Research Article

POLLEN ANALYSIS AND ANTIOXIDANT ACTIVITIES OF HONEY FROM MODERN BEEKEEPING IN THE WORODOUGOU REGION, COTE D'IVOIRE

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ABSTRACT

The purpose of this study is to evaluate the botanical origin, as well as the antioxidant activities of honeys derived from hives installed in cashew orchards in Séguéla (Côte d'Ivoire). Pollen analysis of four samples revealed that three honeys are multifloral (MC, MF, MW), while only one (MB) is monofloral (Lannea acida). In addition, this pollen analysis identified 73 plant taxa belonging to 32 families pollinated by bees. Species richness ranges from 1 to 31 taxa per honey sample. The most dominant plant families are Fabaceae, Euphorbiaceae and Asteraceae. All honeys analyzed have antioxidant activities. Bobi (MB) honey has both the best antiradical activity (IC$_{50}$ = 62.12 μg / ml) and the good reducing power (9.18%). In addition, all honeys derived from Séguéla unlike commercial honey, are dark shades reflecting their high content of phenolic compounds and flavonoids. All its physicochemical parameters comply with the standards proposed by the Codex Alimentarius Commission. It is therefore clear that honeys from modern beekeeping in Séguéla meet the quality criteria required for the marketing and consumption of honey.

INTRODUCTION

Beekeeping is the breeding of bees for the production of honey (Sfich *et al*., 2017). Honey production has evolved from honey hunting to traditional beekeeping with traditional beehives to modern beekeeping, which nowadays employs modern hives (Kelomey *et al*., 2015).

Beekeeping is an important sector of the agricultural economy, both because of the role played by bee populations in pollination and in the production of honey (François, 2012).

Total world honey production is about 1.2 million tonnes per year. Reliable statistics are difficult to obtain because beekeeping is mostly small-scale (FAO, 2010).

In Africa, the beekeeping industry has experienced a boost since 2013, in terms of production and export volumes as well as innovative techniques. It accounted for about 9% of world production, or 155,789 tonnes, according to the most recent FAO data, which corresponds to a 10% increase since 2000 (Bénédicte, 2017).

The beekeeping sector, however, is little developed in Côte d'Ivoire despite strong local and international demand because there are very few beekeeping farms with relatively low apiculture production. There are small-scale initiatives, such as a UNDP program (UNDP, 2017), but the sector has never had sufficient investment to develop. As a result, the production of honey remains quite low.

Honey has several nutritional and therapeutic properties and is used for the treatment of many diseases (Nija 1998, Peter 2006). Honey is also valuable as a marketable commodity in both domestic and international markets and plays an important role in some cultural traditions (Canini *et al*., 2005).

However, honey development stages are complex and likely to be altered by human activities, whether voluntarily or not (Lequet, 2010). Therefore, the absence of good quality honey on the national market constitutes a brake for the promotion of this sector. Indeed, most Ivorian beekeepers are confronted with multiple problems. These problems are due to the lack of mastery of apicultural practices. This sometimes leads to the closing of some beekeeping operations. Faced with this situation, it is necessary to characterize honeys to ensure good quality. It is in this perspective that the present study is carried out.

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The objective of this work is to evaluate the quality of honeys from hives installed in cashew orchards in Séguéla (Ivory Coast) by modern beekeepers. It is a specific way of identifying in a first time, the botanical origin of the honeys through their pollen analyzes to determine the appellations and to evaluate in a second time the antioxidant activities of the honeys

MATERIAL AND METHODS

Geographical location of the study area

The honey samples analyzed in this study come from Séguéla, a city located in the northwest of Côte d'Ivoire (West Africa). The city of Séguéla is located at latitude 7 ° 57'36 " North and longitude 6 ° 40'22 " West and is the capital of the Worodougou region and the Woroba district. With a population of 136,091 inhabitants (RGPH 2014), the city of Séguéla is distant from the economic capital (Abidjan) of 516 kilometers. The Worodougou region is bounded to the north by the Denguélé and Savanes regions, to the east by the Bandama valley region, to the west by the Bafing and Eighteen mountains regions and to the south by the regions of Haut Sassandra and Marahoué (Figure 1).
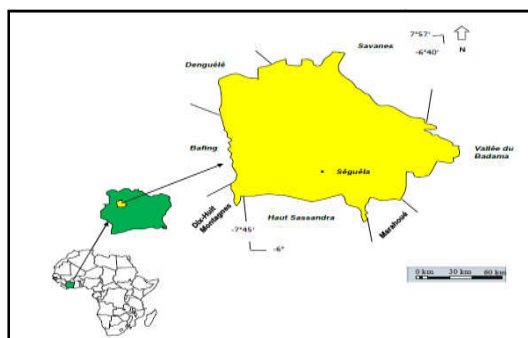

Figure 1 Location of the study area (Séguéla, Côte d'Ivoire)

Biological material

During this study, different honey samples were collected. The samples collected are four in number (Figure 2). Among these 4 samples, three come from different localities in the region of Séguéla, this is the Bobi (MB) honey sample, the Foronan honey sample (MF) and the honey sample. from Wongué (MW). The fourth sample comes from a supermarket in the Daloa (TM) Mall.

Figure 2 Honeys samples

Sampling

The three honey samples (MB, MF and MW) were obtained in cashew orchards from cooperatives in three villages in the Séguéla region (Table 1). They were collected directly in 1 liter cans with automatic crimping. MC honey comes from a supermarket in Daloa Mall. All four (04) honey samples collected were sent to the laboratory for analysis. The harvest period for MB, MF and MW honey is between April and March of 2017.

Table 1 Geographical origin of honey samples

Honey sample code	Locality of origin	Region
MB	Bobi	Séguéla
MC	Daloa (Trade Center)	Daloa
MF	Forona	Séguéla
MW	Wongué	Séguéla

Pollen analysis of honeys

The technique used for the pollen analysis of the 4 honey samples is the Erdtman Method (1960). This methodology includes the following steps: - Acetolysis of the honey samples: For each sampled honey, 20 milliliters or 20 grams were taken, then mixed with 20 ml of pure acetic acid. This mixture is named M1 and allowed to stand for 30 minutes. In another jar, a mixture (M2) of 18 ml of acetic anhydride + 2 ml of sulfuric acid is produced. The mixture M2 must be done gently, stirring under an aspirating host, because the reaction is exothermic. After 30 minutes of the first mixture (M1), the mixture M1 + M2 is produced to give an acid mixture, which is left to react for 24 hours. After this time, the acid mixture is heated in a water bath at a temperature below 40 ° C for a few minutes, until boiling. The slightly cooled mixture is then filtered through a fine sieve to minimize debris from honey harvesting. It is centrifuged at 3000 rpm for 10 min, then underwent multiple washings, firstly 3 times with alcohol (95 °) and then 3 times with distilled water, by successive centrifugations to carefully remove the reagents. . The last pellet collected after centrifugation is added a few drops of glycerin and stored for microscopic examinations.

Determination and counting of pollen grains: A sample of 20 to 40 microliters of the pellet collected by centrifugation for each honey sample is mounted between slides and lamellae for microscopic observation. For each sample of honey, three sets of preparation were made; and each assembly constituting an essay. The observations were made using a bilocular light microscope at magnifications 400X and 1000X. The set of slides prepared for each sampled honey are then explored one by one in order to identify the pollens found there, and then counts are made as and when on horizontal lines from one periphery to another of the preparation so that counted pollens are representative of the entire palynological population of each slide. The determinations were made by comparison with the collections of pollen grain reference slides and the illustrations of the works available in the laboratory. Pollen identification may not be often pushed to the genus or species. The pollen content is determined by the following formula: T p = (n × 10³ × d × 10) / 20 with T p: pollen content; n p: number of pollen on a blade and density of honey.

Determination of the relative pollen density of taxa: The relative density is expressed as the percentage quotient of the absolute density of a pollen type on the sum of the absolute densities of all types of pollen in the sample. It is calculated for each sample of honey with at least 1200 grains of pollen. The assessment was made using the Feller-Desmaly and Parent (1989) method, which distinguishes dominant pollen (> 45%);

accompanying pollen (16-45%); important isolated pollens (3-15%); and isolated pollens (<3%).

Dosing of total polyphenols

The method of Wood *et al.* (2002) was used for the determination of total polyphenols. A volume of 2.5 ml of Folin-Ciocalteu reagent diluted to the tenth was added to 30 .mu.l of extract. The mixture was kept for 2 minutes in the dark at room temperature, then 2 ml of sodium carbonate solution (75 g / l) was added. Then, the mixture was placed for 15 minutes in a water bath at 50 ° C and then rapidly cooled. Absorbance was measured at 760 nm, with distilled water as white. A calibration line was made with gallic acid at different concentrations. The analyzes were carried out in triplicate and the concentration of polyphenols was expressed in milligram gallic acid equivalent per gram of extract (mg EAG / g of honey).

Determination of total flavonoids

The total flavonoid assay was performed according to the method described by Marinova *et al.*, 2005. In a 25 mL vial, 0.75 mL of 5% (w / v) sodium nitrite (NaNO 2) was added to 2.5 ml of extract. To the mixture, 0.75 ml of 10% (w / v) aluminum chloride (AlCl 3) was added, and then the whole was incubated for 6 minutes in the dark. Once this time had elapsed, 5 ml of sodium hydroxide (1N NaOH) was added thereto and the volume was made up to 25 ml. The preparation was vigorously shaken before the determination of total flavonoids at 510 nm with the UV-visible spectrophotometer. The content of total flavonoids was expressed in milligram equivalent quercetin per gram of extract (mg EQ / g of honey). The tests were performed in triplicate.

Antioxidant activities of honey

ABTS method (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid)

This method is based on the ability of the compounds to reduce the radical-cation ABTS + ° (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid). The test was performed according to the method described by Choong *et al.*, (2007). The ABTS + radical-cation was produced by reaction of 8 mM ABTS (87.7 mg in 20 ml of distilled water) and 3 mM of potassium persulfate (0.0162 g in 20 ml of distilled water) in a ratio of 1: 1 (v / v). The mixture was then incubated in the dark at room temperature for 12-16 hours. This ABTS + solution was diluted with methanol to obtain a solution whose absorbance was 0.7 ± 0.02 at 734 nm. Thus, a test portion of 3.9 ml of this diluted ABTS + solution was added to 100 µl of the test compound. After stirring, the mixture was incubated for 6 minutes in the dark (T = 30 ± 2 ° C) (Feng-Lin *et al.*, 2010). The residual absorbance of the ABTS + ° radical was then measured at 734 nm with the UV-visible spectrophotometer. The tests were performed in triplicate.

The percentage inhibition of the ABTS radical is calculated according to the following equation: % I = [(Abscontrôle - Absextrait) / Abscontrôle] x 100 % I: percentage of inhibition of the extract Abscontrol: diluted ABTS absorbance, Absextrait: absorbance ABTS diluted + sample The results were expressed in Trolox µmol equivalent per gram of extract (µmol Eq Trolox / g of honey) according to the following

formula: Concentration (µmol Eq trolox / g extract) =% I / (4.99) 4.99: guideline coefficient of the standard line (% inhibition as a function of trolox concentration). DPPH (2,2'-diphenyl-1-picrylhydrazyl) method. The measurement of the antiradical activity of the four honey extracts was carried out by the 2,2'-diphenyl-1-picrylhydrazyl test (DPPH) according to the method of Parejo *et al.* (2000) with some modifications. A range of concentrations (0-1000 µg / ml) of honey extract or gallic acid (reference antioxidant) is prepared. A volume of 2.5 ml of this solution is mixed with 2.5 ml of DPPH (100 µM) prepared in methanol. After homogenization, the mixture is incubated at room temperature (25 ° C.) in the absence of light. After 15 minutes of incubation, the absorbance is read at 517 nm against a "white" which contains only methanol. The percentage inhibition of the DPPH radical is calculated according to the following equation: Inhibition of DPPH (%) = (1- (OD test / ODblanc)) x 100.

The IC50 which is the concentration of honey extract or gallic acid responsible for 50% inhibition of the DPPH radicals. It is determined on the graph representing the percent inhibition of DPPH as a function of the concentrations of the extracts of honey and gallic acid.

Statistical analysis

The descriptive analysis of the results is performed with the Microsoft Office Excel 2013 software, to determine averages, standard deviations and correlation coefficients. One-way analysis of variance (ANOVA), followed by Turkey's HSD test (the largest significant difference), was applied using the Statistica 7.1 software to highlight significant differences at the p-score. <0.05 between samples for each parameter. Each sample was assigned alphabetical letter. For samples that show no significant difference, they are represented by the same letters.

RESULTS AND DISCUSSION

Pollen analysis of honeys Pollen spectra of honeys

The pollen analysis of the 4 honey samples made it possible to inventory 64 taxa determined up to the species level, 6 taxa at the genus level and 3 taxa at the family level. A total of 73 pollen taxa belonging to 32 families of plants were determined on all the honeys analyzed without counting the indeterminate taxa. Some samples have taxa in common and different taxa. Species richness ranges from 1 to 31 taxa per sample. The pollen spectra of the 4 honey samples (MB, MC, MF and MW) show their diversity and their pollen richness. This pollen richness ranges from 81 to 827 (Tables 2, 3, 4 & 5). MW has the highest value (827) while MC has the lowest value (81).

The pollen abundance of each sample is characterized by the relative density of each taxon expressed by a rate (%) (Tables 2, 3, 4 & 5). On the pollen spectrum of the 4 samples, only two mention Western Anacardium with very low pollen densities (MF: 6.26% and MW: 0.24%) (Tables 4 & 5) while three of the samples (MF MW, MB) are supposed to come from beehives arranged in fields of cashew nuts.

Distribution of families according to pollen taxa

According to their distribution rate in the 4 samples, the 32 families were classified into three classes. The class of families

with a percentage greater than 10%: Fabaceae (15.07%), Euphorbiaceae (15.07%) and Asteraceae (12.33%). Also, there is the class of families with a percentage between 5 and 10%: Arecaceae (5.48%), Caesalpinaceae (9.59%), Anacardiaceae (9.59%), Poaceae (6, 85%), Combretaceae (5.48%) and Mimosaceae (8.22%). And finally the class of families with an incidence of less than 5%: among others: Amaranthaceae (4.11%), Ulmaceae (4.11%), Cycadaceae (2.74%), Moraceae (2.74%), Annonaceae (4.11%), Lamiaceae (1.37%), Myrtaceae (1.37%), Vitaceae (2.74%) (Figure 3).
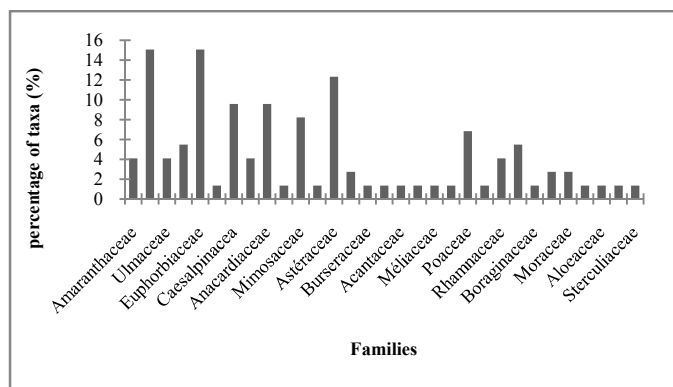

Figure 3 Distribution of families according to pollen taxa

Categories of honey pollens according to density

Of all samples, only MB showed dominant pollen (≥ 45%). The dominant pollen of MB is that of Lannea acida with 51.03%; the accompanying pollen is Mangifera indica (20%); the important isolated pollens are Hymenocardia acida, Elaeis guineensis, Canavaria virosa and all other taxa are rare isolated pollens. MB can then be called Lannea acida honey. Bobi honey is therefore a monofloral honey, it is taxonomically poor (Table 7). Commercial honey contains no dominant pollen but only important isolated pollens (Aspilia africana, Cassia, Indigofera, Lophira alata etc.) and rare isolated pollens (Ageratum conyzoides, Bauhinia rufescens, Khaya senegalensis, Pluchea ovalis etc.). The taxon Lannea acida that was present and dominant in the MB is not listed. MC is a polyfloral honey or all flowers. It is moderately rich in pollen taxa (Table 7). The Foronan (MF) sample contains no dominant pollen, but an accompanying pollen (Elaeis guineensis with 18.59%), important isolated pollen (Alchornea cordifolia, Western Anacardium, Encephalartos natalensis, Lannea acida etc.) and rare isolated pollens (Bidens senegalensis, Canavaria virosa, Celtis integrarifolia, Chlorophyton tuberosum, Cissus rufescens etc). MF is a polyfloral honey or all flowers. It is moderately rich in pollen taxa (Table 7).The Wongué (MW) sample also has no dominant pollen, but only other types of pollen (Table 7). It has the largest number of pollen taxa (31 taxa). MW is also a polyfloral or all-flower honey and is moderately rich in pollen taxa. MC MB MF MW honeys belong respectively to classes I, II, III, IV. MC and MB are very poor and pollen-poor while MF and MW are rich and very rich in pollen respectively (Table 6).

Appearance of some grains of pollen

Pollen analysis of the honey samples revealed some isolated pollen grains. The pollen of Alchonea cordifolia (Euphorbiaceae) has a more or less rounded shape with three pores (Figure 4). That of western Anacardium (Anacardiaceae)

has an oval shape with a single pore (Figure 5). The pollen of Ficus sycomorus (Moraceae) has a spherical shape and has no furrows or pores (Figure 6); Aspilia africana (Asteraceae) has a spherical shape with multiple furrows and pores (Figure 7). The pollen of Isoberlinia doka (Caesalpiniaceae) has a more or less rounded shape and has an ornamentation on its surface with a single pore (Figure 8), Elaeis guineensis (Arecaceae) has a triangle shape and does not have pores (Figure 9).

Table 2 Pollen Spectrum of Bobi Honey (MB).

N°	Pollen taxa	Families	Pollen abundance	
			Density	Rate (%)
1	*Amaranthus vivridis*	Amaranthaceae	2	1.38
2	*Canavaria virosa*	Fabaceae	5	3.45
3	*Celtis integrifolia*	Ulmaceae	3	2.07
4	*Elaeis guineensis*	Arecaceae	5	3.45
5	*Erythrina senegalensis*	Fabaceae	3	2.07
6	*Grewia fallax*	Tiliaceae	3	2.07
7	*Hymenocardia acida*	Euphorbiaceae	5	3.45
8	*Irvingia gabonensis*	Irvingiaceae	3	2.07
9	*Isoberlinia doka*	Caesalpiniaceae	3	2.07
10	*Lannea acida*	Annonnaceae	74	51.03
11	*Mangifera indica*	Anacardiaceae	29	20
12	*Parkia biglobosa*	Mimosaceae	3	2.07
13	*Psidium guajava*	Myrtaceae	2	1.38
14	*Ricinus comminus*	Euphorbiaceae	2	1.38
15	Unidentified		3	2.07
	Pollen richness		145	100

Table 3 Pollen Spectrum of Daloa (Trade Center) Honey (MC).

N°	Pollen taxa	Families	Pollen abundance	
			Density	Rate (%)
1	*Achyranthes aspera*	Amaranthaceae	3	3.7
2	*Ageratum conyzoides*	Asteraceae	2	2.47
3	*Aspilia africana*	Asteraceae	3	3.7
4	*Bauhinia rufescens*	Caesalpiniaceae	2	2.47
5	*Cassia sp.*	Caesalpiniaceae	3	3.7
6	*Cissus quadrangularis*	Vitaceae	5	6.17
7	*Commiphora africana*	Burseraceae	8	9.88
8	*Daniellia oliveri*	Fabaceae	9	11.11
9	*Detarium microcarpum*	Fabaceae	2	2.47
10	*Euphorbiaceae undiff*	Euphorbiaceae	2	2.47
11	*Hyptis pectinata*	Lamiaceae	2	2.47
12	*Indigofera sp.*	Fabaceae	6	7.41
13	*Justicia anselliana*	Ancataceae	2	2.47
14	*Kedrostis foetidissima*	Cucurbitaceae	2	2.47
15	*Khaya senegalensis*	Meliaceae	2	2.47
16	*Lophira alata*	Ochnaceae	6	7.41
17	*Milletia sp.*	Fabaceae	5	6.17
18	*Pluchea ovalis*	Asteraceae	2	2.47
19	*Poaceae undiff*	Poaceae	2	2.47
20	*Rhyncosia malacophylla*	Fabaceae	2	2.47
21	*Strychnos spinosa*	loganiaceae	3	3.7
22	*Suaeda fruticosa*	Amaranthaceae	2	2.47
23	*Vernonia sp.*	Asteraceae	2	2.47
24	*Ziziphus mucronata*	Rhamnaceae	2	2.47
25	Unidentified		2	2.47
	Pollen richness		81	100

Table 4 Pollen Spectrum of Forona Honey (MF)

N°	Pollen taxa	Families	Pollen abundance	
			Density	Rate (%)
1	*Alchornea cordifolia*	Euphorbiaceae	24	4.7
2	*Anacardium occidentale*	Anacardiaceae	32	6.26
3	*Bidens senegalensis*	Asteraceae	2	0.39
4	*Canavaria virosa*	Fabaceae	3	0.59
5	*Celtis intégrifolia*	Ulmaceae	3	0.59
6	*Chlorophyton tuberosu*	Poaceae	5	0.98
7	*Cissus rufescens*	Vitaceae	2	0.39
8	*Combretaceae undiff*	Combretaceae	6	1.17

9	*Combretum grandiflorum*	Combretaceae	24	4.7
10	*Cordia senegalensis*	Boraginaceae	2	0.39
11	*Crassocephalum -crepidioides*	Asteraceae	17	3.33
12	*Elaeis guineensis*	Asteraceae	95	18.59
13	*Encephalartos natalensis*	Cicadaceae	51	9.98
14	Entada abyssinica	Mimosaceae	3	0.59
15	*Ficus sycomorus*	Moraceae	15	2.94
16	*Hymenocardia acida*	Euphorbiaceae	3	0.59
17	*Hymenostegia afzelii*	Euphorbiaceae	3	0.59
18	-*Isoberlinia doka*	Caesalpiniaceae	2	0.39
19	*Lannea acida*	Annonaceae	23	4.5
20	*Mangifera indica*	Anacardiaceae	57	11.15
21	*Pennisetum subangustum*	Poaceae	2	0.39
22	*Phoenix reclinata*	Arecaceae	14	2.74
23	*Pluchea discoridis*	Asteraceae	3	0.59
24	Poaceae undiff	Poaceae	5	0.98
25	*Securinega virosa*	Euphorbiaceae	5	0.98
26	*Spondias mombin*	Anacardiaceae	5	0.98
27	*Tephrosia bracteolata*	Fabaceae	2	0.39
28	*Terminalia superba*	Cambretaceae	71	13.89
29	*Ziziphus mauritiana*	Rhamnaceae	29	5.68
30	Unidentified		3	0.59
	Pollen richness		511	100

Table 5 Pollen Spectrum of Wongue Honey (MW)

N°	Pollen taxa	Families	Pollen abundance Density	Rate (%)
1	*Adenium obesum*	apocynaceae	5	0.6
2	*Ageratum conyzoides*	Asteraceae	2	0.24
3	*Aloe sp.*	Aloeaceae	5	0.6
4	*Anacardium occidentale*	Anacardiaceae	2	0.24
5	*Cassia occidentalis*	Caesalpiniaceae	21	2.54
6	*Celtis intégrifolia*	Ulmaceae	18	2.18
7	*Citrus sp.*	Rutaceae	9	1.09
8	*Crassocephalum crepidioides*	Asteraceae	2	0.24
9	*Daniellia oliveri*	Fabaceae	23	2.78
10	*Dichrostachys cinerea*	Mimosaceae	2	0.24
11	*Elaeis guineensis*	Arecaceae	29	3.51
12	*Encephalartos natalensis*	Cycadaceae	2	0.24
13	*Entada abyssinica*	Mimosaceae	2	0.24
14	*Euphorbia heterochroma*	Euphorbiaceae	6	0.73
15	*Euphorbiaceae undiff*	Euphorbiaceae	5	0.6
16	*Ficus sycomorus*	Moraceae	3	0.36
17	*Hymenocardia acida*	Euphorbiaceae	317	38.33
18	*Indigofera* Sp.	Fabaceae	2	0.24
19	*Lannea acida*	Annonaceae	146	17.65
20	*Mangifera indica*	Anacardiaceae	17	2.06
21	*Parkia bicolor*	Mimosaceae	2	0.24
22	*Parkia biglobosa*	Mimosaceae	2	0.24
23	*Piliostigma thonningii*	Caesalpiniaceae	14	1.69
24	Poaceae undiff	Poaceae	2	0.24
25	*Securinega virosa*	Euphorbiaceae	51	6.17
26	*Spondias mombin*	Anacardiaceae	20	2.42
27	*Tamarindus indica*	Caesalpiniaceae	9	1.09
28	*Terminalia superba*	Combretaceae	32	3.87
29	*Triplochiton scleroxylon*	Sterculiaceae	6	0.73
30	*Ziziphus mauritiana*	Rhamnaceae	68	8.22
31	Unidentified		3	0.36
	Pollen richness		811	100

Table 6 Quantitative Analysis and Classes of Honeys

Honey	Number of pollen (number of pollen / 20 micro liters)	Density (g / ml)	Pollen content (pollen / 10g honey)	Class	Wealth in pollen
MB	145	1.18	85.500	II	Poor
MC	81	1.06	42.930	I	Very poor
MF	511	1.11	283.605	III	Rich
MW	827	1.22	504.470	IV	Very rich

Table 7 Honey pollen category by density and honey name

Honey	Categories of pollen Dominant pollen (> 45%)	Accompanying pollen (16-45%)	Isolated important pollen (3-15%)	Rare isolated pollen (< 3%)	Designation of honey
MB	01 taxon: *Lannea acida*	01 taxon	03 taxa	09 taxa et Unidentified taxa	Monofloral honey *Lannea acida*
MC	Absence	Absence	10 taxa	14 taxa et Unidentified taxa	Polyfloral Honey
MF	Absence	01 taxon	07 taxa	20 taxa et Unidentified taxa	Polyfloral Honey
MW	Absence	02 taxa	04 taxa	24 taxa et Unidentified taxa	Polyfloral Honey

Figure 4 *Alchonea cordifolia* (Euphorbiaceae)

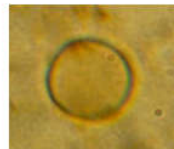**Figure 5** *Anacardium occidentale* (Anacardiaceae)

Figure 6 *Ficus sycomorus* (Moraceae)
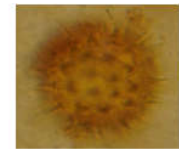

**Figure 7** *Aspilia africana* (Asteraceae)

Figure 8 *Isoberlinia doka* (Caesalpiniaceae)

Figure 9 *Elaeis guineensis* (Arecaceae)

Total polyphenol content of honeys

The total polyphenol contents of the four honey samples are between 7.1 ± 0.1 and 25.8 ± 1.56 mg EAG / g of honey. MC (7.1 ± 0.1 mg EAG / g) and MB (12.5 ± 0.5 mg EAG / g) showed low levels compared to other honeys, while MF gave the value the highest (25.8 ± 1.56 mg EAG / g). The statistical analysis shows a significant difference ($p < 0.05$) between honeys MB, MC and the other samples (MF, MW). There are no significant differences between MF and MW (Figure 10).
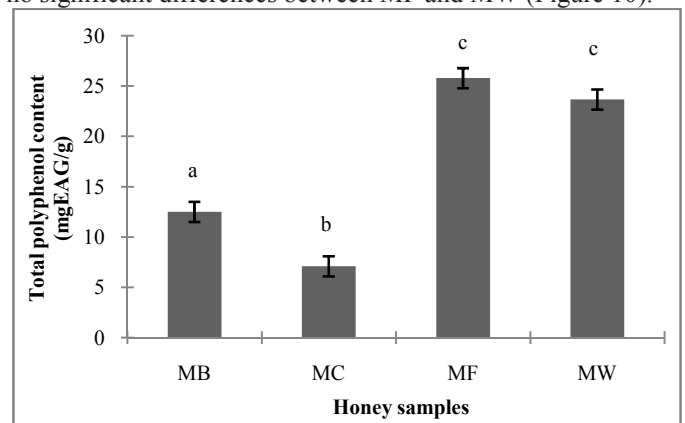
Figure 10 Total polyphenol content of honey samples

Total flavonoid content of honeys

The results of the present study show that the total flavonoid contents of the four honey samples studied ranged from 0.08 to 0.18 mg EQ / g of honey. The commercial honey sample (MC) recorded the lowest grade while the Wongué honey (MW) contains the highest grade. The statistical analysis shows significant differences (p <0.05) between the 4 samples studied (Figure 11).
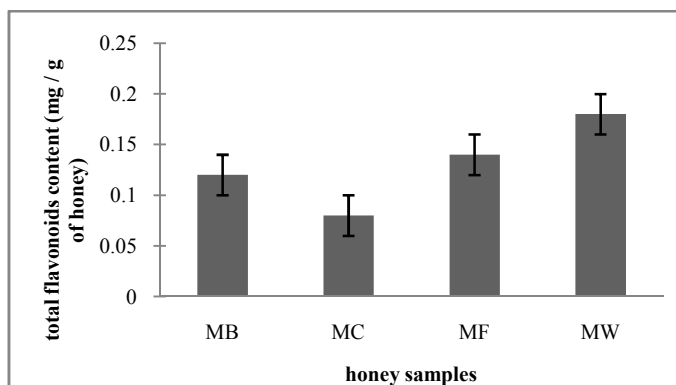
Figure 11 Total flavonoid levels of honey samples

Correlation between flavonoids and phenolic compounds in samples

Figure 12 shows a correlation (R² = 0.7669) between the total phenol content and the flavonoids of the different honey samples.
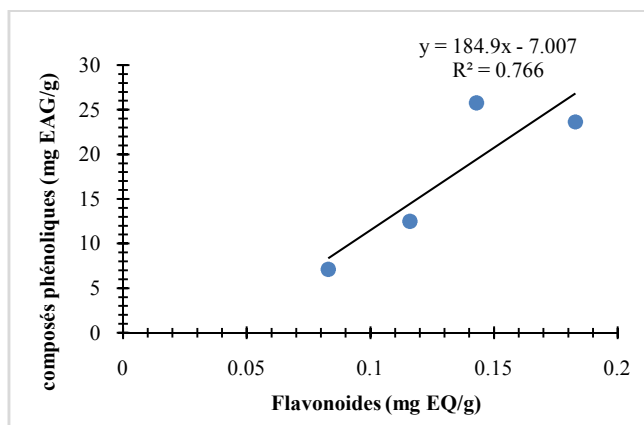
Figure 12 Correlation between flavonoid contents and total phenolic compounds Antioxidant activities of honey

Reducing power (ABTS)

The reducing power of the honey samples analyzed is shown in Figure 13. These results show that Bobi honey (MB) has a better reducing power (9.18%) followed respectively by Foronan honeys (MF) and Wongue (MW) with 8.38% and finally commercial honey (MC) with 6.84%. Statistical analysis revealed significant differences (p <0.05) between the reducing powers (ABTS) of some honey samples. In addition, the MW and MF samples are statistically identical, so they have the same reducing powers.
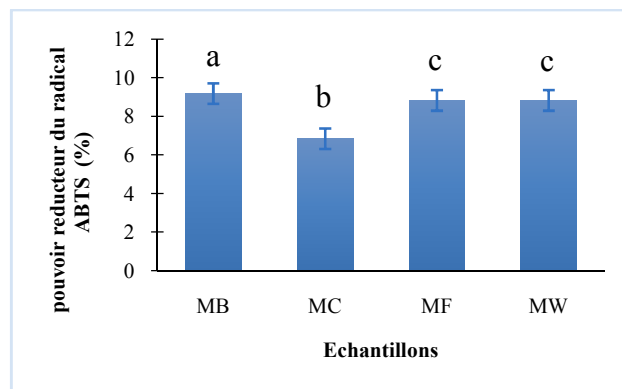
Figure 13 Reducing power of honey samples

Anti-radical activity (DPPH)

The results obtained in Figure. 14 show the antiradical power of the four samples studied vis-à-vis the DPPH radical. The IC50 values of the samples analyzed ranged from 62.12 to 505.46 μg / ml of honey. The MB sample is the most active with IC 50 = 65.12 μg / ml of honey, the other three honey samples (MC, MF, MW) have approximately the same anti-radical activity with IC 50 of approximately 500 μg / ml of honey.
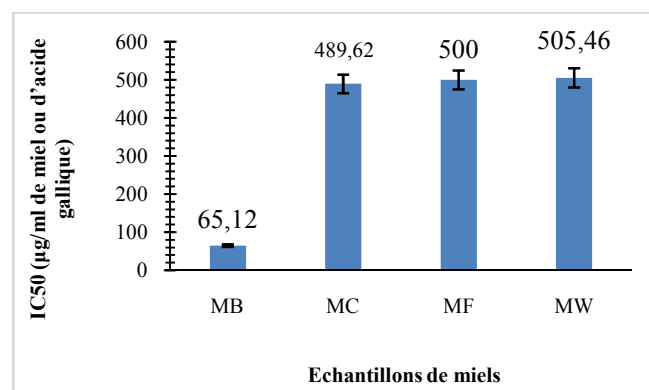
Figure 14 IC$_{50}$ of honey samples

DISCUSSION

The 73 taxons obtained following the pollen analysis of the 4 honey samples reflect a high diversity and specific richness. This specific diversity is greater than that found by Tossou *et al*., (2011) which is 43 taxa for honeys sold in the city of Cotonou (Benin) and that obtained by Lobreau-Callen *et al*., (1986) which is only 4, 7 and 16 taxa, respectively in the honeys of Manta, Boukounbe and Kandi, localities located in the North of Benin. But, it remains lower than the 121 taxa identified by Tossou *et al*., (2005) in honeys harvested in the classified forest of the Lama in southern Benin. This difference in taxonomic diversity can be explained by the number of samples analyzed, their harvest period and the floristic diversity of the types of training used as sources of bee feeding. This idea is shared by Comlan *et al*., (2012) who asserts that this decrease in the number of honey taxa enumerated by this work compared to the others would be related to several reasons. It is due to the fact that these honey taxa are only those determined during pollen analyzes; whereas for the other works mentioned above, these values were obtained by completing the number of honey species identified in the honeys analyzed by that of the species recorded during field observations.

Species richness varying from 15 to 31 taxa per sample remains higher than that of 20 taxa per honey sample in Sudan and Sahelian zones in West Africa (Lobreau-Callen and Damblon, 1994).

The low pollen density of western Anacardium present on the pollen spectra of two of the three samples from cashew plantations may be due to several factors: the harvest period, the flowering period of cashew plants, the season, hive layout in plantations, nest opening and orientation, plant biodiversity and bee behavior. This is supported by Lobreau-Callen and Damblon (1994) who assert that in their natural habitat, bees exploit differently the flowery vegetation available to them near hives. In addition, they appear less selective and exploit a very large proportion of the available melittophilic or amphiphilic flora. They add, moreover, that in the tropical savannah regions, the pollen spectrum of honeys corresponds only to the plant formations surrounding hives that are sometimes representative of the main vegetation types in the region. This low pollen density of western Anacardium observed in the samples could also be explained according to Lagacherie and Cabannes (2001) by the fact that, many factors, including the potential melliferous (itself variable) of a plant species, influence honey production.

This explains the fact that a sometimes attractive species may sometimes become unattractive to bees because of the possibility of a better choice. Of the 4 samples analyzed, only MB is a monofloral honey with a dominant pollen which is that of Lannea acida (51.03%). The other three honeys are polyfloral honeys or all flowers. The presence of Lannea acida as a dominant pollen in the MB can be justified by the abundance of flowering of this species near the hives during the feeding period of the bees and also because it may be more attractive than other species in the area. This is in agreement with Lobreau-Callen and Damblon (1994) who assert that in the tropical regions, blooms are very abundant and diversified near hives. In addition, bees select near the hives the most attractive species with small or relatively large flowers and then spend very little energy in their feeding activities. In this case, these insects exploit only a very small fraction of the existing flowering vegetation. The pollen spectra of such honeys thus give only a very partial image of the vegetation (Lobreau-Callen and Damblon, 1994). This would explain the low number of pollen taxa present in Bobi honey (15 taxa).

On the other hand, the relative density of Lannea acida varies in other samples whereas it is dominant pollen in another sample. Tossou *et al*., (2011) state that the relative density of pollens per taxon may vary from one sample to another within the same appellation or from one appellation to another. Thus, a dominant pollen in a sample may be an accompanying pollen, a large isolated pollen or an isolated pollen in another.

In terms of the distribution of families according to pollen taxa in honeys, the present study has shown that Euphorbiaceae, Asteraceae and Fabaceae are the families most represented in honey taxons followed closely by Caesalpiniaceae, Mimosaceae, Anacardiaceae and Poaceae. The results are consistent with the work of Comlan *et al*., (2012) who states that Euphorbiaceae, Asteraceae, Rubiaceae, and Leguminosae (Caesalpiniaceae, Fabaceae, Mimosaceae) are the most common families in honey taxa; with the exception that in this work Rubiaceae are replaced by Anacardiaceae and Poaceae.

According to the results of the pollen analysis of the various samples of honey harvested, we notice at first, an important variation in pollen richness. Lobreau-Callen *et al*., (1986) have shown that these differences do not appear to be related to the type of honey studied or the type of foraging bee. The pollen poverty of the Commerce and Bobi samples (81 and 145 respectively compared to 511 for Foronan and 827 for Wongué) certainly reflects the fact that we would be dealing with honeys made mainly from nectar (Comlan *et al*., 2012). Similarly, it is conceivable that the presence of large pollen would somehow limit the number of pollen grains in relation to the space available on a slide. In addition to the impact of the pollen count method, the total count of pollens for each honey sample is a general indication that the bees' foraging strategy can vary depending on multiple complementary factors. such as: the production of flowers, the quality of their production of pollen and nectars, the very selective nature of the plants harvested, the competition between bees, etc.

The levels of phenolic compounds in honey samples range from 7.1 ± 0.1 to 25.8 ± 1.56 mg EAG / g honey or from 710 to 2580 mg EAG / 100g honey. These results are lower than those reported by Bouyahya *et al*. (2017) on Moroccan honeys (124.60 ± 1.12 and 75.14 ± 0.78 mg EAG / g of honey) and higher than those obtained by Mouhoubi -Tafinine *et al*., (2016) on Algerian honeys (171.72 to 5351.22 mg EAG / 100g). These results are different from those of this study. This difference may be due to several factors, among others, the geographical location of the different floral sources, since the main source of these compounds is the nectar, the climatic conditions, the plant flora of the honey region and the plant secretions (Alvarez-Suarez *et al*., 2010, Boussaid *et al*., 2014). In addition, the techniques and the experimental analyzes influence the results of the phenolic compounds contents of the samples (Zalibera *et al*., 2008). Similarly, the content of phenolic compounds can also increase under the effect of the intensity of UV radiation, infection of pathogens and parasites, and also by temperature variations. Meda *et al*., (2005) noted that honeydew honeys have higher concentrations of these compounds compared to other types of honeys. Dark, opaque and untreated honeys have high levels of phenolic substances (Wilczynsk, 2014). The dark samples (MB, MF, and MW) of this study actually have higher levels of phenolic compounds. Unlike the lighter sample (MC) which contains smaller amounts of these compounds. MW and MF have statistically the same polyphenol contents, this is explained by their geographical origins which are probably close.

The flavonoid contents of the 4 honey samples ranged from 0.08 ± 0.01 to 0.18 ± 0.02 mg EQ / g honey or 8 to 18 mg EQ / 100g honey. These results are similar to those of Islam *et al*., (2012) on honeys from Bangladesh (3.63-15.5 mg EQ / 100g). The latter have obtained very high flavonoid contents which vary between 109.49 and 12.76mg EQ / 100g. The difference between the results of the flavonoid contents would be due to the same factors mentioned above in the case of the contents of phenolic compounds of the honeys. According to Meda *et al*., (2005) and Alvarez-Suarez *et al*., (2010), the intense color of dark honeys is related to the flavonoid content, which is found

in this study. Indeed, the darkest honey (MW) represents a higher content of flavonoids (18 mg EQ / 100g).

There is a correlation (R^2 = 0.76) between the polyphenol content and the flavonoid content of the samples. This correlation is greater than that reported by Mouhoubi *et al.* (2016) (R^2 = 0.59) and substantially similar to that presented by Akif and Debbou, (2017) with R^2 = 0.83.

The reducing power of the ABTS exerted by the different samples analyzed varies from 6.84% (MC) to 9.18% (MB). These results obtained, different from those of Wilczynska *et al.*, (2014) on honeys from Poland (6 to 79%). These differences of activity can be interpreted by their origins as well as their chemical compositions, in particular phenolic compounds and flavonoids, which differ from one sample to another.

The IC50 values of the analyzed samples are between 62.12 and 505.46 µg / ml of honey. These values also show that among the samples analyzed, it is Bobi honey which has the strongest anti-radical activity and therefore the most active (IC50 = 62.12 µg / ml of honey). Thus, it is sufficient to have a low concentration of this honey (MB) to destroy 50% of the DPPH radicals. These results are different from those of Bouyahya *et al.* (2017) who found in their study of Moroccan honeys an IC50 = 48.15 µg / ml of honey for the Zoumi sample.

CONCLUSION

The present study on honeys derived from modern beekeeping in Séguéla made it possible to know the botanical origin of these honeys, and their antioxidant activities.

Pollen analysis of the four samples revealed that three honeys are multifloral (MC, MF, MW), while only one (MB) is monofloral (Lannea acida). In addition, this pollen analysis identified 73 plant taxa belonging to 32 families pollinated by bees. Species richness ranges from 1 to 31 taxa per honey sample. The most dominant plant families are Fabaceae, Euphorbiaceae and Asteraceae.

All honeys analyzed have antioxidant activities. Bobi (MB) honey has both the best antiradical activity (IC50 = 62.12 µg / ml) and the good reducing power (9.18%). In addition, all honeys derived from Séguéla unlike commercial honey, are dark shades reflecting their high levels of phenolic compounds and flavonoids.

In order to improve the present study, our research is currently focused on identifying and quantifying the nutrients of these different honeys. In addition, studies on the organoleptic analyzes of these honeys and their microbiological quality will be considered.

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