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COMPARATIVE PHYSICO-CHEMICAL COMPOSITION OF NATURAL HONEY COLLECTED FROM TRADITIONAL BEE-BREEDERS IN OGUN STATE.

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ABSTRACT

Comparative studies on the physico-chemical composition of natural honey produced by traditional bee- breeders from the three senatorial districts in Ogun state were investigated. These districts were: Ogun-East, Ogun-Central and Ogun-West consisting of nine, six and five local government areas respectively. Double-stage multistage clustered sampling was used to carry out the studies on the selected six (6) local government areas that were finally chosen for the studies. Fresh natural honey samples were collected in sterile pet bottles and were analyzed for hydroxymethyfurfural, reducing sugar, pH, specific gravity, refractive index, total solids, titratable acidity, moisture and ash contents, and sucrose. Values range of 43.4 to 53.1(ppm); 70.2 to 76.4 (%); 2.73 to 3.74; 1.255 to 1.402; 1.446 to 1.448; 73.5 to 77.5(%); 0.62 to 1.63; 22.5 to 26.5 (%); 1.2 to 2.8 (%) and 3.23 to 4.72 (%) for hydroxymethyfurfural, reducing sugar, pH, specific gravity, refractive index, total soluble solids, titratable acidity, moisture, ash contents and sucrose respectively were recorded. Results obtained from the study however, showed that moisture and ash contents do not conform with the standards (Codex Alimentarius Commission and European Union) in that, values obtained were higher the those of the standards. Conversely, data obtained for titratable acidity, refractive index, reducing sugars, sucrose and to some extent the hydroxymethyfurfural were in agreement with the standards. Generally, all the honey samples investigated were highly acidic with high total soluble solids (sugars) and hence, a good-preserve.

Keywords: Physico-chemical Composition, Natural Honey, Senatorial District, Hydroxymethyfurfural.

INTRODUCTION

Honey is produced by the honeybee and is a natural super-saturated sugar solution, which mainly composed of a complex mixture of sugars. Besides this, it is also contains minor constituents like protein enzymes (invertase, glucose-oxidase, catalase and phosphatise), amino and organic acids (gluconic acid, acetic

acid), vitamins (ascorbic acid, niacin, flavonoids and carotenoid-like substances and minerals) and traces of lipids (Blasa *et al*., 2006). The composition of which depends on plant species visited by honeybee, the environmental, processing and storage conditions (Sudhanshi *et al*., 2010). Blossom or nectar honey is derived from the sugary excretion of some homopterous insects on the host plant or from the exudates of the plants (Abd El-Aleem, 2002).

Honey has been traditionally used as sweeteners in beverages and particularly for different purposes. It has a great potential to serve as alimentary supplement in medical therapies and a natural food without the addition of any substance in its elaboration (Azeredo *et al*., 2003). Nowadays, the concern and awareness on the use of artificial sweeteners and its cumulative consequences made the elites in Nigeria to use natural honey to sweetened their beverages, basically coffee and tea. This has consequently increased the demand for honey, especially in the urban areas and cities. This couple with the increasing population size made the honey breeders within the cities to tap from the increasing demand for natural honey and expand their apiaries, in order to meet the market demand. Honey consumption had been reported to be effective at increasing the total plasma anti-oxidant and reducing capacity in human (Gheldof *et al*., 2003). In recent years, much attention has been centred on the use of natural dietary anti-oxidants as an effective protection against oxidative damage. Enzymes in honey serve as anti-oxidant by promoting the removal of oxygen (Oszmianski and Lee, 1990). Minor components of honey (i.e. enzymes) make it different from other substances, but some treatment like processing and prolonged storage usually reduce it enzymatic activity (Hudobro *et al*., 1995).

The most used parameters as an indicator of freshness of honey are hydroxymethyfurfural (HMF) or 5-hydroxymethy-2-furaldehyde, diastase and invertase. However, HMF and invertase are included as international quality standards for honey but invertase is considered better than diastase as a freshness index because it is more sensitive (Sancho *et al*., 1992). The official honey standards approve major nine parameters have to be determined, including reducing sugars, sucrose, fructose-glucose ratio, moisture, ash, water-soluble solids, acidity, diastase activity and hydroxymethyfurfural (HMF) or 5-hydroxymethy-2-furaldehyde (CAC, 1998; EUC, 1996). These tests are laborious and time consuming. HMF or 5-hydroxymethy-2-furaldehyde is Honey is produced by the honeybee and is a natural super-saturated sugar solution, which

mainly composed of a complex mixture of sugars. Besides this, it is also contains minor constituents like protein enzymes (invertase, glucose-oxidase, catalase and phosphatise), amino and organic acids (gluconic acid, acetic acid), vitamins (ascorbic acid, niacin, flavonoids and carotenoid-like substances and minerals) and traces of lipids (Blasa *et al*., 2006). The composition of which depends on plant species visited by honeybee, the environmental, processing and storage conditions (Sudhanshi *et al*., 2010). Blossom or nectar honey is derived from the sugary excretion of some homopterous insects on the host plant or from the exudates of the plants (Abd El-Aleem, 2002).

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and time consuming. HMF or 5-hydroxymethy-2-furaldehyde is a cyclic aldehyde formed from fructose and glucose during dehydration. High HMF content indicates deterioration of honey which may be due to unsuitable conditions during storage and/or heating of honey (Feather *et al*., 1982; Hoseney, 1984).

The comparative physico-chemical characterization of different honey from some parts of the world has been reported (Mohammed and Babiker, 2009; and Abd El-Aleem, 2002). Information is however scanty in this aspect on natural honey from Ogun State, Nigeria. The present study reports the physico-chemical composition of natural honey produced by traditional bee-breeders from the three senatorial districts in Ogun State.

MATERIALS AND METHODS

Materials Collection

Samples of natural honey used for this study were collected at random, from different breeders in the three senatorial districts, Ogun state. Honey samples were strained with cleaned muslin cloth and packaged in sterile pet bottles obtained from the Nigeria Distilleries limited, Ota, Ogun state and stored (25 ± 2^0C) prior to analysis.

Sample Preparation

The preparation of honey samples were performed according to the procedure described by Louveaux *et al.* (1978). Ten grams of honey were dissolved in 20 ml of distilled water. This mixture was then poured into centrifuge tube of 15 ml and centrifuged for about 5 min, at low speed. Distilled water was again added to the sediment, repeating the previous operation. The supernatant was decanted and stored in sterile pet bottles prior to further analyses.

METHODS

Moisture Content

The moisture content was determined based on the refractometric method. In general, the refractive index increases with the increase in the solid content. The refractive indices of honey samples were measured at ambient temperature (25±2^0C) using the Abbe refractometer and the readings were further corrected for a standard temperature of 20^0C by adding the correction factor of 0.00023/^{0}C. Moisture content was determined in triplicate and the percentage moisture content values corresponding to the corrected refractive index values were calculated using Wedmore's table (AOAC, 1990).

Ash Content

The ash content was determined by placing 2 to 3 honey samples in a crucible in a muffle furnace and heating at 640^{0}C for 6 hrs. Measurements of ash were done in triplicate and the mean was expressed in percentage (%).

$$\% \text{ Ash content} = \frac{weight\ of\ ash}{weight\ of\ sample} \times 100$$

Total Solid and Reducing Sugar

The total solids of the honey samples were measured by refractometry using Abbe hand-held refractometer and the results were expressed in ^{0}Brix. All measurements were done at ambient temperature (25±2^{0}C) and the reading was corrected for a standard temperature of 20^{0}C by adding the correction factor of 0.00023/^{0}C (AOAC, 1990). The total solid content (%) in the honey samples was calculated by using the formula described by Amin *et al*. (1999), as:

$$\%\ Total\ solid = 100 - Moisture\ content$$

The reducing sugar was determined according to the procedures described by Bogadanov, *et al.* (1997).

Specific Gravity, Refractive Index, pH and Hydroxymethyfurfural

Specific gravity was estimated by measuring refractive index (RI) using Abbe-refractometer. For acidity (as formic acid), 10g honey was dissolved in 75 ml distilled water and titrated with 0.1N NaOH. The pH values were measured using a pH meter (Elico pH analyser, Elico Pvt Ltd., Mumbai) for a solution of honey prepared. The HMF content was determined the Winkler method described by Finola *et al*. (2007). Five grams of each of the samples were treated with a clarifying agent (Carrez), the volume was completed to 50ml and the solution was filtered. The absorbance of the filtrate was measured at 284 and 336nm against the aliquot treated with $NaHSO_3$.

STATISTICAL ANALYSIS

Statistical Package for Social Sciences Version 16.0 for Windows (SPSS Incorporated, Chicago, Illinois, USA) was used to conduct one-way analysis of variance (ANOVA) for determining significant difference among means. Statistically significant difference ($p<0.05$) among means were separated using the Duncan Multiple Range Test (DMRT) (Larmond, 1977).

RESULTS AND DISCUSSION

Results (Table 1) of the physico-chemical analysis carried out on the natural honey collected within some selected Local Government Area (LGA) in the three Senatorial Districts of Ogun State, showed that hydroxymethyfurfural (HMF) were in the ranges of 51.5 to 53.1 (ppm), 43.8 to 45.2 (ppm) and 43.4 to 49.6 (ppm) for Ogun-East, Ogun-Central and Ogun-West respectively. The results indicated that all the honey samples were in agreement with Codex standards (≤65.0 ppm level) and but do not conform to EUC (EU) standards of ≤40.0 ppm level. However, values obtained in this study were extremely higher that the values (2.15-4.16 ppm) reported earlier by Azeredo *et al*. (2003) for honey samples of *Apis mellifera* of different floral origin but in agreement with the findings of Finola *et al*. (2007). The HMF content is an indicative of natural honey freshness (Terrab *et al*., 2002). Azeredo *et al*. (2003) gave likely reasons for the low values of HMF as; immediate analyses conducted on honey samples at reception, meaning that no samples were adulterated with commercial sugar or had been subjected to high temperature. From this point of view, majority of the samples analyzed are fresh, if the information given by the bee-breeders that supplied the honey used for the studies are genuine. However, the amount of HMF contained in these samples does not represent a sanitary risk.

Table 1: Physico-chemical Composition of Some Natural Honey

Sample	HMF	RS	pH	SG	RI	TS	TTA	MC	Ash	SC
Ogun-East										
Ijebu-Igbo	53.1^{a}	73.8^{bc}	3.69^{b}	1.384^{b}	1.448^{a}	74.5^{b}	1.58^{a}	25.5^{b}	2.2^{b}	3.45^{c}
Sagamu	51.5^{a}	74.5^{a}	3.67^{b}	1.382^{b}	1.448^{a}	75.0^{b}	1.63^{a}	25.0^{c}	2.8^{a}	3.23^{c}
Ogun-Central										
Abeokuta	43.8^{c}	71.3^{cd}	2.73^{c}	1.402^{a}	1.446^{a}	77.5^{a}	0.64^{c}	22.5^{d}	1.8^{c}	4.56^{a}
Ifo	45.2^{bc}	70.2^{cd}	3.73^{b}	1.377^{b}	1.449^{a}	75.5^{b}	0.62^{c}	24.5^{c}	1.2^{c}	4.34^{ab}
Ogun-West										
Ayetoro	49.6^{ab}	72.1^{cd}	3.74^{a}	1.255^{c}	1.447^{a}	73.5^{bc}	0.63^{c}	26.5^{a}	2.4^{b}	4.57^{a}
Ilaro	43.4^{c}	76.4^{a}	3.69^{ab}	1.399^{a}	1.446^{a}	77.5^{a}	0.73^{b}	22.5^{d}	1.4^{c}	4.72^{a}

HMF= hydroxymethyfurfural (ppm); RS = Reducing Sugar (%); S.G = Specific Gravity; RI = Refractive Index (at 25^{0}C); TS =Total Solid (%); TTA = Titratable Acidity (mg formic acid/kg); M.C = Moisture Content (%); Ash (%); SC = Sucrose (%).

Values are means of triplicate determinations. Means in the same column with different superscripts are not significantly different ($p<0.05$).

The reducing sugar values were in the range of 70.2 and 76.4% which were far higher than the minimum values specified by both the CAC and EUC (EU) which stands at ≥ 65.0. These values were however in line with the findings of Azeredo

et al. (2003); hence it conforms to both standards. The higher values recorded for the reducing sugars may not be unconnected with the fact that, honey is actually a solution with a high concentration of sugars (Azeredo *et al.,* 2003). All the honey collected from the three senatorial districts were strongly acidic of which the honey sample at Abeokuta in the Ogun-Central being the most acidic with pH value of 2.73 and followed by 3.73 at Ifo in the same district. However, pH values obtained from other districts were not significantly different form one another ($p<0.05$) in terms of their acidity, which were in agreement with the previous findings by Azeredo *et al*. (2003) with mean pH values of 3.65. On the other hand, TTA values that were obtained from these honey samples were in the range of 0.62 to 1.63 mg formic acid/kg which was in line with the standards of the CAC and EUC. Though, these TTA values were in agreement and conform to standards but were not significantly different ($p<0.05$) within each senatorial district under consideration.

Percent moisture in the analyzed honey samples ranged from 22.5 to 26.5%. This is above the ≤ 21% water, the maximum amount allowed by the International regulations (CAC, 1998 and EUC, 1996). Finola *et al*. (2007) had reported that the water content of natural honey depends on various factors, some of which are the harvesting season, degree of maturity reached in the hive and climatic factors. The maximum amount of water contained by honey is regulated for safety against fermentation.

The ash content of the honey samples ranged from 3.45 to 4.56%. The ash contents of all the samples of honey were higher than the maximum limits for International regulations. This high dispersion observed in the honey's ash content may indicate that the harvest process and/or the beekeeping techniques used by the producers are non-uniform. However, it has also been proposed that the ash content of honey depends on the material collected by the bees during the foraging on the flora (Ojeda De Rodriguez *et al*., 2004).

Table 2: Quality Standards on Some Physico-chemical Composition of Honey

Quality Parameters	CAC	EUC
Hydroxymethyfurfural (ppm)	≤ 60.0	≤ 40.0
Reducing sugar (%)	≥ 65.0	≥ 65.0
pH	-	-
Refractive index @ 25^{0}C	-	-
Total soluble solid (%)	-	-
Titratable acidity (mg formic acid/kg)	≤ 50.0	≤ 40.0
Moisture content (%)	≤ 21.0	≤ 21.0
Ash (%)	≤ 0.60	≤ 0.40
Sucrose (%)	≤ 5.0	≤ 5.0

Sources: CAC = Codex Alimentarius Commission (1998); EUC = European Union Commission (EU = European Union) (1996)

CONCLUSION

The physicochemical analytical results of the commercial natural honeys, produced in three senatorial district of Ogun state, indicate a good level of quality. Both the HMF and TTA values were mostly low, indicating honey freshness. However, all the honey samples contained above 21% water, the maximum amount allowed by some international regulations (CAC, 1998 and EUC, 1996). It may be advisable to standardize and rationalize the beekeeping practice and techniques among the traditional bee-breeders in Ogun state, to further contribute to improved natural honey quality and values.

REFERENCES

Abd El-Aleem, W.M. (2002). Quality Evaluation for Some Kinds of Honey Produced in Egypt. Master of Science (Agric.), Thesis in Food Science, Faculty of Agric., El-Minia University, Cairo, Egypt.

Amin, W.A., Safwat, M and El-Iraki, S.M. (1999). Quality Criteria of Treacle (Black Honey). *Food Chemistry*, 67: 17-20.

AOAC (1990). *Official Methods* of *Analysis* of AOAC. 13th Edition. Association of Official Analytical Chemists, Washington D.C., USA.

Azeredo, L.C., Azeredo, S.R. and Dutra, V.M.C. (2003). Protein Content and Physicochemical Properties in Honey Samples of *Apis mellifera* of Different Flora Origin. *Food Chemistry*, 80: 249-254.

Blasa, M., Candiracci, M., Accorsi, A., Piacentini, M.P., Albetin, M.C. and Piatti, E. (2006). Raw Mellifera Honey is Packed Full of Antioxidant. *Food Chemistry*, 97: 217-222.

Bogdanov, S. (1999). Honey Quality, Methods of Analysis and International Regulatory Standards: Review of the International Honey Commission. *Mittelungen aus dem Gerbiette der Lebensmittel unter suchung und Hygiene*, 90: 108-125.

Bogdanov, S.; Martin, P., and Lüllmann, C. (1997). Harmonised Methods of the European Honey Commission. *Apidologie Extra Issue, 53-55*.

Codex Alimentarious Commission, CAC (1998). Codex Alimentarious Draft Revised for Honey CAD CX P 5/102, CI, 1998/12-S 1998, FAO: Rome, Italy.

European Union Commission, EUC (1996). Proposal for a Directive of the European Council Relating to Honey. EUD Document 96/0114: Bruxelles, Belgium.

Feather, M.S; Harris D.W. and Nichols S.B. (1982). Routes of Conversion of D-xilose, Hexouronics Acids and L-ascorbic acid to 2-furaldehyde, *Journal of Organic Chemistry* 37*: 1600–1606.*

Finola, M.S., Lasagno, M.C. and Marioli, J.M. (2007). Microbiological and Chemical Characterization of Honeys from Central Argentina. *Food Chemistry*, 100: 1649-1653.

Gheldof, N., Wang, X.H. and Engeseth, N.J. (2003). Buckwheat Honey Increases from Antioxidant Capacity in Humans. *Journal of Agriculture and Food Chemistry*, 51: 1500-1505.

Hoseney, R.C. (1984). Chemical Changes in Carbohydrates Produced by Thermal Processing. *Journal of Chemical Education, 61: 308–312.*

Huidobro, J. F., Santana, F. J., Sanchez, M. P.; Sancho, S. M. and Simal- Lozano, J. (1995). Diastase, Invertase and *B*-glucosidase Activities in Fresh Honey from North-West Spain. *Journal of Apiculture Research, 34 (1): 39-44.*

Larmond, E. (1977). Methods for Sensory Evaluation of Food. Published by the Food Research Central Experimental Farm, Department of Agriculture, Ottawa, Ontario, Canada.

Louveaux, J., Maurizio, A and Vorwohl, G. (1978). Methods of Melissopalynology. *Bee World*, 59: 139-157.

Mohammed, S.A and Babiker, E.E. (2009). Protein Structure, Physicochemical Properties and Mineral Composition of *Apis mellifera* Honey Samples of Different Floral Origin. *Australian Journal of Basic and applied Sciences,* 3(3): 2477-2483.

Ojeda De Redriguez, G., Sulbaran De Ferrer, B., Ferrer, A and Redriguez, B. (2204). Characterization of Honey Produced in Venezuela. *Food Chemistry*, 84: 449-502.

Oszmianoki, J. and Lee, C.Y. (1990). Inhibition of Polyphenol Oxidase Activity and Browning by Honey. *Journal of Agriculture and Food Science*, 38, 1892-1895.

Sancho, M. T; Muniategai, S.; Huidobro, J.F. and Simal, J. (1992). Aging of Honey. *Journal of Agriculture and Food Chemistry, 40: 134-138.*

Subhanshu, S., Satyendra, G. and Sharma, A. (2000). Physical, Biochemical and Antioxidant Properties of Some Indian Honeys. *Food Chemistry*, 118: 391-397.

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