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Physico-Chemical, Microbiological and Antioxidant Properties of Some Local Honey Samples from Senegal

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Abstract

Honey is one of the most pleasant food substances for humanity. It has many properties and contributes to the improvement of human nutrition. In Senegal, honey is an object of many speculations as for its origin and its physico-chemical and microbiological qualities. In this context, our study aims to evaluate the quality of local honey. Our study focused on six honey samples. Physico-chemical and microbiological analyses were carried out to evaluate the quality of the honey samples. The water content of the honey samples varied from 7.86 to 14.96 %. As for the ash content, the results varied from 0.13 to 0.45 %. The pH of the honey samples varies from 4.60 to 5.05, except for sample E5 which is 6.68. The total acidity values of the honey samples ranged from 10 to 26.5 mEq/kg. The IC50 values for the evaluation of the antiradical activity of the honey samples ranged from 145.05 to 189.40 µg/mL. The honey samples were generally compliant to the microbiological parameters studied, with the exception of samples E2 and E3 for which we had the presence of coliform for both samples and the presence of yeast and mold for sample E2 only.

Given the results of this study, it would be necessary to do a more comprehensive study on as many local samples as possible.

Keywords: Quality Control, Honey, Chemical Analysis, Microbiological Analysis

Introduction

Honey is a natural sweet substance produced by Apis mellifera bees from the nectar of plants or secretions from living plant parts or sap-sucking insects (Codex-Alimentarius-Comission, 1981). This noble product of the hive, which has been used since ancient times, is one of the most popular foodstuffs for humans.

Honey is a high-energy food, it is a living product that undergoes some changes over time leading to the loss of its essential qualities (Bouet Kouanou et al., 2020).

It is a complex mixture of various molecules with different proportions, which gives it both therapeutic nutritive and antioxidant properties (Lequet Laudine, 2020; Brischoux et al., 2013).

Honey can contribute to the improvement of human nutrition; it is also a significant potential source of income for the rural population due to its rather valuable market value on national markets.

Honey production in Senegal remains very negligible compared to the existing honey potential. The presence of varied natural resources in the rural areas of the coast could, however, offer the possibility of developing national honey production, and also avoid its massive importation.

Currently, in Senegal, honey is subject to a certain number of speculations, as for its origin and its physico-chemical and microbiological qualities. In addition, the consumer is confronted with the high price of this noble product and is unable to differentiate between an authentic product and a falsified one. The absence of a regulation that would oblige beekeepers to make a systematic control of their products leads to fraud and endangers the health of the consumer.

In this global context and given the nutritional value as well as the market value of honey, our work can be seen as a contribution to the study of the quality and evaluation of the antioxidant activity of local honey.

Methods

The framework of the study

The physico-chemical analyses (pH, humidity, anti-oxidant activity, ash content, acidity) were performed at the Laboratory of Analytical Chemistry and Bromatology of the Faculty of Medicine, Pharmacy and Odontology (FMPO).

Microbiological analyses (total mesophilic germs, coliforms, staphylococci, yeasts and molds) were carried out at the Bacteriology Laboratory of the Aristide le Dantec Hospital (HALD).

Sampling

Our study focused on six honey samples collected during January 2020 as presented in Table 1. The samples were kept at 25 °C away from light.

Table 1. List of honey samples collected as well as their origin and type of packaging

Sample	Provenance	Packaging
E1	Dakar / FMPO	Glass flacon
E2	Dakar / Bop	Plastic flacon
E3	Fouta	Plastic flacon
E4	Dakar / HLM 1	Plastic flacon
E5	Dakar / HLM 2	Glass flacon
E6	Dakar /Pharmacie	Glass flacon

Physical-chemical analysis of honey

- **Humidity determinations**

It consists in determining the percentage of mass lost by the honey after having put it in the oven for 120 min at 105 ± 2 °C. 5 g of honey was weighed in a crucible and held in the oven set at 105 ± 2 °C for 120 min. Humidity is expressed as follows:

Humidity (%) = (M1 – M2) *100 / M0

- M0 = test weight (g)
- M1= crucible mass and test sample (g)
- M2= mass after drying

- **Determinations of ash content**

The ash content is based on the incineration of honey in an oven (Amri et al., 2007). 5 g of honey was heated at 600 ºC for 1 h. Incinerate 3 g of honey in a muffle oven at 550 °C for 1 h. The ash content is calculated as:

Ash (%) = (M1 – M2) *100 / M0

- M0 = test weight (g)
- M1 = residual mass after incineration (g)
- M2= empty crucible mass

- **Determination of pH**

The pH of honey is determined by a potentiometric method using a glass electrode specific for H+ ions. The pH meter is first calibrated with buffer solutions of pH 4 and 7. Then 10 g of honey is dissolved in 100 mL of distilled water (10 % honey solution). Finally, the pH of the solution is

obtained by immersing the electrodes of the pH meter in the 10 % honey solution (Belhaj et al., 2015)

- **Determination of acidity**

The free acidity is the amount of acid titratable with sodium hydroxide solution (NaOH) at pH 7 in the presence of bromothymol blue (BBT) as an indicator of the end of equivalence. The acidity measured is that of a 10 % honey solution (Lydia et al., 2017).

- Use a 10 % honey solution. The pH is first measured with a pH meter.
- Fill the burettes with 10 mL of 0.05 N NaOH.
- Take 25 mL of this solution.
- Add 2-3 drops of BBT. The reaction medium turns yellow.
- Titrate with 0.05 N NaOH until the BBT turns dark green.
- Note the equivalent volume of NaOH (Veq).

Free acidity (milliequivalents/kg of honey) = 1000 * Veq * N / M

- Veq= volume at equivalence
- N = normality of 0.05 NaOH
- M= mass of test sample

- **Determination of antiradical activity by DPPH (2,2-diphenyl-1-picrylhydrazyl)**

This method is based on measuring the ability of antioxidants to trap the DPPH radicals. Indeed, DPPH is characterized by its ability to produce stable free radicals. This stability is due to the delocalization of free electrons within the molecule. The presence of these DPPH radicals'- results in a dark violet coloration of the solution, which absorbs at around 517 nm. The reduction of DPPH radicals by antioxidant agent results in a discoloration of the solution (Bouyahya et al., 2018)

The scavenging capacity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined by the method of (Bouyahya et al., 2018) with some modifications. Thus, 0.2 mL of the honey sample at different concentrations (30, 60, 120, and 240 µg/mL) are added to 1.8 mL of the ethanolic solution of 0.11 mM. DPPH- After an incubation time of 30 minutes in the dark and at room temperature (23 ± 2 °C), the absorbance is measured at 517 nm against an ethanol blank. Ascorbic acid used as a positive control underwent the same treatment as the honey samples.

The percentage inhibition (PI%) of the DPPH radical (DPPH-) was calculated as follows:

PI (%) = (A0 - A1) *100 / A0

- A0 = absorbance of the DPPH solution- alone;
- A1 = absorbance of the DPPH- solution after addition of the extract

Microbiological analyses

- **Enumeration of the total mesophilic germs**

It consists of counting all the germs present in the honey samples that are capable of living between 20 and 40 °C. It is done by surface seeding of a Mueller Hinton agar (MH). A stock solution is prepared from 25 g of honey and 225 mL of distilled water (Souad, 2019). This solution is diluted 1/100 with distilled water. Then, an MH agar is surface inoculated with 10 microliters of the diluted solution. This medium is incubated at 37 °C for 24 to 36 h. The results are expressed in Colony Forming Units (CFU): one colony corresponds to 104 germs/mL.

- **Search for specific pathogens**

- Search for coliforms

A liquid culture medium BT (Thiogluconate Broth) is inoculated with 10 mL of honey and incubated at 37 °C for 18 to 24 h.
Then two Eosin-Methylene Blue (EMB) media are inoculated with 100 µl of the BT broth. EMB1 is incubated at 37 °C for total coliforms while EMB2 is incubated at 44 °C for fecal coliforms. Incubation is done in an oven for 18 to 24 h.

- Search for Staphylococcus aureus

A liquid culture medium BT (Thiogluconate Broth) is inoculated with 10 mL of honey and incubated at 37 °C for 18 to 24 h.
Then a Chapman medium (hypersalted agar medium with 7.5 % NaCl containing mannitol) is inoculated with 100 µL of BT broth. After incubation at 37 °C for 18 to 24 h, the identification of the Staphylococcus aureus will be made by the coagulase test in case of a positive culture.

- Search for yeasts and molds

A liquid culture medium BT (Thiogluconate Broth) is inoculated with 10 mL of honey and incubated at 37 °C for 18 to 24 h. Then, a Sabouraud medium is inoculated with 100 µL of BT broth and incubated at 22 °C for 5 days.

Statistical analysis

All measurements were made in triplicate and presented as mean ± standard deviation. For the evaluation of the anti-radical activity, regression analyses were performed (Figure 1) using the linear and logarithmic equations for the determination of the concentration of the extract that inhibits 50 % (IC50) of the DPPH- radical. These analyses were performed using Excel version 2016.

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Results

Physico-chemical analyses

The results of the physico-chemical analyses of the studied honey samples are presented in Table 2. The values obtained by our samples were compared with the standard of the European Union (EU) and the codex Alimentarius.

Table 2. Results of the physico-chemical analysis of honey samples

Physico-chemical parameters	E1	E2	E3	E4	E5	E6	Standard EU	Standard Codex
Humidity (%)	12.51 ±0.14	10.40 ±0.22	7.86 ±0.20	8.98 ±0.18	14.96 ±0.19	13.24 ±0.27	≤ 21	≤ 21
Ash (%)	0.45 ±0.05	0.30 ±0.03	0.13 ±0.10	0.15 ±0.05	0.28 ±0.02	0.28 ±0.02	≤ 0.6	< 0.6
Acidity (mEq/kg)	19.05 ±0.36	10.00 ±0.40	26.5 ±0.84	10.04 ±0.25	12.02 ±0.27	11.5 ±0.56	≤ 50	≤ 40
pH values	4.66 ±0.05	4.60 ±0.04	4.94 ±0.05	5 ±0.06	6.68* ±0.10	5.05 ±0.07	]3.5 – 5.5 [	] 3.5 – 5.5 [
* non-conforming								

Humidity contents of Honey samples ranged from 7.86 % to 14.96 % and were within the limit set by the European Union and Codex Alimentarius. Regarding the ash content, the results ranged from 0.13 to 0.45 % and were below the standard value of 0.6 %. The pH of the honey samples ranged from 4.60 to 6.68 and agreed with the Codex Alimentarius recommendations and the EU except for sample E5. The total acidity values of the honey samples were between 10 and 26.5 mEq/kg and were within the normal range set by the Codex Alimentarius and the European Union.

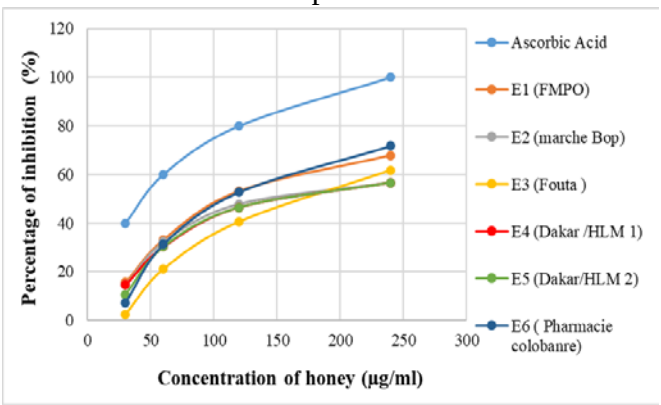

Figure 1. Percentage of DPPH radical inhibition of different honey samples

The IC50 values of the honey samples ranged from 145.05 to 183.08 µg/mL. According to Figure 1, there is an increase in the percentages of DPPH radical inhibition as a function of the concentrations tested for all honey

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samples, with the lowest IC50 value indicating a high free radical scavenging capacity.

The E1 honey sample shows the most interesting antioxidant activity with lower IC50 values of 114.17 and 145.05 µg/mL according to the logarithmic and linear models, respectively Table 3.

Table 3. Concentrations of honey capable of inhibiting 50 % of the DPPH radical and the Coefficient of determination of the linear and logarithmic regression lines

Samples	Linear Model		Logarithmic Model	
	IC50 (µg/mL)	R2	IC50 (µg/mL)	R2
E1	145.05	0.90	114.17	0.99
E2	179.99	0.82	154.23	0.98
E3	183.08	0.93	163.27	0.98
E4	182.99	0.85	160.16	0.99
E5	182.34	0.88	159.45	0.98
E6	145.39	0.89	114.44	0.99
Ascorbic Acid	37.50	0.90	42.42	1

Microbiological analyses

The results of the microbiological analyses of the honey samples are presented in Table 4.

Table 4. Results of microbiological analysis of honey samples

Samples	total mesophilic germs (CFU/mL)	Staphylococcus aureus	Coliforms	Yeasts and molds
E1	04	Absence	Absence	Absence
E2	15	Absence	Presence	Presence
E3	19	Absence	Presence	Absence
E4	05	Absence	Absence	Absence
E5	< 1	Absence	Absence	Absence
E6	< 1	Absence	Absence	Absence
Norme	< 10	Absence	Absence	Absence

The honey samples were generally compliant for the microbiological parameters investigated except samples E2 and E3 where there is presence of coliforms and total mesophilic germs are greater than 10 CFU/mL.

Discussion

Humidity content is an important element in assessing the degree of maturity of honey and its suitability for storage (shelf life). Generally, a high-water content causes the fermentation of honey and the loss of its flavor and quality (Machado De-Melo et al., 2018). The examination of the results shows that Humidity contents of the analyzed honey samples varied between 7.86 and 14.96% and were lower than 21% which is the maximum limit fixed by the European Union (European Directive 2001/110 EC, 2003) and the Codex

Alimentarius (Codex-Alimentarius-Comission, 1981). Thus, the honey samples studied showed good prospect to be preserved.

Regarding the ash content, all the honey samples analyzed showed values within the limit set by the international standards which is a maximum of 0.6 %. According to Bogdanov, (1999) the ash content is related to the botanical and geographical origins of honey and is higher in dark honey. Currently, this parameter tends to be replaced by the measurement of electrical conductivity (Bogdanov, 1999).

The pH values of the studied honey samples, except for E5 (6.68), were between 4.60 and 5.05, hence their acidic character. These values are following the Codex Alimentarius and the EU standards of the pH between 3.5 and 5.5.

The acidity of the honey results from the oxidation of glucose into gluconic acid by glucose oxidase (Lydia et al., 2017). It is an important factor of inhibition of the growth of micro-organisms and the stability of honey. The high pH value of the E5 sample may be due to adulteration with ordinary sugar syrup resulting in a pH higher than normal. The total acidity values of the honey samples ranged from 10 to 26.5 mEq/kg and are within the normal range set by the Codex Alimentarius and the European Union. The total acidity of honey is due to the presence of inorganic ions such as phosphates and chlorites and free or combined acids in the form of lactone (Léopold et al., 2008). It increases as honey ages or is altered by fermentation.

IC50 values of honey samples ranged from 145.05 to 183.08 µg/mL and were far higher than those reported by (Bouyahya et al., 2018) (61 - 80 µg/mL). As a result, the honey samples analyzed in this study have less antioxidant activity. The free radical scavenging capacity of honey would be due to the presence of phenolic compounds because a proportionality relationship was found between the antioxidant potential of honey and polyphenol content (Beretta et al., 2005) the antioxidant properties of honey would derive from the therapeutic properties attributed to this food; moreover, in this study, it was found that samples of the honey packaged in glass bottles had higher antioxidant activity than those packaged in plastic, it would seem that the packaging has an impact on the antioxidant activity.

Concerning microbiological parameters, the honey samples studied were generally compliant except for E2 and E3 samples where the presence of total coliforms, yeasts and molds and a total mesophilic germ higher than 10 CFU/mL were noted. These results are similar to those obtained by Coulibaly B et al., (2019) on honeys collected in Worodougou region, Séguéla (Côte d'Ivoire) and in the town of Daloa.

Fecal coliform testing is performed to monitor fecal contamination of food due to poor hygiene conditions. It is often referred to as E. coli testing because of its rapidity. It should be noted here that the group of fecal coliforms

is defined "administratively" and not on taxonomic bases; they are not necessarily E. coli and E. coli does not necessarily represent all fecal coliforms.

Conclusion

The general objective of this study was to contribute to the promotion of the health of Senegalese populations through the consumption of quality local honey. It concerned six (06) honey samples which were analyzed from the physico-chemical and microbiological points of view.

From the physico-chemical point of view, the majority of honey samples analyzed met the standards set by the Codex Alimentarius Commission. Indeed, the humidity of the samples varied between 8 and 15% and the pH was between 4.6 and 5.05 except for one sample where a pH of 6.68 was found. The free acidity of the honey studied was between 10 to 26.5 mEq/kg.

The study of the antioxidant activity of honey samples by the DPPH radical scavenging method showed that the honey samples had a relatively low antioxidant activity compared to that of the reference sample (ascorbic acid). Microbiologically, the honey samples were generally compliant except E2 and E3 samples where the presence of total coliforms, yeasts and molds and a total mesophilic germ greater than 10 CFU/mL were noted.

Given the results of this study, it would be relevant to widen and deepen the investigations by a significant increase in the number of samples and by carrying out as complete an analysis as possible. This should make it possible to obtain much more exhaustive data on the quality of honey consumed in Senegal.

What is already known on this topic

- ✓ Honey is a living product that undergoes over time several changes leading to the loss of its essential qualities.
- ✓ Honey can contribute to the improvement of human nutrition

What this study adds

- ✓ Study of the antioxidant activity of honey samples
- ✓ Implementation of a protocol for the detection of specific pathogens in honey

Authors' contributions

Souleymane Aidara and Assane Dieng have designed the work, wrote, corrected, and validated the manuscript. Souleymane Aidara carried out the physico-chemical and microbiological analyses in the laboratory and wrote the first draft of the manuscript; Amadou Diop corrected and validated the

manuscript interpretation of the data and revised the first draft of the manuscript.

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