

Physico-chemical Evaluation of the Authenticity of Honey Marketed in Southwestern Nigeria

Abel Adebayo Ayansola¹ and Adedoyin Davies Banjo²

¹Entomology Unit, Natural History Museum, Obafemi Awolowo University, Ile-Ife, Nigeria.

²Department of Plant Science & Applied Zoology, Olabisi Onabanjo University, Ago-Iwoye, Nigeria.

ABSTRACT

There is a growing concern in Nigeria about the authenticity of honey being sold in local markets. Regrettably however, there is dearth of information on the quality of honey being marketed in Nigeria in general and southwestern Nigeria in particular. Previous workers obtained their honey samples from bee farms which made them to conclude that Nigerian honeys are not adulterated. Thus the quality of retailed honey in the local markets had not been evaluated. This explains the rationale behind this study. The authenticity of honey marketed in the six states of southwestern Nigeria (Lagos, Ogun, Oyo, Osun, Ondo, and Ekiti) was evaluated to ascertain the quality status and extent of adulteration of honey using the following physico-chemical parameters : Moisture content, Ash content, Total Solids, Total titratable acidity, Glucose content, Fructose content, Sucrose content, and Hydroxymethylfurfural content. Honey samples from the six states give the values of most of their physico-chemical parameters within the acceptable range of the International Honey Commission except for the Sucrose content and Hydroxymethylfurfural content. The Hydroxymethyl furfural contents for all the six states had their values above the acceptable range. Lagos, Ogun, Ondo and Oyo States had their sucrose content above the permissible maximum value of the International Honey Commission. The high Hydroxymethylfurfural values indicates heat-treatment of honey being sold in local markets in southwestern Nigeria thus rendering the honey nutritionally and medicinally valueless which is a form of adulteration. The high sucrose content is an indication of honey adulteration with sugar syrup. This confirms the insinuations about adulteration of marketed honey in southwestern Nigeria.

KEY WORDS: Hydroxymethylfurfural, Adulteration, Titratable acidity, Sucrose, Glucose, Fructose.

INTRODUCTION

The best known hive product is honey, which is valued both as a food and as a folk medicine. The greatest nutritional attribute of honey is that it consists of simple sugars, these sugars do not need to be digested but are assimilated directly by the body. This makes honey a quick energy source. Honey has numerous uses and functional applications worldwide such as in food systems, religious and cultural ceremonies as well as in human and veterinary medicine [1], [2]. In Nigeria, honey is used as a substance of prayer during naming, marriage and house dedication ceremonies. Divine supernatural power is attributed to its production and sweetness. Its use for prayer is believed to invoke the divine spirits of sweetness and happiness that will result in the happiness, greater successes and divine favour for the person being prayed for. Thus honey is widely used to conjure love, affection and favour [3].

Heat-treatment of honey after extraction reduces the moisture level and destroys yeast cells. Heating liquefies crystals in the honey. Heat-exposure does also result in product deterioration, as it increases the level of hydroxymethylfurfural (HMF) and reduces enzyme (e.g. diastase) activity. The heat does also affect sensory qualities and reduces the freshness and medicinal properties [4]. It is therefore necessary to assess the quality of honey on sale in the market with a view to determining their hydroxymethylfurfural content.

At present, there is a growing concern among honey consumers in southwestern Nigeria about the authenticity of honey being purchased from the markets. Consumers who buy honey and pay for honey prices want honey. They do not want sugar water mixed with the honey. Unfortunately, extending honey with sugar water is practised by either some fraudulent beekeepers and/or some honey sellers in local markets. Majority of what has been sold locally in Nigeria has been suggested to be adulterated honey [5]. Reports have indicated that such adulterated honey do not have any nutritional and medicinal values [6].

However, previous works on honey quality assessment in Nigeria had focused on honey collected from bee farms only. This had led to an erroneous conclusion that Nigeria's honey is free of adulteration [7], [8] and [9]. It is however interesting to note that Tchoumboue et al. [10] who screened both honey from local markets and from University Bee Research farm in Cameroon found adulterated honey samples from only those collected from local markets, but those from bee farms were found to be authentic. It therefore becomes very necessary to evaluate marketed honey in southwestern Nigeria with a view to determining the extent of

*Corresponding Author: Abel Adebayo Ayansola, Entomology Unit, Natural History Museum, Obafemi Awolowo University, Ile-Ife, Nigeria. Phone number: +2348033529966. Email: aaayansola@yahoo.com

adulteration or otherwise. Such result would confirm or allay the fears of consumers. This explained the rationale behind the present study.

MATERIALS AND METHODS

Collection of samples:

Ripe honey samples were obtained from six states within southwestern Nigeria: Ekiti, Lagos, Ondo, Ogun, Osun and Oyo states between December 2009 and March 2010. The honey samples were randomly purchased from different retail outlets in each state. 18 samples were taken from each state. In all 108 samples were taken from the six states. The honey samples were immediately labelled (with location and date) and were kept in a black cupboard until needed for laboratory analysis. The analyses were done at Central Science Laboratory of Obafemi Awolowo University, Ile-Ife.

Honey quality was assessed using the physico-chemical methods of Association of Analytical Chemists [11].

Samples of honey from different study areas were compared in terms of (a) Moisture content (b) Titratable Acidity (c) Ash content (d) Total solids (e) Hydroxymethylfurfural content (f) Glucose content, (g) Fructose content and (h) Sucrose content

Determination of Total Titratable Acidity

20g of each sample was measured into a suitable dish and diluted with 40ml distilled water. 2ml phenol – phthalein was added to it as an indicator and was titrated with 0.1N NaOH to the first persistent pink. Total titratable acidity was reported as percent lactic acid by weight, 1ml 0.1 N NaOH = 0.0090g lactic acid [11].

Total titratable acidity = Titre value × % lactic acid.

Determination of Moisture Content

The moisture content of each sample was determined as follows; 3g of the sample was weighed and placed into a pre – weighed crucible. The sample was dried to constant weight in an oven at 105°C for 4hrs under vacuum [11].

$$\text{Moisture content} = \frac{M1 - M2}{M1 - M0} \times 100$$

Where Mo = Weight of empty crucible

M1 = Weight of the fresh sample + crucible

M2 = Weight of the dried sample + crucible

Determination of Total Solids

The percentage total solid of each sample was determined using the equation:

$$\text{Total solids (\%)} = 100 - \text{Moisture content}$$

Determination of the Ash content

One gram of each honey sample was separately weighed out into a porcelain crucible previously ignited and weighed. The sample was charred by igniting the sample on a hot plate in the fume cupboard. The crucible were then placed in the muffle furnace and maintained at 600°C for 6hrs. They were then cooled in a dessicator and weighed immediately [11]

The percent Ash was calculated as:

$$\text{Ash (\%)} = \frac{(\text{Wt of crucible + ash}) - (\text{Wt of empty crucible})}{\text{Sample weight}} \times 100$$

Determination of Hydroxymethylfurfural (HMF)

10g of each honey sample was dissolved in 20ml distilled water and thereafter transferred to 50ml volumetric flask and made up to 50ml. 2ml of each sample was introduced into 2 test tubes and 5.0ml solution of p-toluidine was added to each tube. Thereafter, 1ml barbituric acid was added to a tube and 1ml water was added to the other tube (blank). The absorbance of the test sample was read against the blank at 550nm using the spectrophotometer (Spectronic 20D model). The HMF was calculated using the equation:

$$\text{HMF} = \frac{\text{Absorbance} \times 19.2 \times \text{Dilution Factor}}{\text{Cell path length}}$$

Where Cell path = 10 – Constant

Dilution Factor = 150 – Constant

Absorbance = variable

Determination of Glucose and Fructose content(%)

1ml of each honey sample was mixed with 9mls of distilled water. This was shaken vigorously to dissolve and the mixture was then centrifuged. The clear solution (20µl) was injected with a microsyringe into High Performance Liquid Chromatography (HPLC) analysis. The run time was 10minutes. The HPLC used was Agilent 1200 series.

Determination of Sucrose (%)

Reagent : (a) Concentrated Sulphuric acid (Conc. H₂SO₄)

(b) 5% Phenol

Equipment : Ultraviolet – Visible Spectrophotometer

Method of Extraction : Phenolic-di-Sulphuric acid Method.

Procedure : 1g of Honey of each sample was weighed in a 50ml beaker with the aid of mettle – toledo analytical balance. 19ml of distilled water was added to the sample. The mixture was vigorously shaken and poured into a sample bottle. This was covered and left overnight for proper extraction to take place. 1ml of 5% Phenol was added to 1ml of the sample. Then 5ml of concentrated sulphuric acid (Conc. H₂SO₄) was fastly added to the sample. The mixture was shaken vigorously and allowed to cool. The total sucrose was read with the aid of Ultraviolet – Visible Spectrophotometer at 490nm.

RESULTS

Moisture content of honey samples

The mean percentage moisture content of the honey samples from the six states of southwestern Nigeria ranged from 11.480% - 15.830% with a mean value of 14.228% (Table I). The mean value of 11.480% obtained from honey samples collected from Lagos state was significantly different from that of Ogun, Ondo and Ekiti states.

A maximum value of 20% moisture content is the internationally recommended standard [12]. The findings revealed that all the honey samples in the study had moisture content within the internationally acceptable range.

Ash content of honey samples

The mean ash content of honey samples collected from the six states ranged from 0.140-0.933% with a mean value of 0.414% (Table I). The ash contents were within the internationally acceptable range of 0.06%-0.49% [12] except those from Lagos state (0.708%) and Osun state (0.933%). The ash content of honey samples from Osun state was significantly different from that of the remaining five states.

Total Titratable Acidity of honey samples

The total titratable acidity calculated as % lactic acid of the honey samples showed that honey samples from Ekiti, Lagos, Ogun, Ondo, Osun and Oyo states had mean percentages of 0.102, 0.067, 0.058, 0.063, 0.117 and 0.053 respectively (Table I). The mean value of the total titratable acidity was 0.077. Total titratable acidity from Osun state was the highest (0.117) while that of Oyo state was the least (0.053). However, both were significantly different from each other. All the honey samples were within the internationally acceptable range of 0.11%-0.46% [12].

Total solids of Honey samples

The total solids of honey samples from the six states in southwestern Nigeria were presented in Table I. The mean total solids ranged from 84.373% - 87.132% with a mean value of 85.772%. Total solids was least (84.170) in Ondo state and highest (88.520) in Lagos state. The total solids of honey samples from Lagos state was significantly different from that of Ekiti, Ogun and Ondo states. The total solids were within acceptable international range of 80-90%.

Hydroxymethylfurfural (HMF) content of honey samples (mg/100g)

The hydroxymethylfurfural (HMF) content of honey samples from the six states were presented in Table I. The mean HMF values ranged from 6.668-14.305mg/100g with an overall mean of 9.861. The HMF values of all the samples were above the internationally acceptable range (0.75-4.29). All the honey samples in this study had very high HMF values. The HMF value of honey samples from Oyo state was significantly different from that of Ogun and Osun state.

Glucose content of honey samples (%)

The mean glucose content of honey samples from the six states ranged from 0.078-14.320% with an overall mean of 6.847% (Table II). The glucose content of honey samples from the six states were very low and very much below the internationally recommended range of 23.0-32.0%. The glucose content of honey samples from Ekiti state was significantly different from that of Ogun state. However, the glucose values of Lagos, Ondo, Osun and Oyo states were not significantly different from each other.

Fructose content honey samples (%).

The mean fructose content of honey samples from the six states were presented in Table II. The mean fructose values ranged from 42.647-74.555% with an overall mean value of 57.949%. However, all the honey samples from the six states had their values above the internationally recommended range of 31.2-42.4%. The fructose values from Ekiti and Osun states were significantly different from those of other states.

Sucrose Content of honey samples (%)

The mean sucrose content of honey samples from the six states ranged from 2.272-4.495% with an overall mean of 3.4808% (Table II). Ogun state recorded the highest percentage (4.495) while Ekiti state had the least (2.272%). The sucrose values of Ekiti and Osun states were significantly different from those of other states. The result revealed that only Ekiti and Osun states had sucrose content within the internationally acceptable range of 0-2.8%. The other states: Lagos, Ogun, Ondo and Oyo states had their sucrose content above the permissible maximum value [12].

Table I : Mean values of physico-chemical composition of honey samples.

States	Moisture content(%)	Ash content(%)	Total Titratable acidity(%)	Total Solids(%)	Hydroxymethylfurfural content (HMF) mg/100g
Ekiti	15.626 ^b ± 4.225	0.265 ^{ab} ± 0.369	0.102 ^{ab} ± 0.08110	84.373 ^a ± 4.225	11.795 ^{ab} ± 9.803
Lagos	11.480 ^a ± 1.515	0.708 ^{bc} ± 0.754	0.067 ^{ab} ± 0.03777	88.520 ^b ± 1.515	9.840 ^{ab} ± 2.759
Ogun	15.528 ^b ± 2.601	0.173 ^{ab} ± 0.166	0.058 ^{ab} ± 0.02639	84.472 ^a ± 2.601	6.668 ^a ± 1.959
Ondo	15.830 ^b ± 1.909	0.265 ^{ab} ± 0.300	0.063 ^{ab} ± 0.02582	84.170 ^a ± 1.909	9.215 ^{ab} ± 3.271
Osun	14.035 ^{ab} ± 1.740	0.933 ^c ± 0.498	0.117 ^b ± 0.04676	85.963 ^{ab} ± 1.740	7.345 ^a ± 2.140
Oyo	12.868 ^{ab} ± 2.521	0.140 ^a ± 0.158	0.053 ^a ± 0.02733	87.132 ^{ab} ± 2.521	14.305 ^b ± 6.432
Mean Total	14.228 ± 2.896	0.414 ^{ab} ± 0.498	0.077 ± 0.04834	85.772 ± 2.896	9.861 ± 5.513

Mean with the same letter(s) in the same column are not significantly different at $P \leq 0.05$ according to the new Duncan Multiple Range Test (DMRT).

Table II : Mean values of physico-chemical composition of honey samples.

States	Glucose Content (%)	Fructose Content (%)	Sucrose Content (%)
Ekiti	14.320 ^b ± 23.931	69.157 ^{ab} ± 26.997	2.272 ^a ± 0.381
Lagos	5.402 ^{ab} ± 2.743	74.555 ^b ± 23.039	3.110 ^b ± 0.585
Ogun	0.078 ^a ± 0.192	47.397 ^{ab} ± 29.374	4.495 ^c ± 0.325
Ondo	7.155 ^{ab} ± 5.154	44.738 ^{ab} ± 13.194	4.322 ^c ± 0.345
Osun	4.060 ^{ab} ± 4.747	69.203 ^{ab} ± 22.960	2.345 ^a ± 0.233
Oyo	10.067 ^{ab} ± 4.304	42.647 ^a ± 24.962	4.342 ^c ± 0.380
Mean Total	6.847 ± 10.652	57.949 ± 25.924	3.4808 ± 1.025

Mean with the same letter(s) in the same column are not significantly different at $P \leq 0.05$ according to the new Duncan Multiple R Range Test (DMRT).

DISCUSSION

The reason for testing honey for quality control purposes is to reveal the possible presence of artificial components or adulterants, as well as to address processing and market needs [13]. This requires not only determining the moisture and ash content, but also the levels of hydroxymethylfurfural (HMF), acidity, total solids and apparent sugars. According to the definition of the Codex Alimentarius [12] and other international standards [14], honey shall neither contain any food ingredient than honey nor shall any particular constituent be removed from it. The honey shall not have begun to ferment. Honey shall not be heated or processed to such an extent that its essential composition is changed and/or its quality impaired.

Water content is also important to the quality of the honey. Harvesting honey with too high water content leads to spoilage by fermentation, resulting in a product with an off-taste and high levels of yeast [15]. To prevent the growth of the naturally- occurring yeasts and the subsequent fermentation of the honey, the water content of the honey should be below 20 per cent [12] Such honey is said to be mature or ripened. Yeasts cannot grow in ripe honey because of osmotic imbalance; there is no water available to the yeast cells for growth [16]. This explains the absence of yeasts in the sampled honeys in this study as all the honey samples had very low moisture content ranging from 11.48% to 15.83%.

The ash content of the honey samples from various study areas showed that the ash contents were within the internationally recommended range (0.06% - 0.49%) [12] except for those from Lagos (0.71%) and Osun state (0.93%). Adebisi [8] recorded a range of between 0.09 and 0.518 which is very similar to what was obtained from Ekiti, Ogun, Ondo and Oyo states. The ash content is a measure of the mineral elements in the honey samples [9]. A variation observed in the ash content between states can be explained by the differences in floral origin of the honey samples [10].

The acid content of honey is relatively low but it is important for the honey taste. Most acids are added by the bees [17]. The main acid is gluconic acid, a product of glucose oxidation by glucose oxidase.

Total titratable acidity was highest in samples from Osun state with 0.12% and least in samples obtained from Oyo state (0.05%). The total titratable acidity were within the internationally acceptable acidity range (0.11% - 0.46%) [12]. The mild acidity level has been shown to slow down or prevent the growth of many species of bacteria [18]. Variations observed in the honey samples may be attributed to the plant source and season of production.

The total solids of honey samples were within the internationally acceptable range for total solids (80 – 90%) [12]. The total solid is a measure of dissolved solids in honey samples. A reduction or absence of total solids in honey samples is an indication that further processing has been done on the honey samples [9].

All the honey samples in this study had high values of Hydroxymethyl furfural content. The Hydroxymethylfurfural values of all the samples were above the internationally acceptable range (0.75 – 4.29mg/100g). The high values indicate that the honey samples had been heated and / or adulterated with processed sugar. This finding contradicts the findings of Lawal *et al.* [9] who reported very low values of Hydroxymethyl furfural for all honey samples in his study.

The Hydroxymethyl furfural measures the quality of honey via the formation of 5- hydroxymethyl furfuraldehyde by acid hydrolysis of its sucrose with the formation of red colour [9]. Hydroxymethylfurfural (HMF) is a major honey quality factor that indicates honey freshness and overheating. In fresh honeys there is practically no hydroxymethyl furfural but it increases upon storage, depending on the acidity of the honey and on the storage temperature [6].

Some European bee federations (Germany, Belgium, Italy, Austria, Spain) market a part of their honey as “quality honey”, having a maximum of 1.5mg/100g. In international trade, a maximum value of 4.0mg/100g has proven satisfactory [6]. In long term routine honey control at the Institute for Honey Analysis (IHA) during the last 10 years, more than 90% of the raw honey samples and more than 85% of the retail honey samples had less than 3.0mg/100g [19]. The latest European Union (EU) standard proposal demands a maximum of 4.0mg/100g [12].

In all the six states, glucose content of honey samples was very low and less than internationally recommended range of 23.0 – 32.0%. The fructose content of honey samples in all the six states had their values far above the recommended range of 31.2 – 42.4%. Fructose and glucose are the major sugars found in honey worldwide. This result is in conformity with the findings by Krell [13] that the majority of sugars in honey samples are glucose and fructose, which represent 80-95% of total sugars found in honey. In this study, fructose was greater than glucose in all the samples from all the six states. This agrees with the findings of Crane [20] who reported that fructose is more abundant than glucose in US honeys. The variations observed in the mean values of fructose and glucose in honey samples from the six states may be attributed to differences in floral origin of the honey.

This study revealed that only Ekiti and Osun states had sucrose content within the internationally acceptable range of 0 – 2.8%. The remaining states: Lagos, Ogun, Ondo and Oyo states had sucrose content above the acceptable permissible maximum value. This implies that there was adulteration of honey with sugar syrup in the aforementioned states.

CONCLUSION

The result of the physico-chemical studies revealed that adulteration of honey with sugar is being practiced in some states in southwestern Nigeria (Lagos, Ogun, Ondo and Oyo states). Heat-treatment of honey appeared to be rampant in southwestern Nigeria as revealed by the high hydroxymethylfurfural content of honey samples.

REFERENCES

- [1] Cooper, R.A., P.C. Molan and K.G. Harding, 2002. The sensitivity to honey of Gram – positive cocci of clinical significance isolated from wounds. J. of Applied Microbiology, 93: 857 - 863
- [2] Eileen, D. M., 2003. The importance of honey. National Honey Board, 650 : 123 – 135. Retrieved January 10, 2011 from <http://www.honey%20bee%20products.htm>
- [3] Morris, K., 2006. Honeybees in Ghana’s traditional communities. J. of American Apitherapy Society, 13:12-17.
- [4] NHB (National Honey Board), 2007. Honey and Bees. Retrieved January 11, 2008, from <http://www.honey.com/consumers/kids/beefacts.asp>.
- [5] Omode, P.E. and S.A. Ademukola, 2008. Determination of Trace metals in southern Nigeria Honey by use of Atomic Absorption Spectroscopy. Spectroscopy Letters, 41 : 328 – 331.

- [6] Bogdanov, S. and P. Gallmann, 2008. Authenticity of honey and other bee products : State of the Art. ALP Science Technical – Scientific information 2008, Nr.520 (On-line). Retrieved June 22, 2008, from http://www.db -alp.admin.ch/de/.../docs/pub_BogdanovS_2008_16946.Pdf?
- [7] Agwu, C.O.C. and G. Okeke, 1997. Pollen analytical and thin-layer chromatographic study of honey from three savanna zones of northern Nigeria. *Nigerian J. of Botany*, 10:25-36
- [8] Adebisi, F.M., I. Akpan, E.T. Obiajunawa, and H.B. Olaniyi, 2004. Chemical/Physical characterization of Nigerian honey. *Pakistan J. of Nutrition*, 3: 278-281.
- [9] Lawal, R.A., A.K. Lawal and J.B. Adekalu, 2009. Physico-chemical Studies on Adulteration of Honey in Nigeria. *Pakistan J. of Biological Sciences*, 12 (15): 1080-1084.
- [10] Tchoumboue, J., J. Awah-Ndukum, , F.A., Fonteh, N.D. Dongock, J. Pinta, and Z.A . Mvondo, 2007. Physico- chemical and microbiological characteristics of honey from sudan-guinean zone of West Cameroon. *Africa J. of Biotechnology*, 6: 908-913.
- [11] AOAC, 1997. Official Methods of Analysis of the Association of Analytical Chemists. 15th Edition, AOAC, Washington, DC.
- [12] Codex Alimentarius Commission, 2001. Revised Codex Standard for Honey, Codex STAN 12-1981, Rev.1 (1987), Rev.2 (2001); FAO/WHO, 215 pp.
- [13] Krell, R., 1996. Value added products from beekeeping. *FAO Agricultural Services Bulletin*, 124. Rome, Italy: FAO. 409pp.
- [14] EU Council, 2002. Council Directive 2001/110/EC of 20 December 2001 relating to honey. *Official J. of the European Communities*, L10:47-52.
- [15] Russmann, H., 1998. Hefen und Glyzerin in Blütenhonigen – Nachweis einer Gärung oder einer abgestoppten Gärung. *Lebensmittelchemie*, 52:116-117.
- [16] Ruoff, K. and S. Bogdanov, 2004. Authenticity of honey and other bee products. *Apiacta*, 38: 317-327.
- [17] Echigo, T. and T. Takenaka, 1974. Production of organic acids in honey by honeybees. *J. of Agricultural Chemical Society*, 48: 225-230.
- [18] Ruiz-Argueso, T., Z. Rodrigue, and A. Navarro, 1973. Gluconic acid-producing bacteria from honeybees and ripening honey. *J. of General Microbiology*, 76:211-216.
- [19] Lullman, C., 1998. Annual Reports of the Institute for Honey Analysis (1989-1997), 145pp
- [20] Crane, E., 1990. *Bees and Beekeeping*. Heinemann Newnes, Oxford, 614pp