

## Research Article

# Physicochemical Characteristics and Microbiological Quality of Honey Produced in Benin

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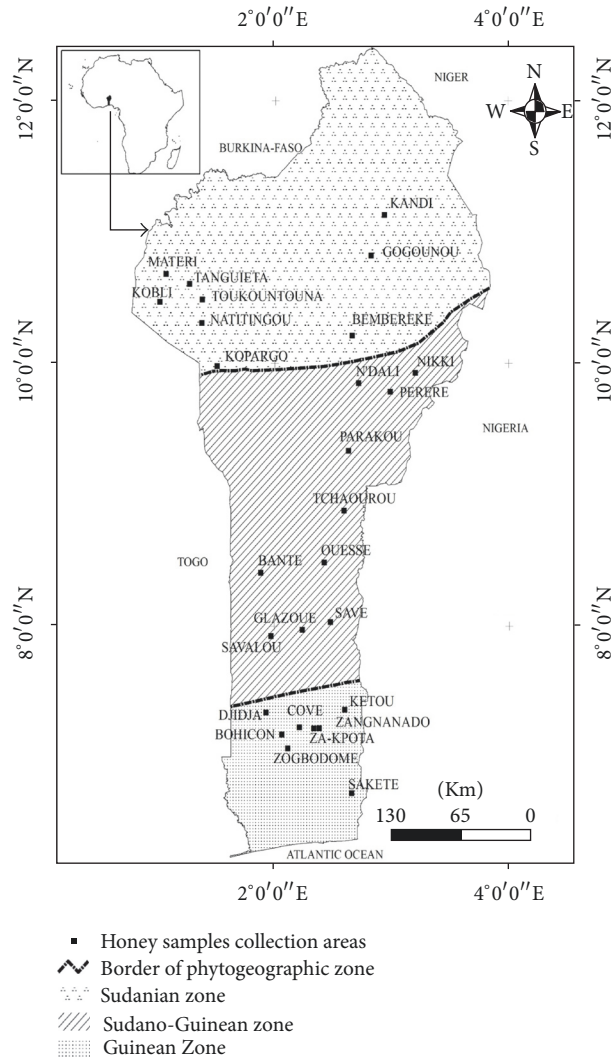
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Honey is a very complex biological product. It has great diversity, giving it a multitude of properties, both nutritionally and therapeutically. This study aimed to study the physicochemical and microbiological characteristics of honeys collected during the dry and rainy seasons in the different phytogeographical areas of Benin. The study revealed that all honeys had pH, water content, electrical conductivity, ash content, free acidity, total sugars, and reducing sugars, respectively, ranging within 3.65–4.09; 12.07–13.16%; 530.25–698.50  $\mu\text{s}/\text{cm}$ ; 0.42–0.53%; 35.67–40.52 meq/kg; 60–70%; and 58–70%. Moisture content, total sugars, and reducing sugars varied very significantly ( $p < 0.05$  to  $p < 0.001$ ) from one area to another and from one season to another. However, only the production season has a significant influence ( $p < 0.05$ ) on the pH of the honey. With regard to the ash content, free acidity, and electrical conduction, no significant difference ( $p > 0.05$ ) between the zones or between the seasons was observed. The results of the microbiological characterization showed that there is heterogeneity in the microbial load. These results have shown that these honeys meet international standards and their characterization will make it possible to obtain Beninese quality labels.

## 1. Introduction

Honey is a natural sweet substance produced by bees (*Apis mellifera*) from the nectar of flowers or tree exudates [1]. The composition of honey mainly depends on climatic and environmental conditions and the diversity of the plants from which they are harvested [2, 3]. Honey contains at least 200 substances mainly carbohydrates and water. It also contains minerals, proteins, free amino acids, enzymes, vitamins, organic acids, flavonoids, phenolic acids, and other phytochemicals [4]. In addition, honey is valuable for the treatment of cardiovascular diseases, cancer, cataract, and

several inflammatory diseases as well as wound healing. The therapeutic actions of honey are due to its antioxidant and antimicrobial properties [5]. The quality of honey is mainly determined by its sensory, physicochemical and microbiological characteristics. The criteria for the physicochemical quality of honey are well specified by the European Community Guidelines 2001/110 [6]. The main criteria of interest are moisture, electrical conductivity, ash, reducing and nonreducing sugars, free acidity, diastase activity, and hydroxymethylfurfural (HMF) content. Nevertheless, the European Union legislation has not specified the standards of microbial and hygienic contamination of honey. The chemical composition



Source: Topographic Bottom IGN, 1992

FIGURE 1: Geographical location of studied area and sampling sites.

of honey varies from a sample to another and usually contains major and minor elements. To determine honey varieties, some pollen, physicochemical, microbiological, and sensorial analysis must be taken into account. Many studies have been reported on the physicochemical and microbiological characteristics of honey worldwide [7–9]. In Benin most of studies are limited first to pollen analysis and phytogeographical characterization of honeys sold in Cotonou [10] and recently to the evaluation of the physicochemical characteristics of honey marketed in Cotonou [11]. Unfortunately, studies on microbial quality are lacking. Thus, physicochemical and microbiological assessment and the factors that may affect it are needed, for both quality control and to develop sales arguments [12]. Then the study aimed to evaluate the physicochemical and microbiological characteristics of honey samples collected in the three phytogeographical zones of Benin.

## 2. Materials and Methods

**2.1. Study Area.** Samples of honey were collected in three phytogeographical zones (Sudanian, Sudano-Guinean, and Guinean) of Benin (Figure 1). Benin is located between the parallel  $6^{\circ}15'$  and  $12^{\circ}25'$  and extends on an area of  $112,622 \text{ km}^2$ . It is limited to the north by Niger and Burkina Faso, to the south by the Atlantic Ocean, to the west by Togo, and on the east by Nigeria. Benin presents a diverse range of climates characterized by the relative weakness of the annual precipitation which vary from 900 to 1300 mm per year. In the Guinean zone from the coast ( $6^{\circ}25'N$ ) to the latitude of  $7^{\circ}30'N$ , there is four seasons (two rainy and two dry seasons). It has an annual rainfall average of 1,200 mm with an average of 250 days of rain. The Sudano-Guinean zone is located between  $7^{\circ}30'N$  and  $9^{\circ}45'N$  with a unimodal (May–October) rainfall regime and the average annual rainfall varies from 900 to

TABLE 1: Characteristics of the different areas of the phytogeographic Benin.

| Characteristics             | Areas  |  |  |
|-----------------------------|--|--|--|
|                             | Sudanian   | Sudano-Guinean   | Guinean  |
| Latitude                    | 8°10'–12°10'   | 7°30'–8°40'  | 6°15' and 7°30'E   |
| Longitude                   | 1°15'–3°45'  | 1°40'–3°45'E   | 1°45'–2°45, N  |
| Pluviometry and temperature | 900–1100 mm<br>24°C–31°C   | 900–1100 mm<br>25°C–29°C   | 1200–1300 mm<br>25°C to 29°C   |
| Seasons                     | Unimodal regime  | (i) Two rainy seasons<br>(ii) Two dry seasons  | (i) Two rainy seasons<br>(ii) Two dry seasons  |
| Moisture in the air         | 18%–99%  | 31% to 98%   | 69%–97%  |
| Soil                        | Drained soils<br>Hydromorphic soils<br>Breastplates<br>Ferralitics<br>Lithosols  | Ferruginous soils  | Ferralitics, alluvial, and vertisols   |
| Plant formations            | (i) Savannas<br>(ii) Forest galleries<br>(iii) Dry forests to Combretaceae   | (i) Clear forests<br>(ii) Wooded savannas or shrubs<br>(iii) Forests   | (i) Moist forests semideciduous<br>(ii) Swamp forests<br>(iii) Coastal savannas  |
| Characteristic species      | (i) <i>Isoberlinia doka</i><br>(ii) <i>I. tomentosa</i><br>(iii) <i>Adansonia digitata</i><br>(iv) <i>Pterocarpus erinaceus</i><br>(v) <i>Erythrophleum guineense</i><br>(vi) <i>Amblygonocarpus andongensis</i><br>(vii) <i>Swartzia madagascariensis</i> | (i) <i>Daniellia oliveri</i><br>(ii) <i>Parkia biglobosa</i><br>(iii) <i>Terminalia glaucescens</i><br>(iv) <i>Anogeissus leiocarpa</i><br>(v) <i>Acacia campylacantha</i><br>(vi) <i>Terminalia macroptera</i><br>(vii) <i>Isoberlinia doka</i><br>(viii) <i>Detarium microcarpum</i> | (i) <i>Ceiba pentandra</i><br>(ii) <i>Azelia Africana</i><br>(iii) <i>Triplochiton scleroxylon</i> and <i>Anogeissus leiocarpus</i><br>(iv) <i>Antiaris toxicaria</i><br>(v) <i>Milicia excelsa</i><br>(vi) <i>Terminalia superba</i><br>(vii) <i>Celtis zenkeri</i> |

1110 mm, distributed approximately over 113 days on average. The Sudanian zone is located between 9°45'N and 12°25'N with 900 to 1100 mm as annual rainfall average, distributed over 145 days [13] (Table 1).

**2.2. Sampling.** Honey samples were collected twice (during the dry season and the rainy season) at the maturity phase of production in 30 beekeepers (10 per zone). Thus, sixty different honey samples were collected aseptically for this study. Once collected, samples were kept in sterile vials, hermetically sealed, labeled, dated, and stored at room temperature (25–30°C) until analysis. The samples collection periods vary according to the zones. In the Sudanian and Sudano-Guinean zones, all samples were collected in 2015 from November to April (dry season) and from June to September (rainy season), whereas, in the Guinea area, samples were collected from November to March and July to September (for dry season) and from April to July and September to October for the rainy season in the same year (Figure 1).

**2.3. Physicochemical Characterization of Honey Samples.** The physicochemical parameters were determined according to the methods described by Bogdanov et al. [14] recognized by the International Honey Order and adapted by several authors [11, 15–17].

**2.3.1. Water Content.** The determination of the water content of the honey was carried out according to the differential weighing method. Thus, 5 g of honey is placed in an oven at 103°C for 2 hours [14].

**2.3.2. Free Acidity.** The free acidity of honey is the sum of all the free acids expressed in milliequivalents per kilogram of honey. The samples (10 g) were dissolved in distilled water (250 ml) and titrated with 0.1 M sodium hydroxide solution at pH = 8.3 of honey are dissolved in a beaker and stirred with a magnetic stirrer. The solution is then titrated with 0.001 M sodium hydroxide solution to pH 8.3. The free acidity, expressed in milliequivalents or millimoles of acid per

kilogram of honey, is equal to the volume of soda  $0.01 \text{ M} \times 1000$  [14].

**2.3.3. pH.** The pH was measured using a portal pH meter (HI 9025-HANNA) with a sample of honey diluted in 10% of distilled water [14].

**2.3.4. Ash Content.** The ash content was determined by heating 3 g of honey in a muffle furnace at  $600^\circ\text{C}$  for 2 hours. After cooling, the ash content is determined [14].

**2.3.5. Electrical Conductivity.** The electrical conductivity at 1/5 was determined according to the method described by Bogdanov et al. [14] using a conductometer (Cond 3210 WTW). The measurements were carried out at  $20^\circ\text{C}$  in a 20% aqueous solution with respect to the dry matter of the honey. The value of the conductivity was directly determined by the cell in the solution after immersion. The results were expressed in micro-Siemens per centimeter ( $\mu\text{S}/\text{cm}$ ).

**2.3.6. Total Sugars.** The total sugar content of the samples was determined by spectrophotometric assay according to the method described by Fox and Robyt [18]. In test tubes, 1 g of each sample is dispersed in 10 ml of 25% (v/v) DMSO. The mixture is incubated in a boiling water bath for 15 min and then 0.1 ml of this mixture in 9.9 ml of distilled water. A concentration range of the standard solution of D-glucose (0.05 mg/ml) was used to establish a standard sugars curve. At different glucose concentrations were added 0.5 ml of 5% phenol and 2 ml of 75%  $\text{H}_2\text{SO}_4$ . After 15 min incubation in a boiling water bath and then 15-minute incubation in the dark, the absorbance was read at 492 nm on the spectrophotometer (Biomate 3S, UV-Visible Spectrophotometer). For the determination of the total sugar content, 0.5 ml of 5% phenol was added to 0.5 ml of the previously prepared sample. After homogenization, 2 ml of 75%  $\text{H}_2\text{SO}_4$  was added. The mixture is then treated as the standard used to plot the calibration curve. The test is done in duplicate. The concentration of total sugars was deduced from the standard curve with D-glucose as the reference sugar (Figure 5).

**2.3.7. Reducing Sugars.** To a mixture of solution A (0.5 g of DNS in 10 ml of distilled water) and solution B (1.06 g of NaOH in 10 ml of distilled water), 17.4 g of sodium and potassium tartrate was added. The solution was then adjusted to 28.57 ml with distilled water. A volume (0.1 ml) of the sample previously dispersed in DMSO mixed with 0.4 ml of distilled water was reacted on a water bath boiling for 8 minutes with 1 ml of the DNS reagent. After cooling in an ice bath for 3 min, the absorbance was read at 546 nm on the spectrophotometer. The reducing sugar concentrations were calculated from the calibration curve using fructose as the standard (Figure 6).

**2.4. Microbiological Characterization of Honey Samples.** Total viable count, total coliform, sulfite-reducing anaerobic bacteria, *Salmonella* spp., yeast, and mold were investigated. Thus, 10 g of each sample was homogenized in 90 ml of sterile distilled water and serial decimal dilutions ( $10^{-1}$  and  $10^{-2}$ )

were made with the same solvent. All microbial tests were performed in duplicate.

**2.4.1. Total Viable Count.** The evaluation of the Total Mesophilic Aerobic Flora (TMAF) was carried out according to the indications of standard NP-3788: 2002. Briefly, 1 ml of each decimal dilution ( $10^{-1}$  and  $10^{-2}$ ) was aseptically deposited in sterile Petri dishes. Then 10 to 15 ml of Plate Count Agar (PCA OXOID CM0463) maintained at super atmospheric pressure at a temperature of  $20^\circ\text{C}$  was added and then the inoculum and the culture medium were perfectly homogenized. After complete solidification, plates were turned over and incubated at  $30^\circ\text{C}$  for 72 hours. TMAF colonies have a lenticular appearance.

**2.4.2. Total Coliform (ISO 4831: 2006).** The total coliform search was carried out according to ISO 4831: 2006. 1 ml of each decimal dilution ( $10^{-1}$  and  $10^{-2}$ ) was poured aseptically into sterile plates. Purple crystal, bile-lactose neutral red agar (VRBL), melted and cooled in a water bath at  $45^\circ\text{C}$ , was added to the inoculum at a rate of 15 ml per dish. The mixture was then homogenized by rotary movements. After solidification of the first layer, a second 5 ml layer of VBRL was added. Control of the sterility of the medium was carried out in a Petri dish with approximately 15 ml of VBRL. The total coliform count was done directly after incubation at  $30^\circ\text{C}$  for 24–48 hours. Fecal coliforms are characterized by a small mass of fluorescent colonies with a diameter of 0.5 mm [19].

**2.4.3. Sulfite-Reducing Anaerobic Bacteria.** The sulfite-reducing anaerobic bacteria research was carried out by the technique of enumeration in a solid medium in tubes. To 1 ml of each decimal dilution ( $10^{-1}$  and  $10^{-2}$ ), respectively, contained in sterile tubes, 20 ml of the melted Sulfite Tryptone Neomycin agar was added and cooled in a water bath at  $45^\circ\text{C}$ . After homogenization and solidification, the tubes were incubated anaerobically at  $37^\circ\text{C}$  for 24–48 hours. Colonies characteristics of sulfite-reducing anaerobic bacteria appear black in the tubes [19].

**2.4.4. Yeasts and Molds.** The counting of yeasts and molds was carried out by the spreading technique carried out by using 0.1 ml of the stock solution and its decimal dilutions ( $10^{-1}$  and  $10^{-2}$ ) on Sabouraud agar. Plates were incubated at room temperature for 5 days. Creamy white or whitish colonies characterize yeasts and fluffy colonies or rough molds.

**2.4.5. Salmonella.** The search for *salmonella* was done in four stages.

(a) *Preenrichment.* 25 g of each sample of honey was added to 225 ml of sterile buffered peptone water. The homogeneous solution ( $10^{-1}$ ) thus obtained was incubated at  $37^\circ\text{C}$  for 20 hours.

(b) *Enrichment.* The enrichment was carried out on the selective enrichment media Rappaport-Vassiliadis broth and selenite-cystine broth. 0.1 ml and 2 ml of the preenrichment

were inoculated into 10 ml of Rappaport-Vassiliadis broth and 20 ml of selenite-cystine broth. The tubes were, respectively, incubated at 41°C and 37°C for 24 hours.

(c) *Isolation*. Isolation was done on selective medium Hektoen Agar. The isolation medium was melted and cooled in a water bath at 45°C and then poured into a Petri dish. After solidification, it was cultured at the surface, using a platinum loop from the enrichment media. The plates were incubated for 24 hours at 37°C. After incubation, the colonies appear bluish with or without black center (production of H<sub>2</sub>S).

2.5. *Statistical Analysis*. The Microsoft Excel 2010 spreadsheet was used for the input and processing of microbiological and physicochemical data. The Statistical Analysis System 9.2 software (SAS v. 9.2) was then used for statistical analysis. These analyses consisted of analyses of variance with two factors, namely, season and climatic zone and a principal component analysis in relation to the geographical area. In addition, the interaction between geographical zone and season was estimated to evaluate the relations between the physicochemical parameters and the geographical zones. The mean values were then compared with each other using the Student-Newman-Keuls test at 5%.

### 3. Results and Discussion

3.1. *Physicochemical Characteristics*. The physicochemical characteristics (pH, water content, electrical conductivity, and mineral content) of the honeys are illustrated in Figure 2 and summarized in Tables 3 and 4.

3.1.1. *pH*. The pH values obtained ranged from 3.7 to 4.1 with an average of 3.8 (Table 3, Figure 2). These results confirm the acidic nature of honey [20]. Our results are similar to those (3.5 to 4.7) reported in Algeria [21], India, Brazil, Spain, and Turkey [8, 22]. Our values are lower than those obtained in several countries including Benin [11], Poland [23], Portugal [24], Nigeria [25], and Cameroon [12]. The acidity of honey is due to a large number of organic acids. The main acid is gluconic acid which is in equilibrium with its lactones or its esters and inorganic ions such as phosphates and chlorides. There are also formic, tartaric, maleic, citric, succinic, butyric, lactic, and oxalic acids as well as various aromatic acids [26]. The results of the analysis of variance indicate that the season significantly influences ( $p < 0.05$ ) the pH of the honey (Table 3). Thus, independently of the area, pH levels were significantly higher during the rainy season than in dry (Table 4). The results obtained are in contrast to those of Mbogning et al. [12] on the physicochemical characteristics of honeys in the western Sudan-Guinean zone and in Cameroon. Those authors assert that the pH of dry season and rainy season honey was not significantly different. During the rainy season, the most significant pH values were obtained in the Sudanian zone while the lowest values were obtained in the Guinean zone (Table 4). No significant difference ( $p > 0.05$ ) was observed for the different study areas (Table 3). These results are different with the observations in Cameroon [12, 27]. In the Sudanian zone,

rainy season honey showed a higher pH than dry season ones. The variation in pH could be due to the flavor of the bee, the salivary secretion of the bee, and the enzymatic and fermentative processes during the processing of the raw material, it being understood that the acids found in the honey come from the flowers and digestive secretions of bees [27].

3.1.2. *Water*. The water content of the honey analyzed varied from 12.1 to 13.2% with an average of 12.6% (Table 4, Figure 2). These values are well below the 18% recommended for tropical honeys [28]. The low water levels obtained reflect a mature harvest of the different honey samples [29]. These results are identical to those reported in Algerian [21] and Northern Ethiopian honeys [30]. However, values ranging from 14.6 to 17.2% (India) and 16.0 to 18.6% (Azerbaijan) were recorded on honey samples [8, 31]. The variation in water content is due to different environmental conditions such as climate, floral origin of honey samples, water content of nectars, processing techniques, and storage conditions [14, 20, 32]. Consequently, moisture content is a complex function of a large number of variables such as extraction and handling practices and hygroscopic nature, which in turn depends on climatic conditions, the time of year, the initial moisture of the nectar, the degree of maturation, and its geographical origin [33]. The results of the analysis of variance (Table 3) show that the water content varies significantly ( $p < 0.05$ ) according to the zones. Thus, the Student-Newman-Keuls test (Table 4) revealed that water contents were significantly higher in the Sudanian zone ( $12.6 \pm 0.1$ ) and lower in the Sudano-Guinean zone ( $12.1 \pm 0.1$ ). Our results corroborate those of Mbogning et al. [12], who proved that the water content of honeys in the Sudano-Guinean zone in Cameroon varies significantly ( $p < 0.05$ ) according to the regions. These variations would certainly be due to the fact that beekeepers do not have refractometers to test the water content of honey before harvesting. Also, the water content highly varies ( $p < 0.01$ ) according to the seasons. Thus, rainy season honey had higher water contents ( $12.9 \pm 0.2$ ) than dry season honeys ( $12.3 \pm 0.1$ ).

3.1.3. *Electrical Conductivity*. The electrical conductivity of honeys is closely related to their concentration of mineral salts, organic acids, and proteins. The studied honeys show electrical conductivities varying within 530.3–698.5  $\mu\text{s}/\text{cm}$  with an average value of 620.6  $\mu\text{s}/\text{cm}$  (Table 4, Figure 2). The values obtained corroborate those reported in Algerian honey [9]. Electrical conductivity is a good criterion for determining the botanical origin of honey [34]. This parameter is also used for the classification of monofloral honeys [35]. The zone and season do not significantly affect ( $p > 0.05$ ) the electrical conductivity of honey samples (Table 3). Our observations on the honey samples studied are contrary to those of Mbogning et al. [12]. Indeed, they noticed disparity between the different regions and a very significant influence of the season on the electrical conductivity of the honeys of Cameroon. However, the electrical conductivity was slightly strong for dry season honeys ( $635.7 \pm 29.7 \mu\text{s}/\text{cm}$ ) than those of the rainy season ( $605.5 \pm 32.8 \mu\text{s}/\text{cm}$ ). The variability of

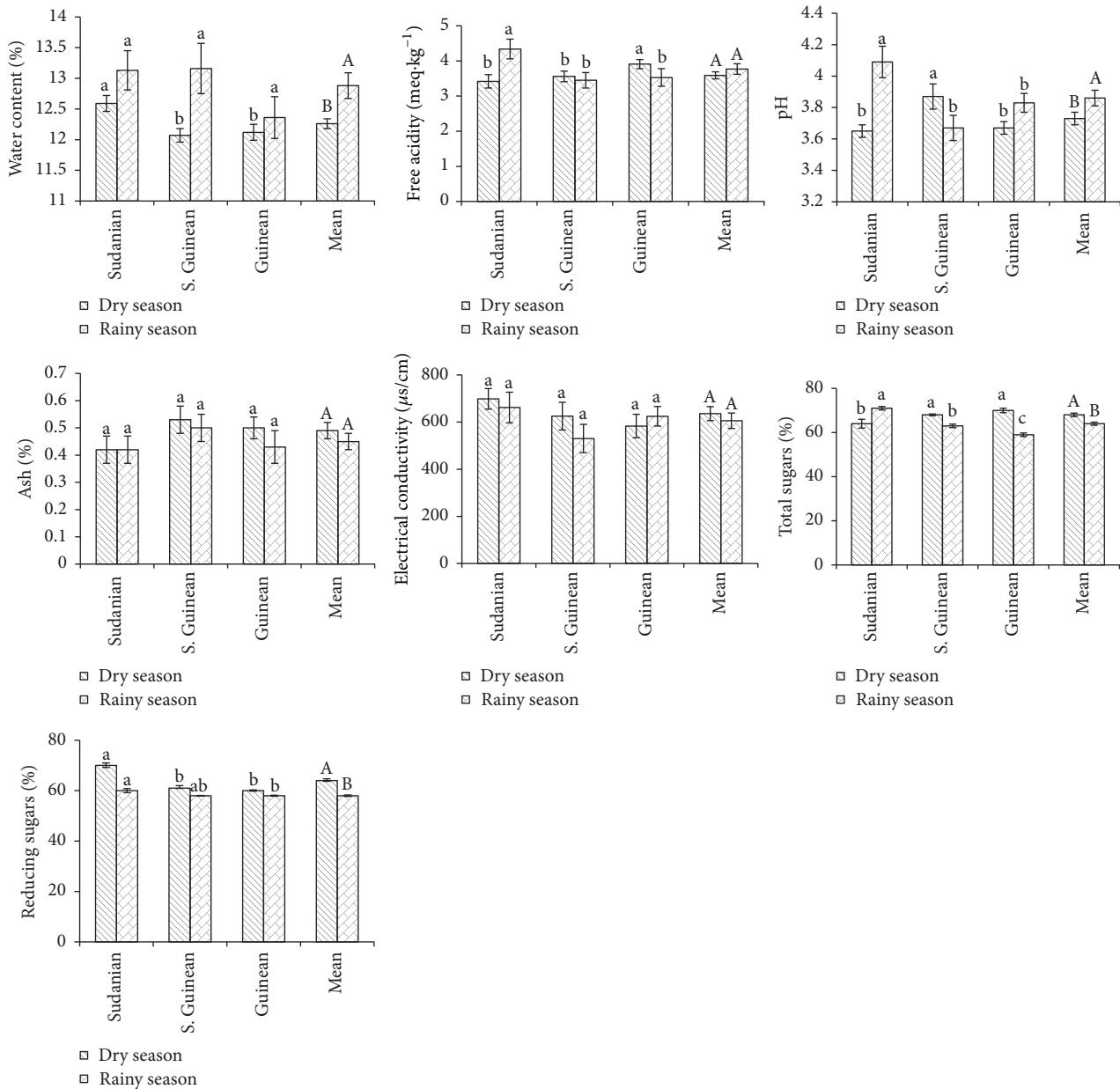


FIGURE 2: Water content, free acidity, pH, ash content, electrical conductivity, and total and reducing sugars of honeys according to zones and seasons. The means with different letters are significantly different with probability level of 5% according to Student-Newman-Keuls test.

the results could be due to fluctuations of mineral salts, organic acids, and proteins concentrations [34]. The values obtained in this study are in line with the European Directives standards ( $\leq 0.8$  ms/cm for nectar honey and  $\geq 0.8$  ms/cm for honeydew honey) [36]. All the samples of honey analyzed have conductivities not exceeding 0.8 m/cm, thus making it possible to classify them as honey obtained from the nectar of the flowers.

**3.1.4. Ash.** The ash represents the mineral residue of the honey after incineration. The determination of the ash offers

the possibility of knowing the overall mineral content of the honey [37]. According to Vanhanen et al. [38], ash is fundamentally and quantitatively dependent on soil and climate characteristics of the honey region of origin. It is also considered as a quality criteria that indicates the possible botanical origin of honey. Its value in the analyzed samples varied from 0.4 to 0.5% with an average value of 0.5% (Table 4, Figure 2). The ash values of our samples are in the range of 0.1–0.5% obtained on the honey samples collected in Algeria [31]. The permissible limit of ash content of honeys nectar is 0.6% [20] and 1.2% for honeydew honey [39]. The ash

values found were below 0.6% and are consistent with the limit allowed by [28] for nectar honeys. We note no significant differences ( $p > 0.05$ ) between zone and season (Table 3).

**3.1.5. Free Acidity.** The free acid values of the honey analyzed ranged within 35.7–40.5 meq/kg (Table 4, Figure 2). The values obtained were lower than the 50.4 meq/kg obtained in Algeria [40] and higher than the 17.6 meq/kg reported in Azerbaijan [31]. The acidity of honey is mainly due to the presence of organic acids, particularly the gluconic acid and inorganic ions such as phosphate and chloride [25]. No significant differences ( $p > 0.05$ ) were observed between the zones and between seasons. However, significant differences ( $p < 0.01$ ) were observed among dry season samples (Table 3). The highest value was recorded in samples from the Guinean zone ( $40.5 \pm 0.9$ ) and lower in samples from the Sudanian zone ( $35.7 \pm 0.9$ ). Globally, our samples showed acceptable acidity levels (50 meq/kg) according to the Codex Alimentarius criteria [28]. The low free acid values obtained in the present work are a good indicator of conservation since strong acidity promotes the degradation of hexoses to hydroxymethylfurfural [41, 42].

**3.1.6. Total Sugars and Reducing Sugars.** All honey tested contained total sugars and reducing sugars at different concentrations (Table 4, Figure 2). The total and reductive sugars obtained are within the limits of acceptable values, which shall not be less than 60 g/100 g of flower honey and 45 g/100 g of honey honeydew [28]. The grades obtained for total and reducing sugars vary within 60–70% (flower honey) and 58–70% (honey honeydew). These results confirm that sugars are the major constituents of honey. Total sugars obtained are comparable to those of certain honey from Pakistan (61.7–72.4%) and Brazil (67.6–72.4%) [43, 44] but are lower than those reported in Iran (74.0–81.8%), Algeria (69.1–82.1%), Burkina Faso (73.9–85.5 g/100 g), and Cameroon (77.9–83.1%) [45–47]. These values are higher than those of honey collected in western India (42.8–60.6%) [48]. As for reducing sugars, our results are similar to those obtained with Indian (62.2–70.2%) and Pakistan (57.7–70.5%) honeys [8, 44] but higher than those reported in Algeria (34.5–50.3%) [49]. However, these values are lower than those of Portuguese honeys which range within 64.5–80.0% [24]. The results of the analysis of variance (Table 3) show that the total sugars and reducing sugars significantly ( $p < 0.05$  to  $p < 0.001$ ) vary from a climatic zone to another and from a season to another. The Student-Newman-Keuls test showed that, during the dry season, reductive sugar levels are significantly higher in the Sudanian zone while they are low in the Guinean zone. Conversely, the highest levels of total sugars are recorded with the Guinean zone honeys. During the rainy season, the same test indicates that the total sugars and reducing sugars had the most significant values in the Sudanian zone while the lowest values were obtained in the Guinean zone. The values of total and reducing sugars were higher in honeys of the dry season than in the rainy season. (Table 3). These elements appear to be more available in the plant in the dry season than in the rainy season. Thus, we can

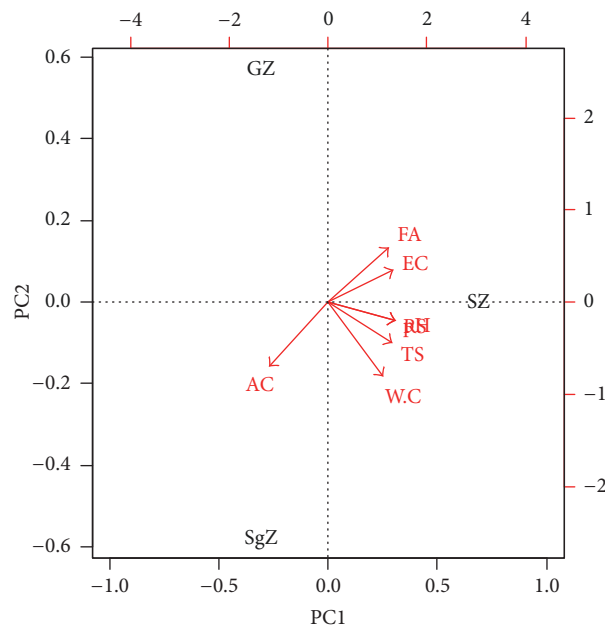


FIGURE 3: Factorial axis plane showing the simultaneous projection of physicochemical parameters of honey from different geographical areas. SZ: Sudanian zone; SgZ: Sudano-Guinean zone; GZ: Guinean zone; AC: ash content; EC: electrical conductivity; WC: water content; FA: free acidity; pH: hydrogen potential; TS: total sugars; RS: reducing sugars.

say that rains are a disadvantage to the concentration of sugars in honey.

**3.1.7. Relationship between the Different Physicochemical Parameters.** The results of the Principal Component Analysis (PCA) show that the first two main components account for all the variability related to the concentration and the content of the various parameters evaluated in the honeys of the different study zones. The honey of the Sudanian zone is characterized essentially by an electrical conductivity, total and reducing sugar agent, and high pH as opposed to honeys in the Guinean and Sudano-Guinean zones which have very high ash content (Figure 3). The honey of the Guinean and Sudanian zone shares a relatively high level of acidity. On the other hand, the water content is high for the Sudanian zone and the Sudano-Guinean zone. Globally, the honey of the Sudanian zone is richest in all the physicochemical parameters evaluated except the ash content. Similarly, the relationship between the physicochemical characteristics of the honeys and the seasonal and geographic interaction was realized. The PCA showed that the first two main components account for 62.8% of the variability related to the physicochemical parameters in honeys according to seasons and zones. Thus, only honey produced in the dry season in the Guinean zone contains a higher ash content than the other zone independently of the seasons (Figure 4). The Sudanian zone honey in the dry season displays a high acidity, a high rate of reducing sugar, and high electrical conductivity. The honey produced in the Sudanian rainy season has a high water content, high pH, and electrical conductivity. Honey

TABLE 2: Results of the microbiological analysis.

| seasons | Areas      | TMAF (cfu/g)       | Yeasts and molds (cfu/g) | Microorganisms          |                                    |                               |
|---------|------------|--------------------|--------------------------|-------------------------|------------------------------------|-------------------------------|
|         |            |                    |                          | Total coliforms (cfu/g) | Sulfite-reducing anaerobic (cfu/g) | <i>Salmonella</i> spp.(cfu/g) |
| Dry     | Sudanian   | $1.81 \times 10^2$ | $9.09 \times 10^2$       | <10 cfu/g               | <10 cfu/g                          | Absence                       |
|         | S. Guinean | $1.90 \times 10^2$ | $1.34 \times 10^3$       | <10 cfu/g               | <10 cfu/g                          | Absence                       |
|         | Guinean    | $2 \times 10^2$    | $8.18 \times 10^2$       | <10 cfu/g               | <10 cfu/g                          | Absence                       |
|         | Mean       | $1.90 \times 10^2$ | $9.54 \times 10^2$       | <10 cfu/g               | <10 cfu/g                          | Absence                       |
| Rainy   | Sudanian   | $2.90 \times 10^2$ | $1.09 \times 10^3$       | <10 cfu/g               | <10 cfu/g                          | Absence                       |
|         | S. Guinean | $2.45 \times 10^2$ | $1.2 \times 10^3$        | <10 cfu/g               | <10 cfu/g                          | Absence                       |
|         | Guinean    | $2.27 \times 10^2$ | $1 \times 10^3$          | <10 cfu/g               | <10 cfu/g                          | Absence                       |
|         | Mean       | $2.54 \times 10^2$ | $1.1 \times 10^3$        | <10 cfu/g               | <10 cfu/g                          | Absence                       |

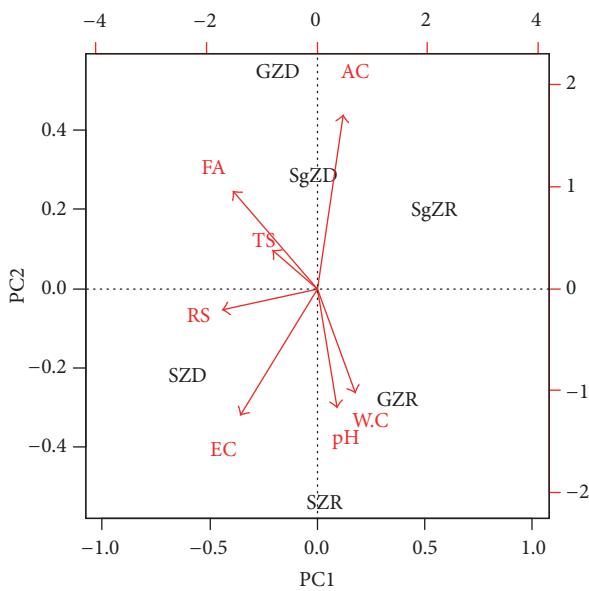


FIGURE 4: Factor axis plane showing the simultaneous projection of physicochemical parameters of the honey of the different zones in relation to the seasons. SZD: Sudanian zone dry; SgZD: Sudano-Guinean dry; GZD: Guinean zone dry; SZR: Sudanian zone rainy; SgZR: Sudano-Guinean rainy; GZR: Guinean zone rainy; AC: ash content; EC: electrical conductivity; WC: water content; FA: free acidity; pH: hydrogen potential; TS: total sugars; RS: reducing sugars.

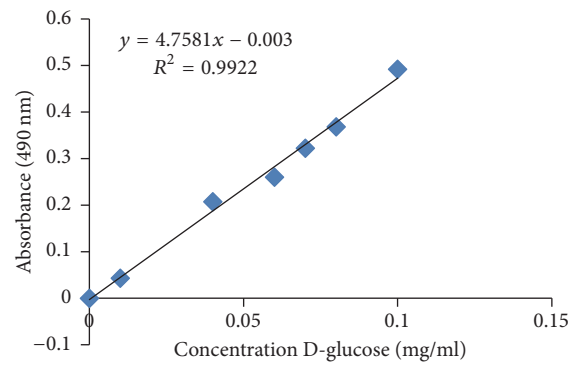


FIGURE 5: Calibration curve of D-glucose.

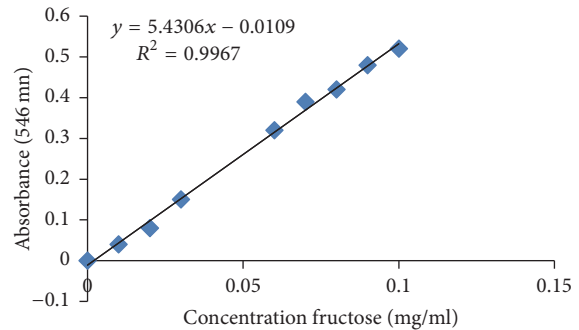


FIGURE 6: Calibration curve of fructose.

from the Guinean zone in the rainy season has relatively high water content, pH, and relatively high electrical conductivity. The honey produced either in the rainy or in the dry season in the Sudano-Guinean zone has an acidified and a relatively high content ash.

3.2. *Microbiological Contamination.* The level of microbial contamination of the different honey samples is shown in Table 2. The contamination with Total Mesophilic Aerobic Flora varies within 180–290 cfu/g. This result conforms to the French standard (ECOC0300092V standard in France, 2003), which suggests levels below 1000 cfu/g. The analysis

of the table revealed a high level of Total Mesophilic Aerobic Flora in the rainy season honey samples ( $2.54 \times 10^2$  cfu/g), while the low rate was recorded in the dry season samples. Those observations are higher than the 10 cfu/g reported in Algeria [50] and the 100 CFU·g<sup>-1</sup> in Romania [51] but lower than results obtained in Nigeria [52] and Cameroon [53]. Total coliforms are absent in our analyzed samples. This indicates good hygienic practice by beekeepers. The absence of fecal coliforms was also observed in some honey samples in Spain [54], Argentina [55], and Morocco [56]. However, total coliforms were reported (between 1 cfu/g and 3.10 cfu/g) in Nigeria [52]. The presence of sulfite-reducing anaerobes is



TABLE 3: Analysis of variance (F value) of the physicochemical parameters in the different phytogeographical zones and according to the seasons. The values in parentheses represent the probabilities.

| Sources of variation | DF | Values                |                     |                    |                    |                         |                      |                        |
|----------------------|----|-----------------------|---------------------|--------------------|--------------------|-------------------------|----------------------|------------------------|
|                      |    | Water content         | pH                  | Free acidity       | Ash content        | Electrical conductivity | Total sugars         | Reducing sugars        |
| Seasons              | 1  | 9.22**<br>(0.003)     | 5.08*<br>(0.026)    | 0.23 ns<br>(0.634) | 0.74 ns<br>(0.391) | 0.47 ns<br>(0.493)      | 11.62***<br>(0.001)  | 100.70***<br>(<0.0001) |
| Areas                | 2  | 3.10*<br>(0.048)      | 1.68 ns<br>(0.191)  | 1.18 ns<br>(0.309) | 2.26 ns<br>(0.109) | 1.96 ns<br>(0.145)      | 3.37*<br>(0.038)     | 42.61***<br>(<0.0001)  |
| Rep                  | 2  | 12.07***<br>(<0.0001) | 0.03 ns<br>(0.866)  | 1.58 ns<br>(0.208) | 0.11 ns<br>(0.746) | 1.62 ns<br>(0.205)      | 0.46 ns<br>(0.497)   | 6.00*<br>(0.016)       |
| Season × area        | 2  | 1.48 ns<br>(0.230)    | 9.49***<br>(0.0002) | 6.42 ns<br>(0.002) | 0.33 ns<br>(0.721) | 0.81 ns<br>(0.447)      | 29.38 ns<br>(<0.001) | 19.34***<br>(<0.0010)  |

DF: degree of freedom; ns =  $p > 0.05$  (not significant); \*  $p < 0.05$  (significant); \*\*  $p < 0.01$  (highly significant); \*\*\*  $p < 0.001$  (very highly significant).

TABLE 4: Physicochemical characteristics of honey samples (means  $\pm$  standard error) according to seasons and phytogeographical zones.

| Seasons | Areas      | Water content<br>(%) [ $<20$ ]* | pH<br>[3.2–4.5]* | Free acidity<br>(meq·kg <sup>-1</sup> )<br>[ $<50$ ]* | Ash content<br>(g/100g)<br>[ $<0.6$ ]* | Electrical<br>conductivity<br>( $\mu$ S/cm)<br>[ $\leq 0.8$ ms/cm]* | Total sugars<br>(g/100 g)<br>[ $>0.6$ ]* | Reducing sugars<br>(g/100 g)<br>[ $>0.6$ ]* |
|---------|------------|---------------------------------|------------------|---|--|---|--|---|
| Dry     | Sudanian   | 12.59 $\pm$ 0.13a               | 3.65 $\pm$ 0.04b | 35.67 $\pm$ 0.94b                                     | 0.42 $\pm$ 0.05a                       | 698.50 $\pm$ 43.63a   | 64 $\pm$ 0.02b                           | 70 $\pm$ 0.01a                              |
|         | S. Guinean | 12.07 $\pm$ 0.11b               | 3.87 $\pm$ 0.08a | 39.72 $\pm$ 1.05a                                     | 0.53 $\pm$ 0.05a                       | 625.25 $\pm$ 58.81a   | 68 $\pm$ 0.004a                          | 61 $\pm$ 0.01b                              |
|         | Guinean    | 12.12 $\pm$ 0.13b               | 3.67 $\pm$ 0.04b | 40.52 $\pm$ 0.92a                                     | 0.50 $\pm$ 0.04a                       | 583.25 $\pm$ 49.77a   | 70 $\pm$ 0.01a                           | 60 $\pm$ 0.003b                             |
|         | Mean       | 12.26 $\pm$ 0.08B               | 3.73 $\pm$ 0.04B | 38.63 $\pm$ 0.60A                                     | 0.49 $\pm$ 0.03A                       | 635.67 $\pm$ 29.66A   | 68 $\pm$ 0.008A                          | 64 $\pm$ 0.007A                             |
| Rainy   | Sudanian   | 13.13 $\pm$ 0.32a               | 4.09 $\pm$ 0.10a | 39.43 $\pm$ 1.39a                                     | 0.42 $\pm$ 0.05a                       | 661.75 $\pm$ 64.96a   | 71 $\pm$ 0.008a                          | 60 $\pm$ 0.008a                             |
|         | S. Guinean | 13.16 $\pm$ 0.41a               | 3.67 $\pm$ 0.08b | 37.75 $\pm$ 0.89a                                     | 0.50 $\pm$ 0.05a                       | 530.25 $\pm$ 59.94a   | 63 $\pm$ 0.008b                          | 58 $\pm$ 0.001ab                            |
|         | Guinean    | 12.36 $\pm$ 0.34a               | 3.83 $\pm$ 0.06b | 37.53 $\pm$ 0.81a                                     | 0.43 $\pm$ 0.06a                       | 624.50 $\pm$ 41.57a   | 59 $\pm$ 0.008c                          | 58 $\pm$ 0.002b                             |
|         | Mean       | 12.88 $\pm$ 0.21A               | 3.86 $\pm$ 0.05A | 38.24 $\pm$ 0.61A                                     | 0.45 $\pm$ 0.03A                       | 605.50 $\pm$ 32.80A   | 64 $\pm$ 0.007B                          | 58 $\pm$ 0.003B                             |

The means with different letters are significantly different with probability level of 5% according to Student-Newman-Keuls test. \* Standard values according to Codex Alimentarius 2001.

an indicator of contamination of honey. Our results indicate the absence of these in the analyzed honey samples (Table 2). Similar observation was made concerning *Clostridium* in Morocco [50]. On the other hand some studies indicated the contamination of honey samples by sulfite-reducing anaerobes particularly *Clostridium botulinum* (between 3 cfu/g and 23 cfu/g) in Cameroon [48], Sweden, Norway, and Denmark [43, 57].

The fungal microbial load of the analyzed samples varied from  $8.18 \times 10^2$  to  $1.34 \times 10^3$  cfu/g. Reference [56] revealed levels below 100 cfu/g in the Moroccan honey. Gomes et al. [15] and Popa et al. [51] reported rates below 40 cfu/g in honey in Algeria and Romania. In the study conducted by Omafuvbe and Okanbi [52], no mold contamination was detected.

#### 4. Conclusion

This study describes the physicochemical and microbiological characteristics of 60 samples of honey collected during the dry and rainy season in the Sudanian, Sudano-Guinean, and Guinean zones of Benin. Apart from the reducing sugar content, the other physicochemical parameters of the honey analyzed are within the range of recommended values by the Codex Alimentarius and the European Directive. pH, water content, total, and reducing sugars are significantly influenced by region and season. Water levels and pH were significantly higher during the rainy season in all climatic zones, while total and reducing sugars were higher in dry season honeys. The differences observed in this work constitute a basis for the data for the definition of quality standards for honeys in the different phytogeographical zones of Benin. Total viable count, yeasts, and molds, characteristics of commercial quality, are present in our honey samples, but these are not in any way a danger to human health.

#### Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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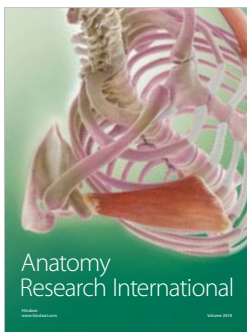
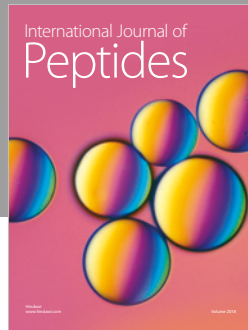
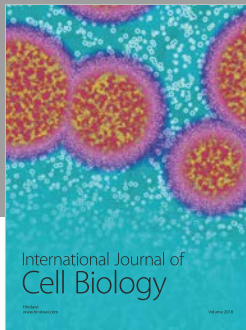
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