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Microbiological, Physicochemical and Sensory Characterization of Honey, a Natural Healthy Product in Burkina Faso

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Authors' contributions

This work was carried out in collaboration among all authors. Author TF designed the study, analysis, interpretation and manuscript preparation. Authors KB, SA, ZO, YWN, OH, CH, ZC and SA contributed to data collection, analysis, interpretation and manuscript preparation. Author SA had the role of supervision. All authors read and approved the final manuscript.

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ABSTRACT

Honey is a natural product produced by bees from the nectar of flowers. It is a very healthy food whose multiple properties significantly improve health and prevent many diseases. However, some practices can affect its quality, hence the objective of the study was to assess the honey safety

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from 6 honey-producing regions in Burkina Faso. The physicochemical, microbiological and sensory characteristics were determined using standard methods.
The densities ranged from 1.39 to 1.44; the pH, 5.73 to 6.56; the total acidity, 13.00 to 83.00 meq/kg; the Brix degree ranged 86.00 to 88.50%; the moisture,11.86 to 18.83%, the electrical conductivity, 101.00 to 155.00 µs/cm and the Hydroxymethylfurfural (HMF), from 14.67± 0.00 to 90.52±0.35.
Microbiological analysis showed the total counts varied from $1.21.10^5 \pm 1.18.10^4$ to $3.50.10^3 \pm 3.50.10^3$ to $1.21.10^5$ CFU/mL; yeast and mold rates were below 10^3 CFU/mL, spore contamination is also noted in some honey samples and was between $2.23\times10^1 \pm 2.51$ to $1.38\times10^2 \pm 7.63$CFU/mL), *Salmonella, Shigella* and coliform were not detected in the honey samples.
Sensory analysis revealed that the organoleptic characteristics of honey varied from one region to another. All the honey was differently appreciated by the tasters.

Keywords: Honey; microbiological quality; physicochemical quality; sensory quality; Burkina Faso.

1. INTRODUCTION

Honey is a natural product produced by bees of the species *Apis mellifera* from flower nectar and as well as honeydew, they collect, transform and store them in the combs of the hive [1]. It has been consumed by man since ancient times and traditionally used as a sweetener and for therapeutic purposes [2]. Honey is a very healthy food whose multiple properties significantly improve health and prevent many diseases. It prevents arthritis and helps reduce cholesterol levels. It increases energy and strengthens the immune system. It is a great ally in cleansing the body due to its antibacterial and antiviral properties [3, 4, 5].

This product was considered a preferred food by wealthy families. Thus, beekeeping began to experience significant growth in various regions of the world.

In Burkina Faso, this activity has an important place in the rural populations life. Indeed, agriculture and animal husbandry, which were the only main activities, must have to reckon with beekeeping as important sources of income [6].

Honey presents physicochemical and microbiological characteristics that make it possible to determine its botanical origin, its quality or its adulteration [7]. Among its physicochemical characteristics, Electrical Conductivity (EC) and pH are used to differentiate the geological botanical origin of honey, while parameters such as Hydroxymethylfurfural (HMF) content reflects its age and thermal past [7,8].

Honey the best known and the most consumed bee product. It is fashionable to say that honey is a "nutraceutical" (food-medicine), and that it is a "natural product" in our time when consumers are wary not without reason of foods available on the market. As a result, quality control is necessary for consumer satisfaction. The composition of honey can vary widely depending on the region, season, bee variety, plant source of nectar and storage time in the honeycomb as well as the mode of harvesting and post-harvest storage [9]. Honey is often adulterated or passed to heat either to increase its quantity or lifespan, in order to derive more benefits from it. These practices can be dangerous for people suffering from diabetes or even toxic for consumers, so it is necessary to assess the quality of honey consumed by the population in Burkina Faso for the preservation of it health. This study evaluated the physicochemical, microbiological and sensory characteristics of honey produced from six producing regions in Burkina Faso.

2. MATERIAL AND METHODS

Honey samples were collected from six producing regions in Burkina Faso: Cascade, Est, Boucle du Mouhoun, Sud-Ouest, Nord and Centre. In each region, a quantity of 350 mL of honey was twice collected into sterilized and labelled bottles.

2.1 Physicochemical Analysis of Honey

The evaluation of honey quality in Burkina Faso was assessed through the determination of the physico-chemical characteristics of samples from six regions of Burkina Faso.

The density of honey was determined using the method described by Qamer et al. [10]. Ten (10) mL of each sample were weighed using an electronic model scale and 10 mL of distilled

water were weighed. The density of the honeys was calculated according to the following formula:

$$d = \frac{m0}{m}$$

mo: mass of the sample; **m**: mass of water pH of samples were determined according to the AOAC method [11] using a Hanna model electrode pH meter. Ten (10) g of each honey were dissolved in 45 mL of distilled water and homogenized. The pH of the solution was read on the display of the pH meter.

The total acidity of the honey samples was determined by the volumetric titration according to AOAC 962.19 [11]. It was deduced from the volume of NaOH (0.05 N) added to the honey solution until a pH of 8.5 respectively is obtained. A 2.5 g of honey were dispensed into 25 mL distilled water in a 250 mL beaker and stired well. The initial pH of the solution was measured and then titrated with NaOH (0.0 5 N) to pH 8.5. The result was expressed in mEq of acid per kilogram of honey according to the following formula:

$$\text{Total acidity} = \frac{(\text{VNaOH} - \text{V b}) * 50}{\text{m}}$$

VNaOH= volume of NaOH solution poured in the presence of the sample in mL;**Vb**= volume of the NaOH solution for blank titration ; **m**= mass of honey.

Brix degree measurement was made using an ATC model refractometer. One (1) mL of the sample was used and the reading was made directly on the screen of the device after a countdown of ten seconds then expressed as a percentage (%) [11].

The moisture content of the samples was estimated using the thermogravimetric method [11]. The principle of this method is based on the physical elimination of water from the sample by heating in an oven. Five (5) g of honey were weighed and placed in an oven. The oven was heated to a temperature of 105° C for 24 hours. After which, the samples were removed, cooled in a desiccator for 30 minutes, then weighed. The moisture content was determined according to the following formula:

$$\text{H (\%)} = \frac{\text{M} - \text{m}}{\text{M} - \text{m0}} * 100$$

m0 = the weight of the empty capsule (g); **M** = the weight of the capsule and the sample before drying; **m** = the weight of the capsule and the sample after drying

The electrical conductivity (EC) of the honey samples was carried out according to the method described by Bogdanov et al. [12]. The measurements were carried out at 20°C in an aqueous solution. The reading was taken directly after immersing the conductivity cell in the solution. For this, 20 g of each honey sample were taken and dissolved in a beaker containing 100 mL of distilled water. This solution was placed in a bath equipped with a thermostat to have a temperature of 20°C. Finally, the conductimetry cell (Schott brand) was immersed in the beaker to measure the electrical conductivity. The results are displayed directly on the screen and were expressed in milliSiemens per centimeter (mS/cm).

Hydroxymethylfurfural (HMF) concentration was determined by the bisulphite method described by Bogdanov et al. [13] with slight modification. This method is based on the determination of the absorbance of HMF at 284 nm and 336 nm using the spectrophotometer, in order to avoid interference from other components. The HMF concentration was calculated using the formula:

$$\text{HMF(mg/Kg of honeyl)} = \frac{(\text{A284} - \text{A336})}{\text{P}} \times 149.7 \times 5$$

A284: Absorbance at 284 nm;
A336: Absorbance at 336 nm;
P: test portion

2.2 Microbiological Analysis of Honey

A volume of 10 mL of each sample was added under sterile conditions to 90 mL of a sterile physiological solution (9 ‰ NaCl) and homogenized. A ten-fold serial dilution of each honey sample was prepared. Mueller Hinton Agar (MH), Eosin with Methylene Blue (EMB), Sabouraud chloramphenicol media were prepared according to the manufacturer's instruction. Each culture media was sterilized in an autoclave at 121°C for 15 minutes.

The total counts were performed on Mueller Hinton agar after 24 to 48 hours of incubation at 37°C [14].

The Yeast and molds were counted on Sabouraud agar after 72-120 hours at 25°C [15].

The coliforms were counted on Eosin Methylene Blue (EMB) agar after 24 to 48 hours of

incubation at 30°C for total coliforms and 44°C for thermotolerant coliforms [16]. Colonies of total coliforms are red colonies surrounded by an opaque halo and those of thermotolerant are purple sand surrounded by a red halo.

The spores forming-bacteria were counted on the MH agar after 24 to 48 hours of incubation at 37°C [17].

Salmonella and *Shigella* were researched according to the ISO 6579 standard [18]. The research was carried out in 3 stages which are: pre-enrichment, selective enrichment and isolation.

For the pre-enrichment, a volume of 25 mL of each honey sample was taken aseptically and introduced into 225 mL of peptone water and then incubated for 24 hours at 37°C.

The selective enrichment was done by transferring, using a sterile pipette, 1 mL of the pre-enriched liquid medium into 09 mL of Rappaport-Vassiliadis (RV) selective liquid medium. Incubation done at 37°C for 24 hours.

The Isolation was made on *Salmonella-Shigella* (SS) agar, from the stock enrichment solution. Starting with a streak, the incubation was done at 37°C for 24 hours. The reading of the dishes and identification of *Salmonella* and *Shigella* is presented as follows: colonies most often blue-gray with a black center on SS agar.

For microbiological analysis, the results retained came from the counting of dishes containing between 15 and 300 colonies according to the ISO 7218 standard [19]. The number N of microorganisms present in the samples was calculated as the average weight of two successive decimal dilutions using the following formula:

$$N = \frac{\sum C}{V * d * (n1 + 0.1 n2)}$$

∑C= Sum of colonies counted on the dishes kept after two successive decimal dilutions;
V= Volume of inocula applied to each box;
d= dilution corresponding to the first dilution retained;

(**n1+0.1 n2**): n1 the number of plates of the first dilution and n2 that of the second dilution. For plates with a sum of colonies fewer than 15:

$$N = \frac{\sum C}{V * d}$$

For the box in which there was no colony observed less than 1 (<1/d)

2.3 Analysis of the Sensory Quality of Honey

Sensory analysis is the technique that uses the human senses to know and describe the organoleptic characteristics of a product. For this, 60 people aged, 20-45 years were selected to form the jury, giving particular importance to their repeatability and their ability to describe, to perceive the sensory quality of honey. Six honey samples were subjected to different tests. For each honey, the tasters had a corked glass bottle with a capacity of 100 mL containing 10 g of honey and were asked to describe the color, texture and smell. A glass also containing 10 g of honey was given to each person to describe the flavor and aroma of the honey samples respectively.

Between each honey, the subjects rinsed their mouth with mineral water and eating an apple wedge. All honey samples were presented anonymously with a two-digit code so as not to influence tasters.

2.4. Data Analysis

Analysis of variance (ANOVA) was performed and means were compared using Turkey's test at $p<0.05$.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Characteristics of Honey

The results of the physicochemical analysis of honey samples from the six regions of Burkina Faso are presented in Table 1.

The density or specific gravity of the different honey samples varied from 1.39 to 1.44 with a difference significative. Density values obtained were comparable to 1.42 for honey with moisture content of 17.5% at 20°C reported by Hoyet [20] and generally varies from 1.39 to 1.44. The variation in honey density could be explained by poor storage conditions. For example, if the container used in keeping the honey is poorly closed and the room is too humid, honey harvested prematurely, less ripe, will have a lower density.

The pH values of the honey samples studied ranged from 5.73 to 6.56 with a difference significative. The pH of honey samples from Ouahigouya (E5OU) and Ouagadougou (E6OA) had no significant difference ($p>0.05$). The pH obtained from our research were higher than 3.58-4.84 reported by Nombré et al. [7] for honey imported and sold in Burkina Faso. But our results are consistent with those reported by Hoyet [20] who reported that honey is acidic and the pH fluctuates between 3 and 6. According to Soria et al. (2004) [21], the pH of honey can exceed 6. The pH values of analyzed honey did not fall within the range of 3.5-5.5 stipulated for honey by the Codex Alimentarius [22].

The Total acidity of honey is the non-cyclic form of gluconic acid, the acidity values ranged between 13.00 and 83 meq/kg. Almost all the samples (83.33%) comply with the Codex Alimentarius standard [22] against 16.67% which had acidity slightly above the standard. These values are similar to those of Rabeharifara [23] on the characterization of Malagasy honey for authentication and to that of Kologo [24] on the evaluation of the physicochemical quality of some honey sold in Ouagadougou. A strong acidity of honey is likely to cause the degradation of hexoses into Hydroxymethylfurfural. Consequently, the analyzed honey samples with Total acidity above 16.67% are the most exposed to degradation due to their free acid content.

The brix values of the honey varied from 85.50±0.50 to 86.00±0.00. Despite their geographical differences, the honey samples analyzed showed almost similar brix values. It corresponds to the mass of sugar in grams (sucrose) contained in 100 g (i.e. approximately 100 mL of the solution).

The results of moisture content are similar to those found on honey sold in the city of Ouagadougou (10.10±0.09 to 22.51±0.40) [24] and that of Meda et al. [25], for Burkina honey collected directly from beekeepers (15.1 to 21.9). The honey samples studied had a moisture ≤ 21% set by the Codex Alimentarius [22]. This shows that the water content of the honey samples complies with the Codex Alimentarius Standard [22]. Indeed, the water content is a quality criterion used mainly to estimate the degree of maturity of honey, and provides information on the stability of the product against fermentation during storage. According to Bogdanov [26], stable honey should contain lower than 18% water. 83.33% of our honey samples were stable. The honey water content can be affected by many parameters including the harvest season, the initial humidity of the nectar and honeydew, the degree of maturity reached, as well as the geographical origin [27]. The high water content can lead to a growth of yeast and molds, causing fermentation, flavor losses and low shelf life [9].

The electrical conductivity values of all the samples were lower than the maximum value of the codex alimentarius (<800 µs/cm), the highest value was obtained with the sample from Orodara (E1OR) and the lowest was obtained for the Fada (E2FA) sample, these values were lower than those reported by Belhaj et al. [28] on natural honeys of Moroccan origin. Electrical conductivity is a good indicator of the botanical origin of honey and is used during routine checks instead of ash content. It depends on the mineral content and the acidity of the honeys. These results can be explained by the acidity of honey. The higher the acidity, the higher the corresponding conductivity [29].

The hydroxymethylfurfural contents of the different honey samples ranged between 11.68±00 and 90.52±0.35. The highest value was obtained with Gaoua (E4GA) sample and the lowest value with Ouagadougou (E6OA) sample. 80% of our samples were above the limit of Codex Alimentarius standard (80 mg/kg) The values were similar to those found by Nombré et al. [7] for honey samples from Burkina Faso harvested from 2001 to 2007 and analyzed in 2010 (13.4 to 1169.0 mg/kg) and Kientega [30]. The high content of hydroxymethylfurfural could be explained by decomposition of fructose, due to the poor storage conditions, aging and prolonged heating of these honeys [9, 10, 31].

3.2 Microbiological Characteristics of Honey

The results of the microbiological analysis of the honey samples are presented in Table 2.

3.2.1 Total counts

The total counts results showed a non-significant difference for the different honey samples. The highest value was obtained for Fada (E2FA) sample with a value of $1.21.10^5 \pm 1.18.10^4$ CFU/mL and the lowest for the Ouahigouya (E5OU) sample with a value of $3.50.103 \pm 3.50.10^3$ CFU/mL. All honey samples had values not exceeding 10^5 CFU/mL as defined by the

Codex Alimentarius standard [22]. We can deduce that the honey from the six regions was conform as regarding this standard.

3.2.2 Yeasts and molds

Yeasts and Molds found in honey samples comply with the current standard for unpasteurized fresh products (<10^3 CFU/mL). The conformity of results obtained reflects good conservation and storage of our honeys. However, maintaining its mold and yeast populations at acceptable levels will reduce the risk of poisoning and fermentation of our honey samples. These yeasts and molds come from pollen and from the legs, tongues and crops of bees, contaminated through contact with floral nectaries and possibly ripe fruit [32].

3.2.3 Total Coliforms (TC) and Thermotolerant Coliforms (CTh)

The results of TC and CTh of the six honey samples all gave a value of < 1 UFC/mL, the standard in force on unpasteurized fresh products requires that the number of UFC/mL is < 10^2. Therefore, we can say that our results are consistent with the standard. Since no coliform was detected in all the honeys, these results indicate that the extraction and storage of the honeys were carried out under good hygienic conditions.

3.2.4 Spore-forming bacteria

The results showed that no spore-forming bacteria was observed in the sample of Fada (E_2FA) and Ouahigouya (E_5OU). The sample of Gaoua (E_4GA) and Orodara (E_1OR) gave similar values and had the highest values observed compared to the others.

3.2.5 *Salmonella* and *Shigella*

The results showed a total absence of *Salmonella* and *Shigella* in 25 mL of honey in all the samples analyzed. These results obtained is an indication of good hygienic conditions observed through harvesting to the packaging of the honey samples. *Salmonella* and *Shigella* are indicators of fecal contamination, the source of contamination by these germs can be bee, the environment, the personnel when handling the honey or the equipment. According to the study conducted by Belhaj et al. [28], their honey samples only inhibited *Salmonella* and *E. coli*. This has shown that Gram-positive bacteria are more sensitive than Gram-negative bacteria and that several studies have shown that Gram-positive bacteria with a thick and dense wall resist better to strong pressure exerted by high concentrations in sugars than Gram-negative bacteria with a thin and loose wall [33].

3.2.6 Organoleptic and sensory characteristics of honey

The results of the analysis of the honey-sensory quality are presented in Table 3.

The Fig. 1 presents the acceptability of honey from six regions of Burkina.

The results showed that Orodara (E1OR) honey was really like and the Ouagadougou honey (E6OA) was the unless appreciated by the consumers.

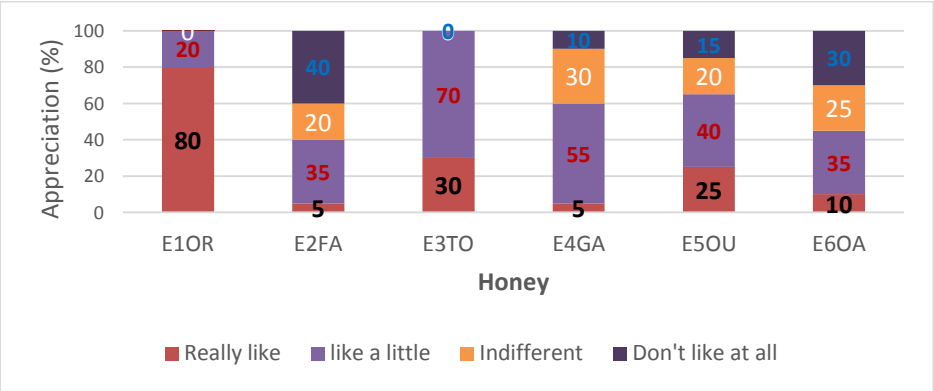

Fig. 1. Acceptability and grading of honey

Table 1. Physicochemical characteristics of honey

Samples	Density	pH	TA (meq/kg)	°Brix (%)	Moisture (%)	CE (µS/cm)	HMF (mg/kg)
E_1OR	1.41±0.01^{abc}	5.73±0.00^{a}	83.00±5.00^{de}	86.00± 0.00^{a}	18.83±1.83^{c}	155.00±1.00^{e}	82.93±0.00^{ef}
E_2FA	1.42±0.00^{cd}	6.56±0.03^{e}	13.00±5.00^{a}	88.00±0.00^{cd}	11.86±0.46^{a}	101.00±2.00^{a}	14.67±0.00^{a}
E_3TO	1.39±0.00^{a}	6.33±0.00^{d}	18.46±0.50^{ab}	87.50±0.50^{bc}	16.83±0.00^{bc}	118.00±1.00^{b}	77.54±10.18^{e}
E_4GA	1.42±0.00^{bc}	5.92±0.01^{b}	23.00±5.00^{abc}	87.95± 0.05^{cd}	16.96±0.07^{bc}	145.00±1.00^{d}	35.33±1.5^{c}
E_5OU	1.40±0.00^{ab}	6.01±0.00^{c}	29.66±2.88^{bcd}	88.50±0.50^{d}	17.17±0.28^{bc}	138.00±0.00^{c}	25.75±0.00^{b}
E_6OA	1.44±0.00^{dc}	6.01±0.01^{c}	36.00±2.00^{cd}	86.75±0.05^{ab}	15.98±0.08^{b}	139.00±1.00^{c}	90.52±0.35^{f}
Codex Standard	-	3.58-4.84	< 50	-	< 21	≤ 800	< 80

Legend: Values bearing exponent of different letters are significantly differents according to Turkey's test; E1OR: Orodara samples; E2FA: Fada samples; E3TO: Tougan samples; E4GA: Gaoua samples; E5OU: Ouahigouya samples; E6OA: Ouagadougou sample

Table 2. Microbiological characteristics of honey samples

Samples	FAMT (UFC/mL)	LM (UFC/mL)	TC (UFC/mL)	CTh (UFC/mL)	FS (UFC/mL)	SS (in 25 mL)
E_1OR	$5.14.10^{4}$ ±$3.35.10^{4}$ ^{a}	$4.06.10^{1}$±3.05^{cd}	< 1	< 1	$1.38.10^{2}$±7.63^{d}	Absent
E_2FA	$1.21.10^{5}$±$1.18.10^{4}$ ^{a}	< 1± 0.00^{a}	< 1	< 1	< 1±0.00^{a}	Absent
E_3TO	$4.51.10^{4}$ ±$3.48.10^{4}$ ^{a}	$2.03.10^{1}$±2.51^{b}	< 1	< 1	2.23.101±2.51^{b}	Absent
E_4GA	$8.74.10^{4}$±$3.75.10^{4}$ ^{a}	$2.31.10^{1}$±6.55^{e}	< 1	< 1	$1.38.10^{2}$±3.60^{d}	Absent
E_5OU	$3.50.10^{3}$±$3.50.10^{3}$ ^{a}	$2.83.10^{1}$±7.63^{bc}	< 1	< 1	< 1±0.00^{a}	Absent
E_6OA	$6.69.10^{4}$±$3.80.10^{4}$ ^{a}	$5.00.10^{1}$± 5^{d}	< 1	< 1	$6.93.10^{1}$±4.04^{c}	Absent
Standard	10^{5}	< 10^{2}	< 10^{2}	-	-	Absent

Legends: Values bearing exponent of different letters are significantly differents according to Turkey's test ; E1OR: Orodara samples; E2FA: Fada samples; E3TO: Tougan samples; E4GA: Gaoua samples; E5OU: Ouahigouya samples; E6OA: Ouagadougou sample; FAMT: Total counts; LM: Yeasts and Moulds; TC: Total coliforms; CTh: Thermotolerant coliforms; FS: Spore-forming bacteria; SS: Salmonella and Shigella

Table 3. Organoleptic characteristics of honey

Samples	Sensory profile					Acceptability
	Colors	Aromas	Texture	Sweet taste	Acid taste	
E_1OR	Light brown (100%)	Not good (55.2 %)	Cloudy (75.9%)	Sweet (44.8%)	Not sour (89.7%)	Neither pleasant nor unpleasant (37.9%)
E_2FA	Very dark brown (95%)	Good (62.1%)	Clear (51.7%)	Sweet (58.6%)	Sour (86.2%)	Pleasant (62.1%)
E_3TO	Brown (75%)	Very Good (41.4%)	Clear (51.7%)	Sweet (55.2%)	Very Sour (62.1%)	Pleasant (51.7%)
E_4GA	Brown dark (60%)	Good (60.4%)	Clear (47.9%)	Sweet (56.3%)	Sour (79.2%)	Pleasant (49%)
E_5OU	Brown (95%)	Good (62.5%)	Clear (62.5%)	Sweet (54.2%)	Sour (77.1%)	Pleasant 57.1%)
E_6OA	Brown dark (85%)	Good (60.4%)	Cloudy (54.2%)	Sweet 58.3%)	Sour (72.9%)	Pleasant (57.1%)

Legends: E1OR: Orodara samples; E2FA: Fada samples; E3TO: Tougan samples; E4GA: Gaoua samples; E5OU: Ouahigouya samples; E6OA: Ouagadougou sample

4. CONCLUSION

This study allowed us to evaluate the microbiological quality of honey from six regions of Burkina Faso. The analysis of the physicochemical quality of the honey shows compliance with the results of the Codex Alimentarius standard. However, there is a non-compliance with the result of the Total acidity of the honey of Orodara, which gave a value higher than the standard of the codex. The microbiological quality analysis indicated the absence of *Salmonella* and *Shigella* in all the honey and a low load of total mesophilic aerobic flora, yeast and mold, spores, and coliforms in the honey samples. This complies with the recommendations of the standard for unpasteurized fresh products. In general, the physicochemical and microbiological quality of the honey meets the standards. However, the presence of molds and spores in some samples can pose a health threat to consumers. Given the literature, honey is an antimicrobial product, and microorganisms are not likely to live there for long, nevertheless, we recommend that beekeepers and honey houses ensure the sanitation of hives, and the production environment and to make a good choice of honey packaging.

In view of the results obtained on honey from six regions of Burkina, certain parameters remain to be studied. From this, as perspective, it is possible to look for chemical contaminants, evaluate the nutritional quality, and analyze the organoleptic quality of honey and other hive products from different regions of Burkina Faso.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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