

Organoleptic, Physico-Chemical and Bacteriological Characterization of Two Main Varieties of Honey from Haut-Katanga

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Publication Date: 2025/10/30

Abstract: The objective of this study was to analyze some physicochemical parameters (density, pH, water content and sugar content), organoleptic (taste, color and odor) and bacteriological (counting of *Escherichia Coli* colonies) of the two main varieties of honey from Haut-Katanga in the laboratory, in order to determine their qualities. Thus, the results obtained showed that likasi honey is much more altered than Lubumbashi honey. The water content contained in these two samples is consistent with food codex standards i.e. less than or equal to 20%. The pH for both varieties remains within codex standards food because ranging from 3.88 to 4.5 while remaining in the range of the standard which wants it to be between 3.5 and 4.5. So both of ours varieties of honey are acidic. The sugar content remains lower than the standard of 57.5 and 60% instead of 78 to 80% as required by the standard. The density of our two varieties of honey of 1.39 and 1.45 remains in conformity with the standard of the food codex which requires it to be in the range of 1.39 and 1.45g/m³. Both samples analyzed showed a high level of *Escherichia coli* or 3800 and 210 CFU/10g contrary to the standard which requires zero presence of these pathogenic germs in honey. Regarding the organoleptic parameters, we arrived at the following results: Both varieties of honey are brown in color and smell floral with a sweet flavor. These results therefore confirm the hypothesis that, two main varieties of honey from Haut-Katanga sold in Kinshasa honeys present alterations linked to poor production, conservation and transport conditions which would justify a low sugar content due perhaps to an accidental addition of another syrup in these honeys and a strong presence of *Escherichia Coli* indicating proof of a fecal contamination probably occurred in the process of either production, storage or transport of these honeys. This alteration in the sugar content in an isolated manner without modification of the other physicochemical and organoleptic parameters shows that there was no deliberate intention to fraudulently modify the quality of this honey for economic purposes but rather this calls into question the process of production, conservation and transport of this precious resource and this is also supported by the strong presence of pathogenic germs of fecal origin in the samples analyzed. Thus, this study recommended to other researchers to do the microbiological analysis of the production environment of these honeys in order to find the origin of possible contaminations detected in the laboratory while also taking into account other microbiological parameters not analyzed in this work. And from the Physicochemical point of view this study encouraged other researchers to deepen certain physicochemical aspects not taken into account in this work such as the refraction rate, the hydroxymethylfurfural content (HMF content), the concentration of heavy metals, the concentration of pesticide and veterinary drug residues. To the national authorities responsible for this sector, we propose the establishment of an environmental monitoring system in the two main sites (Likasi and Lubumbashi) with a view to controlling upstream the environmental conditions of production, conservation and transport of this honey in Haut-Katanga and strengthening downstream the quality control mechanisms before marketing and consumption of this honey in Kinshasa.

Keywords: Characterization, Organoleptic, Physico-Chemical, Bacteriological, Honey.

How to Cite: Lucien Mbalala Mosengo; Divin Muepu Mudinga; Archal Ngandote Mutemusa; Daniel Mudinga Mudinga (2025) Organoleptic, Physico-Chemical and Bacteriological Characterization of Two Main Varieties of Honey from Haut-Katanga. *International Journal of Innovative Science and Research Technology*, 10(9), 3084-3092. <https://doi.org/10.38124/ijisrt/25sep1523>

I. INTRODUCTION

Currently, several questions arise regarding the quality of food consumed by the world's population in general. Health issues have been a major concern since the dawn of time, as human life depends on them. With the advancement of science, many products and foodstuffs undergo quality control through in vitro experiments to determine their quality and compliance with various standards in force.

Indeed, honey is a commodity produced by honeybees from flower nectar or certain secretions from or found on living parts of plants, which they forage, transform, combine with specific materials, store, and allow to ripen in the hive's combs. This commodity can be a thick, fluid, or crystallized (Donadieu, 2003).

Honey, a completely natural sweet substance, is one of the products from the hive that has been used for millennia by many civilizations for its nutritional qualities and therapeutic uses. Medical uses are also mentioned in various pharmacopoeias, particularly for treating infected wounds or for boosting energy. (Gonnet M, 1982).

Nowadays, with the rise of natural medicines and in the face of certain pathologies resistant to conventional treatments, honey can be an asset thanks to its therapeutic and, above all, antibacterial properties.

In light of all these virtues mentioned above, a phenomenon that has become almost commonplace in the honey sector is currently being observed: "honey fraud."

In the current context of our developing countries in general and the DRC in particular, growing concerns about the integrity of food products still persist. Thus, it may happen that the much-vaunted honey from the deep country presents an alteration in its composition resulting from fraudulent techniques to make it more economically profitable, or alterations linked to poor production, conservation, or even transport conditions of this resource. Hence, it is necessary to conduct analyses to effectively detect the quality of these honeys.

In light of the concerns raised by fraud and production conditions in the beekeeping sector, our research question is: Do the two main varieties of honey from Upper Katanga (from Likasi and Lubumbashi) sold in Kinshasa meet the standards recommended by the Food Codex?

In response to the question posed above, we hypothesize that: The two main varieties of honey from Upper Katanga (from Likasi and Lubumbashi) sold in Kinshasa exhibit alterations related to poor production, storage, and transportation conditions, and not alterations related to fraudulent techniques for economic profitability.

II. OVERVIEW OF THE ENVIRONMENT, MATERIALS, AND METHODS

➤ Overview of the Environment

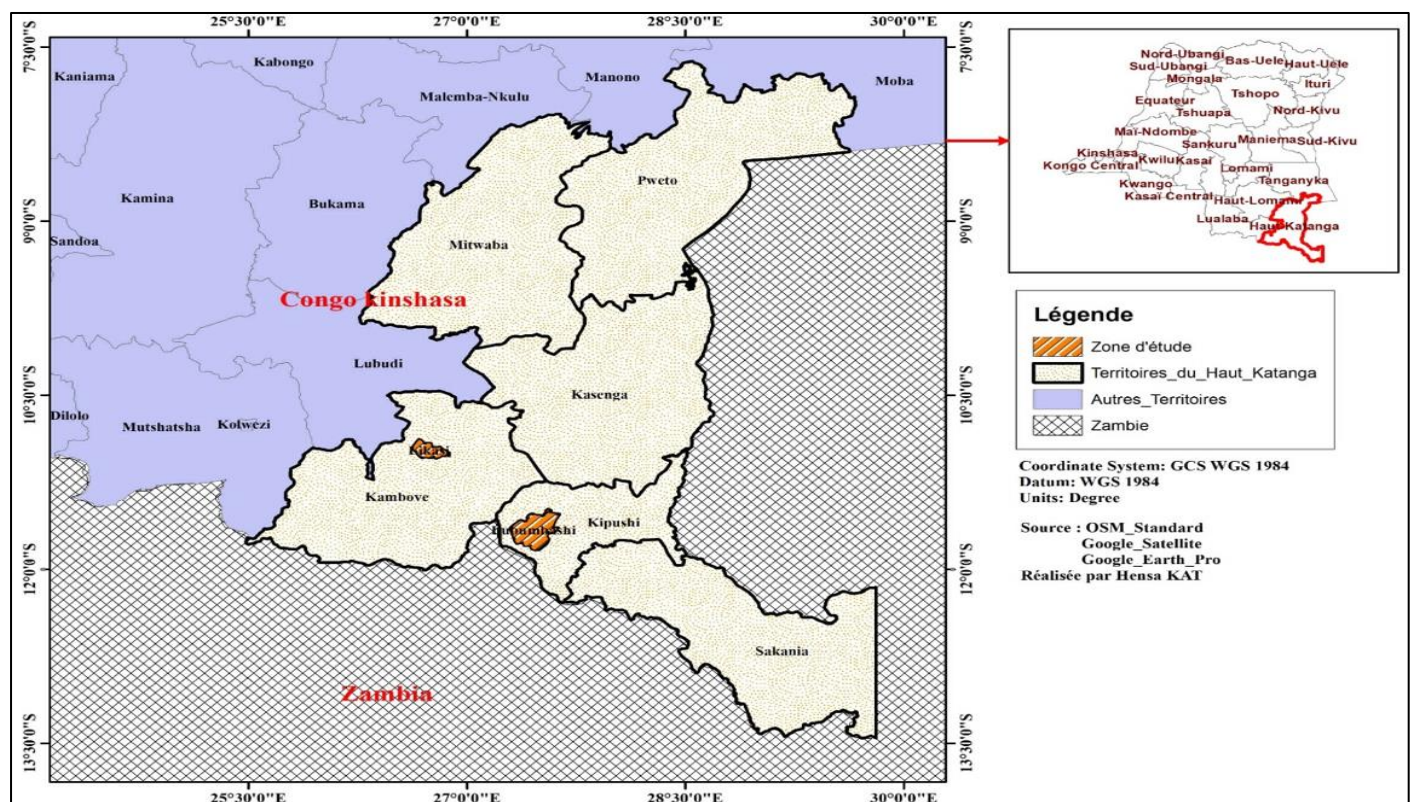


Fig 1 Map the Cities of Likasi and Lubumbashi in the Province of Upper Katanga

Source: Lucien Mbalala

III. MATERIALS USED

As part of our research, it is necessary to conduct in vitro analyses. To do this, we used the following equipment:

- Incubator: promotes the development of young organisms or microorganisms by protecting them from sudden changes in temperature, humidity, oxygen, and CO₂;
- Beaker: container used for many laboratory applications. It is the most commonly used piece of glassware in a laboratory because it allows for many stirring tasks: solution preparation, heating, titration. It indicates the volume of the contents.
- pH meter: electronic device that displays the pH.
- Pycnometer: used to measure the density and specific gravity of a liquid at a given temperature.
- Viscometer: designed to measure the viscosity of fluids.
- Magnetic stirrer: device used to mix two components homogeneously.
- Magnetic stir bar: Used to stir a solution as described: Place the magnetic stir bar in the container on a magnetic stirrer, ensuring that the bar is centered on the stirrer plate.
- Balance: Used to measure mass.
- Membrane filter: Used to filter organic solvents and aqueous solutions.
- Vacuum pump: Used to create a vacuum in a closed chamber system. Membrane pumps are distinguished by their ability to penetrate a vacuum of 1000 to 1 millibar with excellent vapor resistance.
- Petri dish: Used in microbiology for culturing microorganisms, bacteria, or cells of higher organisms.

IV. METHODOLOGY

➤ Methods

To carry out this work, we used the following methods:

- Descriptive method: This allowed us to describe the honey, its origin, and its characteristics.
- Analytical method: This was based on the methods described by (Bogdanov S et al, 1995, 1999, 2002, and 2004) as well as (Jeanne F., 2005) for each parameter below.
- *Hydrogen Potential (pH):*
The hydrogen potential (pH) was determined for honey samples from Lubumbashi and Likasi.
 - ✓ Principle: This involves assessing the acidity or basicity of a product.
 - ✓ Apparatus: The pH of the various honeys was determined using a device called a pH meter (Starter 400 Series).
- *Procedure:*
For the honeys, 2g of the sample was taken and placed in a clean, dry beaker;
 - ✓ 15 ml of distilled water was added and gently mixed to homogenize the solution;

- ✓ The pH meter was previously calibrated with buffer solutions of pH 4.01 and pH 7.00;
- ✓ The pH was measured by dipping the pH meter electrode into the honey solution diluted in distilled water;
- ✓ The pH reading on the device was waited for to become stable;
- ✓ The value displayed on the pH meter was noted;
- ✓ The steps were repeated 1 to 4 times to obtain reproducible results.
- ✓ Standard: It is important to note that the pH of honey is generally between 3.4 and 6.1.

• Humidity (Water Content)

- ✓ Principle: Moisture was determined for both varieties of honey. This involved drying a sample at a temperature of 105°C for 24 hours and determining the amount of evaporated water.
- ✓ Apparatus: To determine the water content, a precision balance capable of weighing to the nearest 0.0001 g was used. A spatula, a crucible (PO), and an oven were also used.

• Procedure:

- ✓ 5g of honey samples (PE) were weighed into a crucible (PO) previously dried in the oven for 24 hours;
- ✓ For each sample, three tests were performed.
- ✓ The crucibles were removed from the oven and then placed in the desiccator;
- ✓ After cooling to room temperature (between 25°C and 30°C);
- ✓ Finally, the crucibles are weighed (MP).
- ✓ Expression of results: The mass percentage of water and volatile matter is obtained according to the following formula:

$$\% \text{ Humidity} = \frac{\text{PE} - (\text{PF} - \text{PO}) \times 100}{\text{PE}}$$

• Knowing that:

- ✓ PE: Test sample;
- ✓ PO: Weight of the empty crucible;
- ✓ PF: Final weight.

• Density

The density was determined for both honey samples.

- ✓ Principle: The measurement principle is based on the ratio between the density of the sample and that of a reference substance at a temperature of 20°C. Apparatus: The pycnometer. Procedure:

After measuring the weight of the empty pycnometer;

The weight of the pycnometer is then measured with a honey sample at 20°C; the result is recorded.

- ✓ Expression of the results: The density is obtained using the following formula:

$$D = (P_f - P_i)/99.202$$

Where:

P_i : mass of the empty pycnometer,

P_f : mass of the pycnometer with sample,

99.202: mass of water contained in the pycnometer at 20°C.

- *Sugar Content*

The sugar content was determined for both honey samples.

- ✓ Principle: Sugar analysis provides information on the floral origin. It is an analysis that also shows that the honey has not been adulterated with sugar. Several types of sugars are analyzed, such as glucose, fructose, sucrose, maltose, etc.
- ✓ Apparatus: A refractometer is used.

- *Procedure:*

For honey, 5g of the mixture was taken and placed in a clean, dry beaker, followed by the addition of 15ml of distilled water;

The mixture was left stirring for 48 hours to allow the solution to become homogenized. After this step, two drops of this mixture were placed on the refractometer to read the raw percentage value on the instrument's display.

Sugar content was measured using a refractometer because a refractometer is an instrument that measures the refraction of light through a liquid, which allows the sugar concentration to be determined;

The reading on the instrument was waited for to stabilize;

The value displayed on the display was noted and then compared to the standard.

Standard: It is important to note that the sugar content of honey is generally between 78 and 80%.

- *Escherichia Coli*

For honey, 10g of sample was taken and placed in a clean, dry beaker;

100ml of distilled water was added and gently mixed to homogenize the solution.

A sterile membrane filter with a diameter of 47mm and an average pore size of 0.45µm was used.

The filter was then incubated at 37°C for 2 to 4 hours on a previously prepared Tryptone Soya Agar (TSA) culture medium.

The filter was then transferred to another culture medium (TBX) for 24 hours. The media were prepared by diluting the TSA and TBX in distilled water, bringing them to a boil, sterilizing them, cooling them, and then placing them in Petri dishes.

To obtain reliable results, three replicates of each culture were performed. Once the incubation time was over, the blue-colored bacterial colonies were counted.

The average number of bacteria found in the three replicates is considered the number of colony units formed, or Col.

The average number of bacteria found in the three replicates is considered to be the number of colony forming units (CFU) of *E. coli* in 10g of honey.

- ✓ Standards: From a microbiological perspective, mesophilic bacteria in honey must be less than 30 CFU/g and must not contain fecal coliform bacteria or human pathogenic microorganisms (germs, yeasts, fungi).

- *Techniques*

This study used the following techniques:

- ✓ Documentary technique: This technique helped us consult the various documents related to our study. These included written documents and reports, including general works, and academic work (DEA, theses, undergraduate dissertations, etc.).
- ✓ Direct observation: This technique was used to perform organoleptic analyses using sight, smell, and taste. It should be noted that for vision (color), we also referred to the Pfund scale.

- *Statistical Analysis*

All determinations were performed in triplicate, and the data were processed using Microsoft Excel 2019 software.

V. RESULTS AND DISCUSSION

➤ *Presentation of Results*

This article presents and interprets the results of our laboratory experiments on two control honey samples, one from Lubumbashi and the other from Likasi. The analyses were conducted in the Organic Analysis and Synthesis Laboratory (LASORG) of the Faculty of Sciences of the University of Kinshasa (UNIKIN), and the physicochemical and bacteriological results were compared to the standards of the Food Codex, 2001, and the organoleptic results were assessed to develop an overall profile of the two honey varieties studied. The two varieties analyzed presented the following results:

Table 1 Organoleptic Tests of Likasi and Lubumbashi Honey

Parameters	Likasi	Lubumbashi
Color	Brownish	Brownish
Odor	Floral	Floral
Flavor	Sweet	Sweet

Source : Personal Observation (Lucien Mbalala)

Here, the organoleptic results show that our two honey varieties are brownish in color, between white and very dark

brown, or between 0 and 140 on the Pfund scale, and have a floral aroma with a sweet flavor.

Table 2 Physicochemical Analyses of Likasi and Lubumbashi Honey

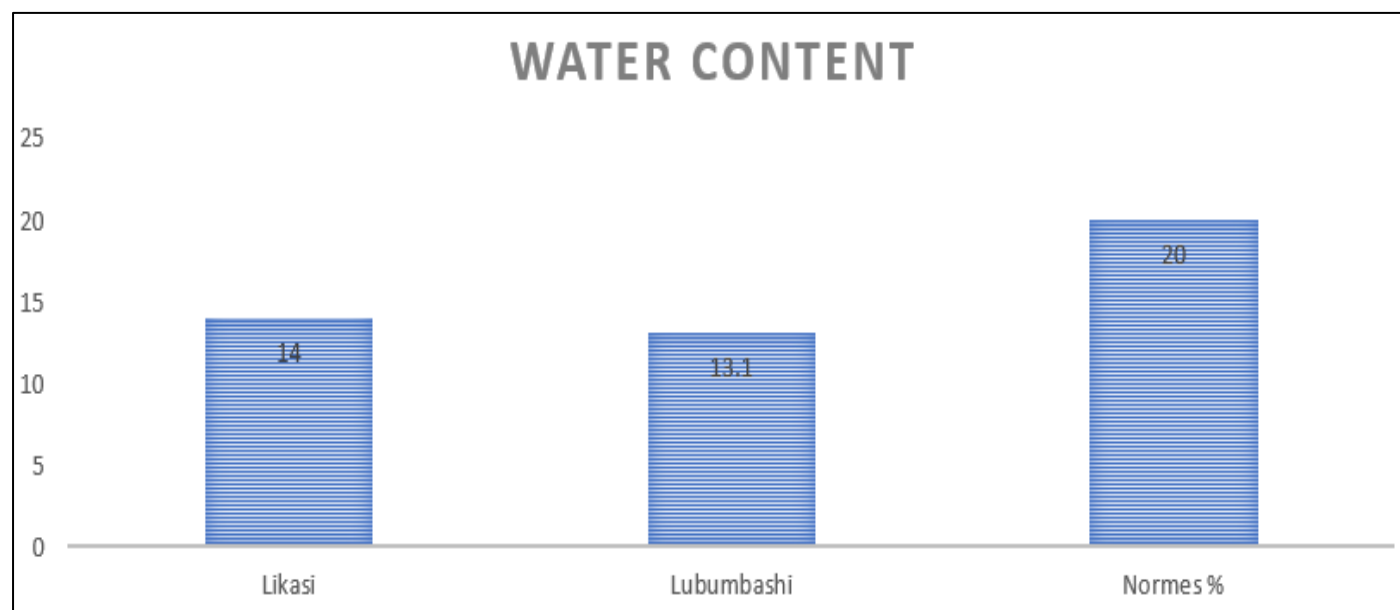
Parameters	Likasi	Lubumbashi	Food Codex Standards, 2001
Water content	14	13,1	$\leq \text{à } 20\%$,
Hydrogen Potential	3,88	4,02	Between 3,5 and 4,5
Sugar Content	57,5	60	Between 78 and 80 %
Density	1,39	1,40	Between 1,39 and 1,45 g/m ³

Source: Laboratory Results (Lucien Mbalala)

Table 3 Water Content

Parameters	Likasi	Lubumbashi	Standard
Water Content	14	13,1	$\leq \text{à } 20\%$

Source: Laboratory Results (Lucien Mbalala)



Graph 1 Water Content

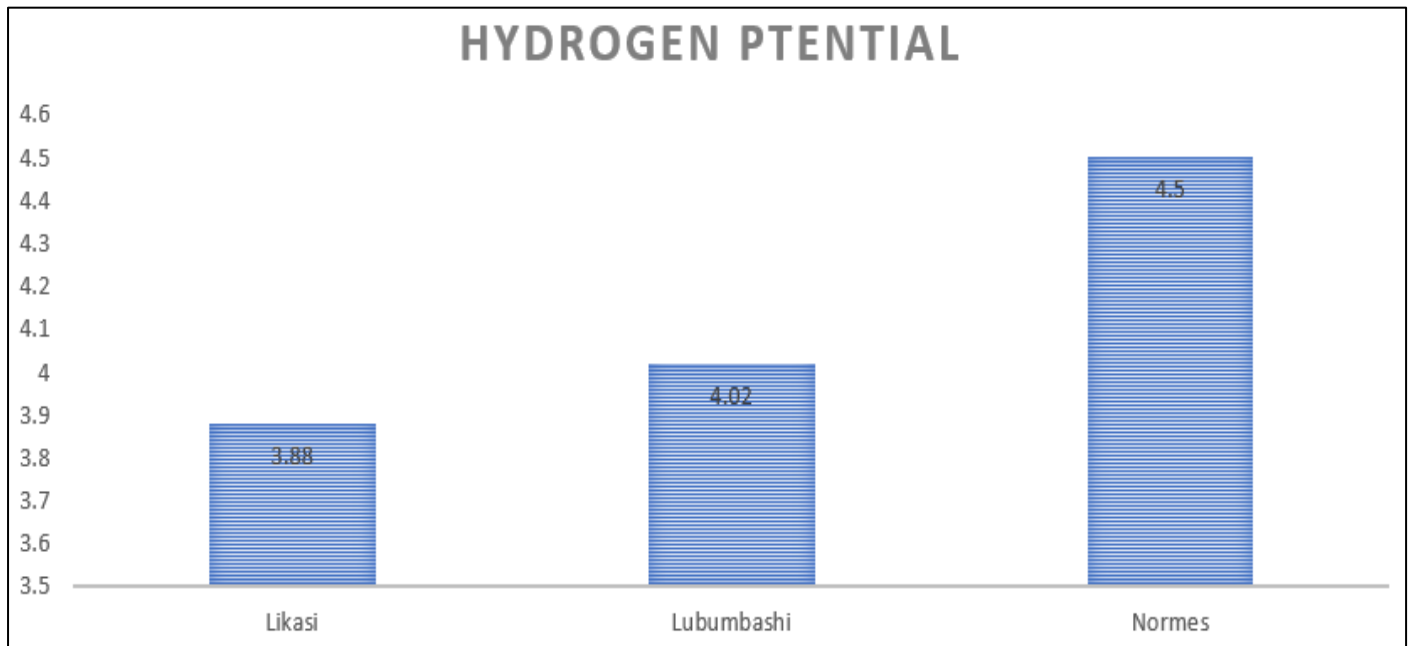
The results obtained show that the water content contained in these two samples complies with the standards

of the Food Codex. According to the standard, the water content must be less than or equal to 20%.

Table 4 Hydrogen Potential

Parameters	Likasi	Lubumbashi	Standard
Hydrogen Potential	3,88	4,02	Between 3,5 and 4,5

Source: Laboratory Results (Lucien Mbalala)



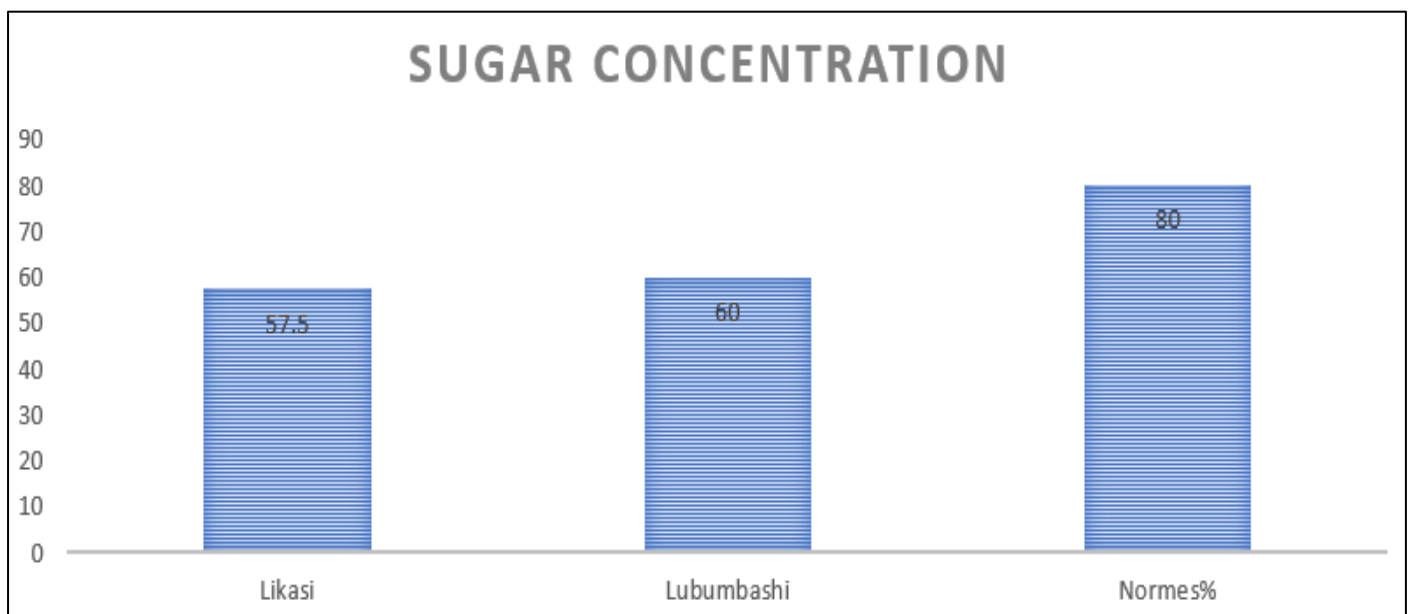
Graph 2 Hydrogen Potential

The results show that both samples analyzed have a pH in accordance with the CODEX food standards ranging from 3.88 to 4.5. Therefore, both of our honey varieties are acidic.

Table 5 Sugar Concentration

Parameters	Likasi	Lubumbashi	Standard
Sugar Content	57,5	60	78 to 80 %

Source: Laboratory Results (Lucien Mbalala)



Graph 3 Sugar Concentration

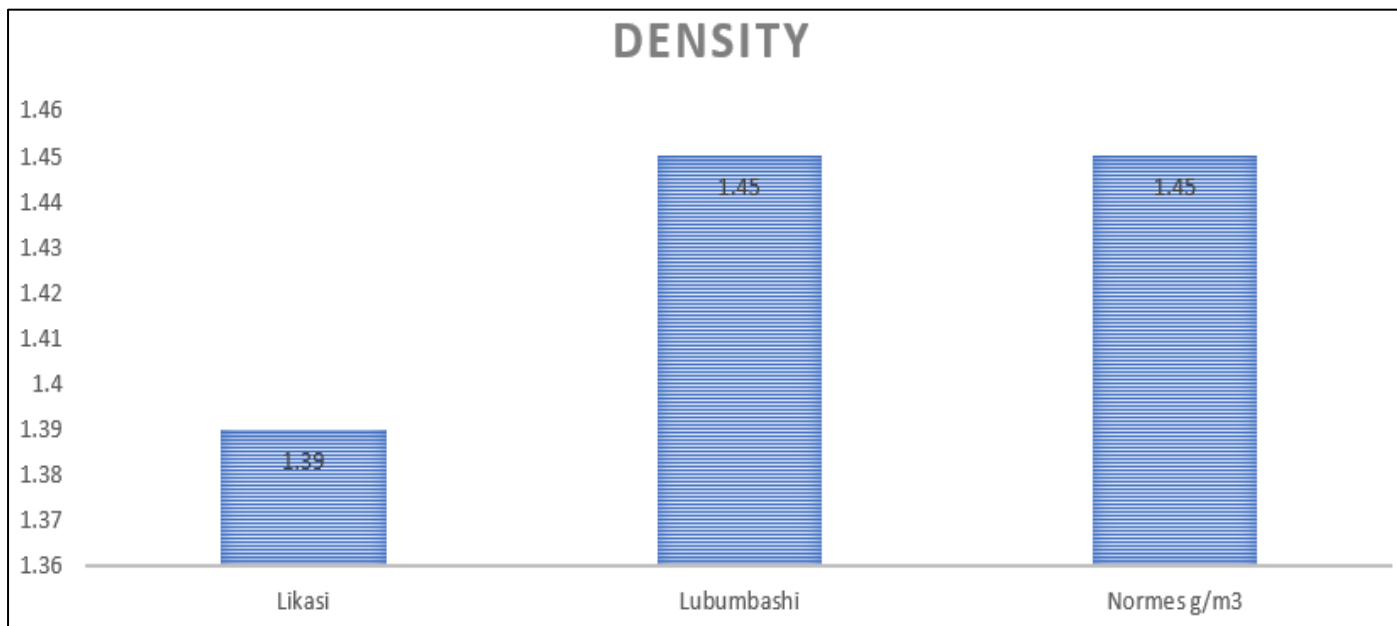
Here the results indicate that our two varieties of honey have a sugar content lower than the standard, which already

explains an alteration at the level of our two varieties of which the Likasi honey seems more altered.

Table 6 Density

Parameters	Likasi	Lubumbashi	Standard
Density	1,39	1,45	Between 1,39 and 1,45 g/m ³

Source: Laboratory Results (Lucien Mbalala)



Graph 4 Sugar Density

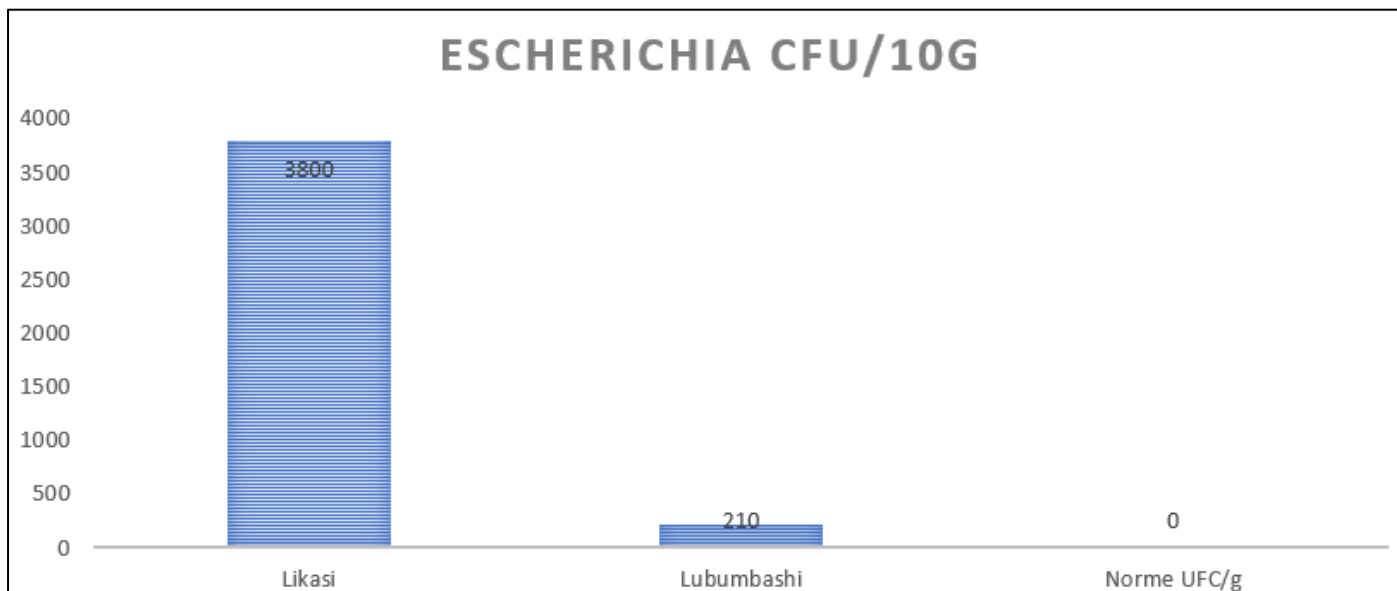
The results tell us that the density of our two varieties of honey complies with the food codex standard, which requires it to be in the range of 1.39 and 1.45g/m3.

Table 7 Bacteriological Analysis of Likasi and Lubumbashi Honey

Parameters	Likasi	Lubumbashi	Standard
Escherichia CFU/100g	3800	210	0 UFC/g

Source: Laboratory Results (Lucien Mbalala)

Both samples analyzed show a high level of Escherichia coli, which justifies fecal contamination in the two varieties of honey, of which Likasi honey is the most contaminated.



Graph 5 Escherichia CFU/10g

VI. INTERPRETATION OF RESULTS

The results obtained show that the water content is inversely proportional to the density, hence the higher the water content, the more viscous the honey and the lower the water content, the less viscous the honey. Thus, Likasi honey

with 14% water content is slightly more viscous with a density of 1.39g/m3 than that of Lubumbashi with 13.1% water content or 1.45g/m3 density. But in general, both varieties have low water contents and densities that comply with standards, which confirms their degree of chemical

stability, fermentation and possible crystallization during storage (Küçük et al, 2007).

Both varieties exhibited acidic pH values of 3.88 for Likasi honey and 4.02 for Lubumbashi honey. These values remain consistent with Food Codex standards and also confirm the results found by (Abersi et al., 2016), thus determining the plant origin of our two honey varieties.

The sugar content of our two varieties remains relatively low and does not comply with the standard, i.e., 57.5% for Likasi honey and 60% for Lubumbashi honey, whereas the standard requires that the latter be between 78 and 80%. However, sugars represent the main components of all types of honey. Reducing sugars, mainly fructose and glucose, are the major constituents of honey and alone represent nearly 90% of the sugars in honey (Bruneau, 2009). However, their low presence in our two varieties is an indicator of honey adulteration due to the addition of external syrups, particularly sugar (Abersi et al., 2016).

Microbiologically, our two varieties exhibited high concentrations of *Escherichia coli*, with 3,800 CFU/10g for Likasi honey and 210 CFU/10g for Lubumbashi honey. These results once again confirm a qualitative alteration, especially for Likasi honey.

This high presence of *Escherichia coli* in our two varieties confirms fecal contamination, which further reinforces the hypothesis that the production, storage, and transportation conditions of these two varieties of honey remain very precarious, especially for Likasi honey.

For our two main varieties, all the samples analyzed were nectar honeys, and they have a brownish color. Our results confirm those of Muepu (2023) only for Kwilu honey.

Regarding the different honeys, their odors vary considerably but evaporate very quickly. They are of vegetal, floral, or fruity origin, which can be strong or not, delicate, heavy, or vulgar. A smoky or fermented odor is a defect (Mokeddem, 1997). The odor is extremely variable and depends on the flowers. For our samples, the odor is floral. The two main varieties from Haut-Katanga analyzed retain a sweet flavor.

VII. CONCLUSION AND SUGGESTIONS

Honey has been a long-known food. Its preventive and curative medicinal properties have also been recognized since ancient times. Its economic value continues to rise despite technological advances in the agricultural sector, hence the importance of our study. The overall objective of this study was to analyze several physicochemical (density, pH, water content, and sugar content), organoleptic (taste, color, and odor), and bacteriological (*Escherichia coli* colony count) parameters of the two main varieties of honey from Haut-Katanga in the laboratory, in order to determine their qualities. The results obtained demonstrated that:

- The water content of these two samples complies with the Food Codex standards, i.e., less than or equal to 20%.
- The pH for both varieties remains compliant with the Food Codex standards, ranging from 3.88 to 4.5, while remaining within the standard range of 3.5 to 4.5. Therefore, both of our honey varieties are acidic.
- The sugar content remains below the standard, i.e., 78 to 80% (57.5 and 60%), instead of the standard of 78 to 80%.
- The density of our two honey varieties, 1.39 and 1.45, complies with the Food Codex standard, which requires it to be in the range of 1.39 and 1.45g/m³.
- The two samples analyzed showed high levels of *Escherichia coli*, i.e., 3,800 and 210 CFU/10g, contrary to the standard, which requires the absence of these pathogens in honey. Regarding the organoleptic parameters, we obtained the following results:
- Both varieties of honey are brownish in color and have a floral aroma with a sweet flavor.

In conclusion, these results confirm the initial hypothesis that the two main varieties of honey from Haut-Katanga sold in Kinshasa exhibit alterations related to poor production, storage, and transportation conditions, which would explain a low sugar content, possibly due to the accidental addition of another syrup to these honeys, and a high presence of *Escherichia coli*, indicating evidence of fecal contamination that likely occurred during the production, storage, or transportation of these honeys. However, alterations related to fraudulent techniques for economic profitability were not detected, as the results show that the essential physicochemical parameters analyzed comply with the Food Codex standards, with the exception of sugar content. This alteration in the sugar content in isolation without modification of the other physicochemical and organoleptic parameters shows that there was no deliberate intention to fraudulently modify the quality of this honey for economic purposes but rather calls into question the production, the preservation, and transportation process of this precious resource, and this is also supported by the high presence of pathogenic germs of fecal origin in our analyzed samples.

• Therefore, we Propose the Following:

- ✓ For any researcher who wishes to further these studies on the quality of honey from Haut-Katanga, we suggest conducting a microbiological analysis of the production environment of these honeys in order to detect the origin of any contamination discovered in the laboratory by our study. We also suggest taking into account other microbiological parameters not analyzed in this work.
- ✓ We also suggest conducting other physicochemical analyses not addressed in our study, such as refraction rate, hydroxymethylfurfural content (HMF content), heavy metal concentration, and pesticide and veterinary drug residue concentration. - To the national authorities responsible for this sector, we propose the establishment of an environmental monitoring system in the two main sites (Likasi and Lubumbashi) with a view to controlling upstream the environmental conditions of production,

conservation and transport of this honey in Haut-Katanga and strengthening downstream the quality control mechanisms before marketing and consumption of this honey in Kinshasa.

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