Research Article
Volume 16:1, 2025

Journal of Veterinary Science & Technology

ISSN: 2157-7579 Open Access

Microbiological Characteristics of Cameroonian Honey and Public Health Significance

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Abstract

From January to December 2022 a study was carried out in Cameroon to have the microbiological profile of honeys produced in the most productive areas, according to the transformational levels. For that, 150 samples were collected proportionally to the weight production of each are with respectively 90 samples in the bimodal forest, and 30 in the western highlands and Sudanoguinean agroecological zones. These samples were analysed *via* the plate count methods and the Analytical Profile Index (API 20E-BIOMERIEUX®) according to the manufacturer's guidelines. The results obtained showed that all the samples were contaminated, 48.66 ± 33% of them having load level above the recommended ones. Risks factors associated with the contamination of honeys revealed a strong association ($p<0.001$) between the microbial agent and the technological level. A multilinear regression analysis showed that there is a high correlation of contamination of honey at the market level with those at the hive and extraction levels ($F(1,4)=96,63$, with $p<0.01$; $R^2=0.96$). In general, type of hives and extraction method influence the probability of contamination, straw hives and traditional methods having a positive influence. The honey from market were more contaminated than those at the extraction level, followed by those from the hives, while honey from the Sudanoguinean area were more contaminated followed by those from the bimodal forest, and those of the western highlands being the less. These results bring a new insight in the risk factors of contamination of honeys in Cameroon, and may help to put a place a training program to promote good beekeeping practices in order to ameliorate the quality of hone so as its productivity.

Keywords: Cameroon • Honey • Microbial characteristics • Risks factors • Agroecological zones

Introduction

With up to five (5) aeroecological zones, Cameroon is a country in the Central African region characterised by a great diversity of ecosystems [1]. Honey is of great importance in the country's agricultural sector, Cameroon being the first honey productive country in the Central African sub-region [2]. However, if beekeeping practices and characteristics of honey have been studied by previously, so far they have been done in few parts of the country [3]. Moreover, these studies are old and not only these characteristics have never been studied for the whole country, but they have mainly focused on commercial honeys without studying their variation through the transformation chain. The aim of this study was therefore to obtain a general overview of beekeeping practices and the physicochemical characteristics of honey throughout the transformation chain. Thus, four mains physicochemical (pH, electric conductivity, total sugars and moisture) have been studied on honey collected at the level of the hive, after the extraction process and from the honey collected at the various markets, in order to get a general idea of how these variables are changing from production to consumption.

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Received: 09 August, 2023, Manuscript No. JVST-23-109880; **Editorassigned:** 14 August, 2023, PreQC No. JVST-23-109880 (PQ); **Reviewed:** 29 August, 2023, QC No. JVST-23-109880; **Revised:** 22 January, 2025, Manuscript No. JVST-23-109880 (R); **Published:** 29 January, 2025, DOI: 10.37421/2157-7579.2025.16.283

Materials and Methods

Sample size for microbiological evaluation

The determination of honey samples to be collected was carried out using the Thrusfield formula and the method applied was that of the French ministry of agriculture for the quality control of honey by instruction DGAL/SDSPA/ 2019-94 du 01/02/2019 [4]. According to the production weight of each region the size obtained was allocated, and samples were taken randomly in the hives, after extraction for the same honey, and in the markets. From the work done by Tchoumboue et al. The expected prevalence was set at 73.47% and to precision of 7% (Table 1).

$N=(Z^2P(1-P))/d^2$

With:

N=Sample size,

Z=Critical value of the normal distribution at the required confidence level, (1,96)

p=Sample proportion (73.47%),

d=Margin of error or precision (7%)

Agroecological zones	Honey production (2019) in tons	Weight (%)	Sample size	Sample size allocated per technological level		
				Level 1 (Hives)	Level 2 (Extraction)	Level 3 (Markets)
Soudano-guinean	987	13.77	30	10	10	10
Bimodal forest	4522	63.12	90	30	30	30
Western highlands	1655	23.09	30	10	10	10
Total	7164	100	150	50	50	50

Table 1. Honey production and minimal sample size allocation.

(INS, 2019; MINEPIA-DEPCS, 2020) at each level, a quantity of honey of approximately 100 g was sampled placed in sterile tubes, labelled and taken to the laboratory stored at 4°C before analysis.

Microbial evaluation

To evaluate the microbiological characteristics of honey, the serial dilution and the Standard Plate Count (SPC) method were used. 1 ml of the 10^{th} dilution of the stock solution prepared by dilution 25 ml of honey in 225 mL of Buffered Peptone Water (BPW) was aseptically transferred into sterile Petri dishes in triplicate and approximately 20 mL of melted Mueller Hinton agar (45°C) was added. The sample and agar were mixed then incubated at 37°C for 24 hours. The results were expressed in Colony Forming Unit (CFU).

Total coliforms

1 ml of sample from the 8th dilution was pipetted and placed in Petri dishes containing Hecktoen Enteric agar (GranuCult-prime-MERCK®) prepared according to the manufacturer's instructions. The samples were then incubated at 37°C for 24 hours and the colonies numbered.

Identification of bacterial species

Two biochemical methods were used, a classic one based on presumptive identification in different culture. That is *Escherichia coli* (yellow to reddish color colonies), *Salmonella* spp. (green with black center colonies) and *Shigella* (black colonies). The second method used was the identification through the Analytical Profile Index (API 20E®-BIOMERIEUX) according to the manufacturer's instruction.

Yeast content

It was done through the Sabouraud dextrose agar which was prepared according to the manufacturer's instructions (BIOKAR®) and allowed to cooled to 45°C. 1 mL of honey samples from the third dilutions was pipetted and placed in petri dishes then 20 mL of the culture medium was poured and gently mixed to ensure uniformity. Thy were incubated at room temperature for 7 days and results obtained [5].

Analysis

The data were collected and registered in Microsoft excel 2020™ and analysed with SPSS 20 IBM™. Results were expressed in the form mean ± standard deviation at a level of significance of 95%. Comparison of means was done using one-way Analysis of Variance (ANOVA) and Duncan post-hoc test used to separate means when they were comparable. *Chi square* was used to measure the association between the technological level and the prevalence of contamination in the agroecological zones and the strength of this association was evaluated by the measurement of the odds ratio. A multinomial logistic regression was used to identify risk factors susceptible to induce the contamination of honey within the different agroecological zone, the type of extraction method and the type of beehives.

Results

A total of 150 honey samples were collected from each of three main areas corresponding to the agroecological zones of the bimodal forest (90), western highlands (30) and Sudanoguinean (30) as presented in Figure 1 below.

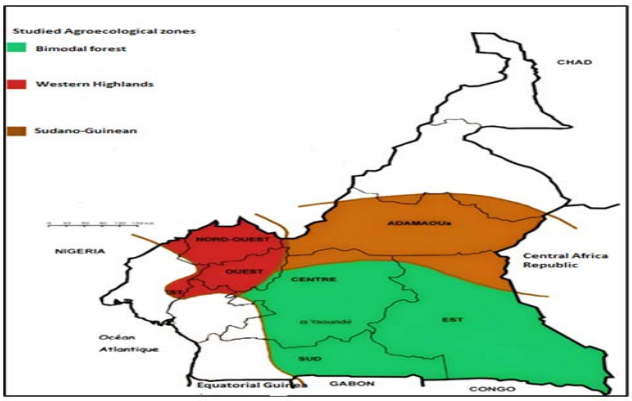

Figure 1. Studied areas (1,2,3).

Honey contamination evaluation between the different Agroecological zones

Our study showed that 100% of the samples were contaminated with at least one type coliform either in the hive, after extraction or in the markets. The mean of contamination was more than 100 UFC/g for faecal and total coliforms in all the different levels (Table 2). An overall prevalence of 48.66 ± 33% of honeys with contamination levels above the limited 100 UFC/25 g contamination to *E. coli* and *Salmonella* defined by the European Commission was obtained with respectively 23 ± 32% of sample from hives, 50.66 ± 33% of the extraction level, and 72.33 ± 34% of the markets while yeast contamination was under the recommended 100 UFC/g [6].

Bacteria specie/group	AEZ	Prevalence/AEZ (%)	Overall prevalence (mean)	p-value
Faecal coliforms* (UFC/g)	WH	196.53 ± 52.31	193,6 ± 50,5	0,812
	S-G	194.36 ± 46.82		
	BF	190.08 ± 52.59		
Total coliforms* (UFC/g)	WH	114,19 ± 31,40	111,3 ± 38,6	0,269
	S-G	102,98 ± 41,79		
	BF	116,86 ± 42,68		
Escherichia coli	WH	53^{a} ± 50,70	76 ± 42,9	0,002
	S-G	90^{b} ± 30,50		
	BF	79^{b} ± 43,00		
Salmonella spp./*Shigella* spp.	WH	17^{ab} ± 38,00	27 ± 44.7	0,012
	S-G	10^{a} ± 30,00		
	BF	34^{b} ± 47,80		
Staphylococcus aurcus	WH	50 ± 51.00	57 ± 49,7	0,115
	S-G	43 ± 50,40		
	BF	63 ± 48,50		
Aspergillus spp.	WH	47^{a} ± 50,70	59 ± 49.4	0,002
	S-G	80^{b} ± 40,70		
	BF	56^{a} ± 50		
Penicillium spp.	WH	7^{a} ± 25,40	21 ± 40,6	0,035
	S-G	33^{b} ± 47		
	BF	20^{ab} ± 40,20		
Yeast	WH	20^{a} ± 40,70	43 ± 49,7	0,013
	S-G	53^{b} ± 50,70		
	BF	48^{b} ± 50,20		

Note: WH: Western Highlands; S-G: Sudano-Guinean; BF: Bimodal Forest

*: mean value for faecal and Total coliforms

^{a,b}. variables with different letters are significantly different between the AEZ

Table 2. Microbiological contamination of honeys between the agroecological zones.

Furthermore, a deep analysis of Table 2 shows that there was a significant difference ($p<0.05$) between the microbial contamination of honey by five microbial agents (Yeast, *Penicillium* spp., *Aspergillus* spp., *Salmonella* spp./*Shigella* and *Escherichia coli*) and the agroecological zones. The prevalence of microbial agents was higher in the Sudanoguinean (51.50 ± 41.55%) followed by the bimodal forest area (50 ± 46.62%) and finally by the western highlands (32.33 ± 47.98%).

The analysis of the trend of the microbial contamination according to the technological level presented in Table 3, showed a similar trend for all the areas with an increase of the prevalence of contamination from the Hive to the market level. Moreover, there was a highly significant difference between the technological level and the prevalence of contamination within the region, except for the Sudanoguinean where the mean prevalence of contamination was not significantly different at the market level compared to the hive and extraction ones, though it was higher.

Variables	Bimodal forest			Western highlands			Sudano-guinean			Prevalence (%)	p- value
	H	E	M	H	E	M	H	E	M		
Faccal coliform (UFC/g)	202.96 ± 66.24	183.10 ±45.78	179.84 ± 46.51	181.46 ± 54.95	211.36 ± 50.04	196.76 ± 52.87	234.77^{b} ± 39.17	174.13^{a} ± 33.15	174.17^{a} ± 41.33	100%	0.812
Total coliforms (UFC/g)	128.19 ± 46.05	106.80 ± 31.54	115.60 ± 47.29	101.53 ± 38.53	121.47 ± 24.38	119.57 ± 28.80	1.03 ± 46.59	1.06 ± 34.32	9.89 ± 47.41	100%	0.269
E. coli	0.40^{a}± 0.49	0.97^{b} ±0.18	1.00^{b} ±0.00	0.20^{a} ± 0.42	0.60^{ab} ± 0.51	0.80^{b} ± 0.42	0.70^{a} ± 0.48	1.00^{b} ± 0.00	1.00^{b} ± 0.00	76%	0.002
Salmonella spp./*Shigella* spp.	0.10^{b} ± 0.30	0.33^{b} ± 0.47	0.60^{a} ± 0.49	0.00^{a} ± 0.00	0.10^{ab} ± 0.31	0.40^{b} ± 0.51	0.00^{a} ± 0.00	0.00^{a} ± 0,00	0.30^{b} ± 0.48	27%	0.012
Staphylococcus aureus	0.40^{a} ± 0.49	0.67^{b} ± 0.47	0.83^{b} ± 0.37	0.10^{a} ± 0.31	0.50^{b} ± 0.52	0.90^{c} ± 0.31	0.20^{a} ± 0.42	0.40^{ab} ± 0.51	0.70^{b} ± 0.48	57%	0.115
Aspergilus spp.	0.10^{a} ± 0.30	0.77^{b} ± 0.43	0.80^{b} ± 0.40	0.40 ± 0.51	0.30 ± 0.48	0.70 ± 0.48	0.70 ± 0.48	0.90 ± 0.31	0.80 ± 0.42	59%	0.002
Penicilium spp.	0.00^{a} ± 0.00	$0,17^{a}$ ± 0.37	0.43^{b}± 0.50	0.00 ± 0,00	0.00 ± 0.00	0.10 ± 0.20	0.10^{a} ± 0.31	0.30^{ab} ± 0.48	0.60b ± 0.51	21%	0.035
Levure	0.50^{ab} ± 0.50	0.30^{a} ± 0.46	0.63^{b} ± 0.49	0.40 ± 0.51	0.10 ± 0.31	0.10^{a} ± 0.31	0.30^{ab} ± 0.48	0.70 ± 0.48	0.60^{b} ± 0.51	43%	0.013
Prevalence %	26%	54%	72%	21%	29%	53%	37%	58%	69%		
p-value	0.000	0.000	0.000	0.007	0.004	0.000	0.002	0.000	0.053		

H: Hive level; E: Extraction level; M: Market level
a,b,c: variables with different letters are significantly different.

Table 3. Microbiological contamination according to the technological level within the different AEZ.

A study of risk factors associated with honey contamination showed firstly that there is correlation between some microbiologic agents (Table 4). A strong relationship ($p<0.001$) was found between the presence of total coliforms and faecal coliforms, *E. coli* and *Aspergillus* spp. so as *Salmonella*/*Shigella* spp., and *Aspergillus* spp. and *Penicillium* sp. so as faecal coliforms demonstrating that there is a high probability of having these pathogens together in different samples irrespectively of their origin or technological level [7].

The one-way regression analysis showed that hive type and treatment highly influenced the likelihood of honey with Odds Ratios scores higher for these two technological levels for almost all the microbial agents except the yeast in all the agroecological zones (Table 5).

Bacterial species/ groups	Faecal coliform	Total coliform	*E. coli*	*Salmonella/Shigella*	*Staphylococcus aureus*	*Aspergillus*	Penicillium
Total coliforms	0.45**						
E. coli	0.01	-0.1					
Salmonella/Shigella	0.12	0.15	0.33**				
Staphylococcus aureus	0.07	0.04	0.11	0.15			
Aspergillus	-0.30**	-0.21*	0.26**	0.07	0.06		
Penicillium	-0.14	-0.1	0.20*	0	0.1	0.39**	
Yeast	-0.1	0.03	0.11	0.04	0.09	-0,09	0.15

Note:* : significantly different at 5%; **: Significantly different at 1%

Table 4. Pearson correlation table between the microbial agents.

Variable	Technological level	For the three ecological zones			
		n_i	Prevalence% (IC 95%)	OR (IC 95%)	p-value
E. coli	H	21	42 (28;56)	18.18 (7.09;47.61)	0.0001
	E	45	90 (81;99)	0.24 (0.68;0.8)	
	M	48	96 (90;100)	0.08 (0.01;0.35)	
Salmonella spp/*Shigella* spp	H	3	6 (0;13)	8.84 (2.55;30.30)	0.0001
	E	11	22 (10;34)	1.37 (0.62;3.06)	
	M	25	50 (36;64)	0.16 (0.07;0.35)	
S. aureus	H	16	30 (17;43)	5.20 (2.49;10.86)	0.0001
	E	30	583 (44;72)	0.88 (0.44;1.76)	
	M	41	82 (71;93)	0.18 (0.08;0.42)	
Aspergillus spp.	H	14	28 (15;41)	7.29 (3.41;55.62)	0.001
	E	35	70 (57;83)	0.48 (0.23;0.99)	
	M	39	78 (66;90)	0.27 (0.12;0.58)	
Penicillium spp.	H	1	2 (2;6)	2 (2.63;20.0)	0.0001
	E	8	16 (5;27)	1.48 (0.60;3.61)	
	M	21	42 (28;56)	0.13 (0.05;0.33)	
Levure	H	21	44 (30;58)	0.92 (0.46;1.85)	0.103
	E	17	34 (20;48)	1.79 (0.88;3.62)	
	M	26	52 (38;66)	0.59 (0.29;1.17)	

Note: n_i: number of positive cases, with a population of 50 at all the three levels (N=50)

Table 5. Risk factors of honey contamination odds ratio.

A multinominal regression analysis on the type of hives and treatment influencing the contamination (Table 6) showed that hives made of straw were at least ten times more likely to be contaminated by *Aspergillus* spp. and *E. coli* compared to other types of hives. Likewise, traditional extraction method which consisted of grinding honeycombs and filtering them through a sieve was three times and five time to more prone to contamination by *Penicilium* sp. and by yeast compared to honey processed through modern methods [8].

In general, except for *staphylococcus aureus*, hives made of straws presented higher risk of contamination compared to those made of wood (Langstroth and KTBH), the same observation being

done for traditional extraction method, except for *E. coli* and *Salmonella/Shigella* spp.

Variables	Type of hives			p-value	Extraction method		p-value
	Straw hive	Langstroth and derivatives	KTBH and derivatives		Traditional	Modern	
E. coli	2.5 (0.52;12.14)	0.25 (0.03;2.44)	0.17 (0.07;2.45)	0.17	2^{-8} (2.03^{-9};1.98^{-7})	7.88^{-9} (7.87^{-9}; 7.87^{-9})	0.24
Salmonella spp./ *Shigella* spp.	1 (0.09;11.03)	9.28^{-9} (9.28^{-9};9.28^{-9})	9.28^{-9} (9.28^{-9};9.28^{-9})	0.43	0.14 (0.01;1.67)	0.90 (0.18;4.48)	0.12
S. aureus	1 (0.16;6.138)	7 (1.02;47,97)	2.1 (0.39;11.43)	0.19	1.20 (0.23;6.39)	1.09 (0.24;5.03)	0.98
Aspergillus spp.	10 (1.67;60)	3.64^{-9} (3.64^{-9};3.64^{-9})	3.64^{-9} (0.00;b)	0.001	1.143 (0.15;8.59)	0.48 (0.08;2.66)	0.39
Penicllium spp.	1 (0.00;b)	1 (1;1)	1 (0.00;b)	0.31	2.909 (0.27;31.21)	1.04 (0.09;11.52)	0.43
Yeast	0.46 (0.01;2.25)	0.46 (0.07;2.98)	0.56 (0.11;2.81)	0.68	5.25 (0.80;34.43)	1.05 (0.17;6.6.46)	0.043

Table 6. Multinomial logistic regression of honey contamination according to the type of hives and extraction methods.

Moreover, a multilinear regression analysis on honey contamination between showed one more a strong relationship between the probability of contamination and different levels ($F(1,4)=96,63$, with $p<0.01$; $R^2=0.96$) as presented in Table 7 and Figure 2.

	Coef	SE	t-stat	$t_{0.025}$	$t_{0.975}$	Coeff stand	p-value	VIF
b	15,96	2,32	6,88	8,57	23,34	0	0.006	
X_1	-0.09	0.2	-0.44	-0.73	0.55	-0,07	0,6	2,11
X_2	0.76	0,12	6,37	0,38	1,14	1,03	0,007	2,11

Table 7. Multilinear regression parameters.

The logistic multilinear regression equation found was then:

$y=15,96-0,09x_1+0,76x_2$

with

y=honey at the market level with at least one infectious agent

x_1=honey at the hive level

x_2=honey at the extraction level

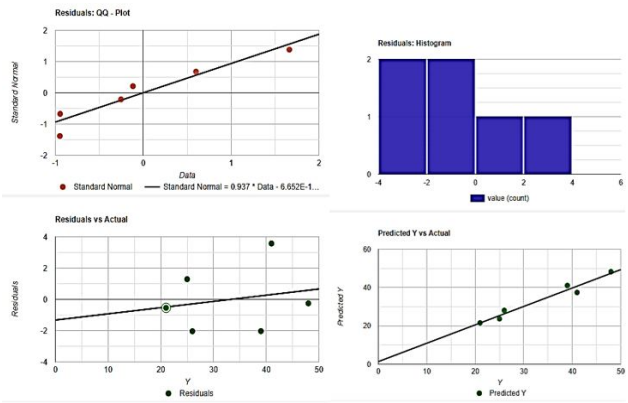

Figure 2. Diagrams of logistic regression correlation.

Discussion

This study revealed a 100% prevalence rate of honey, similar with a study done by Tatsadjeu et al. in the sudanoguinean agroecological zone who find a 100%contamination rate of honey. This result is explained by the influence of the different risk factors (agroecological zone, extraction method, type of hives and technological level) identified in our study which positively influence the probability of contamination.

Honey from straw hives extracted traditionally were more probe to be contaminated than those made of wood. Furthermore, market samples were more contaminated than those from the hives and at the extraction level. This result is similar to a study done in the wester highlands by Tchoumboue et al. where they found that 73.47% of honeys sold at the Dschang were contaminated while those from the university apiaries had no contaminants [9].

This study brings a contribution on the idea that honey like any other food product is not always free of microbial agent as it was long time supposed. If it is true that honey has antibacterial properties it has also been proven that under some conditions, it can harbour pathogenic agents. In fact, microbes that can withstand the normal physicochemical properties of honey can grow in it.

Therefore, some can be found in honey for a period of 8-20 days under 20°C, but will be eliminated or inhibited by the antibacterial effect of the honey [10].

Therefore, as our samples were all analysed within 72 hours after collection, this can also explain the higher number of bacterial found at all levels [11]. However, this also leads us to suggest that newly harvested honey should not be directly consumed but should undergo a certain period of maturation under proper storage conditions so that the product will be as safe as possible. The possibility of honey adulterations is also to be taken into consideration as honey from this level were the most contaminated.

Higher concentrations of microbial found in this study is similar with other ones where they were ten times higher [12,13]. However, this high concentration can also be linked to the analysis method which was a plan count which has be demonstrated to overestimate the bacteria abundance. Thus, in reality the contamination [14] rate can be lower, even if it does not reduce the health hazards presented by this contamination.

Conclusion

The objective of this study was to have a general view of microbial quality of honey in Cameroon confirmed the hypothesis that the trend on contamination is related to the zone so as the technological level. For the first time to the best of our knowledge, we have shown that honey collected from hives is progressively contaminated throughout the transformation chain with higher levels of extraction and in the markets. Therefore, beekeeping practices so as physicochemical characteristics of honey should be investigated to a better understanding of the risk factors apart of those obtained in this study.

Recommendations

We recommend to the beekeepers to move from conical hives made of straw to Kenyan and Langstroth one made of wood which have a better productivity to as better microbial quality shown in this study. Treatment of honey harvested shall also be investigated in order to ensure a better competitively of the sector and protect the health of the consumers from all the probable diseases that can occur from the contamination of this honey.

Acknowledgement

We would like to express our gratitude to the ministry of livestock, fisheries and animal husbandry, to the ministry of agriculture and rural development, so as the different institutions who helps us to collect information for this research. The research team is also grateful to all the beekeepers for their collaboration, enthusiasm and willingness to share us information.

Funding's

Not applicable, the study didn't have any funding's and was financed by personal resources.

Conflict of Interest

We declare that we have no any financial or personal interest that inappropriately influences in writing this article.

Ethics Approval

Consent to participate.

All the participants gave freely their consent to participate to this study.

Consent for Publication

Not applicable.

Availability of Data and Material/Data Availability

Data are available and will be provided if required or asked.

Code Availability

Not applicable.

Authors' Contributions

Dr Ngah Osoe Bouli Freddy Patrick Conceived and conducted the study, Professors Mamoudou Abdoulmoumini and Aliou Mohamadou supervised the study, Dr ADAMOU Moise, MOFFO Frederic and Dr WAFO FOKAM Agnes Jorelle did the revision of the manuscript, and Dr HAMIDOU LIMAN did a part of the analysis.

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How to cite this article: Patrick, Ngah Osoe Bouli Freddy, Aliou Mohamadou, Moffo Frederic and Hamidou liman, et al.. "Microbiological Characteristics of Cameroonian Honey and Public Health Significance." *J Vet Sci Technol* 16 (2025): 283.