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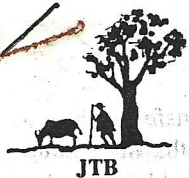
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Quality evaluation of some honey samples from Lagos, Nigeria

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Abstract

The quality of five samples of honey obtained from research bee farm (University of Lagos) and Tejuoso market, Lagos, was evaluated. 80% of the samples exhibited proper maturity based on the low moisture content ($17.20 \pm 0.43\%$). The ash contents were within the limit allowed for floral honey (0.6%) and indicating the cleanliness of honey samples. Low hydroxymethylfurfural content ($8.49 \pm 2.67 \text{ mgkg}^{-1}$) and high diastase activity ($18.62 \pm 3.83 \text{ G}^{\circ}$) indicated high level of freshness. The total acidity which was below 40 meqkg^{-1} indicated absence of undesirable fermentation, while the pH (3.87 ± 0.20) was within the recommended value. The Glucose, fructose and sucrose contents were $26.80 \pm 3.96\%$, $38.88 \pm 0.83\%$ and $1.59 \pm 0.63\%$ respectively. These data suggest that good quality honey of international standard can be obtained in Nigeria.

Key words: Honey, physico-chemical parameters, quality, diastase activity, undesirable fermentation.

Introduction

Most consumers of honey demand for basic quality level and a clear certificate of geographical and botanical origin. This occurred in some European countries and has led to regulations in different countries (Mateo and Bosch-Reig, 1997). In Spain, for instance, the honey regulation states that geographical and botanical origin of the honey must be shown on package labels (Mateo and Bosch-Reig, 1997). It was further reported that the control of honey requires the determination of parameters that could unequivocally establish origin and calls for efforts to improve honey characterization. The composition of a particular honey sample will greatly depend on the composition of the nectar(s) where it originates (Perez-Arquillue *et al.*, 1994). Battaglini and Bosi (1972; 1973) who studied honey sugars by gas chromatography (GC) concluded that honey sugars are related to those present in the raw materials (nectar or honeydew) foraged by bees to make a unifloral honey in such a way that identification of the source could be possible. Sugars contained in nectars are mainly fructose, glucose and sucrose, but vary in relative proportion (Maurizio, 1975; Baker and Baker, 1983), and the diastase activity and the hydroxymethylfurfural (HMF) content are widely recognized parameters in evaluating the freshness of honey (White, 1979). Legal regulations in Spain set a minimum value for diastase activity of eight on Gothe's scale and a maximum HMF content of 40 mg/kg (Perez-Arquillue *et al.*, 1994). In honeys with enzymatic content diastase number of three on Gothe's scale is permissible as long as HMF content does not exceed 15 mg/kg (Perez-Arquillue *et al.*,

1994). Most honeys are acidic as a result of the presence of organic acids (mainly D-gluconic acid) in equilibrium with their lactones, or internal esters, and some inorganic ions such as phosphate, chloride and sulphate, whose corresponding acids are honey constituents (Perez-Arquillue *et al.*, 1994). In Nigeria, scientific investigations into the physico-chemical properties and the microbial agents of honey are lacking. Therefore, the aim of this study was to evaluate and compare the quality of honey from Lagos with the Spanish standards for honey and other published data worldwide based on the physico-chemical parameters with the intention of promoting beekeeping.

Materials and Methods

Sample collection

Four samples were taken directly from beehives in a research bee farm, Zoological Garden, University of Lagos, which were harvested at the same period. After extraction, the samples were aseptically transferred to four sterilized glass bottles, while the fifth sample was sourced from Tejuoso Market, Lagos. The bottles were taken to the laboratory and stored at room temperature for a month until the analysis was carried out.

Physico-chemical analysis

Methodology

Moisture content, sugar composition, ash content, hydroxymethylfurfural, diastase activity, pH, acidity (free, lactone and total acidity), viscosity, the nitrogen content were determined according to the offi-

cial method of Association of Official Analytical Chemists (AOAC, 1990).

Moisture content

Moisture in honey was determined with a Shibuya refractometer reading at 20°C obtaining corresponding percentage moisture from the Chataway table (Chataway, 1935; AOAC, 1990).

Carbohydrate composition

The sugar composition was determined by Gas-Liquid chromatography with flame ionization detector (GC-FID) using column chromatograph HP 5890 series II and an HP 3396A integrator. Results were expressed as grams of each sugar (Glucose, Fructose, Sucrose and Maltose) in 100g of honey (percentage) (AOAC, 1990).

Ash content

Ash percentage was determined by calcination overnight at 600°C in a furnace to a constant weight. It was allowed to cool over calcium oxide in a desiccator and weighed. The percentage ash was calculated.

The pH value

The pH was measured with a pH meter from a solution containing 10g of honey in 75ml of CO₂-free distilled water.

Determination of acidity content

Free, lactone and total acidity were determined by the titrimetric method using a solution containing 10g of sample dissolved in 75ml of water in a 250ml beaker. The solution was titrated with 0.05N NaOH at a rate of 5.0ml/minute. Immediately the pH read 8.50; the addition of NaOH was stopped. 10ml of 0.05N NaOH was then pipetted and was back-titrated with 0.05N HCl from 10ml burette to pH 8.30. The blank was equally determined as above. The total acidity was determined by adding free acidity and lactone acidity (AOAC, 1990). Results were expressed as meq/kg.

Diastase activity

Diastase activity was measured using a buffered solution of soluble starch and honey which was incubated in a specially designed glass tube, shaped to end in an inverted "V", in a thermostatic bath until the end-point was determined photometrically. Results were expressed (as Gothe degrees) as ml of 1% starch hydrolyzed by an enzyme in 1g honey in 1 hour (AOAC, 1990).

Hydroxymethylfurfural (HMF)

Hydroxymethylfurfural was determined by dissolving 10g of unheated honey sample in 20ml of cold

distilled water. The solution was transferred into a 50ml volume flask and made up to the mark. Into each of two test tubes, was added 2.0ml honey solution and 5.0ml P-toluidine solution. 1ml of water (blank) was immediately added to one of the tubes and 1ml of barbituric acid solution to the other. The absorbance of the sample was measured against the blank solution in a 1cm cell at 550nm as soon as the maximum value was reached. For the calibration, a standard solution of 0.300µg of HMF spectrophotometrically assayed at 284nm was used. Results were expressed as mg/kg.

Nitrogen content

One gram of honey sample was weighed into a Kjeldahl flask. Added to it were 10g of potassium sulphate, 0.2g of mercury oxide and 0.05g of selenium. Thereafter 20ml of concentrated sulphuric acid was added to the contents of the flask, which washed down the solids adhering to the neck. The flask was shaken vigorously to mix its contents. The flask was closed with a loosely fitting flat reagent bottle stopper and kept inclined at an angle of 30°-45° in a hood. The flask was heated for digestion at 2.5-3.0 minutes boiling time for 1.5 hours until the solution became colorless or clear; heating was continued for another 30 minutes. After cooling, 40ml of water was added carefully a little at a time to dissolve solids, cooled and thin film of grease was placed on rim of flask. The digest was completely transferred to a 100ml volumetric flask and made up to the mark. Ten milliliters of the solution was transferred to an ammonia distillation flask. 2g of granulated zinc and 50ml of sodium hydroxide-sodium thiosulphate solution were added to it without agitation. The flask was immediately connected to the condenser whose tip was immersed in 50ml of 4% boric acid. The flask was then heated so that the contents boiled gently and distilled for 30-40 minutes. By this time all the ammonia had passed over in the receiver. A few drops of the mixed indicator (Methyl Red-Methylene blue) were added to the receiver and the borate formed was titrated to end point with standard 0.01M hydrochloric acid. The blank was determined exactly as above. The nitrogen content (%) was calculated.

Colour determination

Colour determination was carried out using comparator (colour classifier). Clear blanks were placed in compartments 1, 3 and 5 of the comparator. The sample (honey) was also placed in compartment 2 or 4 of comparator. The comparator was held at convenient distance from eye and viewed by diffused light (day light fluorescent lamp). The sample was

moved from compartment to compartment until the sample equal the match standard.

The undetermined (insoluble matter) category is taken as the difference between 100 and the total sugars plus the moisture content along with the small amounts of protein (nitrogen content %). The viscosity of the samples was determined using U-tube viscometer.

Results and Discussion

The analytical results for 5 samples are summarized in Table 1. The moisture content of honey depends on harvest season with the degree of maturity reached in the hive. This parameter is very important for the shelf-life of the honey during storage. Four out of five samples, yielded moisture between 16.2-18.0% which means a proper degree of maturity. Most of the honey samples showed moisture content of 17.93±1.95% which was similar to that of 17.20±0.43% as reported by Perez-Arquillue *et al.* (1994) but slightly different from the moisture content (15.44±0.36%) of Saudi Arabian honey as reported by Al-Hindi (2005), while the moisture content of Burkina Fasan honey varied from 15.0±0.1 to 25.1±0.0% (Meda *et al.*, 2005). The pH range of the honey samples is 3.68-3.92. Most honeys are acidic, having pH in the range of 3.5-4.5 (Perez-Arquillue *et al.*, 1994). The pH values obtained from the samples agreed with data reported by Pourtallier and Taliercio (1970) who established pH ≤ 4.0 as the normal value as cited by Perez-Arquillue *et al.* (1994) but low when compared with the mean pH of 4.77 Spanish honey (Terrab and Heredia, 2004). The ash contents were relatively low (0.29±0.22%). The range of values for ash content (0.11-0.62%) (Table 1) was within the limit allowed for floral honey (0.6%) and indicating the cleanness of honey

samples and possibly lack of adulteration with molasses.

The total sugars, the fructose and glucose mean values agreed with results of Serra *et al.*, (1987) which were 35.26% fructose and 29.52% glucose. The mean±S.D. values of free and lactone acidity of the samples are 19.26±4.72 meqkg⁻¹ and 2.19±0.90 meqkg⁻¹ respectively. The total acidity was within limits (below 40 meqkg⁻¹) indicating the absence of undesirable fermentation but Terrab and Heredia (2004) reported that Spanish honey showed total acidity below 50 meqkg⁻¹. This showed that Nigerian honey has low acidic content compared to Spanish honey.

The diastase activity and the hydroxymethylfurfural were used in evaluating the freshness of honey because it under goes physical and chemical changes in storage (Schade *et al.*, 1958 and White, 1979). The honey samples showed an approximate diastase number ranging from 14.20 to 24.20G^o and their hydroxymethylfurfural (HMF) content averaged 8.49mgkg⁻¹ with a maximum of 12.0 mgkg⁻¹ (Table 1). Thus all samples were within the Spanish legal regulations for diastase number and HMF content. Legal regulations in Spain set a minimum value for diastase activity of eight Gothe's scale and a maximum HMF content of 40mgkg⁻¹ (Perez-Arquillue *et al.*, 1994). The percentage of nitrogen content is an index of the presence of nitrogenous substances such as proteins.

In Nigeria, most beekeepers and honey hunters lack adequate knowledge about the physico-chemical properties of honey produced. The study on quality evaluation of some honey samples (natural honey from beehives and market sources) prov-

Table 1. Comparison of physico-chemical parameters of Lagos honey samples with published data [II = Standard range (source: Perez-Arquillue *et al.*, 1994)]

Parameters	Range	Mean±S.D	II
Moisture content (%)	16.20-21.21	17.93±1.95	13.4-22.9
pH	3.68-3.92	3.87±0.20	3.42-6.10
Ash content (%)	0.11-0.62	0.29±0.22	0.02-1.028
Glucose (%)	20.30-30.15	26.80±3.96	27.25-44.26
Fructose (%)	38.20-40.12	38.88±0.83	22.03-40.75
Sucrose (%)	1.12-2.68	1.59±0.63	0.25-7.57
Maltose (%)	3.32-4.05	4.35±1.11	2.74-15.98
Other disaccharide (%)	0.80-2.17	1.53±0.60	0-1.76
Higher carbohydrate (%)	2.0-4.40	3.22 ±1.02	0.13-8.49
Free acidity (Meqkg ⁻¹)	14.00-26.80	19.26±4.72	10.6-26.9
Lactone acidity (Meqkg ⁻¹)	1.25-3.23	2.19±0.90	0-18.76
Total acidity (Meqkg ⁻¹)	16.25-29.55	21.65±4.92	8.68-59.49
Nitrogen content (%)	0.14-0.28	0.21±0.07	0.047-0.223
Diastase activity (G ^o)	14.20-24.20	18.62±3.83	10.0-29.0
Undetermined (insoluble matter) (%)	2.79-3.16	3.00±0.14	0-13.2
Hydroxymethylfurfural (HMF) (mgkg ⁻¹)	5.53-12.0	8.49±2.67	0-18.26
Viscosity (mm ² /cSt)	15.00-18.00	15.60±1.34	-----

ide a basis for comparison with international standards. This study has shown that honey harvested from flourishing vegetation, extracted hygienically and stored properly, meets international standards; it confirms the report of Ikediobi *et al.* (1985) that Nigerian honeys (harvested at ARTS, Umuahia), when compared with honey from Greece, Cyprus and U.K, adequately met the specifications laid down by the U.S. food and Drug administration (White *et al.*, 1962). Further survey on the physico-chemical properties of honey samples from other parts of Nigeria is suggested.

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