Original Research Article

Evaluation of pollen and chemical composition of honey samples sourced from open markets in Anambra State, Nigeria to ascertain their authenticity

ABSTRACT

Aims: To ascertain the predominant honey plants that served as major sources of nectar and or pollen to the honeybees and to determine the quantitative presence of some physico-chemical components of the honey samples.

Study design: The honey samples were collected from the various locations based on purposive sampling.

Place and Duration of Study: The samples were collected from seven towns in three Local Government Areas of Anambra State as follows; Ukpor, Usumenyi and Ezinifite (Nnewi South LGA), Nnokwa, Alor and Nnobi (Idemmili South LGA) and Ezinifite (Aguata LGA) between January and April, 2013.

Methodology: The honey samples were dissolved in warm (40^0C) acidified water and subsequently subjected to acetolysis treatment. The recovered residues were suspended in glycerol-alcohol mixture in vials from where samples were collected for routine pollen count and identification. The chemical analysis was carried out according to the analysis of the Association of Official Analytical Chemists with four replicates. The pollen data were converted to percentage, while data from chemical parameters were converted to mean and standard deviation.

Results: A total of 67 pollen types belonging to 39 families were identified. The honey samples were grouped into two based on the botanical origin: three monofloral and four polyfloral honeys. The predominant honey plants include *Hymenocardia acida,* Combtretaceae/Melastomataceae, *Lannea* sp., *Alchornea cordifolia* and *Phyllanthus muellerianus*. The chemical analysis showed that the values of all the parameters (moisture, pH, Sucrose, Protein, Hydroxymethylfurfural, etc) tested were within the acceptable limits of international honey standard. However, the sum of glucose and fructose in three honey samples did not meet the 60g/100g recommended as minimum limit for blossom honeys.

Conclusion: The chemical analysis showed that the honey samples contained acceptable standard concentrations of all the physicochemical parameters (such as HMF, protein, moisture, sucrose, etc.) tested with exception of the sum of glucose and fructose which did not meet the standard in some samples. The predominant honey plants that served as souces of nectar and pollen in the to the bees include *Hymenocardia acida,Lannea* sp*., Phyllanthus muellerianus* and members of the Combretaceae/Melastomataceae families.

Key Words: honey, chemical composition, monoflora honey, polyfloral honey, HMF, pollen, nectar

INTRODUCTION

Honey is a highly delicious and sweetened valuable natural product savored for it nutritional, medicinal and other enumerable health benefits. It also serve industrial purposes such as in the confectionary and pharmaceutical industries for production of valuable products [19]. It is an important source of nutrients in human diets, a preferred table sweetener in most homes. It is affordable and its production si widely spread not only across the different eco-vegetation zones of Nigeria, but the world over. Honey is an exceptionally heterogeneous viscous liquid characterized by varied physicochemical, sensory, nutritional and epitherapeutic properties.

These characteristic properties of honey are attributed to the presence in honey of sugars (mono-, bi-, tri- and polysaccharides), protein, mineral salts, moisture, flavonoids, hydrogen peroxide, phenols, HMF, vitamins, organic acids and electrical conductivity among other constituents [9,18]. Some of these phyto-constituents are central to the bioactive, antioxidant, antimicrobial, therapeutic and wound healing potentials credited to honeys [7,14]. In fact, the medicinal and healing effects associated with Manuka, Tuelang and other honeys may be attributed to the presence of phenolic acids, flavonoids and anthocianinn inherent in the honey types [6, 31]. Generally, the composition of honey is determined to a large extent by the botanical (sources of nectar, extrafloral nectar and pollen grains) and geographical sources as well as climate, soil, honey bee species and other environmental variables surrounding their production [29].

In addition to the knowledge of physicochemical parameters, the characterization of the pollen spectrum of honey makes the honey attract premium price in the international market and guide the ability to make informed choices by consumers, especially individuals allergic to certain pollen types [25]. Because of the importance of honey to health and the associated commercial benefits, it becomes imperative to determine the geographical and botanical origin of the honey so as to differentiate honey produced in different regions and vegetation sources of the world [35] . Studies on pollen analysis of honeys in Nigeria have shown that each ecological region has characteristic honey plants that are sources of nectar and pollen as well as some species that are commonly distributed across most ecological zones of the country [2,4,32,42,43]. Such characteristic plants peculiar to a particular ecological zone can be used as botanical markers to differentiate honey from the different vegetation regions. Good knowledge of these honey plants are important because the present day natural vegetation in the forests and bushland thickets are being demolished indiscriminately due to agricultural expansion, urbanization and industrial establishments. The knowledge provided by pollen analysis may help in apicultural sustenance by reforesting or re-establishment of such known apicultural plants for increased production of honey.

In the past, pollen analysis was the main focus of honey analysis, but recently, other methods such as determination of the physicochemical parameters, DNA method, biomarkers and mineral content have been widely used either alone or in various combinations [51,57]. In this study the honey analysis will be based on the pollen analysis and chemical composition of the honey samples. In Nigeria, several studies have been published on pollen analysis and especially, physicochemical and metal analyses in other to evaluate the constituent, purity and plants used as sources of nectar and pollen of honey from different regions by the honeybees in different eco-regions of Nigeria. In Anambra State, there are few literatures on pollen and physicochemical analyses of honey produced in the state, particularly with respect to the pollen spectra of honeys [45,47,48]. The main objectives of this work were to ascertain the predominant pollen types and chemical composition of the honey samples from Anambra State. This will provide additional information on melissopalynological research and chemical characterization of honeys from the State regarding the sources of nectar and pollen foraged by *Apis mellifera* and whether the quality of honey produced is according to the Codex Allimentarius Commission [22] and EU Council [26].

MATERIALS AND METHODS

Honey sample collection

The study was carried out in Anambra State and the honey samples were sourced from seven towns in three Local Government Areas (LGA) of the state as follows; Ukpor, Usumenyi and Ezinifite in Nnewi South LGA, Nnokwa, Alor and Nnobi in Idemmili South LGA and Ezinifite in Aguata LGA between January and April, 2013. The samples were labelled accordingly and kept at room temperature in the Laboratory prior to analysis. The chemical analyses of the samples were carried out in Devine Laboratory, 12 Ibagwa Road, Nsukka with four replicates, while the pollen analyses were done in the Environment and Palynology Research Unit, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, all in Enugu State, Nigeria.

Pollen analysis

Ten grams of the agitated honey sample were diluted with 35 mls of acidified warm (40^{o}C) water (3 ml Conc. H_2SO_4 and 997 ml distilled water) to dissolve the colloidal matters and sugars. The sample was centrifuged at 2000 rpm for ten minutes to recover the residue and then acetolysed [39,42]. The recovered polliniferous residues were suspended in 2 ml of glycerol-alcohol in vials from where samples were taken for routine pollen count and identification under the light microscope at X400 magnification. Routine pollen counts were done on the entire area (484 cm^2) of the cover slip and identification of pollen grains was aided by photomicrographs in Bonnefille and Riolett [16], Y'bert [55], APLF [10] and pollen slides in the Environment and Palynology Research Unit, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Physicochemical parameters

Determination of proximate components

The honey samples were analyzed for percentage crude protein, moisture, ash, fibre and fat contents. The % crude protein was calculated as Nitrogen (N x 6.25) by Kjeldahl's method. All analyses were carried out according to the methods of Association of Official Analytical Chemists [9,44].

Determination of pH

The pH of the honey sample was measured with a pH meter (Hi 8519 Hanna Instrument). The instrument was standardized with buffer solutions of pH4, pH7 and pH 10. It was then washed with distilled water, dried and immersed in the honey sample until the reading stabilized and was recorded.

Determination of free acidity.

Ten grams of the honey sample was dissolved in 75ml of distilled water and stirred properly until a homogeneous mixture was obtained. Two drops of phenolphthalein indicator were added to the mixture and titrated with 0.1M sodium hydroxide till the first persistent pink colour. The amount of mills of 0.1M sodium hydroxide used was recorded as the titre value. The free acidity which is expressed in milliequvalents of acid per kilogram of honey was calculated as titre value x molarity of NaOH x 4.6 / weight of honey sample used.

Determination of HMF

Five grams of honey were measured into 50ml volumetric flask containing 25mls of distilled water. 0.5ml of Carrez solution 1 (K4Fe (CN)6 3H2O 15%w/v and 1.25ml of Carrez solution 2 (Zn (CH3 (O2) 22H2O) 30% w/v were mixed and diluted to volume with distilled water. It was filtered and 4ml of the filtrate was pipetted into one test tube and 4 ml of 0.27 sodium bisulfate into another and were mixed thoroughly and the absorbance was measured against reference at 284nm and 336nm. HMF in mg/100g honey = (A_{284} – A_{336}) x 14.97.

Carbohydrate content

The percentage carbohydrate content was determined by subtracting the percentage values of the proximate parameters from 100 %.

Carbohydrate = 100% - % (moisture + fat + protein + ash).

Data analyses

Pollen counts were converted to percentage based on the total pollen count from each samples while data from chemical analysis were convereted to mean and standard deviation using IBM SPSS Statistisc 20 package.

RESULTS AND DISCUSSION

Pollen analysis

The results of the pollen analysis of honey samples from Anambra State showed that a total of 67 pollen types belonging to 39 families were identified from both nectariferoius and non-nectariferous plants. This is an indication that the honeys were produced from a wide range of plant sources. In Nigeria and other West African countries and environment, tropical lowland rainforests and forest-savanna mosaic woodland vegetations are known to be refuge to abundant and diversity of melliferous plant species of all habits which serve as sources of nectar, extrafloral nectar and pollen to honeybees [52]. This aptly is demonstrated in the level of pollen abundance and diversity recorded in this study. These results are comparable to the findings of some investigations conducted in honey samples from southeast, Nigeria [3,41,44]. From the results of pollen spectrum, the honey samples can be grouped into two on the bases of their botanical origin. The first group of honey samples were categorized as unifloral honeys in accordance to Codex Alimentarius and EU standard designation [37]. This was because each of the honey samples had one predominant pollen type with percentage pollen ≥ 45.

In the unifloral honeys, Combretaceae-Melastomataceae was predominant (≥ 45) in honey sample from Ukpor and *Hymenocardia acida* predominant (≥ 45) in honey samples from Osumenyi and Ezinifite 1 samples (Table 1). Combretaceae-Melastomataceae comprised complex plants species most of which are trees and lianas of forest and savanna woodlands, while *Hymenocardia acida* is mostly associated with woodland savanna vegetation. Both group of plants are commonly distributed in the study areas. The rest of the honey samples examined were polyfloral honeys because percentage pollen type of contributing plants ranged from Secondary (16 – 45%) to minor pollen (≤3%) [37] (Table 1). The production of uniflora honeys from wild honeybees is usually rare because most honeys derived from the wild are usually multifloral due to the diversity of plant species readily available to the honeybees to select as pollen and nectar sources. Similar unfloral honeys have also been reported in melissopaynological studies conducted on wild honeys from five states of Nigeria [44]. The production of such unifloral honeys may be attributed to the local abundance of Combretaceae-Melastomataceae and *Hymenocardia acida* in the vegetation and occurrence their flowering periods which usually coincided with major active periods of honeybees as well as their selective preference as major sources of nectar and pollen. Incidentally,

177 *Hymenocardia acida* and members of Combretaceae-Melastomataceae are nectariferous and
178 polliniferous plants and have been generally identified in honeys analysed from this region.

179 The honey sample from Nnokwa was commonly dominated by pollen types of *Elaeis guineensis*
180 followed by *Irvingia gabonensis* and *Alchornea cordifolia*. The major honey plants recorded out of the 19
181 pollen types include *Elaeis guineensis, Irvingia gabonensis, Phyllanthus muellerianus, Nauclea latifolia*,
182 Combretaceae-Melastomataceae, *Parkia biglobosa* and *Crossopteryx febrifuga* (Table 1). In that of Alor
183 honey sampl, 24 pollen types were recorded with pollen types of Combretaceae-Melastomataceae
184 families predominating followed by those of *Lannea* sp., *Hymenocardia acida*, *Phyllanthus muellerianus,*
185 *Nauclea latifolia* and *Citrus sinensis*. The nectariferous plants suspected to be main sources of nectar
186 include Combretaceae-Melastomataceae, *Lannea* sp., *Phyllanthus muellerianus, Citrus sinensis,*
187 *Syzygium guineense, Parkia biglobosa, Pentaclethra macrophylla, Senna* sp., and *Parinari* sp. *Alchornea*
188 *cordifolia*, Poaceae, *Elaeis guineensis* and *Nauclea latifoila* were among the important sources of the
189 honey pollen (Table 1).

190 The sample from Nnobi was characterized by pollen types arising from Combretaceae-
191 Melastomataceae, *Lannea* sp., *Phyllanthus muellerianus, Syzygium guineense, Prosopis africana,*
192 *Alchornea cordifolia, Parinari curatelifolia, Senna* sp. and *Hymenocardia acida*. Although 21 pollen types
193 were identified, these plants constituted the major sources of nectar and pollen for the honeybees (Table
194 1). The plants sources of the pollen grains identified in these locations are characteristic members of the
195 flora of the study areas [33]. Comparatively, similar pollen types have been reported in a study by Agwu
196 and Njokuocha [3] in honey samples from Anambra State.

197 A total of 30 pollen types were identified in the honey sample from Ukpor. The most common
198 plant sources of nectar and pollen recorded were Combretaceae-Melastomataceae, *Lannea acida*,
199 *Blighia sapida, Hymenocardia acida,* Poaceae, *Palisota hirsuta, Bombax buonopozense, Prosopis*
200 *africana* and *Phyllanthus muellerianus* (Table 1). In the honey sample from Osumenyi, only 18 pollen
201 types were identified and of these the major plant sources of nectar and pollen include members of
202 Combretaceae-Melastomataceae, *Hymenocardia acida, Elaeis guineensis, Bridelia feruginea, Prosopis*
203 *africana* and *Parkia biglobosa* (Table 1). For Ezinifite 1, a total of 23 pollen types were recorded and the

major pollen and nectar sources include *Hymenocardia acida, Crossopteryx febrifuga*, *Allophyllus* sp., *Senna* sp., Combretaceae-Melastomataceae, *Lannea* sp., *Parinari* sp. and *Psorospermum* sp.. Commonly recorded in this honey sample were pollen grains of anaemophilous plants such as Poaceae, Moraceae, *Alchornea cordifolia, Pinus* sp., Cyperaceae and Amaranthaceae-Chenopodiaceae (Table 1). In Ezinifite II, 42 pollen types were recorded from the honey sample. The most predominant nectariferous and polliniferous plants include Combretaceae-Melastomataceae, *Pterocarpus* sp., *Psorospermum* sp., *Piliostigma thonningii, Hymenocardia acida, Afzelia africana, Lannea* sp., *Elaeis guineensis*, *Phyllanthus muellerianus* and *Mangifera indica* (Table 1). Similar findings in pollen characteristics have been reported by Agwu and Njokuocha [3] and in honeys collected from the forest-savanna vegetation of southeastern, Nigeria by Njokuocha and Nnamani [42].

The plant taxa identified in this study reflected to a large extent the characteristic flora existing in the patches of lowland rainforest and forest-savanna vegetation associated with the study areas. The characteristic taxa of the lowland rainforest recorded in the analyzed honey samples were *Elaeis guineensis, Alchornea cordifolia*, *Bombax buonopozense, Irvingia gabonensis, Canarium sweinfurthii,* Moraceae, *Pentaclethra macrophylla, Olax* sp. and *Brachystegia euricoma.* Similarly, the characteristic elements of the forest savanna mosaic vegetation associated with the study area which were identified in the study include Combretaceae-Melastomataceae, *Hymenocardia acida, Syzygium guineense, Phyllanthus muellerianus, Piliostigma thonningii, Crossopteryx febrifuga, Nauclea latifolia, Parkia biglobosa, Crossopteryx febrifuga, Bridelia ferruginea, Spondias mombin, Lannea sp. and Bligia sapida* among others [49]. Related studies associating honey pollen with the floristic composition of the study area have been reported by previous authors in Nigeria [32,42]. Evidence of anthropogenic activies such as changes in landscape and existence of exotic flora in the study environment were clearly demonstrated by the presence of pollen grains of *Citrus sinensis, Mangifera indica, Pinus* sp., *Senna* sp., *Delonix regia, Triumfetta rhumbiodea, Casuarina equisetifolia* and *Manihot esculenta* which occurred in noticeable quantity in the honey samples. Similar findings have also been reported by Njokuocha and Ekweozor [41] and Njokuocha and Nnamani [42].

231 Table 1. The dominant pollen types in the honey samples according to the percentage frequency
232 (Predominant pollen = ≥ 45%; secondary pollen = 16 – 44%; important minor pollen = 3 – 15%; minor
233 pollen = ≤ 3)

Family	Taxon	**% frequency of dominant pollen types in the honey samples/location**						
		Ukpor	**Osumenyi**	**Ezinifite 1**	**Nnokwa**	**Alor**	**Nnobi**	**Ezinifite 11**
Anacardiaceae	*Lannea* sp.	2.0	1.0	6.0	0	22.2	18.0	5.88
	Mangifera indica	0	0	0	0	0	0	2.25
Arecaceae	*Elaeis guineensis*	2.0	0	0	23.2	0	0	3.42
Chrysobalanaceae	*Parinari* sp.	0	1.0	2.0	0	0	0	0
Combretaceae/ Melastyomataceae		64	11.6	18.0	2.0	36	36.0	10.97
Euphorbiaceae	*Alchornea cordifolia*	2.0	1.0	0	8.4	0	3.6	0
Fabaceae	*Afzelia Africana*	0	0	0	0	0	0	4.34
	Prosopis Africana	2.8	0	0	0	0	6.5	0
	Piliostigma thonningii	0	0	0	0	0	0	2.50
	Pterocarpus sp.	0	0	0	0	0	0	23.52
	Senna sp.	0	7.0	2.5	0	0	0	0
Hymenocardiaceae	*Hymenocardia acida*	4.0	72.0	54.0	0	16.0	2.8	7.55
Hypericaceae	*Psorospermum* sp.	0	0	2.0	0	0	0	18.27
Irvingiaceae	*Irvingia gabonensis*	0	0	0	15.0	0	0	0
Moraceae		0	1.0	0	4.1	0	0	0
Myrtaceae	*Syzygium guineense*	2.0	0	0	6.0	0	10.2	0
Phyllanthaceae	*Phyllanthus muellerianus*	2.9	0	0	2.7	11.6	12.3	2.67
Rubiaceae	*Crossopteryx febrifuga*	0	0	2.0	0	8.0	4.1	0
	Nauclea latifolia	0	0	0	3.6	0	0	0
Rutaceae	*Citrus sinensis*	0	0	0	0	3.5	0	0
Sapindaceae	*Allophyllus* sp.	0	0	5.0	0	0	0	0

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Physicochemical analysis

The results of the proximate analysis showed that the parameters tested in the honey samples conformed with the standards of EU and Codex recommendations for honey produced from nectariferous plants [22,26] (Table 2). These findings are also comparable to the works of previous authors not only in Nigeria [4,19,40,43], but some other parts of the world [12,18]. The variations observed in the values of the moisture, crude protein, ash, pH and free acidity contents of the honeys sourced from different locations of the study area may be attributed to the differences in microclimate and soil properties on which the vegetation of the of the areas depend on for sustenance [21]. The low moisture content, the acidic and free acidity levels of the samples indicate that the honeys have potential for long shelf life and strong inhibitory property against microbial activity. The acidity in honey is attributed to the presence of organic acids such as gluconic acid and inorganic ions [12]. The considerably low free acidity especially in samples from Ezinifite II and Nnokwa is a good indication of good quality honey because high acidity has been reported to facilitate the breakdown of hexoses to hydroxymethylfurfural [5], therefore the level of acidity recorded is an indication of freshness of the honeys.

The ash content of the honey samples was within the permissible limit (0.6 %) from nectariferous plants [22]. Ash content of honey is an indication of the mineral concentration [8]. Quantitatively the ash content of honey is dependent on the soil properties and climatic factors of the honey region of origin. It is also used as quality idex for determination of the botanical origin of honey [53]. The study showed that the honey samples contained a considerable percentage of protein in the range of 0.76 ± 0.01 in honey from Ezinifite II to 1.67 ± 0.01 in honey sample from Ezinifite I. This range is considerably higher than the rough limit of 0.5g /100g of blossom honey, but far below the recommended daily intake of protein [17]. Protein is a very important dietary component, the presence of which may lead to a food to be considered not only as possible source of protein but an essential dietary product. Honey protein is mostly derived from enzymes introduced into the honey by the honeybees such as diastase, invertase, glucose oxidase, catalase and amino acids [19]. Pollen grains which are ever present in considerable quantity and diversity in honeys have been reported to be rich protein natural foods of bees; hence they are important sources of protein in honey. Considerable literature on the physicochemical components of honey in Nigeria and

263 other regions have reported considerable but variable quantity of protein in honey produced from both
264 wild and domestic apiary [17,40,43].

265
266 Table 2 Proximate composition of the honey samples from Anambra State (Mean ± standard deviation)

	Parameters				
Source location	Moisture (%)	Crude protein (%)	Ash (%)	pH	Free acidity (Meq/kg)
Ukpor	15.63 ± 0.03	1.63 ± 0.01	0.56 ± 0.01	3.4 ± 0.2	40.10 ± 0.01
Osumenyi	16.29 ± 0.01	1.58 ± 0.02	0.59 ± 0.02	3.5 ± 0.1	35.0 ± 2
Ezinifite I	15.78 ± 0.01	1.67 ± 0.01	0.86 ± 0.02	3.7 ± 0.2	37.0 ± 1
Nnokwa	16.22 ± 0.01	1.5 ± 0.01	0.53 ± 0.01	3.6 ± 0.1	15.0 ± 2
Alor	17.51 ± 0.02	1.54 ± 0.02	0.56 ± 0.01	3.3 ± 0.2	40.1 ± 0.02
Nnobi	17.63 ± 0.01	1.49 ± 0.01	0.51 ± 0.01	3.7 ± 0.1	31.0 ± 0.8
Ezinifite II	16.43 ± 0.01	0.76 ± 0.01	0.06 ± 0.01	3.5 ± 0.1	0.05 ± 0.02

267

268 Studies have shown that about 80% honey is composed of sugars; and of this glucose and
269 fructose constitute the highest proportion of the sugar components. In the present study, the percentage
270 concentration of glucose and fructose is high and in conformity with the general observation regarding
271 their dominant percentage proportion in comparison to other sugars in honeys (Table 3). Similar findings
272 have been reported in honey samples from Nigeria [19,36], Turkey [20], Egypt [27] and Tunisia [18]. The
273 sum of glucose and fructose in a honey is an important factor for assessing honey quality. According to
274 Codex Alimentarius Commision [22] and EU Council [26] regarding good quality honey, the sum of
275 glucose and fructose must be equal or higher than 60g/100g of honey. However, not all the honey
276 samples met the limits of recommended international standard.

277 The samples from Alor, Ukpor and Osumenyi did not meet the minimum limit set by codex. but for
278 honey samples from Ezinifite I, Nnokwa, Nnobi and Ezinifite II the sum of glucose and fructose were
279 within the acceptable international standard (Table 3). These findings are comparable to those reported
280 by other authors [20,46]. The low values obtained in the sum of glucose and fructose in honey samples
281 from Alor, Ukpor and Osumenyi may be attributed to the nature of nectar sugars, types of enzymes

deposited by the honeybees and the extent of maturity of the honey samples prior to their harvest. In the assessment of good quality honey, it is expected that the value of fructose should be greater than that of glucose [56]. This is in conformity with the results of the present study. This factor also become valuable when considering the fructose/glucose ratio which is an important criterion when considering the crystallization rate of the honey. The study showed that the ratio of fructose/glucose in all the samples were within the range of 1.0 to 1.45 acceptable optimum limit [19,38]. Honey within such fructose/glucose ratio range has very low rate of crystallization and therefore remains in liquid form. Equally influencing the rate of honey crystallization is the glucose/water ratio balance. High glucose and lower water ratio leads to high rate of crystallization, while the reverse leads to low rate of crystallization [15].

The sucrose content of the analyzed honey samples varied from 1.11 ± 0.01 in honey sample from Osumenyi to 2.04 ± 0.02 in honey sample from Ezinifite II (Table 3). According to laid down international honey standard, the sucrose content of a good quality honey should not exceed 5g/100g of honey [22,26]. This indicates that sucrose content of all the honey samples were with the acceptable limits of international standard. It has also been pointed out that even in honey that contains an active sucrose converting enzymes, the sucrose level can never be zero [54]. The findings in this study is comparable to that published by Aljohar *et al.* [6], Czipa *et al*. [23] and Njokuocha [44]. But the percentage values of sucrose in this study was lower than that reported by Aino [4], Nweze *et al*. [46] and Boussaid *et al*. [18].

HMF is one of the important quality criterion used in determining the freshness and purity of honey. It is an indication of overheating or exposure to high temperature and poor storage condition such as prolonged storage under high temperature. The results of the present study showed that the HMF values of the honey samples is very low ranging from 0.38 ± 0.02 in Ukpor sample to 3.89 ± 0.02 in Ezinifite 11 sample. These results are below the limits of 40mg/kg set by Codex Alimentarius Commission [22] for honeys from tropical areas like Nigeria. This shows that the honey samples analyzed in this study may be regarded as being fresh and pure. This finding compared favourably with those of Aljohar et al. [6], Czipa *et al*. [23] and Njokuocha [44]. However, the HMF values are lower compared to the higher

values reported by Njokuocha and Osayi [43] and Boussaid *et al.* [18]. HMF is formed during acid-catalyzed breakdown of hexose and decomposition of 3-deoxosone in Maillard reaction [28].

There is correlation between HMF formation and some honey characteristics such as pH, free acid content, total acidity, lactone and mineral contents as well as floral sources of the honey [50]. Important factors that leads to the HMF formation are heating of sugars from breakdown of hexoses under acidic condition at high temperatures and from oligo- and polysaccharides that can produce hexoses when hydrolyzed [11]. Under certain conditions, HMF may have positive or negative effect on human health. The consumtion of HMFin honey and other food products may cause mutagenic, genotoxic, organotoxic, DNA damaging and enzyme inhibiting effects [30]. But where HMF occurs in the form of 5-sulfoxymethylfurfural it has such benefits as anti-oxidative, anti-allergic, anti-inflamatory and anti-sickling effects, among others on human health [1,34 ,58].

Table 3 The sugar and carbohydrate content of the honey samples from Anabra State

	Parameters 0							
Source location	Glucose (g/100g)	Fructose (g/100g)	Fructose + glucose	Fructose/ Glucose ratio	Glucose/ Water ratio	Sucrose (g/100g)	HMF (mg/100g)	Carbohydrate (%)
Ukpor	25.26 ± 0.01	26.78 ± 0.01	52.04 ± 0.05	1.06 0 ± 0.03	1.62 ± 0.01	1.21 ± 0.01	0.38 ± 0.02	82.51 ± 0.01
Osumenyi	25.41 ± 0.01	26.35 ± 0.03	51.76 ± 0.02	1.04 ± 0.01	1.56 ± 0.01	1.11 ± 0.01	0.43 ± 0.02	80.69 ± 0.01
Ezinifite I	27.41 ± 0.02	33.49 ± 0.02	60.9 ± 0.08	1.22 ± 0.01	1.74 ± 0.01	1.90 ± 0.01	0.43 ± 0.02	81.69 ± 0.01
Nnokwa	30.01 ± 0.01	31.27 ± 0.02	61.28 ± 0.02	1.04 ± 0.01	1.85 ± 0.01	1.90 ± 0.05	0.43 ± 0.02	81.75 ± 0.01
Alor	28.03 ± 0.02	28.46 ± 0.01	56.49 ± 0.01	1.02 ± 0.01	1.6 ± 0.08	1.64 ± 0.02	0.58 ± 0.01	80.39 ± 0.02
Nnobi	27.11 ± 0.01	34.97 ± 0.01	62.08 ± 0.02	1.29 ± 0.01	1.54 ± 0.01	1.77 ± 0.01	0.52 ± 0.01	80.37 ± 0.01
Ezinifite II	36.22 ± 0.02	43.87 ± 0.02	80.09 ± 0.01	1.21 ± 0.01	2.21 ±0.04	2.04 ± 0.02	3.89 ± 0.02	82.75 ± 0.01

Of all the components of honey, carbohydrate constitutes the highest percentage, comprising about 95 - 98% of dry weight of honey [19,20]. Fructose and glucose are the main constituents of carbohydrate found in honey. At least about 22 more complex sugars are present in small amount in honeys, and they include monosaccharide, disaccharides, trisaccharides and oligosaccharides formed during the process of honey ripening by the interactions of honeybee enzymes, acids and temperature [13]. The carbohydrate content of the honey samples analyzed in this study ranged from 80.37% to 82.75% in honey samples from Nnobi and Ezinifite II respectively. Similar findings have been reported by Buba *et al*. [19].

Conclusion

The analysis of the honey samples revealed that the honey samples are fresh and genuine based on the values of the tested chemical parameters which were within the limits of international acceptable limits. The pollen spectrum of the honey samples indicated that the honeys were formed from diverse plants sources, although three of the honey samples (from Ukpor, Osumenyi and Ezinifete) are monofloral, while four samples (from Nnokwa, Alor, Nnobi and Ezinifite II).are mutifloral honeys. The common honey plants identified almost across the samples includes *Hymenocardia acida,* Combretaceae/Melastomataceae, *Lannea* sp., *Alchornea cordifolia and Phyllanthus muellerianus*.

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