

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Physicochemical Attributes of Nigerian Natural Honey from Honeybees (*Apis mellifera adansonii*) (Hymenoptera: Apidae) and its Shelf Life in Storage at Room Temperature

K.A. Fasasi

Department of Biological Sciences, College of Science, Engineering and Technology,
Osun State University, P.M.B. 4494, Osogbo, Osun State, Nigeria

Abstract: Nigerian honey competes globally in the world honey market. The physicochemical parameters of honey samples sourced from colonies of *Apis mellifera adansonii* were studied with the effect of storage-time over a period of two years. This was done by analyzing and evaluating eleven common physicochemical parameters including colour, moisture content, ash content, sugar content, acidity, pH value, hydroxymethylfurfural, diastase activity, nitrogen content, insoluble matter and viscosity of honey samples with the effect of storage-time on the physicochemical parameters using Association of Official Analytical Chemists methods. Most of the honey samples showed proper maturity considering the moisture content ($17.9 \pm 2.0\%$). The total acidity ($21.5 \pm 5.6 \text{ meq kg}^{-1}$) indicated absence of undesirable fermentation after harvest and extraction. The Mean \pm SD pH value (3.9 ± 0.2) of the honey samples was within acceptable standards of Economic European Community (EEC) and Codex. The ash content ($0.4 \pm 0.2\%$) (0.2 - 0.6%) was slightly higher than 0 - 0.50% of EEC and Codex standards. The fructose and glucose mean values were $38.9 \pm 0.8\%$ and $28.3 \pm 2.4\%$, respectively. The Mean \pm S.D value of hydroxymethylfurfural content ($8.5 \pm 2.7 \text{ mg kg}^{-1}$) was low, while the Mean \pm SD value of diastase activity was high depicting freshness. The evaluated physicochemical parameters of two years old honey samples exhibited no significant deviation ($p > 0.05$) from that of the fresh samples. This study showed that natural honey if properly harvested, extracted hygienically, preserved and stored can maintain their stability relatively for at least two years at room temperature without undue interference.

Key words: Physicochemical attributes, Nigerian natural honey, shelf-life, storage-time, room temperature

INTRODUCTION

Honey is levorotatory and nectar exudation of plants sourced from virile vegetation, processed and stored in the honeycomb cells by honeybees in their colonies and contains less than 25% of water, about 0.25% of ash and not more than 8% sucrose (Doner, 1977). It is either produced from floral nectars or honeydew secretions from hemipterous insects (aphids, leaf hoppers and scale insects) that feed on phloem sap of various trees. Bees gather honeydew during periods of low nectar (Crane *et al.*, 1984). The processing procedures of honeydew and nectar by honeybees are the same with different compositions reflected in their final products referred to as honeydew and floral honeys. Foraging bee suck nectar through the proboscis into the honey-sac where enzymes from the hypopharyngeal gland act on it and regurgitate to form honey and then returns to the hive and pass its load of materials to the house bee that ripens by repeated regurgitation (Mace, 1976). The ripened honey is stored as food in the comb cells and capped by the bees which man exploits to his benefit.

Honeybees depend on stored honey in the comb cells when there is a shortage of nectar in the field due to adverse weather conditions (Fasasi *et al.*, 2007). However, man exploits honeybees for honey as food and beeswax for industrial purposes. Ninety percent of the estimated annual world production of honey is consumed by man as honey, while the remaining 10% is used industrially (Ikediobi *et al.*, 1985).

Demands for honey both internationally and locally have necessitated various studies on quality of honey produced in different countries and setting of standards for quality control. Honey surveys were generally carried out using several parameters which included moisture content, granulating tendency, pH, free acidity, lactone content, total acidity, nitrogen content and diastase value. The chemical composition of honey dictates the grades and the market values of honey, either Grade A (14-16% moisture content), B (17-18% moisture content), C (19-21% moisture content) or D (>21% moisture), depending on the physicochemical parameters such as the moisture content, HMF (hydroxymethylfurfural), diastase activities and pH (White and Doner, 1980). The

qualities of 27 samples of rosemary (*Rosemarinus officinalis*) honey from Aragon, Spain, were evaluated and most samples showed proper maturity considering lower moisture content and low honeydew which is an indication of low trisaccharide content found in the samples by Gas-Liquid Chromatography with Flame Ionization Detector (GC-FID) quantification (Perez-Aguillue *et al.*, 1994).

A survey of 21 honey samples representing 19 floral sources using selective adsorption chromatography and analytical method was conducted. The average values of the physicochemical properties found in this survey were 16.72% moisture; 32.29% glucose and 39.28% fructose. This is represented considerably by high glucose, fructose levels, low sucrose level and significant amounts of reducing disaccharide (maltose). Hence, it is apparent that honey is essentially a carbohydrate material, with 95-99% of the total solids being sugars (Doner, 1977). In the previous survey of 21 honey samples, several parameters such as granulating tendency, pH, free acidity, lactone content, total acidity, ash, nitrogen and diastase value, were not included to give full knowledge of the variation of honey with floral sources, age, production area and crop year which are of great value to honey merchants and consumers. The need for a more comprehensive survey forms the basis of current trends of physicochemical analysis of honey in different parts of the world.

"Honey pastes" marketed in Jeddah, Saudi Arabia, were subjected to physicochemical studies and screening for aflatoxins and pesticide residues and the physicochemical parameters studied were mainly moisture content, total sugars, total ash, total nitrogen, fibers, total acidity and pH. The mean values of the examined parameters were 15.44 ± 0.36 , 74 ± 4.30 , 0.40 ± 0.062 , 0.22 ± 0.05 , $6.93 \pm 1.30\%$, 2.53 ± 0.161 meq kg^{-1} and 4.10 ± 0.158 , respectively (Al-Hindi, 2005). Meda *et al.* (2005) evaluated the physicochemical properties of Burkina Faso honey and compared with Codex standards and all samples were observed to be within the limits of the Codex standard for hydroxymethylfurfural, reducing sugars and diastase activity. Only 7.4% (ash), 14% (free acidity) and 22.2% (moisture) of the samples exceeded the Codex-permitted limits with a highly significant correlation between pH and ash content ($R = 0.77$; $p < 0.001$). The training of non-professional beekeepers was suggested so as to improve the quality of Burkina Faso honey. In Spain, the physicochemical attributes and pollen spectrum of 19 unifloral Spanish honeys were studied. The 10 botanically typified honeys based on microscopic pollen analysis were Willow (*Salix* spp.), Sainfoin (*Onobrychis viciifolia*), Chickweed (*Hypocoum* spp.), Crucifer (*Brassica* spp.),

Fruiter (*Prunus* spp.), Thyme (*Thyme* spp.), Blueweed (*Echium* spp.), Spike lavender (*Lavandula latifolia*), French lavender (*Lavandula stoechas* L.) and Vetch (*Vicia sativa* L.) and the samples were found to meet all major national and international honey specifications (Perez-Arquillue *et al.*, 1995).

In Nigeria, however, literature searches revealed a dearth of study on the physicochemical properties of honey and its shelf life. Due to the aforementioned, it is important that studies should be carried out on the physicochemical properties of honey samples stored at room temperature and compared with international standards for honey and other published data worldwide for the benefits of honey consumers and the traders. The study determined and compared the physicochemical parameters of honey samples sourced from Bee Research farm center with international standards. It also determined storage effect on the physicochemical properties of the honey samples.

MATERIALS AND METHODS

Establishment of bee colonies: The studies on behavior, nutrition and pests of honeybees commenced fully in September, 1997 but this particular aspect of the study was between November, 2005 and October, 2007. Bees that were used for the experiments were attracted from the wild into the hives at the Biological Garden, University of Lagos. To start off bee cultures, a paste consisting of 70 mL honey and 35 g of sugar grains was enclosed in a perforated Petri dish. This was placed inside each hive to attract bees into the hive. Five replicates hives were set up to give rise to bee cultures. Within one and half years, the hives were colonized by honeybees forming what was referred to as bee colonies. Specimens of bees were taken from each colony and were identified in the Department of Crop Protection and Environmental Biology, University of Ibadan.

Sample collection: Five honey samples were aseptically collected soon after harvesting and extraction from 5 replicate hives in the Bee Research farm located in the Biological Garden, University of Lagos and analyzed to determine the different phases of physicochemical parameters and the effect of storage on the properties.

Determination of physicochemical characteristics of honey samples: The samples of honey were analyzed according to the official methods of the Association of Official Analytical Chemists (AOAC, 1990) to determine the color, moisture content, sugar composition, ash content, diastase activity, pH, acidity (Free, lactone and

total acidity), viscosity, the nitrogen content and Hydroxymethylfurfural (HMF) and the undetermined (insoluble matter).

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

Color determination: Color determination was carried out using a color comparator (Lovibond 2000 visual comparator, The Tintometer Co. 206 Packets Ct, Williamsburg, VA 23185). Clear blanks were placed in compartments 1, 3, 5 of the comparator. The sample (honey) was also placed in compartment 2 or 4 of comparator. The comparator was held at a convenient distance from the eye and viewed by diffused light (daylight fluorescent lamp). The sample was moved from compartment to compartment until the sample equals the match standard (AOAC, 1990).

Sugar compositions: The sugar composition was determined by Gas-liquid Chromatography with flame ionization detector (GC-FID). Trimethylsilyl derivatives of sugar oximes were baseline separated and quantified in a gas chromatograph HP 5890 series II and an HP 33964 integrator under the following conditions: 3 mm stainless-steel column (1/8-in o.d.) packed with 4% SE-52 on chromosorb WAWDNCS 100/120 mesh, carrier gas flow 25 mL N₂ min⁻¹, FID with H₂ at 30 mL min⁻¹ and O₂ at 400 mL min⁻¹, temperatures (°C): injector 280, detector 290 and column 205, rate 20°C/min to 280°C, held for 20 min, internal standard calibration with xylose. All standard sugars were analytical grade. Results were expressed as grams of each sugar in 100 g of honey (Sugar compositions in percentages) (AOAC, 1990).

Hydroxymethylfurfural: Hydroxymethylfurfural was determined by dissolving 10 g of unheated honey sample in 20 mL of cold distilled water. The solution was transferred into a 50 mL volumetric flask and made up to the mark. Into each of two test tubes, was added 2.0 mL honey solution and 5.0 mL P-toluidine solution. One milliliter of water (blank) was immediately added to one of the tubes and 1 mL of the barbituric acid solution to the other. The absorbance of the sample was measured against the blank solution in a 1 cm cell at 550 nm as soon as the maximum value was reached. For the calibration, a standard solution of 0.300 µg of HMF spectrophotometrically assayed at 284 nm was used. Results were expressed as mg kg⁻¹ (AOAC, 1990).

pH value: The value was measured, using a Crison micro pH meter: 2001 model, from a solution containing 10 g honey in 75 mL of CO₂-free distilled water (AOAC, 1990).

Ash content: Ash percentage was determined by calcinations over night 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 (AOAC, 1990).

Determination of acidity content: Free, lactone and total acidity were determined by the titrimetric method using a solution containing 10 g of sample dissolved in 75 mL of water in a 250 mL beaker. The solution was titrated with 0.05 N NaOH at a rate of 5.0 mL min⁻¹. Immediately the pH read 8.50; the addition of NaOH stopped. Ten mL of 0.05 N NaOH was then pipetted and was back-titrated with 0.05 N HCL from 10 mL 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⁻¹.

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

Nitrogen content: One gram of honey sample was weighed into a Kjeldahl flask. Added to it were 10 g of potassium sulphate, 0.2 g of mercury oxide and 0.05 g of selenium. Thereafter, 20 mL of concentrated sulphuric acid was added to the content 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 min boiling time for 1.5 h until the solution became colourless or clear; heating was continued for another 30 min. After cooling, 40 mL of water was added carefully a little at a time to dissolve solids, cooled and thin film of grease was placed on the rim of the flask. The digest was completely transferred to a 100 mL volumetric flask and made up to the mark. Ten milliliter of the solution were transferred to an ammonia distillation flask. Two gram of granulated zinc and 50 mL 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 50 mL of 4% Boric acid. The flask was heated so that the contents boiled gently and distilled for 30-40 min. 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 0.01 M HCl. The blank was determined exactly as above. The nitrogen content (%) was calculated (AOAC, 1990).

Undetermined (insoluble matter): The undetermined (insoluble matter) category is taken as the difference between 100 and the total sugars plus the moisture content along with smaller amounts of protein (nitrogen content, %) (AOAC, 1990).

Viscosity: The viscosity of honey was determined by using U-table Viscometer (300BS/IP/CF 4414). The Viscometer was calibrated against water, the flow time of which represented 100% viscosity reduction:

$$\text{Viscosity (v)} = k \times t$$

where, k is constant (k = 0.25), T = time (second), the unit for viscosity is mm²/s or Cst (AOAC, 1990).

Effects of storage time on physicochemical properties of natural honey of *Apis mellifera adansonii*: Honey samples (5 kg) of 3 replicates were collected from one year old colony and stored under laboratory condition (26.7±1.9°C and 93.0±5.3%r.h) in closed screw capped jars.

At intervals of 6 months, 0.5 kg sample was taken from each replicate jar and analyzed separately for the physicochemical parameters, as described above, for a total period of 2 years.

Statistical analysis: The mean values of each parameter over time were computed and used to evaluate any departures in characteristics of the starting fresh sample status values. Also, the data were subjected to analysis of variance to determine the extent of departure at 5% significant test.

RESULTS

Physicochemical characteristics of some honey samples:

It was observed from this study that the physicochemical properties of the honey samples conform to the international standards. Also, the study showed that honey samples may probably maintain relative stability in physicochemical properties for at least two years. From this study, it shows that honey can be preserved and stored at room temperature without any significant change in the physicochemical properties. The study indirectly emphasized that the physicochemical analysis of honey harvested may likely reduced possible adulteration.

The analyzed honey samples exhibited color variations from light amber to dark amber (Table 1). The Mean±SD moisture content of the samples was 17.9±2.0% with Mean±SD pH value of 3.9±0.20. It was observed that the honey samples exhibited low ash content (0.4±0.2%)

Table 1: Physicochemical characteristics of natural honey samples compared with international standard values after EEC (1974) and Codex Alimentarius (2001)

Physicochemical parameters	Mean±SD (range)	International standard values
Color	Varies from light amber to dark amber	Depending on botanical origin of nectar (*0)
Moisture content (%)	17.9±2.0 (16.9-19.9)	< 21 (*0)
pH	3.9±0.2 (3.7-4.1)	3.4-6.1 (*)
Ash content (%)	0.4±0.2 (0.2-0.6)	3.20-4.5 (0)
Glucose (%)	28.3±2.4 (26.0-30.7)	0-0.5 (*0)
Fructose (%)	38.9±0.8 (38.0-39.7)	Σ(G+F+M)≥ 65 (*0)
Maltose (%)	4.4±1.1 (3.2-5.5)	Σ(G+F+M)≥ 65 (*0)
Sucrose (%)	1.6±0.6 (1.0-2.2)	Σ(G+F+M)≥ 65 (*0)
Other disaccharide (%)	1.5±0.6 (0.9-2.1)	≤ 5.0 (*0)
Higher Carbohydrate (%)	3.2±1.0 (2.2-4.2)	0.0-1.76 (*)
Free acidity (meq kg ⁻¹)	19.3±4.7 (14.5-24.0)	0.13-8.5 (*)
Lactone acidity (meq kg ⁻¹)	2.2±0.9 (1.2-3.2)	10.6-26.9 (*)
Total acidity (meq kg ⁻¹)	21.5±5.6 (15.8-2.1)	0.0-18.8 (*)
Nitrogen content (%)	0.2±0.1 (0.1-0.3)	≤ 50 (0)
Diastase acidity (G°)	18.6±3.8 (14.8-22.5)	> 40 (*)
Undetermined (Insoluble matter) (%)	3.6±0.8 (2.8-4.5)	-
Hydroxymethylfurfural (HMF) (mg kg ⁻¹)	8.5±2.7 (5.8-11.2)	> 8 (0)
Viscosity (mm ² /Cst)	15.0	10.0-40.0 (*)
		0.1-0.5 (* 0)
		< 8 (0)
		0-4 (*)

Σ: Sum, G: Glucose, F: Fructose, M: Maltose, *EEC, 1974, 0: Codex Alimentarius (2001) (EEC, 1974; Codex Alimentarius, 2001)

which indicate that the samples were clean and devoid of adulteration. The Mean±SD values of Glucose, Fructose and maltose contents of the samples were 28.3±2.4, 38.9±0.8 and 4.4±1.1%, respectively. The Mean±SD total acidity of the samples was 21.5±5.6 meq kg⁻¹ while the nitrogen content and the diastase activities measured 0.2±0.1% and 18.6±3.8 G°, respectively.

The Mean±SD value of the undetermined (insoluble matter) was 3.6±0.8% while the Mean±SD value of Hydroxymethylfurfural (HMF) content of the samples was 8.5±2.7 mg kg⁻¹ with constant viscosity of 15 mm²/Cst. From this study, it was observed that fresh honey samples exhibited good quality and clarity.

Effect of storage time on physicochemical properties of natural honey at room temperature: The honey samples maintained their initial extra-light amber color in storage for only 6 months before changing gradually to dark amber within 24 months. The results showed that the moisture contents increased slightly in storage from 17.5±0.5 to 18.4±0.2% within 24 months (Table 2). The pH of the honey samples increased slightly from 3.3±0.1 to 4.3±0.2. There were no significant variations in sugar compositions for 24 months except that the total acidity increases slightly from 29.2±1.9 to 31.4±2.4 meq kg⁻¹. The diastase activities increased slightly from 20.5±1.8G° to 21.1±2.2G° in storage over 24 months which was insignificant (Table 2).

The Nitrogen content (0.2±0.1%) and the undetermined (insoluble matter) (0.3±0.1%) remained constant in storage throughout the duration of the study. Also, Hydroxymethylfurfural (HMF) exhibits a slight

increase in values within the storage period while the viscosity remained constant (Table 2). It was observed that there was no significant deviation (p>0.05) from the evaluated physicochemical parameters of a honey sample when fresh and two years old.

DISCUSSION

Physicochemical characteristics of natural honey samples and effect of storage time: The color of the analyzed honey samples varied from extra-light amber to dark amber which was still within the prescribed colors by international standards (EEC, 1974; Codex Alimentarius, 2001). The variations in color of the honey samples may probably be due to the nectar sources of the honey from the wild or the age of the honey in the hives during storage by honeybees or prolonged and continuous usage of honeycomb cells for storage of honey by honeybees. The moisture content of the honey samples was 17.9±1.9% which was within the limit (<21%) set internationally and indicated the degree of maturity of the honey samples against possible fermentation. This mean value was lower than those previously reported in Spain (Serra *et al.*, 1987). Moisture content of honey depends on harvest season with the degree of maturity reached in the hive and is one of the most important parameters of the shelf-life of honey during storage (Perez-Aguillue *et al.*, 1994). Most honeys are acidic, having pH in the range of 3.4-4.5 (Doner, 1977; Perez-Aguillue *et al.*, 1994; Mateo and Bosch-Reig, 1997). The mean pH values obtained from the honey samples studied conformed with the data reported (pH≤4)

Table 2: Changes in physicochemical characteristics of honey samples stored at room temperature (25.5±3.5°C) over a period of two years

Parameters (composition)	Fresh	6 months old	12 months old	18 months old	24 months old	International standard values
Colour	Extra light amber	Extra light amber	Light amber	Light amber	Dark amber	Depending on botanical origin of nectar (*0)
Moisture content (%)	17.5±0.5	17.5±0.6	17.6±0.5	18.2±0.1	18.4±0.2	<21 (*0)
pH value	3.3±0.10	3.5±0.2	3.8±0.3	4.1±0.2	4.3±0.1	3.4-6.1 (*) 3.2-4.5 (0)
Ash content (%)	0.4±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0.5±0.1	0-0.5 (*0)
Glucose (%)	30.8±0.6	30.8±0.5	30.8±0.6	30.8±0.5	30.9±0.5	Σ(G+F+M)≥65 (*0)
Fructose (%)	35.2±0.7	35.2±0.2	35.3±0.6	35.3±0.5	35.3±0.6	Σ(G+F+M)≥65 (*0)
Maltose (%)	6.9±0.4	6.9±0.4	6.9±0.4	6.9±0.4	6.9±0.4	Σ(G+F+M)≥65 (*0)
Sucrose (%)	3.6±0.7	3.6±0.8	3.6±0.8	3.6±0.8	3.6±0.8	≤5.0 (*0)
Other disaccharide (%)	1.1±0.2	1.2±0.2	1.2±0.2	1.2±0.2	1.1±0.1	0.0-1.8 (*)
Higher carbohydrates (%)	3.4±0.9	3.4±0.8	3.4±0.9	3.4±0.9	3.4±0.9	0.1-8.5 (*)
Free acidity (meq kg ⁻¹)	21.8±1.0	22.2±1.3	22.3±1.3	22.3±1.4	23.2±1.3	10.6-26.9 (*)
Lactone acidity (meq kg ⁻¹)	7.5±1.0	7.6±1.1	7.6±1.1	7.7±1.1	8.2±1.1	0.0-18.8 (*)
Total acidity (meq kg ⁻¹)	29.2±1.9	29.7±2.4	29.9±2.4	30.0±2.4	31.4±2.4	≤50 (0) ≥40 (*)
Nitrogen content (%)	0.2±0.1	0.2±0.1	0.2±0.1	0.2±0.1	0.2±0.1	-
Diastase activity (G°)	20.5±1.8	20.5±1.9	20.6±1.9	20.7±1.9	21.1±2.2	>8 (0) 10.0-40.0 (*)
Undetermined (%) (insoluble matter)	0.3±0.1	0.3±0.0	0.3±0.1	0.3±0.1	0.3±0.1	0.1-0.5 (* 0)
Hydroxymethylfurfural (HMF) (mg kg ⁻¹)	3.3±0.1	3.4±0.1	3.6±1.5	4.2±0.2	4.4±0.1	<80 (0) 0-40 (*)
Viscosity (mm ² /Cst)	15.0	15.0	15.0	15.0	15.0	-

Σ: Sum, G: Glucose, M: Maltose, *EEC, 1974, 0: Codex Alimentarius (2001) (EEC, 1974; Codex Alimentarius, 2001)

(Doner, 1977; Perez-Aguillue *et al.*, 1994; Mateo and Bosch-Reig, 1997). The range of values for ash content (0.2-0.6%), was in conformity with what was reported in Spain and Libya (Mohamed *et al.*, 1981; Perez-Aguillue *et al.*, 1994) but slightly above the international standards (EEC, 1974; Codex Alimentarius, 2001). Ash contents of honey samples are influenced by the botanical origin and harvesting technique used (Perez-Arquillue *et al.*, 1995). The diastase activity and the hydroxymethylfurfural (HMF) content are acceptable parameters in evaluating the freshness of honey (Schade *et al.*, 1958). Legal regulation in Spain sets a minimum value for diastase activity to be 8G° and a maximum Hydroxymethylfurfural (HMF) content of 40 mg kg⁻¹ (Perez-Aguillue *et al.*, 1994). The honey samples in this study showed appropriate diastase number ranging from 14.8 to 22.5 G° and their HMF content averaged 8.49 mg kg⁻¹ with a maximum of 11.2 mg kg⁻¹. These values are within the range of diastase activities and HMF values (10-29G° and 3.0-18.3 mg kg⁻¹, respectively) for 27 analyzed honey samples in Spain (Perez-Aguillue *et al.*, 1994). The total acidity of the honey samples was 21.5±5.6 meq kg⁻¹ and is within the range of total acidity reported (Doner, 1977; Mohamed *et al.*, 1981; Perez-Aguillue *et al.*, 1994) and conformed to international standards (EEC, 1974; Codex Alimentarius, 2001). The fructose and glucose mean values (38.9±0.8 and 28.3±2.4%, respectively) of studied honey samples varied slightly from the reported results of 36.6±0.6 fructose and 31.2±0.4% glucose in Aragon, Spain (Perez-Aguillue *et al.*, 1994) but compared with low values of 32.9±0.4% for fructose and 25.0±0.5% for glucose obtained in Libya (Mohamed *et al.*, 1981). The mean proportion of sucrose (1.6%) was lower than the 1.97% sucrose reported (Perez-Aguillue *et al.*, 1994). Also, maltose was found at lower levels (4.4±1.1%) than the 7.2±0.2% for 27 samples in Aragon, Spain (Perez-Aguillue *et al.*, 1994) and 5.7% reported in Spain (Serra *et al.*, 1987). The sum total obtained from the percentages of fructose, glucose and maltose of the honey samples was above 65%, the minimum limit set by EEC regulations (EEC, 1974) for reducing sugars. The mean percentages of sucrose of the studied honey were all below 5% which is the maximum limit proposed by FAO/WHO standards of honey (Crane, 1990; Perez-Arquillue *et al.*, 1995; Meda *et al.*, 2005). However, the variations in carbohydrate compositions in different regions indicate different sources of honey from different flora in different regions (EEC, 1974; Codex Alimentarius, 2001). The undetermined values were taken as the difference between 100% and the total sugars plus the moisture content in percentage. However, the undetermined value of the honey samples is 3.6±0.8% and

their viscosity remained constant. All the analyzed samples showed proper maturity considering the low moisture content which was within the Codex Alimentarius standards (Codex Alimentarius, 2001). The hydroxymethylfurfural content was low and the diastase activity was high which showed good freshness of the samples according to international standards (EEC, 1974; Codex Alimentarius, 2001).

The study on the effect of storage time on physicochemical attributes of natural honey at room temperature showed that honey changes colour gradually over time. The gradual change in colour (extra light amber to dark amber) of honey in storage over time may probably be as a result of the presence of propolis particles realized from the constant reuse of old crude beeswax in the hives by bees to store their honey when not harvested early enough. In hives, fresh honey (<6 months old) harvested and extracted from newly formed crude beeswax exhibited clear golden yellow colour or extra light amber colour. When crude beeswax are reused 2 or more times by honeybees to store their honey for two or more years, such crude beeswax becomes coated with propolis substances and the crude beeswax becomes more thick and hardened than the fresh crude beeswax. When honey is harvested and extracted from such crude beeswax, the honey realized will be dark brown in colour compared to honey realized from fresh crude beeswax.

CONCLUSION

The study of physicochemical attributes of honey samples produced by bees raised in Bee Research farm centre in the Biological Garden, University of Lagos, Nigeria, showed that the honey met the required international market standards. Hygienically harvested natural honey had stable moisture content with slight variations in sugar compositions, diastase activities and the HMF (Hydroxymethylfurfural) while maintaining EEC and Codex Alimentarius standards with constant viscosity at room temperature for two years. The study also showed that honey could maintain its stability without significant physicochemical change for at least two years if properly harvested, extracted and preserved hygienically at room temperature without undue interference during processing and storage.

RECOMMENDATION

It is recommended that (a) Bee farmers should maintain high level of hygiene from the field to storage phase of production, (b) Bee farmers and honey merchants should store excess honey produced

seasonally at room temperature to meet the demands throughout the year to enhance price stability, (c) Consumers should store honey, either in use or not, at room temperature instead of refrigeration to avoid crystallization and it will also save or reduce the energy bill and (d) Bee farmers and Honey merchants must analyze honey produced or stored to determine the physico-chemical properties in conformity with European Economic Community and Codex Alimentarius standards before distributions and sales. Lastly, Bee farmers interested in honey production are advised to harvest their honey including the honeycombs seasonally as at when due to minimize presence of propolis in honey.

ACKNOWLEDGMENT

Special thanks to Messers O.O. Oworu and D. Mongbe for their technical assistance on the field.

REFERENCES

- AOAC., 1990. Official Method of Analysis. 15th Edn., Association of Official Analytical Chemists, Washington, DC., USA.
- Al-Hindi, R.R., 2005. Physico-chemical studies and screening of aflatoxins and pesticide residues in some Honey paste marketed in Jeddah, Saudi Arabia. Umma Al-Qura University J. Sci. Med. Eng., 17: 121-126.
- Chataway, H.D., 1935. Honey tables showing the relationship between various hydrometer scales and refractive index to moisture content and weight per gallon of honey. Can. Bee J., 43: 215-215.
- Codex Alimentarius, 2001. Revised standard for honey. Codex standards 12-1981. Rev. 1 (1987), Rev. 2 (2001), FAO, Rome, pp: 1-21.
- Crane, E., W. Penelope and D. Rosemary, 1984. Directory of Important World honey Sources. IBRA, London, pp: 15.
- Crane, E., 1990. Bees and Beekeeping: Science, Practice and World Resources. Comstock Publishing Associates (Cornell University Press), Ithaca, New York, USA., ISBN-13: 9780801424298, pp: 614.
- Doner, L.W., 1977. The sugars of honey-A review. J. Sci. Food Agric., 28: 443-456.
- EEC, 1974. Council directive of 22 July 1974 on the harmonization of the laws of the member states relating to honey. Official J. Eur. Commun., L221: 10-14.
- Fasasi, K.A., S.L.O. Malaka and O.O. Amund, 2007. Sugar syrup as substitute for nectar: Effect on production and density of honeybee, *Apis mellifera adansonii* (Hymenoptera: Apidae) in artificial beehive. Nig. J. Entomol., 24: 48-53.
- Ikedioji, C.O., V.C. Obi and I.A. Achoba, 1985. Beekeeping and honey production in Nigeria. Niger. Field, 50: 49-51.
- Mace, H., 1976. The Complete Handbook of Beekeeping. Wardlock Limited, London, UK., Pages: 191.
- Mateo, R. and F. Bosch-Reig, 1997. Sugar profiles of Spanish unifloral honeys. Food Chem., 60: 33-41.
- Meda, A., C.E. Lamien, J. Millogo, M. Promito and O.G. Nacoulma, 2005. Physicochemical analyses of Burkina Fasan honey. Acta Vet. Bron, 74: 147-152.
- Mohamed, M.A., A.A. Amed and M.M. Mazid, 1981. Studies on Libyan honeys. J. Food Qual., 4: 185-201.
- Perez-Aguillue, C., C. Pillar, A. Agustin, J. Teresa and H. Antonio, 1994. Quality evaluation of Spanish rosemary (*Rosemarinus officinalis*) honey. Food Chem., 51: 207-210.
- Perez-Arquillue, C., P. Conchello, A. Arino, T. Juan and A. Herrera, 1995. Physicochemical attributes and pollen spectrum of some Unifloral Spanish honeys. Food Chem., 54: 167-172.
- Schade, J.W., G.L. Marsh and J.E. Eckert, 1958. Diastase activity and hydroxymethylfurfural in honey and their usefulness in detecting heat alteration. Food Res., 23: 446-463.
- Serra, B.J., P.A. Gomez and G.J. Gonell, 1987. Composition, Physicochemical properties and pollen spectrum of various Unifloral honeys from Spain. Alimentaria, 185: 61-84.
- White, J.W. and L.W. Doner, 1980. Honey composition and properties. Beekeeping in the United States Agriculture Handbook No. 335, pp: 82-91. <http://www.beesource.com/resources/usda/honey-composition-and-properties/>