



## Determination of the Fermentation Parameters of an Artisanal Beer Based on Wax and Honey in the North of Côte d'Ivoire

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### Authors' contributions

This work was carried out in collaboration among all authors. Author KD designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors FJB and MJLA managed the analyses of the study. Author OGOM managed the literature searches. All authors read and approved the final manuscript.

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### ABSTRACT

The aim of this study is to evaluate some microbiological and physicochemical parameters during the production of artisanal beer based on honey and beeswax. Samples were collected at various critical points during the production process. The load of mesophilic aerobic germs and yeasts remains high during production. A total absence of lactic acid bacteria is observed in both types of beer during production. Moreover, Gram staining made it possible to isolate for wax beer, 26% of the Gram negative bacteria of which only one (1) is rod-shaped (5%) and (4) strains are hulls (21%) and 74% of the Gram positive bacteria of which three (3) strains are rods (16%) and eleven (11) strains are hulls (58%). For honey beer, 45% of the Gram-negative bacteria were isolated, of which seven (7) strains are hulls (32%) and three (3) strains are rods (13%), and 55% Gram-positive

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bacteria, of which seven (7) strains are hulls (32%) and five (5) strains are rods (23%). Wax and honey beers contain a high level of reducing sugars at the beginning of exploitation. During the heat treatment, the reducing sugars reduce the microorganisms disappear after 9 min and 11 min heat treatment respectively for wax and honey beer.

*Keywords: Beer; honey; wax; heat; treatment; microorganisms.*

## 1. INTRODUCTION

Human beings have been using fermentation for thousands years to obtain food with improved nutritional value. In Africa, cereals such as sorghum, maize and millet are often made into a drink (beer), the manufacture of which involves an essential step of alcoholic fermentation. These fermented foods and beverages are very popular, contribute to people's diets and are produced locally in households, villages and by small production units (women's cooperatives) [1,2]. In Côte d'Ivoire, SOLIBRA, which produces the national industrial beer, holds more than 50% of the Ivorian market share with a turnover of more than 100 billion and a production of 130 million liters. The other share of the Ivorian market is shared by imported beers and home-made beers [3]. Artisanal beers are one of the most popular alcoholic beverages in the world, as it is produced in both developed and underdeveloped countries. They meet physiological, psychological and sociological needs. They dissipate weariness and depression, provide escape, lead to euphoria, facilitate communication and create celebration [4].

They are at the heart of popular ceremonies and festivities (weddings, baptisms, initiations, funerals). As such, they play a very important socio-cultural and economic role. It is also consumed during field work and given abroad for refreshment and welcome [5,6]. This beverage has nutritional values that contribute to improving the diet of consuming populations. In addition, therapeutic virtues are attributed to its laxative, anti-malarial and anti-hemorrhoidal properties [7,8,5]. Its relatively low cost also makes it an affordable product for all [9,10,11]. Indeed, often attached to traditions of hospitality and conviviality, they are part of the know-how of most families and serve to seal relations between individuals [12]. Given the importance of the market and the improvement in quality and quantity of artisanal beers, research has shown that the production of these artisanal beers has a currency earning activity to the detriment of imported beers; the majority of artisanal beers

are produced in the north of Côte d'Ivoire with increasing consumption [13]. Despite the proliferation of production sites, manufacturing conditions are not changing much. Brewers are confronted with the difficulties of production, conservation and reproducibility of craft beers. At the production level, brewers find the manufacturing process difficult and time-consuming. Numerous studies have been carried out in northern Côte d'Ivoire and several other African countries over the last 20 years on various aspects of traditional beers [14]. However, traditional beer made from honey is still unknown to the majority of the Ivorian population. It is mainly produced in the north. Microbiological and biochemical studies carried out on this beer are almost non-existent. However, given its importance in customary practices in the Korhogo area, it is useful to have data that will be of paramount importance in the development of this beer. Thus, this work was carried out in order to evaluate and analyze certain parameters during the fermentation of an artisanal honey-based beer in the north of Côte d'Ivoire.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

The organic material used is honey and beeswax obtained from a woman in the village of Nangakaha, in the korhogo area. Samples were taken from the different critical points that can bring contaminating microorganisms to beer during artisanal production. The samples were taken aseptically to avoid external contamination and placed in sterile bowls in a cooler containing ice. The samples were then sent to the laboratory of the University of Peleforo Gon Coulibaly for analysis.

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### 2.2 Enumeration of Microorganisms

In this study, 10 g of samples were aseptically collected and introduced into a STOMACHER bag, and then the volume is made up to 100 mL with pre-sterilized Buffered Peptone Water

(EPT). The tenfold serial dilutions were prepared and spread-plated for determination of micro-organism counts. After dilutions, enumeration of total aerobic mesophile was carried out using plates of Plate Count Agar (PCA, Difco 0479-17-3; Difco Laboratories, Detroit, MI, USA) which were incubated at 30°C for 2 days. Lactic acid bacteria (gram positive catalase negative rods, cocci and coccoids) were enumerated by pour plate on DeMan, Rogosa and Sharpe Agar (MRS, Merck 10660; Merck KGaA, Darmstadt, Germany) containing 10 mg/mL cycloheximide (ICN 100183 Biomedical Inc., Aurora, OH, USA) to suppress yeast growth after incubation at 30°C for 3 days in an anaerobic jar with anaerocult A (Merck). Yeasts and moulds were enumerated on plates of Sabouraud Chloramphenicol agar (BIO-RAD, France) which were incubated at 30°C for 3 - 5 days. Enumeration of total faecal coliforms was carried out using plates of Violet Red Bile Lactose agar (VRBL, Merck 10660, Merck, Darmstadt, Germany) which were incubated for 24 h at 30°C for total coliforms and 44°C for faecal coliforms.

### **2.3 Determination of pH and Total Titratable Acidity**

The pH of the honey beer and the honey wax samples was determined directly using a pH-meter (pH-meter P 107, CONSORT, Bioblock, France) after calibration with standard buffers. Total titratable acidity was determined by titrating samples against 0.1 M NaOH using 1% phenolphthalein as indicator as described by and expressed as percentage of lactic acid.

### **2.4 Determination of Reducing Sugars and Alcohol Content**

Reducing sugars were quantified as previously described by [15] while the amount of ethanol was determined according to the method described by [16].

### **2.5 Determination of the Water Content**

The water content is determined by weighing difference of a sample before and after passage in the oven according to the standard (NF V03-707, July 2000).

### **2.6 Determination of the Dry Matter Content**

The solids content is determined by drying in an oven at a temperature of 105°C until a constant weight.

## **2.7 Statistical Analysis**

The data obtained were subjected to analysis of variance (Statistica, 99 Edition, Alabama, USA) and mean differences determined by Duncan's multiple range tests. Significance of variations in the analyzed data was tested at 95% confidence limit.

## **3. RESULTS**

### **3.1 Load of Mesophilic Aerobic Germs, Lactic Acid Bacteria, Total Coliforms and Yeasts**

The micro-organisms listed (mesophilic aerobic germs, total coliforms, yeasts and moulds) come from two types of beer (wax beer and honey beer). On the whole, the micro-organisms evolve in a similar way for both types of beer used. The load of mesophilic aerobic germs is higher in honey beer than in wax beer during production. This load changes in a sawtooth pattern from one critical point to the other in both types of beer. At the start of production, the load is 6.5 log CFU/mL in honey beer and 4 log CFU/mL in wax beer. The yeast load also changes gradually from one critical point to another during the production of the different beers, from 3.2 log CFU/mL to 4.3 log CFU/mL for honey beer, while the load for wax beer changes from 3.5 log CFU/mL to 4.2 log CFU/mL. An absence of lactic acid bacteria is observed in both types of beer during the production process. The total coliform load increases gradually from one critical point to the other in both types of beer. It increases from 1.1 log CFU/mL to 1.2 log CFU/mL in honey beer, while in wax beer it increases from log 0.6 CFU/mL to log 1.3 CFU/mL. The overall results are presented in Table 1.

### **3.2 Macroscopic Identification of Yeasts Isolated from Wax and Honey Beers**

Fifty (50) and thirty-four (34) strains were randomly isolated from honey and wax beer respectively for yeast identification. These strains made it possible to classify the yeasts according to their coloration. Three (3) main colours were described for honey beer and four (4) main colours for wax beer. In general, the dominant colour of both beers is whitish, with proportions of 74% for honey beer and 59% for wax beer. For honey beer, eight (8) strains are yellowish in colour (16%), thirty-seven (37) strains are whitish in colour (74%) and five (5) strains are orange in colour (10%). For wax beer, three (3) strains are

yellowish in colour (9%), six (6) strains are orange (17%), five (5) strains are olive green (15%) and twenty (20) strains are whitish (59%) (Fig. 1).

### 3.3 Microscopic Identification of Mesophilic Aerobic Germs in Wax and Honey Beers

Twenty-two (22) and nineteen (19) strains were randomly isolated in honey and beer wax for Gram stain respectively. The microorganisms, after Gram staining, are observed under a microscope to determine the different forms and groupings of each type of microorganisms in the different beers. Microscopic observations show the proportions of 26% and 74% respectively for Gram negative and Gram positive for wax beer; 45% and 55% respectively for Gram negative and Gram positive for honey beer. For wax beer, of the 26% of Gram-negative strains, only one (1) is rod-shaped (5%) and four (4) are hulls (21%). For 74% of the Gram-positive strains, three (3) strains are stem-shaped (16%) and eleven (11) strains are hulls (58%). For honey beer, of the 45% Gram-negative strains, seven (7) strains are cockles (34%) and three (3) strains are rod-shaped (13%). For the 55% Gram-positive bacteria, seven (7) strains are cockles (33%) and five (5) strains are rod-shaped (22%). The overall results are presented in Fig. 2.

### 3.4 Evaluation of the Physico-Chemical Parameters during the Production of Both Types of Beer

The pH of honey is 3.77 and its titratable acidity is 0.25%. At the second critical point the pH is 3.92 with a titratable acidity of 0.22%. At the third critical point the pH decreases to 3.63 and its titratable acidity increases to 0.35%. The pH of wax is 4.17 with a titratable acidity of 0.18%. At critical point 2, the pH decreases to 4.13 with its titratable acidity increasing to 0.21%. At critical point 3, the pH increases to 4.22 and its titratable acidity decreases to 0.14%. At critical point 4 the pH decreases to 4.18 and its titratable acidity increases to 0.15%. The sugar content gradually decreases from one critical point to another in both types of beer. For honey beer, it goes from 1.76 mg/mL to 1.43 mg/mL at the end of production. For wax beer, the sugar content goes from 2.26 mg/mL and decreases to 1.94 mg/mL at the end of production. The water content gradually changes from one critical point to another in both types of beer. For honey beer it

increases from 62% to 84% and for wax beer from 42% to 76%. The dry matter content decreases rapidly from critical point 1 to critical point 2 and from this critical point onwards it decreases slowly. For honey beer the content increases from 38% to 16% and for wax beer from 58% to 24% (Table 2).

### 3.5 Measures Microbial Growth of Sterilized Beer

Les résultats de la mesure de la croissance microbienne après inoculation dans le bouillon nutritif ont montré la présence de microorganismes pour le témoin (T0) et le traitement thermique à différents temps de stérilisation jusqu'à 9 minutes pour la bière de cire et 11 minutes pour la bière de miel. Après ces minutes de traitement thermique, une absence totale de microorganismes a été observée jusqu'à la fin du traitement thermique (Fig. 3).

### 3.6 Evaluation of the Physico-Chemical Parameters after Fermentation of the Sweet Wort of Both Types of Beer

For honey beer, before fermentation the pH is 3.77 and its titratable acidity is 0.25%, but after fermentation the pH rises to 3.72 with a titratable acidity of 0.28%. For wax beer, the pH is 4.19 before fermentation and its titratable acidity is 0.54%, after fermentation the pH rises to 3.92 with a titratable acidity of 0.6%. Before fermentation, the levels of reducing sugars in honey beer and wax beer are 1.76 mg/mL and 2.26 mg/mL respectively, and after fermentation they rise to 1.55 mg/mL and 2.2 mg/mL respectively. There is no alcohol in both types of beer before fermentation, but after fermentation the alcohol content in honey beer is 2.5% and in wax beer 5.26% (Table 3).

## 4. DISCUSSIONS

The overall load of microorganisms remains high during the production of the different beers. This load is between 4 log CFU/mL and 8 log CFU/mL for mesophilic aerobic germs; the same is true for total coliform loads which are between 1.1 log CFU/mL and 1.3 log CFU/mL. The high load of mesophilic aerobic germs in the different beers would be brought by the unsterilized collection material used by the beekeeper as well as insects attracted by the high sugar content and microorganisms in the environment. The presence of these germs is also linked to the

production of these beers on a family scale using rudimentary utensils and equipment under uncontrolled environmental conditions [17,18,19,20]. These results are more or less identical with those of [21]. According to these authors, the microorganisms present in palm wine at harvest are brought in by unsterilized collection equipment. Moreover, the presence of mesophilic aerobic germs would be due to the addition of water at the beginning of beer production. The variation in the mesophilic aerobic germs load would be related to the competition between microorganisms in this complex environment and also to the addition of chilli powder at the various critical points which would be an antibacterial. The different beers (honey beer and wax beer) analysed indicate the presence of total coliforms and yeasts but a total absence of lactic acid bacteria. The total coliform load varies from log 0.6 CFU/mL to log 1.3

CFU/mL during production. Whereas, the yeast load increases during production and remains almost stable until the end. Indeed, studies on traditional sorghum beers in West Africa show that these traditional drinks constitute a complex biotope composed of several yeast genera and species [22,23]. In these types of beverages, yeasts constitute the dominant flora [24]. The variation in the loads of microorganisms would be related to the period of exploitation of the honey and the rapid changes in physicochemical parameters that create selection pressure on the initial flora. Also, the abundance of nutrients such as amino acids and sugars, honey and beeswax, would allow the growth of many species of yeasts and bacteria. The results obtained are not in agreement with those of [25]. According to these authors, honey is a clean food in the sense that there is no addition of water, sugars, or perfume and clean in the bacteriological sense,

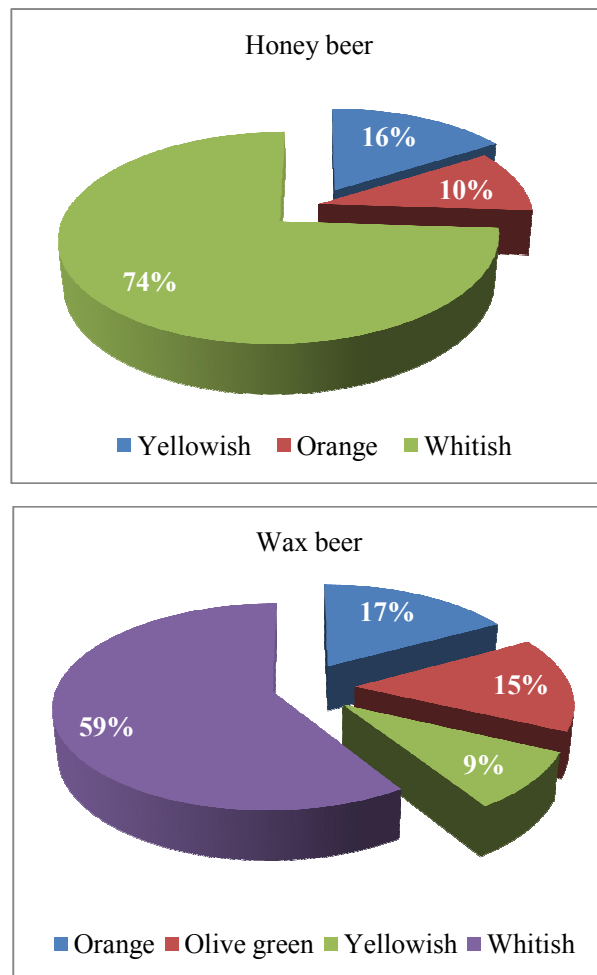
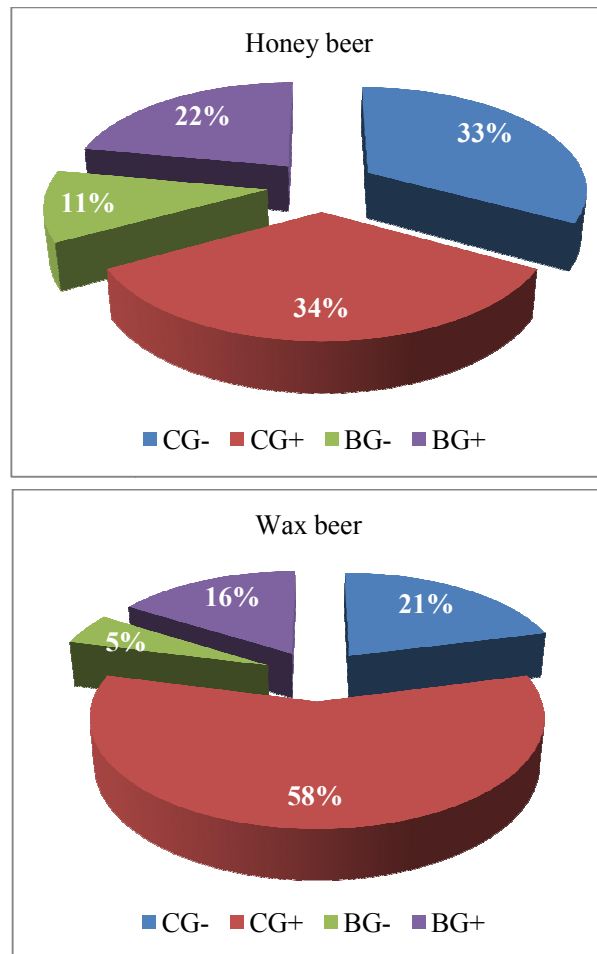


Fig. 1. Proportions of yeast colours in the both types of beer

therefore cannot contain microorganisms. The average values of the reducing sugars remain high from one critical point to another, then after heat treatment they gradually decrease and disappear at T9min in the wax beer while they disappear at T11min in the analysed honey beer. The microbial loads of the beer decrease with the increase of the heat treatment time. The temperature used for the heat treatment of wax beer and that of honey is a treatment to destroy pathogenic microorganisms and reduce the microflora load. The treatment time plays an important role in the elimination of the microflora from the beer. The total elimination of the microorganisms after 9 minutes and 11 minutes respectively for wax and honey beer would be related to the physicochemical composition of the beers but also to the initial load of the microorganisms. The ideal sterilisation time for wax beer is 9 minutes, whereas that for honey beer is 11 minutes. After fermentation of the

various unsterilized musts for 24 hours, the level of reducing sugars decreases thanks to the degradation of the sugars by the microorganisms. This degradation is also marked by a decrease in pH and an increase in ethanol in the different beers. Also, fermentation leads to the production of organic acids and alcohol with the release of CO<sub>2</sub>. The rate of alcohol obtained after fermentation is 2.5% and 5.26% respectively in honey beer and wax beer. The presence of alcohol in the various beers is said to be linked to the yeast load which ferments the sugars into alcohol, with the release of CO<sub>2</sub>. The alcohol content (2.5%) obtained after fermentation of the honey beer is more or less identical with those of [26]. According to these authors, the alcohol content of sorghum beer in West Africa differs from traditional barley beer in several points. This beer has a rather low alcohol content (2-3%).



**Fig. 2. Proportions of the forms of mesophilic aerobic germs after gram staining of the both types of beer**

**Table 1. Microorganisms isolated from freshly produced wax and honey beers**

Microorganisms log (CFU/mL)	Honey beer			Wax beer			
	HB0	HB1	HB2	WB0	WB1	WB2	WB3
Mesophilic aerobic germs	6.5 ± 0.5 <sup>a</sup>	6.3 ± 0.5 <sup>a</sup>	8 ± 0.6 <sup>e</sup>	4 ± 0.2 <sup>f</sup>	5.3 ± 0.4 <sup>h</sup>	5.2 ± 0.4 <sup>h</sup>	5.6 ± 0.4 <sup>h</sup>
Yeasts	3.2 ± 0.1 <sup>b</sup>	3.3 ± 0.1 <sup>b</sup>	4.3 ± 0.2 <sup>f</sup>	3.5 ± 0.1 <sup>b</sup>	3.8 ± 0.1 <sup>b</sup>	4 ± 0.1 <sup>f</sup>	4.2 ± 0.2 <sup>f</sup>
Lactic acid bacteria	0 ± 0 <sup>c</sup>	0 ± 0 <sup>c</sup>	0 ± 0 <sup>c</sup>	0 ± 0 <sup>c</sup>	0 ± 0 <sup>c</sup>	0 ± 0 <sup>c</sup>	0 ± 0 <sup>c</sup>
Total coliforms	1.1 ± 0.02 <sup>d</sup>	1.2 ± 0.02 <sup>d</sup>	1.2 ± 0.02 <sup>d</sup>	0.6 ± 0.01 <sup>g</sup>	1.2 ± 0.02 <sup>d</sup>	1.2 ± 0.02 <sup>d</sup>	1.3 ± 0.02 <sup>d</sup>

*HB: Honey beer; WB: Wax beer*

**Table 2. Physicochemical parameters during the production of the different beers**

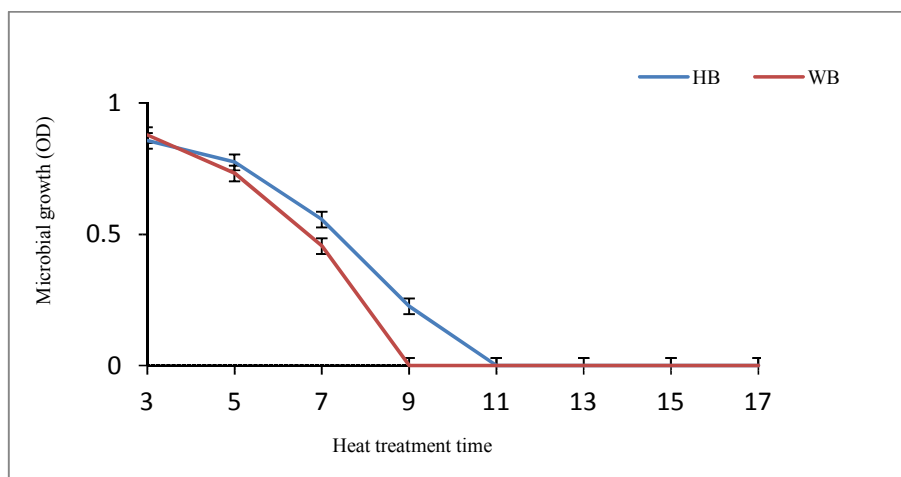
		pH	TA (%)	MC (%)	AC (%)	DMC (%)	RS (mg/mL)
Honey beer	HB0	3.77 ± 0.2 <sup>a</sup>	0.25 ± 0.01 <sup>a</sup>	62 ± 0.5 <sup>a</sup>	10 ± 0.2 <sup>a</sup>	38 ± 0.5 <sup>a</sup>	1.76 ± 0.3 <sup>a</sup>
	HB1	3.92 ± 0.2 <sup>b</sup>	0.22 ± 0.01 <sup>a</sup>	84 ± 0.6 <sup>b</sup>	6.66 ± 0.3 <sup>b</sup>	16 ± 0.4 <sup>b</sup>	1.74 ± 0.1 <sup>b</sup>
	HB2	3.63 ± 0.1 <sup>a</sup>	0.35 ± 0.01 <sup>a</sup>	84 ± 0.6 <sup>b</sup>	6.66 ± 0.3 <sup>b</sup>	16 ± 0.4 <sup>b</sup>	1.43 ± 0.1 <sup>b</sup>
Wax beer	WB0	4.17 ± 0.2 <sup>b</sup>	0.18 ± 0.01 <sup>a</sup>	42 ± 0.4 <sup>c</sup>	13.33 ± 0.4 <sup>c</sup>	58 ± 0.5 <sup>c</sup>	2.26 ± 0.02 <sup>c</sup>
	WB1	4.13 ± 0.2 <sup>b</sup>	0.21 ± 0.02 <sup>b</sup>	72 ± 0.5 <sup>b</sup>	10.33 ± 0.4 <sup>a</sup>	28 ± 0.4 <sup>d</sup>	2.23 ± 0.2 <sup>c</sup>
	WB2	4.22 ± 0.2 <sup>b</sup>	0.14 ± 0.02 <sup>b</sup>	74 ± 0.5 <sup>b</sup>	10 ± 0.4 <sup>a</sup>	26 ± 0.4 <sup>d</sup>	2.21 ± 0.2 <sup>d</sup>
	WB3	4.18 ± 0.2 <sup>b</sup>	0.015 ± 0.02 <sup>b</sup>	76 ± 0.6 <sup>b</sup>	10 ± 0.4 <sup>a</sup>	24 ± 0.4 <sup>d</sup>	1.94 ± 0.2 <sup>d</sup>

*HB: Honey beer; WB: Wax beer; TA: Titratable Acidity; MC: Moisture content; AC: Ash content; DMC: Dry matter content*

**Table 3. Physicochemical parameters before and after fermentation of the two types of non-sterilised beers**

		pH	TA (%)	Al C (%)	RS (mg/mL)	MC (%)	AC (%)	DMC (%)
Honey beer	Before fermentation	3.77 ± 0.2 <sup>a</sup>	0.25 ± 0.01 <sup>a</sup>	0 ± 0 <sup>a</sup>	1.76 ± 0.04 <sup>a</sup>	62 ± 0.5 <sup>a</sup>	10 ± 0.2 <sup>a</sup>	38 ± 0.5 <sup>a</sup>
	After fermentation	3.72 ± 0.1 <sup>a</sup>	0.28 ± 0.1 <sup>a</sup>	2.5 ± 0.1 <sup>b</sup>	1.55 ± 0.04 <sup>a</sup>	84 ± 0.6 <sup>a</sup>	6.66 ± 0.1 <sup>b</sup>	16 ± 0.3 <sup>b</sup>
Wax beer	Before fermentation	4.17 ± 0.1 <sup>b</sup>	0.54 ± 0.02 <sup>b</sup>	0 ± 0 <sup>a</sup>	2.26 ± 0.1 <sup>b</sup>	42 ± 0.4 <sup>c</sup>	13.33 ± 0.4 <sup>a</sup>	58 ± 0.5 <sup>b</sup>
	After fermentation	3.92 ± 0.1 <sup>b</sup>	0.6 ± 0.01 <sup>b</sup>	5.26 ± 0.1 <sup>c</sup>	2.2 ± 0.1 <sup>b</sup>	76 ± 0.5 <sup>b</sup>	6.66 ± 0.1 <sup>b</sup>	24 ± 0.3 <sup>c</sup>

*HB: Honey beer; WB: Wax beer; TA: Titratable Acidity; MC: Moisture content; AC: Ash content; DMC: Dry matter content; Al C: Alcohol content; RS: reducing sugars*



**Fig. 3. Variation in microbial load during heat treatment**

## 5. CONCLUSION

The aim of this study was to analyse the physicochemical and microbiological parameters of wax beer and honey. During artisanal production, microorganisms transform sugars into ethanol with production of CO<sub>2</sub> and organic acids. Some physicochemical parameters (pH, dry matter and ash content) decrease while others (titratable acidity, moisture content in wax beer) increase. The heat treatment time plays an important role in the elimination of microorganisms in wax and honey beers. The total elimination of microorganisms in wax beer is observed after 9 minutes of heat treatment, whereas the elimination of honey is observed after 11 minutes of heat treatment.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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