International Journal of Innovation and Applied Studies
ISSN 2028-9324 Vol. 41 No. 1 Nov. 2023, pp. 137-144

http://www.ijias.issr-journals.org/

Physico-chemical and microbiological characterization of local beers of the city of Kara in Togo

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ABSTRACT: Sorghum beer, commonly called Tchoukoutou in Togo is a popular loved local beer in several African countries. However, the manufacturing conditions of this beer are still artisanal and this drink suffers from a lack of hygiene, reproducibility in the manufacturing chain and analysis of several parameters. Thus, in order to control quality and health safety, the physico- chemical and microbiological parameters of the samples of three different productions of local beers produced in Kara were analyzed. By that way, pH-metry, densimetry, colorimetry, alcoholometry, spectrophotometry, manometry and fresh state followed by counting on agar are methods used to determine respectively pH, density, volatile acidity and total acidity, alcohol content, sugar content, carbon dioxide content and microbiological quality of beer. Four samples of beer have been collected from sellers, and have been analyzed: these are two beers of type «Kabyè-Missine», a beer of type «Tchakpalo» and the fermented must of type «Kabyè-missine». The results showed that these beers have a pH between 1.92 ± 0.01 and 2.67 ± 0.00, a density that varies between 1.0063 ± 0.0001 and 1.0248 ± 0.0003, an alcohol content between 3.45 ± 0.03 and 7.48 ± 0.05. The microbiological analysis revealed that Saccharomyces yeast strains are present in all beverages, and unidentified wild yeast strains are also present in 3 out of 4 beverages. All drinks contain lactic acid and acetic bacteria. This study which is the first on the local drinks of the city of Kara will be deepened by complementary analyses such as the counting of other microorganisms, the detection possibly of methanol, tastings to assess the organoleptic quality of these drinks and the search for stabilization methods.

KEYWORDS: Sorghum-beer, physico-chemical analysis, microbiology, Togo.

1 INTRODUCTION

Red sorghum, also called millet or coarse millet is the second cereal produced in Togo after maize. For the 2020-2021 agricultural season, production was about 275708 tonnes in Togo [1]. This cereal plays a major role in the diet of the population and is currently cultivated in all regions of Togo. This sorghum is used in food to make millet dough or porridge but more than 60% is used for the manufacture of craft beers. This is also true in several African countries where sorghum grain is the main cereal used in the production of traditional beers [2][3]. This artisanal beer is known in many African countries as Tchakpalo in Côte d'Ivoire or Benin, Dolo in Burkina Faso and Mali, Billi-Billi in the Central African Republic and Cameroon [4][5][6]. In Togo, «tchoukoutou» is the beer obtained by the alcoholic fermentation of sorghum which is done in a traditional way. The production of tchoukoutou is characterized by three phases: malting sorghum, brewing sorghum malt and fermentation of must [7]. The production of this traditional beer has a great social and economic importance. Indeed, it is one of the main sources of income for women who produce it by hand [8][9]. Because of its low cost, it allows the most disadvantaged classes who cannot afford to buy industrial beer, to give themselves the luxury of drinking with friends to entertain themselves. This

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beer is mainly produced by the populations of northern Togo, including the Kara region, and is widely used and consumed during traditional ceremonies such as funerals [10] and during initiation rites such as «struggles evala» which are moments of reunion and rejoicing that develop the cooperative spirit. However, many people, especially those who are not used to consuming tchoukoutou regularly, complain of ailments such as stomach aches and diarrhea after consuming this drink. This is because the production process suffers from a crucial lack of precision measuring instruments and good hygiene practices. In addition, fermentation of sweet musts is carried out by inoculation with ferments from previous fermentations, the microbiological composition of which is not necessarily known [11][12]. Hygiene in "tchouk bars" is often questionable and deplorable with the presence of all kinds of waste, flies and standing water nearby (Fokou and al., 2016) [9]. The beer consumed is still in the state of active fermentation: it is therefore a living and unstable environment that has a short shelf life and must be consumed in general within 24 hours otherwise it becomes too acidic [13]. The physicochemical and microbiological parameters of this popular beer in Kara are not known although some studies have been carried out [14]. The difficulty lies in the fact that there is no standardization of production. However, in the city of Kara where such a study has never been conducted, this beer is consumed by both adults and adolescents (or even children). Knowing that alcoholism is a preventable cause of death, it is interesting to study this local beer. Thus, our study focuses on the physico-chemical and microbiological characterization of a typology of local beer: the «kabyè-missine» produced by kabyès and which is the most found and consumed in the city of Kara.

2 MATERIALS AND METHODS

FRAMEWORK STUDY

The experimental work was carried out at the Laboratory of Organic Chemistry and Environmental Sciences (LaCOSE) of the University of Kara (Togo). The analyses were performed at the analytical laboratory of Laffort Oenologie (Bouliac, France).

2.1.1 SAMPLING

In total, four samples of sorghum beers, called «Tchoukoutou», three fermented since about 8 hours and one fermented, of Kabyè-Missine types, were collected at three current production and sales sites, namely the Chaminade district, the district of the south campus and the village of Yadè. The sites were accessible, the producers were from the Kara region and were recommended by several regular consumers of the drink as making very good drinks. The samples were collected in sterile plastic vials with a capacity of 1.5 liters carefully closed by a screw cap. They were packaged in a thermally insulated cooler, transported to the laboratory, and then stored in the freezer at – 8°C until analysis [15].

2.1.2 PHYSICO-CHEMICAL CHARACTERIZATION OF SAMPLES

The following analyses were performed: pH, density, sugar concentration (glucose + fructose), alcohol content, volatile acidity, total acidity and CO_2 concentration.

PH MEASUREMENT

The pH was determined by direct reading using an INOLAB 1770 pH meter that was previously calibrated with commercially available pH = 4 and pH = 7 buffer solutions).

DENSITY MEASUREMENT

The density of local beers was measured using the areometric method using a densitometer [16].

DOSAGE FOR D-GLUCOSE AND D-FRUCTOSE

We used enzymatic method to determine sugar contents (D-glucose and D-fructose) [16].

ALCOHOL CONTENT

The alcoholic strength of beverages is determined by the densimetric method after distillation [16].

VOLATILE ACIDITY

The volatile acidity, which consists of the acids of the acetic series, is determined by titration after separation of local drinks by steam training [16].

TOTAL ACIDITY

The total acidity, which is the sum of the titratable acidities when the pH is brought to 7 by adding a titrated alkaline solution, was determined by titration in the presence of bromothymol blue as a coloured indicator. Carbon dioxide is not included in the total acidity [16].

CARBON DIOXIDE CONCENTRATION

We used manometric method to determine the carbon dioxide content in each sample [16].

STATISTICAL ANALYSIS

Statistical analysis of the data collected was performed by using Statistica 7.1 software. The Fisher LSD comparison test at the 5% threshold was used to classify the averages of the physico-chemical parameters of the «Tchoukoutou». Statistical differences with a probability value less than 0.05 ($P < 0.05$) are considered significant.

2.1.3 MICROBIOLOGICAL CHARACTERIZATION OF SAMPLES

The microbiological analysis of the samples was carried out under a fresh microscope to determine the presence or absence of microorganisms. Then, each sample was seeded on agar of different types: total yeast medium, MIL-BT/BL x10 medium for total bacteria (acetic bacteria and lactic acid bacteria), MIL-BA x10 medium for acetic bacteria. Genetic identification of yeasts strains has been attempted.

3 RESULTS AND DISCUSSION

3.1 PHYSICO-CHEMICAL CHARACTERISTICS OF THE TCHOUKOUTOU SAMPLES ANALYSED

The results presented in Table 1 and Figure 1 show that the pH values of the “Tchoukoutou” samples are between 1.92 ± 0.02. and 2.67 ± 0.02. The LSD test at the significance threshold of 5%, shows that there is no significant difference between the two samples of beer «kabyè- missine» analyzed. On the other hand, there is a significant difference between the samples of «kabyè-missine» and «tchakpalo», and between the unfermented beer and the other samples. Generally, the pH of the samples show that the beers are acidic or even very acidic for the «tchakpalo» which has a pH of 1.92. The pH results of the «kabyè-missine» beers are substantially comparable to the results obtained by Novidzro and al. in 2018 [14] who found pH of the order of 2.78 for the same type of beer collected in Lomé, about 400 km from Kara. On the other hand, the «tchakpalo» of Kara at a much lower pH compared to the studies made in Lomé. Togolese “tchoukoutou” beers are more acidic than artisanal beers from Côte d’Ivoire [5], Burkina Faso [17], Benin [7] or Central African Republic [6]. These local African beverages are, on the whole, more acidic than conventional industrial beers with an average pH between 4.2 and 4.4 [18]. This high acidity can be explained by the production of organic acids both by yeast and by contamination of beverages by acetic bacteria that may be present in the environment. Since these drinks are produced and always handled in the open and with bare hands. Differences in pH levels between countries, beer types and sampling locations reflect a lack of control and standardization of the local beer-making process [19]. Concerning the volatile acidity which is essentially made up of acetic acid, its content is relatively low in all the local beers analyzed, and below 0.90 g of H_2SO_4 per litre of beer, which is the maximum allowed content in wines in France [20]. This acidity should not be too high in alcoholic beverages because it is a sign of deterioration of the drink and in high quantities it gives a pungent and sour taste [21]. The total acidity of the tchoukoutou studied varies between 2.75 and 4.30 g/ L of H_2SO_4 which is in the classical interval of drinks. It is a parameter that makes it possible to appreciate the organoleptic qualities of a drink and to prevent possible contamination by non-acidophile microorganisms. The density of beverages varies between 1.0063 ± 0.01 g/cm and 1.0248 ± 0.00 g/cm. These values are relatively similar to those obtained by

Bayoï and al. in 2016 with the artisanal white beer Kapsiti from Cameroon (1.01g/cm) [22]. Similarly, the sugar content in the local beers analysed varies between 34.7 and 70.0 mg/L of glucose + fructose. It is the unfermented beer that contains the highest level of sugar with a concentration of 81.2 g/ L which would be related to the fact that the yeast, normally absent, did not transform the sugar into ethanol. However, when we observe the alcohol level in this «unfermented» beer, it amounts to 3.45%. Although women, during the process, do not put yeast in this beer called «unfermented», the alcohol level shows us that there is spontaneous fermentation, certainly due to the contamination of microorganisms, yeast in particular in the environment. This is an important point to emphasize because, very often, this beer called «unfermented» is given to drink to children while it has a significant level of alcohol and alcohol is dangerous for children. For the other local beers that are known to be in the process of fermentation, the degree of alcohol varies between 4.15% for the kabyè-missine beer of Yadè to 7.48% for the tchakpalo beer of the Chaminade district. There is a significant difference in the alcohol level between Yadè's kabyè-missine beer and the other two beers. Overall, the alcohol content of these beers is comparable to that of «tchoukoutou» beers analyzed by Novidzro and al., in Lomé in 2018 [14] and «tchakpalo» beers analyzed in 2005 by Amane and al [23]. These local drinks have alcohol levels comparable to those of commercial beers found on the Togolese market: we can therefore reasonably call them local beers. The level of carbon dioxide in all these local drinks varies between 373 and 503 mg/L. This is consistent with the fact that, during the fermentation process, yeasts convert fermentable sugars into ethanol and carbon dioxide. No preliminary study on the CO_2 content in local beers was carried out to give a point of comparison. However, this content is lower than that contained in industrial beers, which however contain varying levels of CO_2 concentration. This difference can be explained on the one hand by the difference in the raw materials used to make beer and on the other hand yeast, conditions of uncontrolled fermentation. The results obtained are given in Table 1.

Table 1. Physico-chemical parameters of the local beers studied

	KY	**KC**	**KTT**	**KTT-NF**
pH	2.55	2.67	1.92	2.24
Total acidity g/L	3.31	4.30	4.25	2.75
Volatile acidity g/L	0.08	0.04	0.02	0.54
Density	1.0188	1.0063	1.0179	1.0248
Alcohol content %	4.15	7.14	7.48	3.45
Sugar content (glucose + fructose) mg/L	64.5	34.7	70.1	81.2
CO_2 mg/L	463	373	430	503

KY (kabyè missine Yadè); KC (Kabyè missine campus); KTT (Tchakpalo Chaminade); KTT-NF (Tchakpalo unfermented Chaminade)

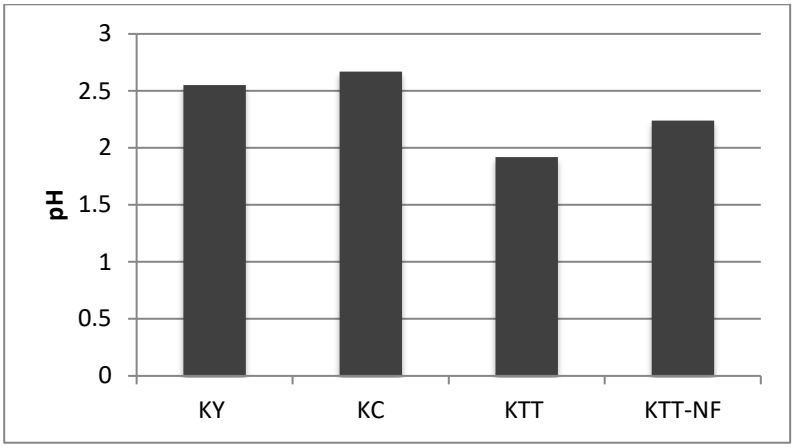

Fig. 1. pH of the different Tchoukoutou studied

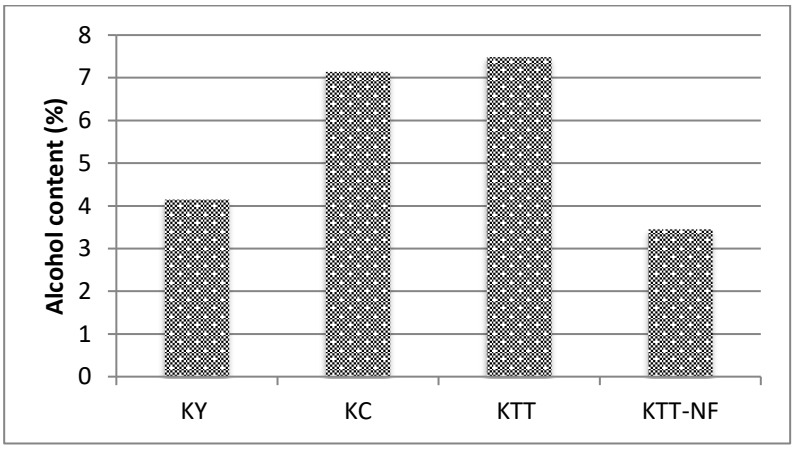

Fig. 2. Alcohol levels of the different Tchoukoutou studied

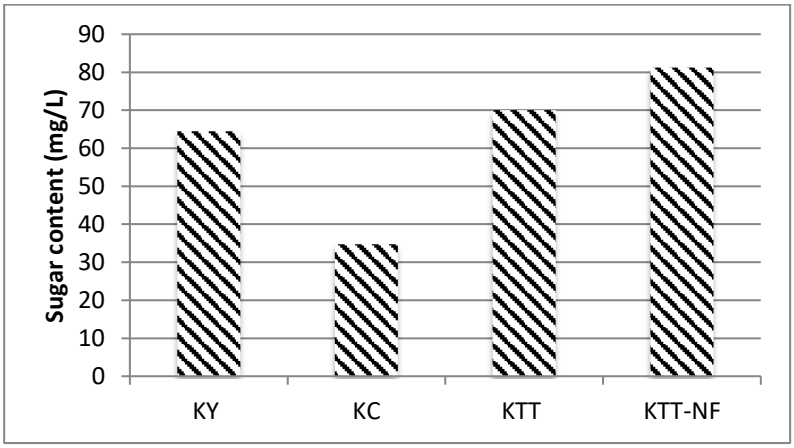

Fig. 3. Sugar levels of the different Tchoukoutou studied

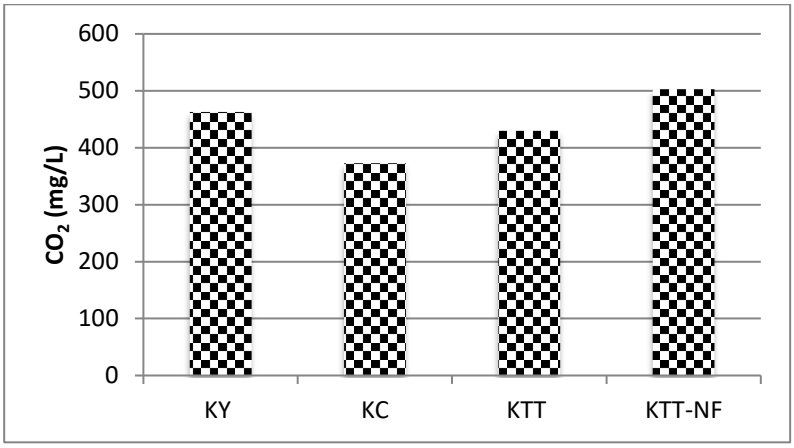

Fig. 4. CO_2 content in the different Tchoukoutou studied

3.2 MICROBIOLOGICAL CHARACTERISTICS OF THE TCHOUKOUTOU SAMPLES ANALYSED

All samples of tchoukoutou analyzed contain yeasts. For Tchoukoutou during fermentation, this observation is normal since yeasts are introduced during the local beer production process by inoculation of a previous fermented beverage that contained yeasts [24]. However, the unfermented beer was not inoculated by any ferment. This assumes that the sweet must has undergone spontaneous fermentation caused by the surrounding microbiota. Regarding yeast strains, the analysis shows that all beverages contain Saccharomyces type yeasts. The samples of Tchakpalo (fermented and unfermented) and Kabyè-missine from the Campus district contain wild yeasts that were not precisely identified, in addition to Saccharomyces-type yeasts. Wild yeasts can be beneficial in contributing to the aroma and organoleptic complexity of alcoholic beverages [25]. All samples contain lactic acid and acetic bacteria. Lactic acid bacteria are, along with yeasts, the microorganisms commonly found in products resulting from the fermentation of cereals [26][27]. The presence of lactic acid bacteria could be favored by the presence of soluble nitrogen compounds, dissolved CO_2 and other factors such as vitamins or pyruvates and malic acid that could be further analyzed to confirm this hypothesis. Lactic acid bacteria transform malic acid into lactic acid, creating an acidic environment that promotes the subsequent process of alcoholic fermentation by yeast. The activity of these bacteria explains the relatively low pH of all samples. The presence of acetic bacteria is harmful to the must in fermentation. These bacteria can act under all pH conditions encountered and they are not inhibited by ethanol (unlike yeast). These bacteria are powerful oxidizing agents that oxidize ethanol into acetic acid causing unpleasant tastes and aromas of acid and sour drinks. Their presence is a sign of contamination of the environment, certainly due to the often deplorable hygiene conditions in the places of manufacture of these craft beers. Table 2 shows the results achieved.

Table 2. Microbiological analysis of local beers studied

Sample Name/Microorganisms	Wild yeasts	Saccharomyces Yeast	Acetic bacteria	Lactic bacteria
KY	Presence	Presence	Presence	Presence
KC	Presence	Presence	Presence	Presence
KTT	Absence	Presence	Presence	Presence
KTT-NF	Presence	Presence	Presence	Presence

4 CONCLUSION

This study focused on the physico-chemical and microbiological analysis of some samples of tchoukoudou from the city of Kara. The results revealed that the parameters are relatively variable from one type of beer to another and from one seller to another. These beers have relatively acidic pH, certainly due to the presence of lactic acid bacteria responsible for malolactic fermentation that acidifies the environment before alcoholic fermentation. The degree of alcohol is quite high but of the same order of magnitude as that of commercial beers sold in Togo. It should be noted that even beer called «unfermented» contains a significant amount of alcohol due to spontaneous fermentation. This should be emphasized because this drink is often given to children to drink. The other parameters made it possible to make points of comparison with similar drinks produced in other countries. From the microbiological point of view, the local beers contain yeasts of the species Saccharomyces and wild yeasts not Saccharomyces. Wild yeasts could not be precisely identified but this will pave the way for further analyses. All beverages contain lactic acid and acetic bacteria that have an impact on the organoleptic quality of beverages. The poor hygiene conditions in which these drinks are made, explain the presence of these bacteria of deterioration, especially acetic bacteria. Another study will verify the presence or not of other microorganisms and will be accompanied by tasting sessions by trained panelists, connoisseurs of local drinks who can give their point of view on the organoleptic qualities of the various local beers.

CONFLICTS OF INTEREST

The authors of this article declare that there are no conflicts of interest in this publication.

AUTHORS CONTRIBUTION

Marie-France Bakaï: Conceptualization of research project, Data collection and analysis, Manuscript writing and editing.

Batcha Ouadja: Fieldwork and sample collection, Data collection and analysis, Laboratory experiments, Manuscript writing and editing.

Marie-Estelle Kipré-Naura: Experimental design and methodology, Data collection and analysis, Manuscript writing and editing.

AKNOWLEDGEMENTS

The authors would like to express their gratitude to the LaCOSE laboratory at the University of Kara, the Laffort Oenologie laboratory and the « tchoukoutou » sellers in Kara city for their collaboration.

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