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Quantitative ^{1}H NMR Characterization of Eight Honey Samples from the Western, Northern and Central Regions of Ivory Coast

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Abstract

This study used quantitative ^{1}H NMR to characterize the chemical composition of eight honey samples from three distinct regions of Côte d'Ivoire. The analysis was carried out according to a standardized protocol in a certified laboratory. This analysis made it possible to identify and quantify thirteen major compounds with varying concentrations depending on the geographical origin. The carbohydrate profile, predominant in all samples, is dominated by fructose and glucose, with respective proportions of 35.6% to 38.9% and 25.8% to 32.4%. Minor sugars, including turanose, sucrose, and mannose, were detected in low concentrations. The analysis also revealed the presence of several compounds of interest for honey quality: carboxylic acids including citric, acetic, lactic and succinic acids; ethanol; as well as proline, an amino acid recognized as an indicator of maturity. 5-HMF, a marker of thermal degradation, and its precursor dihydroxyacetone, complete this detailed chemical profile. The measured levels of these compounds, particularly 5-HMF and proline, directly influence the organoleptic properties and allow for the assessment of the overall quality of the analyzed honeys.

Keywords

Honey, Ivory Coast, ^{1}H NMR, Chemical Composition, Honey Quality

1. Introduction

Beekeeping is experiencing increasing growth worldwide, and particularly in Côte

d'Ivoire. In this country, this activity constitutes an important source of income in the North, West and Central regions, where the honey produced is notably used in the formulation of many therapeutic products [1] [2]. These medicinal properties are explained by its rich and complex composition, including sugars, enzymes, minerals, vitamins, phytocompounds, proteins and amino acids [2]-[4]. However, despite remarkable floral potential and favorable climatic conditions, Ivorian honey production remains modest, undervalued, and insufficiently organized. Consequently, national production does not cover local demand, forcing the country to rely partially on imports [2]. This situation can be explained by several factors: the persistence of unproductive traditional methods, a lack of technical training, insufficient modern equipment and limited structuring of the sector [4]. Thus, honeys produced in Ivory Coast do not benefit from any official recognition (label, certification of origin), which restricts their access to national and international markets [5]. Although studies have already assessed the quality of honey by physico-chemical parameters [6], a specific study on the characterization of the geographical origin of Ivorian honeys would represent a real added value for the sector. This work falls within this objective, its interest lying in the application of the 1H NMR profiling method. To achieve this, we use quantitative 1H NMR, a method for characterizing and quantifying chemical compounds in many food products [7]-[9]. This technique allows us to determine and quantify the chemical composition of honey samples from three distinct regions of Côte d'Ivoire.

2. Study Materials

The study focuses on eight honey samples collected between February and May 2024 from private beekeepers distributed across three regions of Ivory Coast: the North, the West, and the Center (**Table 1**). The collection sites were selected based on their intensity of beekeeping production. All samples, of polyfloral type and of varied botanical origins, were packaged in glass bottles and then kept away from light and at room temperature until analysis.

Table 1. Presentation of the honey samples studied.

Harvesting areas	Sample	Localities	Harvest date	Floral type
CENTER	M_1	Prikro 1	March 2024	*Daniella oliveri*
	M_2	Prikro 2	March 2024	*Manihot glaziovii*
	M_3	Prikro 3	March 2024	Arbustes de la savane
WEST	M_4	Biankouma 1	May 2024	*Coffea arabica - Ceiba pentandra*
	M_5	Biankouma 2	May 2024	*Elaeis guineensis et autres*
NORTH	M_6	Ferkessedougou	April 2024	*Vitellaria paradoxa Adensonia digitata Anacardium occidentale*
	M_7	Lamekaya	May 2024	*Vitellaria paradoxa Mangifera indica Parkia biglobosa Adensonia digitata*
	M_8	Sinématiali	February 2024	*Parkia biglobosa-anacardium occidentale*

3. Method

3.1. Principle of Analysis

The identification and quantification of compounds in the eight honey samples were performed using quantitative ^{1}H NMR. This technique has demonstrated its effectiveness for the authentication and quality control of food products, particularly honey [7]-[9].

3.2. Sample Preparation

For each sample, 2.5 g of honey was dissolved in 10 mL of distilled water. The resulting solution was filtered to remove any insoluble residues. To minimize chemical shift variations and to standardize the signals of organic acids, the pH of the sample was adjusted to a value of 3.1. Tetramethylsilane (TMS), used as an internal reference for chemical calibration of the spectrum, was then added. Each preparation was finally transferred to a specific NMR tube and then sealed for analysis.

3.3. NMR Analysis

Each of the eight (8) NMR tubes is placed in the NMR spectrometer, which generates an intense magnetic field. Radiofrequency waves are emitted to excite the nuclei of the samples being studied. The signals emitted by the excited nuclei are detected and recorded. Processing these signals generates the NMR spectra [11].

3.4. Acquisition of Spectra

Each of the eight tubes was successively placed in the NMR spectrometer. Under the effect of an intense magnetic field, the nuclei of the hydrogen atoms (protons) of the compounds are excited, detected, recorded and processed to generate the characteristic ^{1}H NMR spectra of each sample [11].

4. Results and Discussion

- Overall spectral profile

Direct ^{1}H NMR analysis of the honey samples revealed a complex spectral profile characteristic of the biological matrix. **Figure 1** shows a representative spectrum of these mixtures, where the main signals are observed in the chemical shift region between 1.0 and 5.5 ppm. Each signal (or peak) in this region corresponds to specific protons belonging to different chemical compounds present in the honey.

- Identification and quantification of compounds

Detailed interpretation of the spectra, based on systematic comparison with spectral databases and data from the scientific literature, allowed for the unambiguous identification of thirteen distinct compounds. The quantification of these compounds was performed by integrating the areas beneath the corresponding resonance peaks. This method, the foundation of quantitative NMR, enabled the

precise determination of the relative chemical composition of the eight samples, the results of which are recorded in Table 2.

Table 2. Composition of the eight honey samples (M_1 to M_8) expressed as percentages.

Compounds	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8
Fructose	38.5	36.8	37.3	37.9	38.1	35.6	38.9	37.1
Glucose	25.8	28.9	29.4	29.7	26.8	30.7	32.4	29.0
Sucrose	0.5	0.5	0.5	<0.5	<0.5	1.9	<0.5	<0.5
5-HMF	0.003	0.02	0.02	0.0055	0.004	0.001	0.003	0.0052
Turanose	1.36	2.27	2.27	1.86	2.75	0.84	1.66	1.96
Citric acid	0.02	0.0115	0.011	0.0054	<0.005	0.0083	0.008	0.02
Ethanol	0.2	0.001	0.0021	0.005	0.002	0.14	0.0042	<0.0005
Acetic acid	0.01	0.0021	0.0023	0.005	0.003	0.0112	<0.001	0.006
Lactic acid	0.02	0.0032	0.0054	0.075	0.02	0.015	0.005	0.0058
Succinic acid	0.012	0.0023	0.0025	0.008	0.0024	0.0045	0.003	0.0035
Proline	0.062	0	0	0.06	0.054	0.058	0.046	0
Mannose	<0.05	0.05	0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Dihydroxyacetone	<0.002	0.002	0.002	<0.002	<0.002	<0.002	<0.002	<0.002

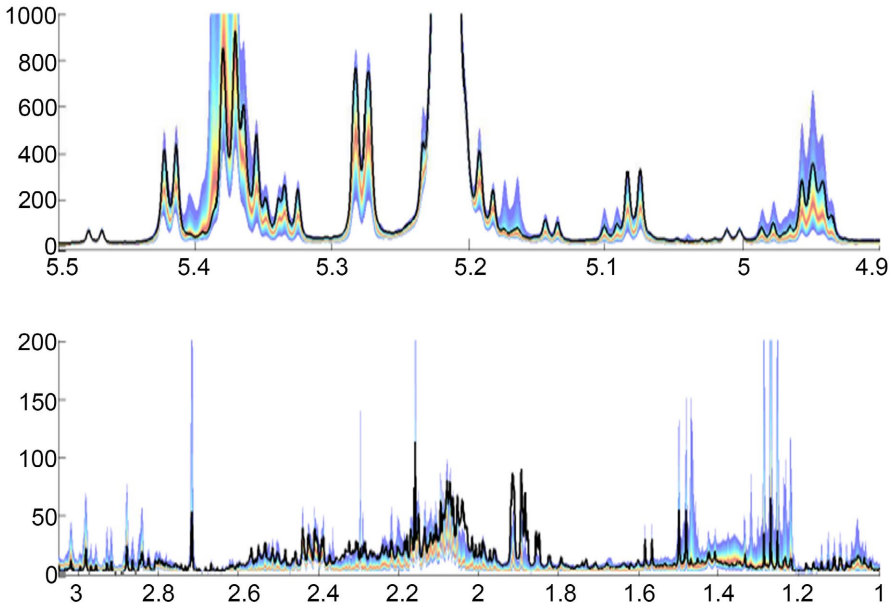

Figure 1. ^{1}H NMR spectrum of honey from the eight honey samples.

Analysis of the Chemical Composition of Honeys

- Sugar composition

^{1}H NMR analysis identified thirteen compounds in the honey samples, including the three major sugars: fructose, glucose, and sucrose. These constitute the main fraction of honey, representing approximately 70% to 80% of its composi-

tion. The fructose (35.6% - 38.9%) and glucose (25.8% - 32.4%) levels measured in the samples are within the limits set by the Codex Alimentarius Commission, which specifies ranges of 32% to 42% for fructose and 26% to 36% for glucose [5] [10]. These values confirm the predominance of these two sugars in natural honeys.

The fructose/glucose (F/G) ratio in **Table 3**, an important indicator of floral origin and crystallization tendency, was calculated for each sample. All samples had an F/G ratio greater than 1.1, characteristic of fructose dominance, typical of natural honeys [5]. A high ratio, such as that of M_1 (1.49), promotes longer storage in liquid form, while a lower ratio, such as that of M_6 (1.16), accelerates crystallization. Furthermore, sample M_7 stands out due to its simultaneously high fructose (38.9%) and glucose (32.4%) content, which could reflect a specific botanical origin.

Table 3. Ratio of fructose to glucose content in honey samples.

Honeys	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8
Fructoses (F)	38.5%	36.8%	37.3%	37.9%	38.1%	35.6%	38.9%	37.1%
Glucoses (G)	25.8%	28.9%	29.4%	29.7%	26.8%	30.7%	32.4%	29.0%
F/G	**1.49**	**1.27**	**1.27**	**1.28**	**1.42**	**1.16**	**1.20**	**1.28**

- Sucrose content and authenticity markers

Sucrose content is a key indicator of honey maturation and authenticity. In a mature and unaltered product, it remains low, as sucrose is hydrolyzed by bee enzymes during honey production in the hive. Regulations, particularly those of the Codex Alimentarius [5], generally set a maximum threshold of 5%. In accordance with this expectation, the majority of the samples analyzed (M_1 to M_5, M_7, M_8) have a very low content (≤0.5%), indicating good maturation (**Table 2**). In contrast, sample M_6 stands out with a significantly higher concentration (1.9%). This abnormal value may suggest premature harvesting, the possible addition of exogenous sugar, or, to a lesser extent, a specific botanical origin, as some honeydew honeys can naturally contain a higher residual level. These observations corroborate the conclusions of Yeboué *et al.* [12].

The presence of other characteristic sugars further supports this assessment. Turanose, a rare sugar synthesized during the enzymatic digestion of nectar and absent from commercial sugars, was detected in all samples (0.84% - 2.75%). Its presence thus confirms the authenticity of the honeys analyzed, with variations in content likely linked to their floral origin.

Finally, mannose and dihydroxyacetone (DHA) were identified in trace amounts in all samples. Since DHA is an abundant marker of heather honey, its low concentrations here rule out a dominant botanical origin of this type.

- Freshness and Aging Indicator

The 5-HMF (hydroxymethylfurfural) content, which ranges from 0.001% to 0.02% in the samples (**Table 2**), is a critical indicator of honey's freshness and heat

history. This compound forms naturally during aging, but its formation is significantly accelerated by excessive heating [13]. The Codex Alimentarius standard establishes a maximum limit of 40 mg/kg (or 0.004%) [5]. In this regard, samples M_2 and M_3, with a content of 0.02%, have a concentration five times higher than this limit and twenty times higher than that of M_6. This result strongly suggests that these honeys have undergone intensified heating, potentially for liquefaction or pasteurization, or that they have reached an advanced stage of aging. Conversely, sample M_6 (0.001%) stands out for its remarkable freshness and has clearly not been exposed to significant heat treatment. The other samples (M_1, M_4, M_5, M_7, M_8) show levels within acceptable to good ranges, in compliance with regulatory limits.

- Composition in alcohols, acids and other compounds

The presence and concentration of certain alcohols and organic acids are key indicators of honey stability and authenticity. In particular, the detection of ethanol and specific acids can reveal a fermentation process, often induced by excessive water content (>18%) or poor storage conditions [13].

Ethanol, a direct by-product of sugar fermentation by yeast, is a recognized marker of degradation [13]. In this study, the levels ranged from 0.0005% to 0.2%. Samples M_1 (0.2%) and M_6 (0.14%) showed abnormally high concentrations, indicating probable fermentation, which represents a major quality defect. This observation is corroborated by the organic acid profile. Acetic, lactic, and succinic acids, produced during fermentation, are present at particularly high levels in sample M_1, confirming its instability. Notably, sample M_4 stands out with a very high concentration of lactic acid (0.075%), suggesting either a specific lactic acid fermentation or a particular botanical origin. Furthermore, citric acid, the presence of which in trace amounts is normal, is found in standard proportions for all samples, indicating no adulteration with acidic syrups.

Analysis of proline, the main amino acid in pollen honey, provides further insight into authenticity. A sufficient level (generally > 0.05%) indicates maturity and raises suspicion of potential fraud through the addition of refined sugars, which do not contain proline. Here, samples M_2, M_3, and M_8 show zero proline content, which could be a serious warning sign of possible adulteration [10]. The other samples have levels that meet expectations (**Table 2**), confirming their authenticity based on this criterion.

5. Conclusion

The aim of this research was to identify probe molecules to characterize the geographical origin of honey from Ivory Coast, using ^{1}H NMR as the main quality control tool.

The analysis of eight samples, taken from the Central, Northern and Western regions of the country, made it possible to validate their typical profile (dominant fructose/glucose, turanose) while identifying critical quality markers (5-HMF, ethanol, proline) revealing specific defects such as fermentation or excessive heat-

ing.

The main contribution of this pioneering work is that it suggests geographical origin influences honey composition more than botanical source, paving the way for reliable honey authentication. Beyond quality control, these unique metabolic profiles offer promising opportunities for the development of Ivorian honey in the functional food and nutraceutical sectors. The small sample size did not allow for a statistical analysis, providing a better visualization of the results.

6. Perspectives

The results obtained open up several strategic perspectives:

- Expand sample collection to all production areas in the country to validate and refine the identified markers.
- Use these chemical signatures as a scientific basis for labeling Ivorian honey, in order to differentiate it on the market.
- Map honey-producing areas and provide technical support to beekeepers in the certification process.

Conflicts of Interest

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

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