

## Article

# Physicochemical Changes occurring during Long-time Fermentation of the Indigenous Alcoholic Sorghum-based Beverages brewed in the Northern Cameroon

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**Abstract:** In Cameroon, alcoholic beverages remain the main consumed drink. In the Northern regions, indigenous sorghum beers are popular and widely consumed in an actively fermenting state by the people. In this study, some physicochemical parameters of the alcoholic sorghum beverages and correlations between them were evaluated during fermentation for 10 days. The indigenous white and red beers were produced at the laboratory scale assisted by experimented producers and some parameters (pH, total acidity, alcohol, sugars, density, total solids, temperature, and conductivity) were measured on the wort and fermented beverages. The pH decreases from 3.2 to 2.4 and 3.11 to 2.41; total acidity increases from 1.07 to 5.1 g/l and 0.5 to 4.6 g/l; alcohol enhances from 0 to 9.5% and 0 to 6.8% (v/v); total solids drop from 13.6 to 5°P and 12.2 to 3.3°P were observed respectively in the white and red sorghum beers. The multivariate analysis showed a good correlation between consumption of sugar, the decrease of total solids and density with the decrease in pH. It was also shown that, a perfect link exist between the production of alcohol and organic acids. The hierarchical analysis showed that indigenous red beer samples fermented for 1 and 2 days and those fermented for 4 to 10 days are related and could be separate in two distinct groups, whereas white turbid beer samples are separated in three different groups, those fermented for 1 to 4 days, those 5 to 6 days and those 7 to 10 days. The results obtained could serve as a guide to better understand the fermentation process of the indigenous alcoholic sorghum-based beverages.

**Keywords:** Cameroon; sudano-sahelian zone; sorghum; long-time fermentation; turbid beers; physicochemical changes; clustering

## 1. Introduction

Fermented beverages hold a long tradition and contribution to the nutrition of many societies and cultures worldwide. Traditional fermentation has been empirically developed in ancient times as a process of raw food preservation and at the same time the production of new foods with different sensorial characteristics, and enhanced nutritional value (Baschali et al, 2017). Based on archaeological and archaeobotanical findings, it is generally believed that over 9000 years ago individuals of the globe were already fermenting beverages (McGovern et al, 2004). It is reported that fermented foods and beverages globally contribute 20 to 40% of the food supply and usually occupy the third position of food consumed by man (Campbell-Platt, 1994). It is therefore not surprising that fermented foods and beverages make a big contribution to peoples' diets in Africa (Sanni, 1993). Cereals such as sorghum, pearl millet and maize are generally used for the production of indigenous fermented beverages widely consumed all over the African continent (Achi and Asumudo, 2019). It is estimated that over 60 million people living in the very hot, drought-prone tropical regions of Africa, use sorghum and millet as part of their staple diet (Correia et al, 2010; Pale et al, 2015). In

Cameroon, sorghum is essentially cultivated in the soudano-sahelian zone with an annual average production about of 500000 tons (FAO, 2010), which places the northern part of the country at the top rank of the cereal production. Due to many problems of storage encountered in northern Cameroon, a significant part of the sorghum produced is used to brew indigenous alcoholic beverages known as *bil bil* which accounts for approximately 80% of the total consumption of alcoholic beverages in these regions of the country (Kubo et al, 2014). They are popular because of the social, religious, nutritional, and therapeutic values that are associated with them (Ezekiel et al, 2017). They are cherished by both rural and urban populations because they are less costly and available everywhere throughout the year (Ezekiel et al, 2017). In the northern part of Cameroon, indigenous sorghum-based alcoholic beverages are sold as street food at some original and convivial places called cabarets or *saré* in the local dialect (Seignobos, 2005). In the famous “Mandara” mountainous range, situated in the far north of Cameroon, two particular and highly culturally symbolic homebrewed sorghum beers are produced and known as red *tè* beer (or male beer) and white *mpedli* beer (or female beer) (Bayoï et al, 2016, 2017). These beers are an integral part of the socio-cultural life of the *kapsiki*, an ethnic tribe belonging to the *Mandara* group. In addition to their colours which are different, the two *kapsiki* beers are produced by two distinct artisanal fermentation processes. The white beer is made by fermenting the mixture obtained from brewed malt flour added to the cooked non-malted flour of sorghum (*fufu*) without the inoculation of any starter culture. The red beer is generally produced by fermenting sweetish wort (*tè kwarhèni*) from malted flour of sorghum, using an artisanal starter culture. Previous studies on the microbiological and physicochemical characteristics of both indigenous beers as well as their artisanal processing technologies have been carried out and well documented. Contrary to European beers made with barley and fermented by selected yeasts during a long time range between 8 to 15 days, indigenous sorghum beers are fermented by artisanal leaven for a time which varies between 10 h to 48 h. As a result, these traditional sorghum beers from northern Cameroon are consumed in an actively fermenting state and the beverages contain large amounts of fragments of insoluble materials. So far, all data from both indigenous beers gave an overview on the properties (or qualities) of alcoholic beverages just at the end of the fermentation step and at the moment of their consumption. Yet, physicochemical analysis of indigenous alcoholic sorghum-based beverages during a long time fermentation has not been documented.

Given that understanding of the changes in physicochemical characteristics is essential to upgrade the traditional processing to commercial scale, this paper aimed to highlight the physicochemical changes that occurred during 10 days of fermentation of the indigenous alcoholic beverages brewed in northern Cameroon and to determine the correlations among physicochemical parameters and the similarity between the different indigenous beers based on fermentation time using multivariate analysis. The importance of this study is to upgrade the production of the indigenous and culturally embedded beverages to the next level which is a small industrial scale.

## 2. Materials and Methods

### 2.1. Vegetal material and artisanal starter

The local “Damougari” Sorghum (*Sorghum bicolor* L. Moench) grains of the red variety and the white variety were purchased from the local market of Maroua, Diamaré, Cameroon. The plant materials were transported to the laboratory of Food Technology in the plastic bag and subsequently verified by the botanical experts of the Department of Biological Sciences of the Faculty of Science. The artisanal starter used for fermentation of the red kapsiki beer was a gifted dried powder graciously given to us by the local beer producers from Mogode, Mayo-Tsanaga, Cameroon.

### 2.2. Analysis of quality of the sorghum grains

To ensure the production of the indigenous beers with good quality, we have performed quality control of the sorghum grains used during processing. Physicochemical and technological parameters of sorghum grains such as water content, germination capacity, germination energy, the temperature of germination, percentage of impurity and 1000-kernel weight were evaluated using

the method described by Analytica-EBC (2014). After analysis, only grains with good physicochemical and technological characteristics were used for the artisanal processing of the traditional sorghum-based fermented alcoholic beverages.

### *2.3. Laboratory preparation of the indigenous alcoholic beverages*

Two of the experimented women previously interviewed for the description of the artisanal processing of both indigenous beers were called to assist us during the production of the kapsiki beers according to the manufacturing flowchart described in our previous studies and following their observations. Production was conducted in the laboratory of Food Technology, Institute of Agricultural Research, Unit of Maroua, Cameroon.

### *2.4. Laboratory processing of the sorghum turbid white beer*

The traditional processing involved stages of malting, brewing and fermentation. A lot (about 1/6 of the total grains used for the beer production) of the white grains of sorghum was soaked and left to settle for 24 h. The grains were germinated for 48 h, dried for 2 days at 45°C using an oven (Memmert GmbH + Co. KG), ground to malt sorghum flour and kept for further use. The greatest quantity of the remaining white sorghum grains was cleaned and mashed in non-malted sorghum flour. The flour was soaked for 72 h and the floury pellet was recovered, mixed with distilled clean water and cooked for about 3 h to obtain a roasted dough called “fufu”. After cooling, the stored sorghum malt flour was added to the dough and the mixture was hand-kneaded until a wort dough was obtained (Bayoï et al, 2016).

### *2.5. Laboratory processing of the sorghum turbid red beer*

Firstly, the red “Damougari” sorghum grains were quenched for 24 h, and germinated for 4 days with water spraying every 12 h, then dried for 3 days at 45°C using an oven. After milling of the dried grains, the malted flour obtained was mixed out with distilled clean water. The mixture obtained was decanted for 3 h at 45°C using a water bath (Memmert GmbH + Co. KG), then the supernatant was separated and kept. The bottom was pre-cooked for 4 h while shaking every 5 min, later mixed with the previous supernatant to give a sour wort after a night spontaneous fermentation. The sour wort was boiled for 7 h and was mixed every 5 min to give sweet wort at the end. The artisanal dried starter was mixed with distilled water and the sweet-sour wort was inoculated with up to 10% (v/v) artisanal starter suspension for alcoholic fermentation (Bayoï et al, 2017).

### *2.6. Yield of wort production*

The yield of wort production was determined by ratio between the quantity of wort produced and the quantity of raw plant material used according to the formula (1) below:

$Y_p (\%) = (M_n/M_r) \times 100$  (1); where  $M_n$ : mass of wort obtained (g);  $M_r$ : mass of raw material used (g);  $Y_p$ : yield of wort production

### *2.7. Samples preparation and fermentation monitoring*

One hundred (100) milliliters of samples of each indigenous beer produced previously were withdrawn eleven times in triplicate at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days of fermentation, for a total of 33 samples collected for each artisanal fermented alcoholic beverage. Samples were immediately centrifuged for 5 min at 1000g and supernatant was successively filtered through a filter paper whattman n° 1 and a membrane filter (0,62 µm diameter). The beers filtrate collected were used for physicochemical analysis for the determination of pH, total acidity, Brix, sugar, density, alcohol content, conductivity, temperature.

### *2.8. Physicochemical analyses of the traditional sorghum-based alcoholic beverages*

The pH and temperature of the samples were recorded before centrifugation and immediately after harvesting using respectively ATC portable pH-meter (Eco Testr) and infrared thermometer (Initio Jeulin 251040). The total soluble solids was expressed as degree Brix, the density and conductivity of filtered supernatant were measured using portable devices, respectively an ATC refractometer (RHB 90), an aerometer (Assistent 6105/3) and a conductimeter (Eco Testr). Total sugar of the filtrates was evaluated by applying the phenol-sulfuric colorimetric method as proposed by Dubois et al. (1956).

The alcohol content of the filtrates was determined with combined results of degree Brix and density according to the equation (2) as described by Pauline et al. (2017) with slight modifications:

Alcohol content (% v/v) = DB - [(D-1) × 1000]; (2) where DB: Degree Brix value (°B), D: Density

Total acidity expressed as percentage (%) of acetic acid and malic acid in the filtrates was titrated against 0.1 N NaOH solution, using phenolphthalein (0.1% w/v in ethanol) as the colored indicator. The following equation (3) was used to determine the total acidity as described by Desobgo et al (2013):

Total acidity = [(normality of 0.1 NaOH X ml volume used of 0.1 NaOH X 1000) / (ml volume of filtrate supernatant)] X k; (3) where k equal at 0.067 for malic acid and 0.8 for acetic acid

### 2.9. Statistical analysis

All the measurement were done in triplicate and the results were presented as mean ± standard deviation using curves and bar charts to illustrate changes of the physicochemical parameters during fermentation. These parameters were subjected to one-way analysis of variance (ANOVA) to determine the mean differences among the beer samples fermented at different times. Whenever significant differences in ANOVA ( $P < 0.05$ ) were detected, the HSD Tukey's multiple range test was applied to discriminate pair of means significantly different at  $P < 0.05$  (Tukey, 1953) in STAGRAPHS software centurion version 16.1 (Technologies Inc., Virginia, USA). Multivariate analysis especially Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) (Huang et al, 2017) were performed to analyze the correlation between different physicochemical parameters of beer samples, and the relationship between both indigenous beer samples, using SPSS Statistical program (SPSS20, IBM Inc., Armonk, New York, USA).

## 3. Results

### 3.1. Physicochemical and technological characteristics of sorghum grains

The characteristics of sorghum grains used for processing of indigenous beers are summarized in table 1. The impurity percent of red (2.5%) and white (2.4%) sorghum grains was not significantly ( $P < 0.05$ ) different. The water content of grains varies from 5.84% to 5.92% and was lower than the standards recommended by brewers (13%). The weight of white grains was significantly higher than the weight of red grains. The weight of 1000 grains ranged between 33.5 g and 42.2 g. The grains of red sorghum variety showed a germination capacity (97.8%) and germination energy (69.0%) significantly higher than those of white sorghum grain (85.8% and 55 %). Both sorghum grains have shown relatively the same temperature during germination step.

**Table 1.** Physicochemical characteristics of some grains of sorghum sampled in the main markets of Maroua town and used for the production of the indigenous beers

Varieties	Impurity (%)	H (%)	GC (%)	GE <sub>4</sub> (%)	GE <sub>8</sub> (%)	W <sub>1000</sub> (g)	TG (°C)
White sorghum	2.4 ± 0.1 <sup>a</sup>	5.8 ± 0.1 <sup>a</sup>	85.8 ± 1.1 <sup>a</sup>	55.0 ± 0.0 <sup>a</sup>	100.0 ± 0.0 <sup>a</sup>	42.2 ± 1.1 <sup>a</sup>	32.3 ± 1.0 <sup>a</sup>
Red sorghum	2.5 ± 0.2 <sup>a</sup>	5.9 ± 0.2 <sup>a</sup>	97.8 ± 1.1 <sup>b</sup>	69.0 ± 1.4 <sup>b</sup>	87.5 ± 3.5 <sup>b</sup>	33.5 ± 0.5 <sup>b</sup>	32.6 ± 1.2 <sup>a</sup>

H: water content; GC: germinative capacity of 200 grains; GE: germinative energy of 100 grains by using 4 ml of distilled water (GE<sub>4</sub>) or 8 ml of distilled water (GE<sub>8</sub>); W<sub>1000</sub>: weight of 1000 grains; TG: Temperature of grains during the step of germination. Mean values preceded by one common letter in the same column are not significantly different ( $p < 5\%$ ) using ANOVA and Tukey's HSD multiple comparison test.

### 3.2. Processing of indigenous beers

Table 2 below presents the yield of wort production during the processing of red and white traditional beers. We observed that the wort yield of white indigenous beer (59 %) was significantly lower than the wort yield of red opaque beer (62 %).

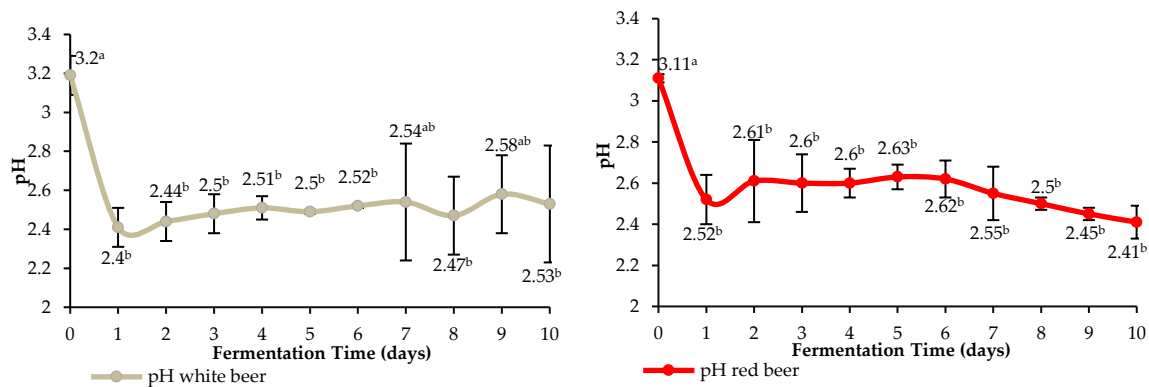
**Table 2.** Wort yield according to the type of sorghum used for the production of indigenous alcoholic beverages

Indigenous beer	White beer	Red beer
Yield of production (%)	59.8 ± 2.9 <sup>a</sup>	62.6 ± 3.8 <sup>b</sup>

Mean values (n= 3 repetitions) preceded by one common letter (a, b) are not significantly different (p < 5%) using ANOVA and Tukey’s HSD multiple comparison test.

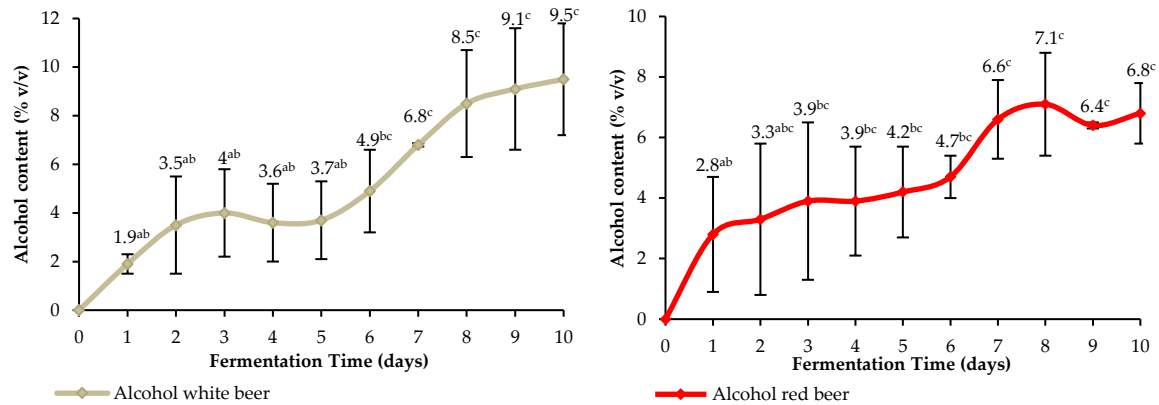
3.3. Evolution of physicochemical parameters during fermentation of opaque beers

Changes in pH during fermentation of the indigenous beers wort are illustrated in figure 1. We observed that the initial pH values were 3.2 and 3.11 respectively for white and red home-brewed beers. After the first day of fermentation, we had a significant pH drop of white and red beers respectively at 2.41 and 2.52. From day 2 to day 6, we noted no significant increase of pH values for both beers, followed by a second slight drop of pH values between day 7 and day 10 of fermentation of the red beer. The alcohol content of the white and red beers home-brewed in Northern Cameroon is shown in figure 2. Alcohol production during fermentation of white described a sigmoid curve with a minimal value of 0 % and maximal value of 9.5% after 10 days of fermentation. We have a significant increase of alcohol content from the first to the third day of fermentation (1.9 - 4%) and which remains steady between the third and fifth day of fermentation (4 – 3.7%). From the sixth to the eighth day, we noted a second and significant rise of alcohol content (4.9 – 8.5%) of white beer samples with a steadiness until the tenth day. The alcohol content during fermentation of red beer describes a curve with two dip points. As white beer, we noticed a significant enhancement in alcohol content after the first day of fermentation red opaque beer (2.8%), followed by a dip and relative constancy from the second to the fifth day of fermentation (3.3-4.2%). Then, we have observed a second dip on the sixth day (4.7%) followed by a second phase of significant increase of alcohol content between the seventh and eighth day of fermentation (4.7-7.1%).

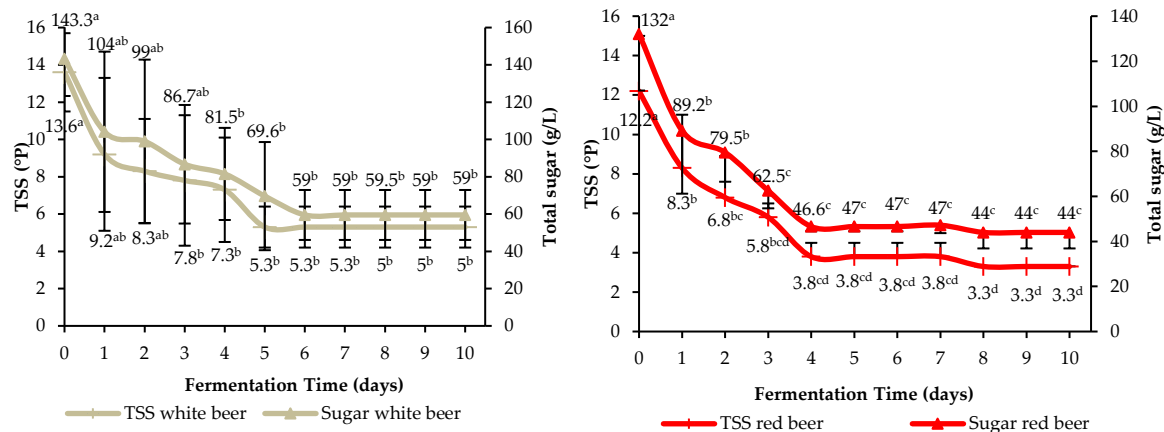


**Figure 1.** Evolution of pH during fermentation of the indigenous sorghum beers. Mean values (n= 3 repetitions) preceded by at least one letter common are not significantly different (P< 0.05) using ANOVA and HSD Tukey’s multiple range test. Bars of the mean values represent standard deviations.





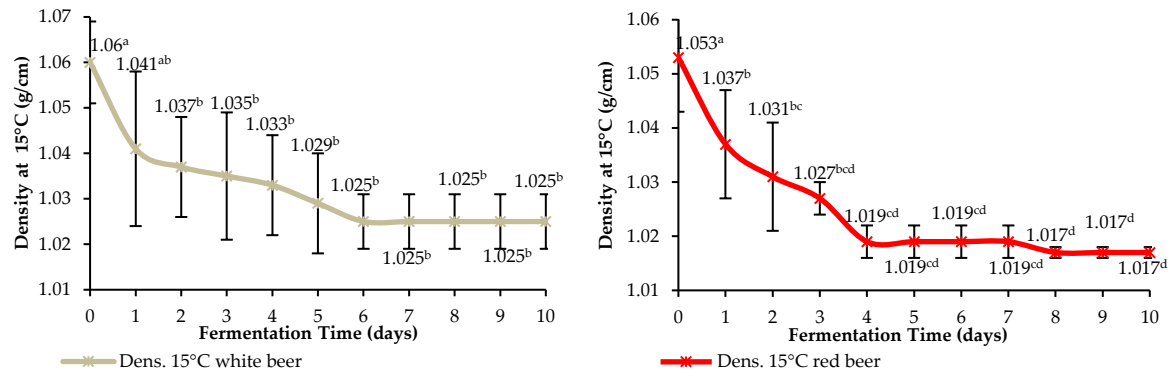
**Figure 2.** Evolution of alcohol content during fermentation of the indigenous sorghum beers. Mean values (n= 3 repetitions) preceded by at least one common letter are not significantly different ( $P < 0.05$ ) using ANOVA and Tukey's multiple range test. Bars under the mean values represent standard deviations.



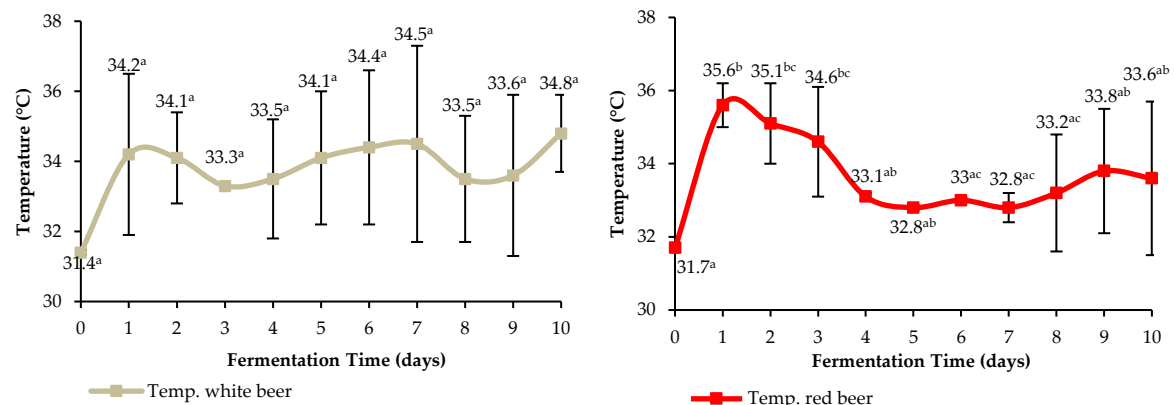
**Figure 3.** Evolution of total soluble solids and sugars during fermentation of the indigenous sorghum beers. Mean values (n= 3 repetitions) preceded by at least one letter common are not significantly different ( $P < 0.05$ ) using ANOVA and Tukey's multiple range test. Bars of the mean values represent standard deviations.

The total solids and sugars contents of indigenous beers samples produced decrease with time of fermentation (Figure 3). The total solids and sugar of the white beers samples varied respectively from 13.6°P to 5°P and 143.3 g/l to 59 g/l. During fermentation, red home-brewed beer samples showed a total solid content varying between 12.2°P to 3.3°P and total sugar ranged between 132 g/l to 44 g/l. The changes in density during the fermentation process of home-brewed beers are presented in figure 4. We noticed a significant decrease of density with time of fermentation for both indigenous beers. Density values of the white beer samples vary from 1.06 to 1.025 g/l and from 1.053 to 1.017 g/l for red beer samples respectively between days 0 to 10 of the fermentation process. We observed a constant density of the beer from the sixth day until the tenth day. Figure 5 shows changes in temperature during the fermentation of sorghum beers. The temperature of the white beer samples during the fermentation process oscillates between 31.4°C before fermentation and 34.8°C on the tenth day of fermentation. The same evolution is observed with the temperature of the red beer samples which move from 31.7°C at the initial time to 33.6°C at the end of the fermentation process. However, we observed a significant enhancement of temperature after the first day of fermentation (35.6°C), following a sudden drop from the second day till the seventh day. Titrable acetic and malic acids content during fermentation of the opaque sorghum beers are shown in figure 6. We have observed a significant increase of acetic and malic acids after a day of fermentation of the white beer

(50 g/l and 4.2 g/l) and red beer (43.3 g/l and 3.6 g/l) samples, followed by a constancy till the tenth day of fermentation of the white beer (only for malic acid) and red beer (both organic acids). We noticed a second increase of acetic acid content of the white beer samples from the seventh day of fermentation (60.1 g/l).

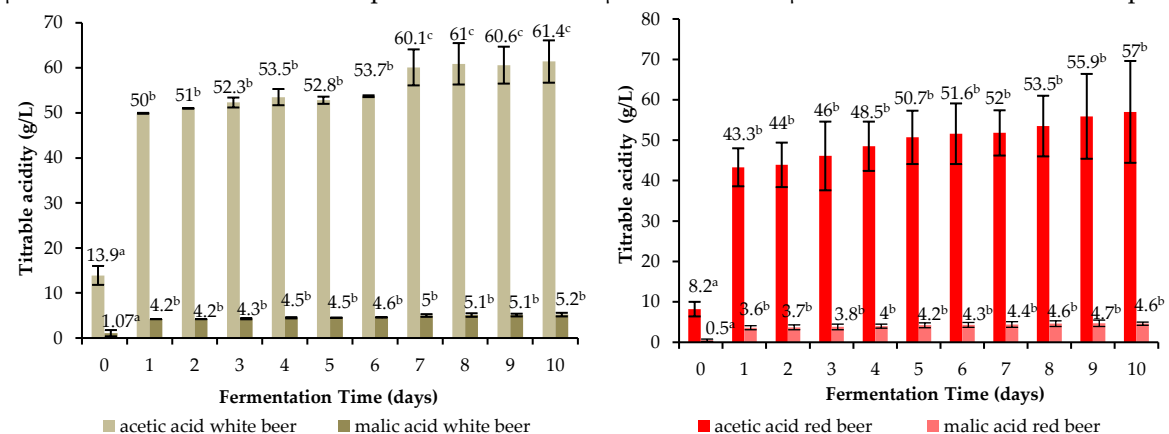


**Figure 4.** Evolution of density during fermentation of the indigenous sorghum beers. Mean values (n= 3 repetitions) preceded by at least one letter common (a, b or c) are not significantly different ( $P < 0.05$ ) using ANOVA and HSD Tukey's multiple range test. Bar of mean values represent standard deviations.

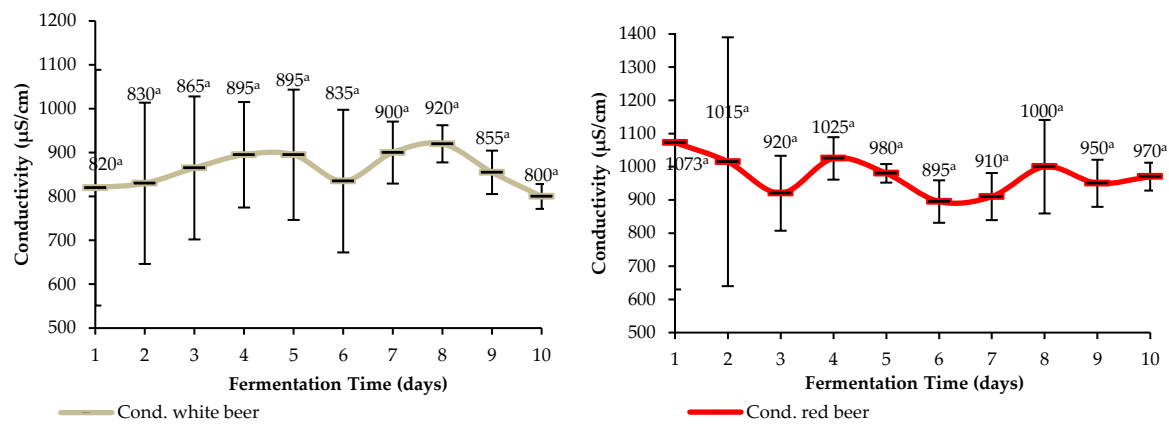


**Figure 5.** Evolution of temperature during fermentation of the indigenous sorghum beers. Mean values (n= 3 repetitions) preceded by at least one letter common are not significantly different ( $P < 0.05$ ) using ANOVA and HSD Tukey's multiple range test. Bar of mean values represent standard deviations.

The conductivity of white and red beer samples are illustrated in figure 7 below. The statistical analysis shows that, there is no significant difference between the conductivity values of both beer samples during the fermentation process. The conductivity values vary between 800  $\mu\text{S}/\text{cm}$  and 920  $\mu\text{S}/\text{cm}$  for the white beer samples and from 898  $\mu\text{S}/\text{cm}$  to 1073  $\mu\text{S}/\text{cm}$  for the red beer samples.



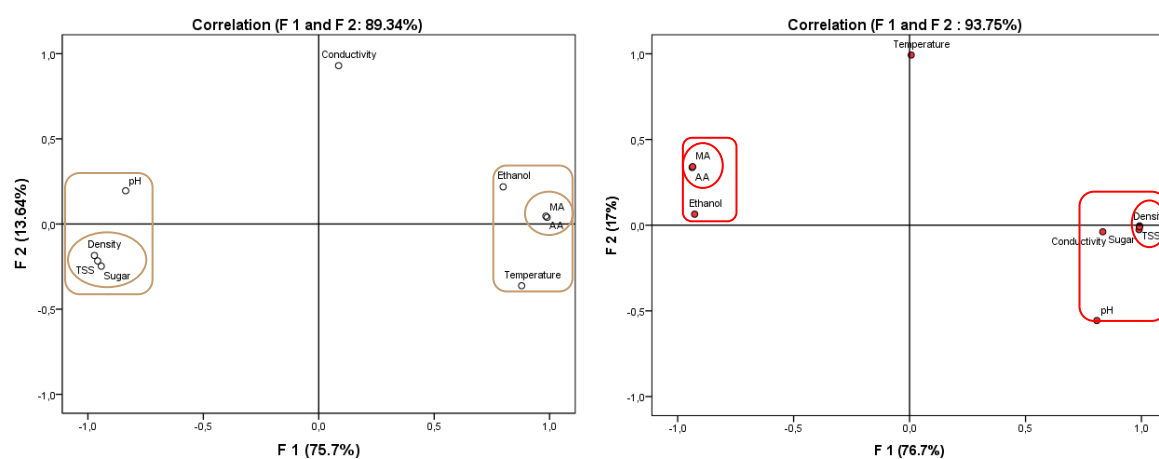
**Figure 6.** Evolution of titrable acidity during fermentation of the indigenous sorghum beers. Mean values (n= 3 repetitions) preceded by at least one letter common are not significantly different ( $P < 0.05$ ) using ANOVA and HSD Tukey's multiple range test. Bar of mean values represent standard deviations



**Figure 7.** Evolution of conductivity during fermentation of the indigenous sorghum beers. Mean values (n= 3 repetitions) preceded by at least one letter common are not significantly different ( $P = 0.05$ ) using ANOVA and HSD Tukey's multiple range test. Bar of mean values represent standard deviations.

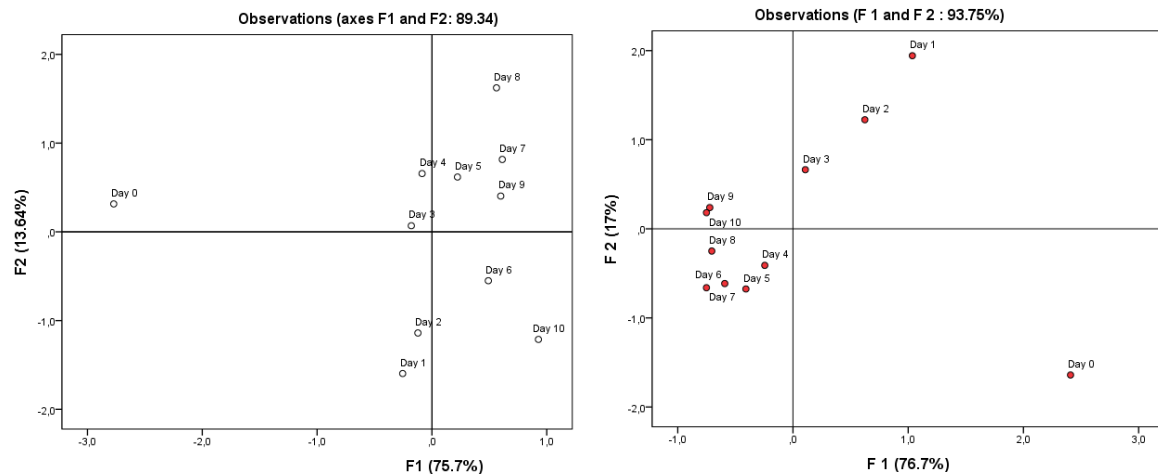
### 3.4. Multivariate analysis of indigenous alcoholic beverages during fermentation

To facilitate the interpretation of the correlations between variables evaluated during fermentation of both traditional beers used, the principal component analysis (PCA) was performed with nine physicochemical parameters of indigenous beer samples. As shown in figure 8, Kaiser-Meyer-Olkin (KMO) measures (0.67-0.721) and Bartlett's test of sphericity ( $P$ -value = 0.000) confirmed that sampling were adequate and the principal component (or factor) analysis was valid. The nine physicochemical variables were reduced to two principal components (F1 and F2) which had eigenvalues larger than one and retained for rotation. F1 accounted for 75.7% and 76.7%, whereas F2 only accounted for 13.64% and 17% of the total variations respectively for the white and red indigenous beers. Combination of F1 and F2 explained together 88.34% and 93.75% of the total variance respectively for home-brewed white beer and red beer samples.



**Figure 8.** Correlation loadings plot of the two main factors resulting from principal component analysis of physicochemical parameters during fermentation of the white beer (left side) and red beer (right side) for ten days. Notes: Rectangles and circles show  $R^2 = 50\%$  and  $98\%$  respectively. Rotation method: varimax with Kaiser normalization (KMO = 0.721 and 0.67 respectively for white and red beer samples;  $P$ -value = 0.000). TSS: total solids soluble; AA: Acetic acid; MA: malic acid.

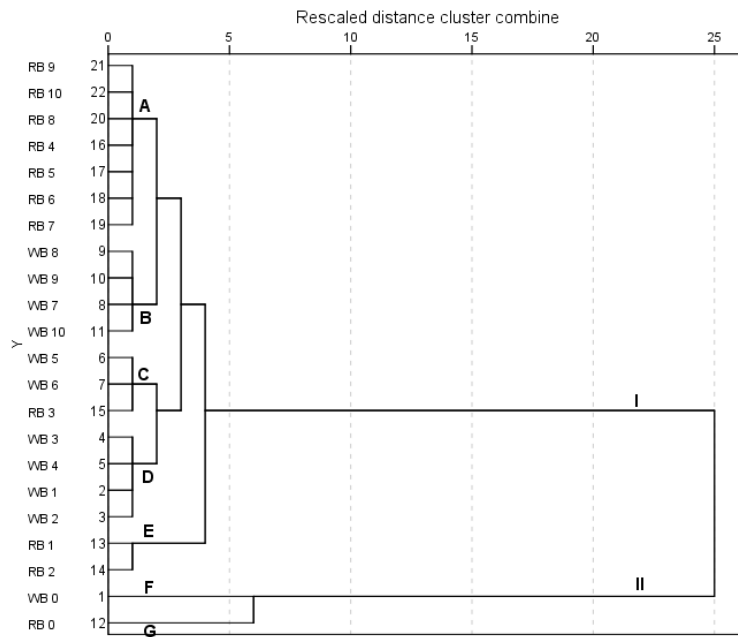




**Figure 9.** PCA scores plots derived using physicochemical values of indigenous white beer (left side) and red beer (right side) samples for ten days of fermentation.

The geometrical figures indicate the correlation levels between physicochemical variables. Rectangles indicate only 50% of the explained variance whereas circles indicate till 98% of the variance explained linearly and show a strong correlation between physicochemical regarded variables. The majority of the variation captured by F1 for all the physicochemical parameters served to distinguish the beer samples during fermentation. The PCA showed that with the traditional white beer, ethanol, temperature and titrable acidity (expressed as acetic and malic acids) were positively loaded on F1 and sugar, TSS, density, pH were negatively loaded on the same factor. Conductivity was the only variable positively loaded on F2. On the contrary, the factor analysis with indigenous red beer samples indicated that variables such as sugar, TSS, density, pH and conductivity positively contributed to F1 and the variables such as titrable acidity (expressed as acetic and malic acids) and ethanol were negatively loaded on F1. Only temperature was positively loaded on F2. Figure 9 presented the scores plots derived from measured physicochemical variables of beer during fermentation. It showed clear discrimination of the observations. But, the plot of the white beer samples appeared to be contrary to the plot of red beer samples. For example, the white beer samples after 1 and 2 days of fermentation were on the negative side of F2 whereas the red beer samples at the same time of fermentation were found on the positive side of F2. It was always the case of beer samples fermented for 6, 7, 9 and 10 days which are found on the negative side and the positive side of F1 for indigenous white beer and red beer respectively.

In an attempt to simplify the interpretation of relationships among indigenous beer samples during fermentation, hierarchical clustering analysis (HCA) was applied to the physicochemical variables by squared Euclidean measurement. The centered-reduced normalization was applied to improve the classification of the samples of both indigenous sorghum-based alcoholic beverages. As shown in figure 10, two main and distinct clusters were identified I and II. The beer samples were divided into seven sub-groups. The red beer samples recovered after 1 and 2 days of fermentation were classified into the same sub-cluster (E) while red beer sampled after 4 to 10 days of fermentation belonged to a different sub-group (A). The white beer samples recovered between 1 to 4 days, and 7 to 10 days of fermentation were grouped into two distinct sub-clusters D and B respectively. However, the white beer samples recovered after 5 and 6 days of fermentation and the red beer samples obtained after 3 days of fermentation were grouped into the same sub-cluster (C). Hierarchical analysis showed that though indigenous beer samples belong to the same cluster, the white beer and red beer before the fermentation process are presented into two different sub-cluster (F and G).



**Figure 10.** Clustering dendrogram of indigenous sorghum beer samples at different fermentation time performed with the hierarchical cluster analysis using centroid method and squared Euclidean measurement. Notes: x-axis and y-axis mean rescaled distance cluster and the beer samples measured. WB: white beer; RB: red beer. Numbers indicate time of fermentation.

#### 4. Discussion

Analysis of sorghum grains used for the beer production have shown that they did not contain enough impurity and were significantly dried. The impurity content of raw material was lower than 5% which is the recommended value of acceptability of grains (Anonyme, 2015). The water content of grains which was lower than 13% means that sorghum grains were well-dried and kept before selling. Grains used for processing of the indigenous alcoholic beverages were above the recommended values (25-29g/1000 grains) indicated by FAO (FAO, 1995). But, they were in the same weight range with the sorghum grains commonly used for the production of indigenous beers in the Northern Cameroon (Nso et al, 2003). Significant gap was observed between germination energy of sorghum grain obtained with 4 and 8 ml of water which confirms the key role played by water during the malting step. With all these physicochemical and technological data, they made us to postulate that sorghum grains used for brewing of the indigenous beers were suitable for malting and the quality of wort that can be obtained after brewing may be considerable. Indeed, the wort yields obtained in this study are higher than those obtained around 56% during the production of sorghum-based turbid beers and indigenous beers based sorghum and banana brewed in West African countries (Dahouenon et al., 2012). However, the wort yields of sorghum beers produced in northern Cameroon are close to those reported around 60% to 64% for the production of plantain sorghum-based beer and alcoholic beverage based pure plantain in Ivory Coast (Ourega et al., 2015). Physicochemical changes during fermentation of both sorghum turbid beers show that it depends on the raw material and production process used, but also on the use or not of a starter. Significant decrease of the pH as observed after a day of fermentation might be explained by the presence of basic amino acids content in the wort. The level of these nitrogen compounds and sugar favour yeast metabolism and has accounted on the type of fermentation occurring. According to Akin (2008), a large content of wort in basic amino acids such as arginine and lysine and their consumption by yeast during metabolism cause release a large number of protons equivalent to the positive charge of the basic amino acids. Furthermore, the production of organic acids and carbon dioxide by consumption of sugars during early lactic fermentation and late alcoholic fermentation respectively contribute in pH reduction from the first hours of fermentation (Valyasevi and Rolle, 2002). This is confirmed by a significant rise of acetic and malic acids content after the first day of fermentation. Nevertheless, the

non-significant increase of pH after the first day is due to some physicochemical modifications that occurred during fermentation. This result agrees with the report of Maoura et al (2005) who showed that pH does not increase significantly during fermentation of bili bili, a sorghum turbid beer home-brewed in Chad. Indeed, the production of alcohol from sugar during fermentation results in a decrease of organic acids dissociation which induces a lower proton release and therefore a slight increase of pH (Akin, 2008). The relatively high contents of residual sugars after fermentation are explained by a limited consumption of sugars by yeasts (Briggs et al, 2004) and the high initial total solids soluble content of the worts. It contributes to increase of osmotic pressure and reduce of the performance of yeast (Lodolo et al, 2008). Changes in alcohol, total solids and sugar contents clearly show and confirm the fact that production of alcohol during fermentation goes with consumption of the reducing sugars contained in the wort by bacteria and yeasts. Changes in temperature during fermentation is associated with the metabolism of the fermentative microorganisms which use sugars and grow up following three particular steps: acceleration, deceleration and stabilization (Sutra *et al.*, 1998). Higher apparent conductivity of the red beer samples compared to the white beer is justified by minerals contents of the raw material used for the production of each indigenous sorghum-based alcoholic beverage. Grains of the red variety used for the preparation of the red turbid beer are richer in calcium, iron, magnesium, and phosphorus than grains of the white variety used for the white home-brewed beer (Sekwati-Monang and Gänzle, 2011). This is due to the high content of the antinutritional factors such as phytate and tannins in the white sorghum grains which decrease the bioavailability of minerals (Aka et al, 2014).

The principal component analysis (PCA) performed on some physicochemical variables of sorghum turbid beers produced in the Northern regions of Cameroon clearly shows that there is a positive correlation between sugars, total solids soluble (TSS), density and pH on one hand and between organic acids and alcohol contents on the other hand, all these variables loaded on the principal component F1. Indeed, factor analysis indicates that consumption of sugars during fermentation is accompanied by a drop in density, total solids soluble (TSS) and especially pH, justifying the acidic character of the indigenous turbid beers at the end of fermentation. While the increase in malic and acetic acids contents during fermentation is accompanied by a rise of the alcohol production. These findings disagree with the report of Panda et al (2015) on home-brewed beers based barley and anthocyanin-rich sweet potato produced in India. They showed that pH and total solids soluble (TSS) loaded on the principal component PC1 were negatively correlated as well as ethanol and organic acids, both loaded on the principal component PC2. Density and sugar content were loaded on the third main component PC3. This difference could be explained by the nature of the raw material (sorghum or barley) and the quality of the starter (traditional or industrial) used for the production of alcoholic beverages. The difference in changes of the physicochemical parameters is confirmed by loading of measured variables on principal component PC1. For example, alcohol and organic acids contents are positively loaded on principal component PC1 for the white sorghum turbid beer samples whereas they contribute negatively to PC1 for the red sorghum turbid beer samples. The same observations are shown for the remaining physicochemical variables. Report of two main clusters shown by hierarchical analysis (Figure 10) confirm that indigenous turbid sorghum beer samples before and after the fermentation process are deeply different and belong to two distinct groups. However, the red and white sorghum beer samples are respectively grouped into two and three main sub-clusters. Indeed, red turbid beers fermented for a short time (1 to 2 days) are clearly distinguished from those fermented for a long time (3 to 10 days). Whereas, the white turbid beers fermented for 1 to 4 days; 5 to 6 days; and 7 to 10 days are separately grouped. These suggested that the samples belonging to each sub-cluster were closely related. This difference is explained by the nature and time of fermentation carried out in each of the sorghum turbid beer produced in the northern region of Cameroon. Alcoholic fermentation of the indigenous red sorghum beer occurs faster (addition of a starter) and lasts more than 24 hours before the beginning of consumption, while alcoholic fermentation of the white sorghum beer is usually preceded by a lactic fermentation which is slow (absence of a starter) because it lasts long for about 48 to 72 hours before consumption of the beverage.

## 5. Conclusions

The level of physicochemical changes during fermentation of the indigenous alcoholic sorghum-based beverages brewed in the northern regions of Cameroon and the correlation between some physicochemical parameters and various fermented alcoholic sorghum turbid beverages were highlighted in this work. It was shown that the physicochemical parameters significantly change just after the first or second day of fermentation after that, there had no significant physicochemical changes. The correlation between the physicochemical variables was demonstrated according to the factor analysis loading plots. Hierarchical clustering analysis grouped sorghum turbid beers based on their physicochemical profiles and time of fermentation in two principal clusters. The results reported could serve as a starting point to well understand the fermentation process during the production of the indigenous alcoholic sorghum-based beverages and to improve the efficiency of the process. However, further experiments based on hazard analysis are needed to study the microbial changes, to determine critical contamination steps during processing which could significantly affect the hygienic quality and physicochemical parameters of the indigenous sorghum turbid beers.

**Author Contributions:** Conceptualization, J.R.B. and F-X.E; Investigation, Methodology, Formal analysis, Writing-Original Draft, J.R.B.; Supervision and Writing-Review & Editing, F-X.E.

**Funding:** This research received no external funding

**Conflicts of Interest:** The authors declare no conflict of interest

## References

1. Baschali, A., Tsakalidou E., Kyriacou A., Karavasiloglou N. and Matalas A-L. (2017). Traditional low-alcoholic and non-alcoholic fermented beverages consumed in European countries: a neglected food group. *Nutrition Research Reviews*, pp 1-24, doi: 10.1017/S0954422416000202.
2. McGovern, P. E., Zhang, J., Tang, J., Zhang, Z., Hall, G. R., Moreau, R. A., ... Wang, C. (2004). Fermented beverages of pre- and proto-historic China. *Proceedings of the National Academy of Sciences*, 101(51), 17593–17598. doi:10.1073/pnas.0407921102
3. Campbell-Platt, G. (1994). Fermented foods — a world perspective. *Food Research International*, 27(3), 253–257. doi:10.1016/0963-9969(94)90093-0
4. Sanni, A. (1993). The need for process optimization of African fermented foods and beverages. *International Journal of Food Microbiology*, 18, 85-95. doi:10.1016/0168-1605(93)90213-z
5. Achi, O. K. and Asamudo, N. U. (2019). Cereal-Based Fermented Foods of Africa as Functional Foods. In J.-M. Mérillon, K. G. Ramawat (eds.). *Bioactive Molecules in Food*, Reference Series in Phytochemistry, Springer Nature Switzerland, pp. 1527-1558, doi.org/10.1007/978-3-319-78030-6\_31.
6. Correia, M. I. T. D., Liboredo, J. C., & Consoli, M. L. D. (2012). The role of probiotics in gastrointestinal surgery. *Nutrition*, 28(3), 230–234. doi:10.1016/j.nut.2011.10.013
7. Pale, S., Taonda, S. J-B., Bougouma, B. and Mason, S. C. (2015). Sorghum Malt and Traditional Beer (Dolo) Quality Assessment in Burkina Faso. *Ecology of Food and Nutrition*, 49(2), 129-141, DOI: 10.1080/03670241003697573.
8. FAO. (2010). *Production de sorgho au Cameroun (tonnes)* (FAO Statistics Division 2010 ed.). Available: <http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor> consulted 28/11/2010
9. Kubo, R., Funakawa, S., Araki S. and Kitabatake, N. (2014). Production of indigenous alcoholic beverages in a rural village of Cameroon. *Journal of The Institute of Brewing*, 120(2), 133- 141, DOI 10.1002/jib.116
10. Seignobos C. (2005). Trente ans de bière de mil à Maroua. In Raimond C. (ed.), Garine E. (ed.), Langlois O. (ed.). *Ressources vivrières et choix alimentaires dans le bassin du lac tchad*, Colloque International Méga-Tchad (FRA), Paris : Editions IRD, Prodig. (Colloques et Séminaires), pp. 527-561.
11. Analytica-EBC (2014).The European Brewery Convention (EBC Press) Release. *Journal of the Institute of Brewing*, 120(3), 284–284. doi:10.1002/jib.146
12. DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry*, 28(3), 350–356. doi:10.1021/ac60111a017

13. Pauline, M., Alexandre, O., Andoseh, B. K., Abeline, M. T. S., & Agatha, T. (2017). Production technique and sensory evaluation of traditional alcoholic beverage based maize and banana. *International Journal of Gastronomy and Food Science*, 10, 11–15. doi:10.1016/j.ijgfs.2017.09.003
14. Desobgo, Z. S.C., Naponni, F.Y. and Nso, E. J. (2013). Characterization of Safrari sorghum worts and beers hopped with *Vernonia amygdalina* and *Nauclea diderrichii*. *International Journal of Innovation and Applied Studies*, 2(1): 83–91.
15. Tukey, J. W. (1953). Section of mathematics and engineering: some selected quick and easy methods of statistical analysis. *Transactions of the New York Academy of Sciences*, 16(2 Series II), 88–97. doi:10.1111/j.2164-0947.1953.tb01326.x
16. Huang, M., Li, Y., Zhan, P., Liu, P., Tian, H., & Fan, J. (2017). Correlation of Volatile Compounds and Sensory Attributes of Chinese Traditional Sweet Fermented Flour Pastes Using Hierarchical Cluster Analysis and Partial Least Squares-Discriminant Analysis. *Journal of Chemistry*, 2017, 1–8. doi:10.1155/2017/3213492
17. Anonyme (2015). Bière de mil, maïs, sorgho. Dossier d'information pour les PME d'Afrique sur la transformation agroalimentaire, CTA – Gret -TPA, 13 pages,
18. FAO (1995). Le sorgho et les mils dans la nutrition humaine, [http://www.fao.org/documents/show\\_cdr.asp?url\\_file=/docrep/T0818F/T0818F00.HTM](http://www.fao.org/documents/show_cdr.asp?url_file=/docrep/T0818F/T0818F00.HTM) Rome,
19. Nso, E., Ajebesome, P. E., Mbofung, C. M., & Palmer, G. H. (2003). Properties of Three Sorghum Cultivars Used for the Production of Bili-Bili Beverage in Northern Cameroon. *Journal of the Institute of Brewing*, 109(3), 245–250. doi:10.1002/j.2050-0416.2003.tb00165.x
20. Ourega, D. A., Ban, L., Kouadio, K., Guessan, F. N., Nemlin, G. J. and Tano, K. (2015). Physicochemical characteristics of plantain beers produced from waste plantain varieties of Côte d'Ivoire (Orishele, French Horn 2 and 1). *International Journal of Innovation and Applied Studies*, 10(2), 666–677.
21. Dahouenon-ahoussi, E., Degnon, R. G., Adjou, E. S. et Sohounhloue, D. C. K. (2012). Stabilisation de la bière produite à partir de matières amylacées locales (*Sorghum bicolor* et *Musa acuminata*) par adjonction de l'huile essentielle de *Cymbopogon citratus*. *Journal of Applied Biosciences*, 51, 3596 – 3607.
22. Akin, H. (2008). *Evolution du pH pendant la fermentation alcoolique de moûts de raisins : modélisation et interprétation métabolique*, Thèse de Doctorat, Institut National Polytechnique, Toulouse, France, 125 pages.
23. Valyasevi, R., & Rolle, R. S. (2002). An overview of small-scale food fermentation technologies in developing countries with special reference to Thailand: scope for their improvement. *International Journal of Food Microbiology*, 75(3), 231–239. doi:10.1016/s0168-1605(01)00711-5
24. D. E. Briggs, C. A. Boulton, P. A. Brookes, and R. Stevens, *Brewing science and practice*. Cambridge: Woodhead Publishing Limited and CRC Press LLC, 2004
25. Lodolo, E. J., Kock, J. L. F., Axcell, B. C., & Brooks, M. (2008). The yeast *Saccharomyces cerevisiae* the main character in beer brewing. *FEMS Yeast Research*, 8(7), 1018–1036. doi:10.1111/j.1567-1364.2008.00433.x
26. Sutra, L., Federighi, M. and Jouve, J-L., (1998). *Manuel de Bactériologie Alimentaire*, 4th Edition, Polytechnica, Paris, France.
27. Sekwati-Monang, B., & Gänzle, M. G. (2011). Microbiological and chemical characterization of ting, a sorghum-based sourdough product from Botswana. *International Journal of Food Microbiology*, 150(2-3), 115–121. doi:10.1016/j.ijfoodmicro.2011.07.021
28. Aka S., Konan G., Fokou G., Dje K. M. and Bonfoh B. (2014). Review on African traditional cereal beverages. *American Journal of Research Communication*, 2(5), 103–153,
29. Panda S.K., Panda S.H., Swain M.R., Ray R.C. and Kayitesi E. (2015). Anthocyanin-rich sweet potato (*Ipomoea batatas* L.) beer: technology, biochemical and sensory evaluation. *Journal of Food Processing and Preservation*, Wiley Periodicals, 10 pages, doi:10.1111/jfpp.12569
30. Maoura, N., Mbaiguinam, M. et Gaillardin, C. (2006). Suivi technique, analytique et microbiologique de la « bili bili », bière traditionnelle tchadienne. *Afrique Science*, 2(1), 69–82.