	5.60±0.73	65.42±0.20	-9
16	18.13±0.06	0.31±0.010	4.08±0.012	30.67±1.15	6.64±0.00	11.23±0.44	65.34±0.36	-3.5
17	17.30±0.10	0.67±0.003	4.27±0.012	35.67±1.53	7.87±0.08	5.10±0.16	64.48±0.52	6.5
18	16.77±0.15	0.13±0.005	4.11±0.000	10.67±1.15	6.12±0.13	7.82±0.41	68.92±0.25	-10
19	17.23±0.15	0.28±0.005	4.07±0.012	29.67±2.08	123.44±0.46	6.39±0.99	63.38±0.40	-3

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