

Biochemical analysis of Senegalese monofloral honeys focusing on antioxidant potential, phenolic and flavonoid contents, and nutritional properties

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ABSTRACT

Honey, a natural product, seems to be in the foreground due to the image of a healthy, dietary product that it conveys. It is therefore considered a 'functional food', which prompts us to question its composition and therapeutic properties. Monofloral honeys come mainly from the nectar of a sole main plant. To explore the potential of monofloral honeys as a functional food, this study was carried out to determine their content of total phenolic compounds, flavonoids, antioxidant compounds and essential nutrients. Total phenol and flavonoid contents were determined using the Folin-Ciocalteu and AlCl₃ methods, respectively. The in vitro antioxidant activity of the honeys was evaluated using the DPPH method. Essential mineral content was determined using the AOAC standard analysis method. The results of this analysis showed that the honeys had an average total phenol content of 834.22 ± 102.88 mg GAE/kg, an average total flavonoid content of 5.37 ± 1.8 mg QE/100 g, an average Na content of 74.72 ± 33.53 mg/kg, an average Fe content of 6.44 ± 0.64 mg/kg and an average K content of 0.019 ± 0.0 mg/kg. However, the average antioxidant activity of the honeys was low, with an *Ic*₅₀ of 17.09 ± 9.11 mg/ml. These results offer interesting opportunities for valorising our honeys in the fight against food insecurity.

Keywords: Monofloral honeys, Total polyphenols, Flavonoids, Antioxidant activity, Nutritional profile, Senegal.

Introduction

Honey is a natural sugar produced by bees of the species *Apis mellifera*. They make it from flower nectar or secretions from living parts of plants, as well as from the excretions of sucker insects deposited on these plants. Bees collect these substances, transform them by combining them with specific secretions they produce, then deposit, dehydrate, conserve and leave them to mature in the honeycombs of the hive, producing nectar or honeydew honey [1]. Indeed, there are two types of nectar honey: monofloral honey and polyfloral honey. Monofloral honeys are produced from the predominant foraging of a particular flower species. In practice, comes from a sole flower species. When the proportion of pollen grains from a sole plant represents more than 50% of the total pollen, the honey is given the name of that plant [2]. The southern part of Senegal has significant honey production potential [3], which could offer an opportunity to develop national production. However, it faces difficulties related to the sale of bee products on the domestic market, due in particular to disloyal competition from many foreign products, fraud and the absence of national quality standards. The establishment of standards is the only alternative for certifying our honey and thus opening up export opportunities in European, American, Asian and other markets. Honey, a product that is in greatly coveted, is a highly complex, top-quality organic foodstuff with high energy value and enormous diversity, giving it various nutritional and therapeutic properties [4].

Indeed, honey contains enzymatic substances (catalase, glucose oxidase, peroxidase) and non-enzymatic substances (ascorbic acid, α-tocopherol, carotenoids, amino acids, proteins, flavonoids, phenolic acids) [5]. For these reasons, many studies have focused on its antioxidant properties [4,6], antiinflammatory [7,8], antibacterial [9,10], antifungal [11], antiviral [12], antidiabetic [13,14], healing [15,16] etc. In addition, studies on essential element content were also conducted [17]. Despite all previous research, Senegalese honeys are poorly exploited in terms of their physicochemical characteristics. It is in this context that a study was conducted to investigate the therapeutic and nutritional potential of monofloral honeys produced in Senegal, particularly in the southern region (Casamance). This study aims to quantify the total polyphenols and total flavonoids present in honeys, to determine their antioxidant capacity and, finally, to identify the mineral elements in honeys.

Materials and Methods

Sampling

Our study was carried out on three honey samples collected during the 2022–2023 season from various modern honey producers in the Ziguinchor region of Senegal. The honeys are classified according to their floral origins and provenance (Table I). The samples were placed in airtight glass jars and kept at 4°C until analysis. All analyses were performed in triplicate. Table I below shows the origins and harvest years of the honey samples studied.

Table 1: Origin and year of extraction of the honey samples studied

Samples	Presumed floral origin	Localities	Year of production
DK	<i>Dialium</i>	Kassele	2023
EU	<i>Eucalyptus</i>	Samatite	2023
MK2	Mangrove	Cataba 2	2023

Quantification of total polyphenols and total flavonoids

Materials

For the assays of total polyphenols and total flavonoids, we used the following equipment and reagents: 20-200 µl and 100-1000 µl micropipettes, 10 ml glass tubes, 10 ml flasks and yellow and blue cones, Vortex mixer (Combi-Spin FVL2400N, BIOSAN), precision balance, Thermo Scientific Evolution 300 UV-Visible Spectrophotometer, Folin-Denis reagent (Merck KGaA, Germany), sodium carbonate (Na₂CO₃, Sigma-Aldrich, USA), gallic acid (Sigma-Aldrich, USA), quercetin (Sigma-Aldrich, USA), sodium nitrite (NaNO₂, Merck KGaA, Germany), aluminium trichloride (AlCl₃, Sigma-Aldrich, USA), Sodium hydroxide (NaOH, Sigma-Aldrich, USA), Ethanol (Sharlau, Spain), Methanol (Sharlau, Spain) and Distilled water.

Methods for determining total polyphenols

The concentration of phenolic content in honeys was determined using the Folin-Ciocalteu method [18] with some modifications. This method was designed and standardized for the quantification of total phenols by [18] and adapted by [19]. 1 g of honey was transferred to a 10 ml volumetric flask using distilled water as the solvent, at a honey solution concentration of 0.1 g/ml. 100 µL of this honey solution was transferred to test tubes and 200 µL of Folin-Ciocalteu reagent was added. Two minutes later, 500 µL of 4% sodium carbonate was added. The tubes were placed in the dark at a temperature of 37°C for 30 minutes. The absorbance was measured at 725 nm using a spectrophotometer. A reagent blank was prepared under the same conditions using distilled water. The results are expressed in mg of gallic acid equivalent/kg of honey (mgGAE/kg), referring to the calibration graph [18,20]

Methods for determining total flavonoid content

The total flavonoid content was determined using the aluminum chloride colorimetric method described by [21]. Aluminum trichloride (AlCl₃) forms a yellow complex with flavonoids. 1 g of honey was transferred to a 10 ml volumetric flask using distilled water as the solvent, at a honey solution concentration of 0.1 g/ml. From this solution, 500 µL was transferred to test tubes and 130 µL of sodium nitrate was added. Then 130 µL of 2% AlCl₃ and, after 6 minutes, 900 µL of NaOH were added. After vortexing, the absorbance was measured at 430 nm using a spectrophotometer. The flavonoid content is expressed in milligrams (mg) of quercetin equivalent per 100 grams (g) of honey (mg EQ/100g), with reference to the calibration graph.

Determination of antioxidant activity (DPPH method)

Material

For the antioxidant activity assay, we used the following material and reagents: 100-1000 µl micropipette, 20-200 µl micropipette, yellow and blue cones, precision balance (Sartorius), 5 ml test tubes, vortex mixer (Combi-Spin FVL2400N, BIOSAN), Thermo Scientific Evolution 300 UV-Visible Spectrophotometer and Chromatographic Cell, 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, USA), Ethanol (VWR Chemicals BDH, France), and L-ascorbic Acid (Sigma-Aldrich, USA).

Methods

The antioxidant activity of all honey samples was measured using the DPPH radical scavenging activity as developed by [22]. 500 µL of honey extract (25-100 mg/ml) was mixed with 1500 µL of DPPH (60 µmol), and the absorbance was measured at 517 nm after 1 hour and 30 minutes of incubation using a spectrophotometer.

Antioxidant activity (AAO) was expressed as a percentage inhibition (PI) and was determined using the following formula:

$$A.A.O (\%) = (White\ Absorbance - honey\ absorbance \times 100) / White\ Absorbance$$

Determination of sodium, iron and potassium content

Material

The material was composed of: porcelain capsules, EW-04805 Series laboratory hot plate, precision balance, desiccator, Chemfree 2000 Type 60 chemical fume hood, Agilent Technologies 200 Series AA Atomic Absorption Spectrophotometer, nitric acid (Sigma-Aldrich, USA), Hydrochloric acid (HCl, VWR Chemicals BDH, France) and Distilled water.

Methods

This analysis is carried out in a first step by mineralization and in a second step by dissolving and measuring the mineral elements using flame atomic absorption spectrophotometry (AAS).

Weigh 1 g of each honey sample in a porcelain capsule. Then calcine at 600°C for three hours, heating gradually from 200°C until white ash is obtained. Allow the capsules to cool in a desiccator, then moisten the ashes with a little distilled water. After cooling in the desiccator, 4 ml of 1/2 diluted nitric acid is added to the ashes. The mixture is brought to the boil on the hotplate (100°C) until the acid has completely evaporated. Calcine again at 600°C for one hour and leave to cool in the desiccator. Then add 10 ml of concentrated hydrochloric acid diluted to 1/2 and filter the solution through a 0.45 µm filter into a 50 ml standard dilution tube, adjusting to the mark with distilled water. A blank reagent solution was prepared following the same protocol as for the samples [23]. The various standards for metallic elements (Fe, Na, K) are prepared in parallel with the samples and conserved at a temperature of 4°C until analysis. The range of concentrations chosen for each metal was determined according to the detection interval of the SAA, which allows calibration lines to be plotted subsequently. The concentration of each metal in the honey samples is calculated in mg/kg, using the following formula: T.S: test sample ; D: dilution factor and C.lue to reading concentration.

$$Mineral\ content\ (mg/kg) = C.lue \times D / T.S \times 1000$$

Statistical analyses

The statistical analysis of our results was performed using R 4.3.3 version 2023, with one-way ANOVA set at p<0.01 and studying the correlation matrices (bivariate analysis).

Results and Discussion

Total polyphenols and total flavonoids

The total polyphenol content (TPT) of our samples ranges from 707.3 to 959.29 mgEAG/kg, with an average of 834.22 ± 102.88. This variation in content could be due to differences in the botanical and geographical origin of the honeys [24].

The TPT of the southern samples ranged from 707.3 mgEAG/kg in Kassèle to 836.08 mgEAG/kg in Samatite and 959.29 mgEAG/kg in Cataba 2 (Table II) with standard linearity ($R^2 = 0.9998$). These results are higher than those obtained by [25] with an average of 613.01 ± 321.41 mgEAG/kg. These results are also close to those of [26] with an average content of 748.88 ± 215.6 mgEAG/kg. Our results are higher than those of [4] for Algerian honeys with a total polyphenol content of 396.71 ± 233.41 mgEAG/kg of honey. They are also higher than those reported by [3] for honeys from Casamance, with levels ranging from 72.5 to 403.9 mg/kg. The highest content was found in sample MK2, characterized by a dark amber color extracted in the department of Bignona in the Mangroves, which is part of the Ziguinchor region and has particular characteristics in terms of geographical isolation, climatic conditions and ecosystem fragility. The mangrove is known for its rich flora and is protected from bushfires and wood cutting. Determining the total phenolic compound content is also considered a promising method for studying the floral origins of honey. [27] believe that botanical and geographical origin affects the concentration of phenolic compounds, pollen distribution and antioxidant activity in honey. Other factors also influence the composition and nature of honey and its characteristics. These include the age of the bee (honey from young bees is particularly clear and less concentrated than that from older bees), the floral origin of the food source, the climate of the environment, the season in which the bees are raised and the honey is produced, the method of honey extraction, and the duration and conditions of storage, which determine the activity of honey enzymes and their efficacy [28]. The ANOVA test performed on the polyphenol content of the samples gives a significant p-value ($p < 0.001$) with a higher concentration of polyphenols in sample MK2. The total flavonoid content of the honeys analyzed ranged from 3.3 to 7.6 mgEQ/100g of honey, with an average of 5.37 ± 1.8 mgEQ/100g of honey. [29], ranging from 1.11 to 7.51 mg/100g, and [30] between 2.07 and 10.15 mg/100g, but lower than that of [31] for values between 12.95 and 30.45 mg/100g of honey and [4] with results between 11.40 and 27.47 mg/100g of honey. However, our results are higher than those of [32] for Moroccan honeys, with values ranging from 2.26 to 4.79 mg/100 g of honey, and [33] for Spanish honeys, with values ranging from 7.24 to 8.26 mg/100 g of honey. These results indicate that Senegalese honey has very high antioxidant potential. In honey, most phenolic compounds are in the form of flavonoids, whose concentration depends on various factors, including the plant species used by bees, plant health, season and environmental factors [34]. The ANOVA test performed on the flavonoid content of the samples gives a significant p-value ($p < 0.001$) with a higher concentration of flavonoids in sample MK2. Also known as Efficient Concentration for 50% (IC_{50}), expressed in mg/ml, this is the concentration of the test sample required to neutralize 50% of free radicals, calculated graphically using Statgraphics 5 plus software and inhibition percentages. Anti-radical activity is inversely related to antioxidant capacity (Inhibitory Concentration = IC_{50}). The lower the IC_{50} value, the higher the anti-radical activity of the honey, as it requires less radical desactivator from the honey to reduce DPPH. These observations have been reported by several authors [20,35]. In general, the results recorded are similar to those obtained by [26], with IC_{50} values ranging from 20.4 to 88.42 mg/ml, and [4] with values ranging from 6.33 to 50.08 mg/ml. The statistical analyses performed show very significant differences

($p < 0.001$), especially with the MK2 sample. Table II below summarises the antioxidant parameters and standard deviations.

Table II: Antioxidant parameters and standard deviations

Antioxidant parameters	Honey sample code		
	MK2	DK	EU
Total polyphenols (mgEAG/kg)	959,29±1,74	836,08±1,19	707,3±0,76
Total flavonoids (mgEAG/kg)	7,68±0,28	5,14±0,05	3,3±0,16
DPPH (%)	57,39±1,13	56,32±1,20	49,63±1,18
inhibitory concentration 50%	8,5±0,42	13,6±0,35	29,7±0,38

A positive correlation was observed between DPPH, PT and FT in honey samples, with a Pearson coefficient < 0.001 . PT showed a weak correlation with antioxidant activity, while FT showed a moderate correlation with DPPH (Table III). However, the correlations are very strong between IC_{50} and total polyphenols and total flavonoids (Table III). This finding is confirmed by other studies conducted by [4, 30 and 34], which show that the antioxidant activity of different types of honey from different geographical origins depended mainly on their concentration of phenolic compounds. Table III illustrates the correlation matrix of antioxidant potential parameters.

Table III: Correlation matrix of antioxidant capacity parameters

	POLYPHENOLS	FLAVONOIDS	DPPH	IC50
POLYPHENOLS	1.000			
FLAVONOIDS	0.991	1.000		
DPPH	0.089	0.166	1.000	
IC50	-0.959	-0.927	0.193	1.000

Determination of mineral content

Sodium (Na) content

They range from 27.5 to 102.12 mg/kg with an average of 74.72 ± 33.3 mg/kg. The average value of the DK sample is significantly higher than that of the others ($p > 0.05$). These results are consistent with those found by [35]. [36]. in Palestinian honeys with an average of 104.67 ± 14.55 mg/kg and [37] in Moroccan honeys with an average of 113.65 ± 58.05 mg/kg. However, our results are significantly lower than those of [4] in Tunisian honeys with an average of 388.32 ± 88.05 mg/kg. These variations are influenced by soil composition, botanical origin, climatic conditions and seasonal variations [38]. Our honeys are rich in sodium and can play a role in trace element supplementation. Figure 1 illustrates the results of the sodium content of our samples. Statistical analyses show very significant differences ($p < 0.001$).

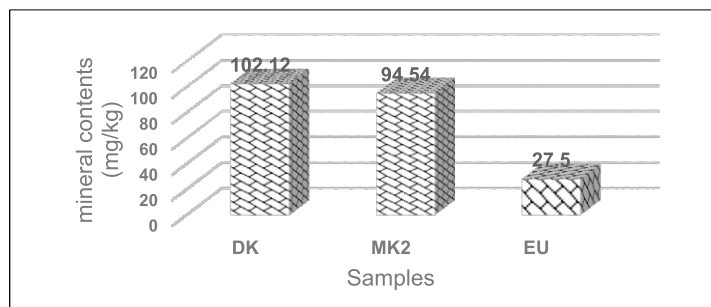


Figure 1: Sodium content of the sample

Iron content

These vary from 5.13 to 7.35 mg/kg, with an average of 6.44 ± 0.64 mg/kg. The average value for the DK sample is significantly higher than that of the others ($p > 0.05$). These results are lower than the maximum limits recommended by the Codex Alimentarius, 2001, which is 15 mg/kg, but are close to those obtained by [39].

in a study on Algerian honeys, where they found contents ranging from 1.95 to 6.37 mg/kg. [36] found similar values to ours in Palestinian honeys, ranging from 2.00 to 10.81, with an average of 5.21 ± 0.49 mg/kg. However, these results remain higher than those found by [2] in honeys from western Algeria, which range from 0.811 to 4.713 mg/kg, and also higher than those found by [20]. In Tunisian honeys, with an average of 1.86 ± 0.97 mg/kg. In a recent study of Croatian honeys. [40]. found average iron contents of 3.03 ± 1.82 mg/kg. These variations in iron content are due to soil types, beekeeping practices and environmental conditions. Statistical analyses show very significant differences ($p < 0.001$).

Figure 2 summarises the iron content of the samples.

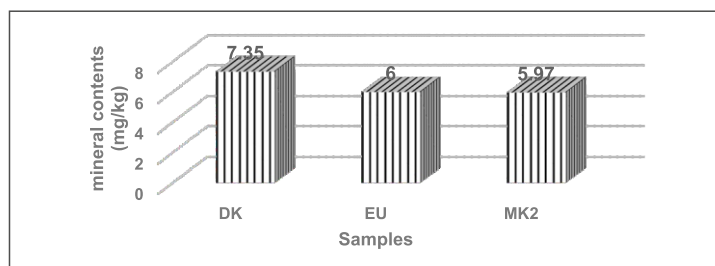


Figure 2: Iron content of samples

Potassium (K) content

This varies between 0.01 and 0.027, with an average of 0.019 ± 0.0 mg/kg. The average value of the EU sample is significantly higher than that of the others ($p > 0.05$). These results are much lower than those reported in studies by several other authors, such as [36]. With an average of 183.86 ± 31.1 mg/kg, [41] with an average of 358.95 ± 95.06 mg/kg, and [37]. with an average of 699.5 ± 296.24 mg/kg. The low potassium content of our honeys can be explained by several factors, namely the source of nectar, the honey production process and environmental conditions (soil conditions and climate type). Concerning the source of nectar, some flowers may naturally contain less potassium, which is reflected in the honey produced from their nectar. In summary, potassium deficiency in honey is the result of complex interactions between the plants visited by bees, the honey production process itself, and environmental conditions. Statistical analyses show highly significant differences ($p < 0.001$).

Figure 3 illustrates the K content of our samples.

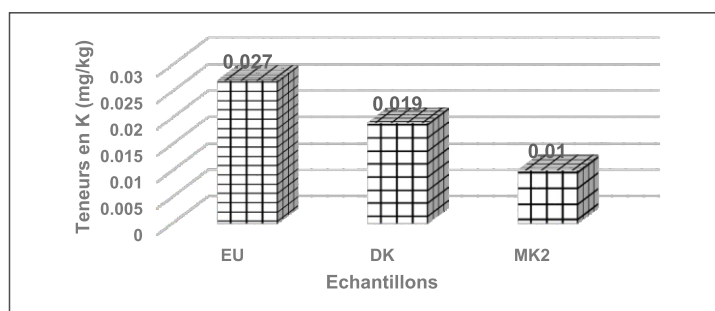


Figure 3: Potassium content of samples

Conclusion

The results obtained in this study reveal the high phenolic and flavonoid content of the honeys analyzed, which are closely linked to significant antioxidant activity. This correlation confirms the essential role of polyphenols and flavonoids in neutralizing free radicals, thereby reinforcing their importance in preventing oxidative stress and associated degenerative diseases. Indeed, the promotion of our honeys as a potential source of natural antioxidants opens up interesting prospects

for the development of nutritional supplements or functional foods. Analysis of mineral content, particularly sodium, iron and potassium, reveals that honey is a significant source of micronutrients essential for nutritional balance. However, further research, particularly in vivo, is needed to better understand the mechanisms of action and biological effects of these compounds.

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Conflicts of interest

The authors declare no conflicts of interest related to the conduct of this study.

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