Original Research Article

Processing and quality of the «red kapsiki», an opaque beer from “Mandara” Mountain in Cameroun

ABSTRACT

Aims: Mandara is a chain of Mountains located in northern Cameroun at the border of the republic of Nigeria. This area is populated by “kapsiki” an indigenous population living. This non-Muslim population is brewing an opaque beer, which has both a symbolic and nutritional. This paper aims at investigating the physicochemical and microbial quality of this beer and highlights its processing.

Study design: The study design used for processing method development is cluster sampling with two degrees of units with at the primary level, the cities surveyed, production sites and markets, and at the secondary level, individuals and groups of individual respondents. The primary units were chosen stratified and reasoned manner.

Place and Duration of Study: In order to describe and follow the process production, a survey was conducted in three kapsiki rural villages of Cameroun, namely Rhumsiki, Rhumzu and Mogodé. Later on, some samples from two urban town close to the area Mokolo.

Methodology: To describe and follow the process production, a survey was on the basis of a questionnaire were conducted. The sample pH, conductivity, density and brix, were recorded onsite using portables devices (conductimeter, densitometer and brix meter).

Laboratory experiment: Titatable acidity, polyphenols, ethanol, specific gravity, viscosity was determined according to accredited methods. Microbial analysis focus on total aerobic mesophilic bacteria, coliforms, Escherichia coli, Staphylococcus aureus, Salmonella and Shigella, Yeasts and molds and sulphite-reducing clostridia were evaluated.

Results: The "red kapsiki" requires for its preparation following steps: malting with quenching, germination and "kiling", decoction, filtration, boiling and sterilizing, cooling, souring and fermentation. The final beverage is opaque, soft and sparkling. The "red kapsiki" present an alcohol content of 3.85 to 4.28 (g/l), a pH between close to 2.40, soluble extract from 6.30 to 7.29 °P, Brix from 7.0 to 7.46 °B, total sugar from 41.8 to 72.9 (g/l), conductivity from 1919 to 1990 (μS/cm), and Specific density (g/cm) a 15°C of 1.25. The color of the "red kapsiki" varies from a pinky brown to reddish according the variety of sorghum used. The microbial analyses indicate the presence of pathogens as Coliforms, Salmonella and Shigella and Yeasts and molds in the beverages which indicate the bad hygienic quality of "the red kapsiki".

Conclusion: Despite its poor hygienic quality, the "red kapsiki", present great potential for local beer producers.

Keywords: Beer, Cameroun, Kapsiki, Microbial, Quality.

1. INTRODUCTION

The artisanal fermentation of cereals into beers and wine-like alcoholic drink is not recent in Africa. The traces of the first artisanal fermentation were found by archaeologists in the Blue Nile region of Sudan [1, 2]. In mountainous area of central African savannah, cereals, mainly millet and sorghum are the most important crops use for fermented beverage [3]. One of this
drink is an opaque alcoholic beer-like beverage made from fermented sorghum grits and
malted maize, mainly used for rituals and for festivities [4]. Two types of this beverage are
produce in "Kapsiki land". They are: "te and mpedii". The first, "te", is the ritual beer.
"Mpedii", is the 'white' beer, which is mainly brewed by women for commercial purpose and has
no ritual significance. "Mpedii" is made by a quick process for immediate consumption [5,
6]. The red "te" beer in which we focus our study is traditionally a man's brew. Its processing
follow a strict procedure, with numerous prohibitions, and red "te" was for long time mainly
used for ritual purposes than festivities. Symbolism was more focused on this beer rather
than commercial and technical [6]. However "red te" has increasingly become a sales
commodity for women both at the village markets and in the cities as it's generally preferred
by the men over "mpedii" [7, 8]. The "red kapsiki" beer locally called "te" often has a high
symbolic content "the message of beer" is by no means uniform. Though most of the
symbolism around beer is a male dominated discourse which concentrates on bonding and
d power, the symbolism is less straight forward and more hidden [6]. Despite of the importance
of the "red kapsiki" amongst this tribe, the beer itself remain unknown in scientific community
and little is known on it processing and quality. This paper aims at valorizing this
opaque beer by describing its processing technique and provides some data in regard with
its quality.

2. MATERIAL AND METHODS

2.1 Field work and sampling

In order to describe and follow the process production, a survey was conducted in three
"kapsiki" rural villages of Cameroon, namely Rhumsiki, Rhumazu and Mogodé. Later on,
some samples from two urban towns close to the area Mokolo, were also collected for
comparison purpose. The choice of this urban area is justified by the fact that Mokolo is the
immediate administrative area populated in majority by "kapsiki" populations. The sampling
method used for processing method study was cluster sampling [9] with two degrees of
units, with at the primary level, the cities surveyed, production sites and markets, and at the
secondary level individual and groups of individual respondents. Two layers were formed:
rural area and urban area. As urban area, Mokolo were chosen because of the possibility of
finding markets as well as production sites. In mountainous Kapsiki land, three cities were
selected: Rhumsiki, Rhumazu and Mogodé. As for the surveyed markets, we conducted a
random choice in Mokolo; in Rhumsiki, Rhumazu and Mogodé, all markets were taken into
account because of the very limited number of markets and their periodical characters.
Producers and women retailers were also randomly chosen in the areas and markets
selected for the survey. A total of 15 production sites and 7 markets were visited, 50
producers and 23 women retailers were interviewed. The interviews were conducted on the
basis of a questionnaire and collected data were processed using the software Winstat
through a counting sheet constructed from the questionnaire.

2.2 On site experiment

For characterization, 40 samples of the "red kapsiki" were collected from 10 sites in
production sites and sales. The sample pH was measured directly onsite using a portable pH
meter. The conductivity, density and brix were also recorded on-site using portables devices
(conductimètre, densitomètre and brix meter). Around 10ml of each sample were introduced
into test tubes and gently shaken. The probe of designated apparatus (pH meter,
conductimètre and densitomètre) was then inserted into the test tube and the values read
directly in the screen of the device. The experiment was repeated four times for each
sample. The mean of each read result were considered. For Total Soluble Solids (% Brix),
the Refractometric method was used to determine the soluble solids in bears samples [10].
The portable refractometer were first thermostated at 20°C using boil water, and regularly
calibrated with cooled distilled water until the screen of the device show 20°C. Soluble solids 
were then obtained from read refractive index on device screen, by reference to a standard 
table.

2.3 Laboratory experiment

2.3.1 Physicochemical analysis

2.3.1.1 Titrable acidity
Titrable acidity (as percentage (w/w) tartaric acid) was determined according to the 
Association of Analytical Chemists [11] method. Acidity was determined by titration with 0.1 
N NaOH, solution and expressed as percentage tartaric acid; bromothymol blue was used as 
an indicator.

2.3.1.2 Total polyphenols
Total polyphenols were assayed by colorimeter using the Folin-Dennis Ciocalteau reagent 
as described by Mangas et al., [12] and the results were expressed as mg/l of gallic acid.

2.3.1.3 Total ethanol
Total ethanol content was preceded by a spectrophotometric micro-method for the 
determination of ethanol after distillation of wine that was made alkaline by a suspension of 
calcium hydroxide [13].

2.3.1.4 Specific Gravity at 20 °C
Specific Gravity at 20°C and Viscosity (poise) at 25°C: These two parameters were 
evaluated as described by Nanda, et al., [14]. The specific gravity determination were done as 
follows: 20 ml of sample was poured into the specific gravity test tube to overflow, then the 
stopper was inserted, then incubated in water-bath at 200°C for 30 minutes. The test tube 
was removed from the water-bath wiped and weighed. Thereafter the sample was boiled. 
Cool water was similarly treated the same way as that of sample, then the specific gravity 
was calculated as the ratio of weight of ash over the weight of fresh sample time 100.

2.3.1.5 Volatile acidity
The volatile acidity (g/l): This was determined using Mathieu method by titration of the 
volatile acids separated from wine by steam distillation and titration of the distillate [15].

2.3.2 Microbial analysis

Assayed 10 milliliters of "4" samples from each location was mixed with 90 ml sterile peptone 
physiological saline solution (1 g Peptone, 8.5 g NaCl and 1000 ml distilled water). Decimal 
dilutions were prepared up to 106 from initial sample as describe by Loyer, & Hamilton [16].

2.1.2.1 Total aerobic mesophilic bacteria
Total aerobic mesophilic bacteria was enumerated on Plate Count Agar (PCA-OXOID) 
supplemented with cycloheximide 0.5% [17]. The plates were incubated at 28°C for 48 to 72 
h

2.1.2.2 Total coliforms and Escherichia coli
Total coliforms and Escherichia coli were accessed on Bubble Lactose Bile with 
Brilliant green (BLBVB - DIFCO). The tubes containing the Durham tubes were incubated at 
30°C during 24 to 48 h. The positive tubes were used to inoculate another test tube 
containing water peptone without indol and were incubated at 44°C for 24 h for E. coli 
determination. E.coli was revealed using Kovac's reagent [18]. Total coliforms and E. coli 
were evaluated by the method of the most probable number.
2.1.2.3 Staphylococcus aureus

*Staphylococcus aureus* was enumerated on Manitol Salt Agar (MSA - Sigma) and revealed by the coagulase test with rabbit's plasma. The plates were incubated at 37°C for 48 h. Faecal *Streptococcus* was enumerated on Blantyre Agar (SL-Merck) supplemented with Cycloheximide at 0.5% after 48 h of incubation at 37°C [19].

2.1.2.4 Salmonella and Shigella; Yeasts and moulds, sulphite-reducing

Salmonella and Shigella were analysed as described by Ribot, et al., [20]. Yeasts and moulds were enumerated on YPD-Chloramphenicol (200 g yeast extract, 10 g peptone, 20 g glucose, 20 g agar, 0.5 g chloramphenicol and 1000 ml distilled water) after 48 to 72 h of incubation at 30°C [18]. Enumeration of sulphite-reducing clostridia were done according to Mossel, [21] method in anaerobic jar. All enumeration in solid media was carried out in triplicate and the plates containing between 33 and 333 colonies were considered. The enumeration in liquid media was evaluated according to De Man most probable number.

3. RESULTS AND DISCUSSION

3.1 Production method of "the red kapsiki"

Sorghum beer is generally made from grain and water sometimes a gelatinous or mucilaginous agent [22]. In Cameroon, most of non-Islamized ethnic groups process it in different forms. The obtained beer is named according to ethnic groups and countries. We can highlight Tchounkoutou in Togo and Benin, Pilô in Ghana and Nigeria, Tchpalo in Ivory Coast, Billi-Billi in Cameroon. The artisanal techniques commonly used include:

3.1.1 Malting

In the case of "red kapsiki" also locally named "tê", as most of beers, the process production starts by the selection of grains. The "red kapsiki" being a noble beer, only good quality grains are considered. Mostly the sorghum variety "Mouskwati" is selected for the "red kapsiki" production. However in raining season, "Dijigari" variety can also be chose.

3.1.1.2 Quenching

After being washed, the grains are immersed in water for 12 hours to 24 hours so as to obtain a moisture content of 35% to 40% for germination. The temperature of water is very important: At high temperature the quenching is rapid. The immersion temperature is close to that of room temperature (around 40°C to 45°C in the region). The grains are then drained on tissue, and then mixed in double layer on cotton cloth bags or on woven mats.

3.1.1.3 Germination

The soft grains are covered and placed in dark area for two to three days for germination. Water is sprayed sometime, when the ambient air is dry or when the temperature is hot. Alternatively the grains are left on the ground and sprayed until the germination process starts and rootlets appear. The high temperature facilitates the beginning of germination. In this case the germination time can lasts four days. It should be noted that the same technique is used at the household level to improve the energy density of slurry [23]. During germination, amylolytic enzymes are produced and protein digestibility of sorghum which is generally low is improved [24]. It was also demonstrated that after three days of malting, there were production of amylolytic enzymes including α-amylase, β-amylase and dextrinase which are all essential for good quality of malt [25].
3.1.4 Drying

This corresponds to the "Klining" and brings moisture of malt to 15-20% without moisture. The malt is dried in sun for one or more days, sometimes less if the processor goes straight to brewing stage. In case of the production of special "red kapsiki" it was noticed that after malting, grains are roasted in firewood and ground coarsely. The obtained powder is kept in dark for two days before brewing.

3.1.2 Brewing

3.1.2.1 Milling

The previously obtained malt is crushed in a mortar or win in urban area, the malt is brought to a motorized milling machine set to crushing mode, to obtain a coarse flour.

3.1.2.2 Pasting

The grind is mixed with water with a gelatinous or mucilaginous agent (okra or sap of various trees that improve flocculation and filtration of insoluble in suspension). After an hour of storage at temperature of 25 to 35 °C, the mixture separate into two phases: the upper liquid phase which is collect and the bottom pasty part which is use as animal feeds. The upper liquid phase already contains a soluble portion of malt sugar.

3.1.2.3 The decoction

The lower phase containing malt flour is cooked slowly to boiling so as to obtain a starch paste (slurry consistency). The upper liquid base is then mixed with 4× water to be more easily saccharified than if it was not being cooked, the diastatic actions being more effective on cooked starch than on raw starch. Alternatively, like for other sorghum beer processing, where the raw grain is added [26, 27, 28] in the case of the "red kapsiki" production may continue with malted sorghum powder. In fact, ground malted sorghum is dissolved in water at the ratio of 1/8 (w/v). After one to three hours of soaking, supernatant is removed and kept for a later use. The remaining mealy material at the bottom of the soaking container is then removed and cooked for three to five hours. It must be notice here that some enzymes produced during the malting stage seem not active. This may be due to the soaking temperature, which is not optimal for enzymes. The mealy deposit is constituted by 60% raw starch. This starch is cooked and lightly cooled before previously removed soaking water probably containing starch digesting enzymes is added. The mixture is then kept warm for one to five hours or let stand overnight at room temperature.

3.1.2.4 Filtration

After the decoction phase, the paste like mixture previously removed become liquid and is filtrate. The dry matter is discarded while the sour mash obtained is kept for the next step of the process. The filtration is mostly done through polypropylene bags, the slurry obtained after decoction is passed through this polypropylene bag. The filtrate now called "liquid sour must" is kept for further processing where the drench is use as animal feed.

3.1.3. Cooking:

The liquid must is concentrated and clarified by skimming. This operation is stopped by several criteria: clarity, color of the must, cold consistency ( syrupy appearance) and also the
flavour of the must. In fact this operation consists of producing a sweetish liquid must which is called "le kwahèni" [4] in Kapsiki dialect.

3.1.4. Fermentation

The sweet must is cooled either spontaneously or by successive decanting and then starter culture is added. Fermentation lasts 12 to 24 hours at room temperature.

General Comments

What is in the Starter culture?

Sorghum based beers are marketed as "gluten free" in the US.
Figure 1: Technological diagram of homemade red Kapsiki beer

Sorghum and Maize grain

Water

Quenching 24h/ home T°C + Decantation + Filtration

Water

Malt flour

Water 1: 9 (w/v)

Quenching at room Temperature then Decantation for 1-3 h

Supernatant

Mealy deposit

Precooking for 3-5 h

Cooling

Second decantation and filtration

Sour wort

Cooking for 5 to 10 hours

Sweet wort

Transfer in new containers

Artisanal starter culture

Red Kapsiki beer

Fermentation for at least 12 hours / at room temperature until supernatant forms.
3.2 Some physico-chemical profile of the "red kapsiki"
Comparing to other African beer as describe by Lyumugabe et al., [18], the "red kapsiki" presented a greater alcohol content (3.65-4.28% v/v), a pH between 2.40±0.19 and 3.26±0.03. Soluble extract varies from 6.30 to 7.29 °P, Brix from 7.0 to 7.46 °B, total sugar from 41.8 to 72.9 (g/l) a conductivity from 1919 to 1990 (µS/cm), and Specific density (g/cm) at 15°C of about 1.25 (Table 1). The color of the "red kapsiki" varies from a pinky brown to reddish according the variety of sorghum used. As most of African sorghum beers, the "red kapsiki" present a touch of fruitiness added to their fermentation odor. This beer is mainly consumed in an actively fermenting state leading to a short shelf life as mentioned for other African beer in literature [28, 29, 30, 31].

Table 1. Physico-chemical profile of the "red kapsiki"

<table>
<thead>
<tr>
<th></th>
<th>Mogodé</th>
<th>Mokolo</th>
<th>Rhumski</th>
<th>Rhumzu</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>2.46±0.08</td>
<td>2.42±0.12</td>
<td>2.40±0.19</td>
<td>3.26±0.03</td>
</tr>
<tr>
<td>Soluble extract (°P)</td>
<td>7.28±1.29</td>
<td>7.29±0.26</td>
<td>7.29±0.26</td>
<td>6.30±1.09</td>
</tr>
<tr>
<td>Brix (°B)</td>
<td>7.0±1.08</td>
<td>7.46±0.83</td>
<td>7.42±0.46</td>
<td>7.0±0.16</td>
</tr>
<tr>
<td>Alcohol (% vol)</td>
<td>3.85±0.58</td>
<td>4.10±0.49</td>
<td>4.06±0.46</td>
<td>4.28±0.78</td>
</tr>
<tr>
<td>Total sugars (g/l)</td>
<td>72.8±1.29</td>
<td>72.9±0.30</td>
<td>72.9±0.40</td>
<td>41.8±0.35</td>
</tr>
<tr>
<td>Conductivity</td>
<td>1919.23±8.12</td>
<td>1990.0±4.08</td>
<td>1990.0±3.53</td>
<td>1929.0±4.02</td>
</tr>
<tr>
<td>Specific density (g/cm)</td>
<td>1.03±0.00</td>
<td>1.33±0.00</td>
<td>1.00±0.00</td>
<td>1.62±0.00</td>
</tr>
</tbody>
</table>

*p<0.05* according to the ANOVA and DUNCAN comparison test.

As present in Table 2, the "red kapsiki" contains a quite good amount of polyphenols. The recorded amount varies from 843±27 mg/l in Mogode samples to 1150±27 in Rhumzu samples. It must be noticed that some of these polyphenols are rare or absent in other "industrial" beer. As indicated by Bröhan et al., [32] when barley malt is used for mashing, around 30% of total beer polyphenols are issued from hops, although added in 100 times lesser quantity than malt. In the case of the "red kapsiki", the sorghum contribution to beer polyphenols could be much higher. In fact sorghum phenolic acids include hydroxybenzoic (mainly protocatechuic and p-hydroxybenzoic acid) and hydroxycinnamic acids (mainly ferulic and p-coumaric acid), [33, 34] both free and bound as esters. Most of them are found in usual lager beers brew (either from barley malt or from hops). Sorghum anthocyanins are unique, as they lack the hydroxyl group at the 3-position of the C ring. Those 3-deoxyanthocyanins such as luteolins and apigenins are used as natural food colorings, because they are more stable than anthocyanins in both organic solvents and acidic solutions. Amount of 1500 mg/l of flavonol were recorded in "red kapsiki". Bröhan, et al., [22] indicate that Flavonols such as apiflorin (leucopagelin) and luteolin (leucoluteolin) are sorghum polyphenols, as precursors of sorghum 3-deoxyanthocyanins. Never reported in beer, they have been found at concentrations up to 4200 mg/kg in sorghum [38]. Other sorghum flavonoids include the flavones apigenin and luteolin [37] the flavones naringenin and eriodictyol [38], the flavonol kaempferol, the dihydroflavonol taxifolin, and the flavan-3-ols (p)-catechin and epicatechin. Hop brings similar flavonoids and flavan-3-ols to wort, in industrial brewing.
Table 3. Microbial profile of the “red kapseki” Sample

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total count (col/ml)</th>
<th>Total coliform (col/ml)</th>
<th>Total coliform (cfu/ml)</th>
<th>Total coliform (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moopol</td>
<td>8.6 ± 0.3</td>
<td>10^{9}</td>
<td>(6.2 ± 0)</td>
<td>10^{9}</td>
</tr>
<tr>
<td>Mopele</td>
<td>12.2 ± 0.2</td>
<td>10^{9}</td>
<td>(7.2 ± 0)</td>
<td>10^{9}</td>
</tr>
<tr>
<td>Rhumulu</td>
<td>10.5 ± 0.4</td>
<td>10^{9}</td>
<td>(6.5 ± 0)</td>
<td>10^{9}</td>
</tr>
<tr>
<td>Mopele</td>
<td>12.2 ± 0.2</td>
<td>10^{9}</td>
<td>(7.2 ± 0)</td>
<td>10^{9}</td>
</tr>
<tr>
<td>Rhumulu</td>
<td>10.5 ± 0.4</td>
<td>10^{9}</td>
<td>(6.5 ± 0)</td>
<td>10^{9}</td>
</tr>
</tbody>
</table>

In conclusion, the results show that the microbial presence found in the produced beers is not significantly different (p > 0.05). The presence of microorganisms is important for the beer production, and a combination of reducing and hydrogen sulfide-producing bacteria was expected to have a synergistic effect in the fermentation process. The results also indicate that the “red kapseki” beer is similar to other traditional beers studied. Further studies are needed to understand the specific bacteria involved in this fermentation process and their role in the beer's characteristics.
been observed that during this time, very little or no increase in the number of contaminating organisms seems to occur [18]. The isolated pathogenic bacteria can originate from the environment including humans, the equipment used and raw material. Among the pathogenic microorganisms could be isolated in craft beers, we can mention *Escherichia coli* and *Bacillus* species. Their presence and persistence in these beverages would not only be linked to a simple fermentation but also to their adaptation ability. Indeed, several studies have shown that some environmental parameters such as low temperatures and pH had the capacity to induce the resistance of these microorganisms to high temperatures [44, 45] and strongly acidic pH [46, 47, 48]. Leyer *et al.* [47] showed that the story of *E. coli* O157:H7 cells to acidic conditions (pH 5) promoted their survival in highly acidic foods such as apple juice. In addition, the surveys of Cheng *et al.* [49] have shown that this adaptation also involved the induction of highly heat resistance. These observations could probably explain partially the persistence of this pathogen and other similar in our beer samples. As for *Bacillus*, the persistence can be explained by their ability to spore-forming but also the capacity of their endospores to induce some of their extraordinary resistances such as heat and acid resistance [44, 46].

The hygienic quality of beer produced depends closely on the conditions of fermentation of the must. Indeed, the levels of total sugar and vitamin C are relatively high in beers obtained from the fermented mash. The recorded values for the red *Kapsiki* are higher than those in the “Tchapaol” [27, 43] where the fermentation is carried out at room temperature with a starter culture based on previous productions. It must also be noted to explain the prevalence of pathogens, that after few days of fermentation, the amount of yeasts decreases because of autolysis. With little or no competition from yeasts for the readily available nutrients, contaminating microorganisms increase rapidly in number and their metabolites may change and spoil the beer. Because of the relatively high temperature of the "red kapsiki" fermentation, these sequential events occur within a short time period. This period does not usually exceed more than 3 days in summer or 5 days in winter before this spoilage occurs. The metabolic activities of mesophilic bacteria are primarily responsible for the spoilage. These bacteria, along with other undesirable bacteria may produce acetic acid, volatile off-flavors, fruity odors, and pellicles which render the taste, odor and texture of the beer unacceptable to consumers.

4. CONCLUSION

The "red kapsiki" as discussed above present a symbolic value for local mandara population. The "red Kapsiki" requires for its preparation following steps: malting with quenching, germination and "killing", decoction, filtration, boiling and sterilizing, cooling, sauvage and fermentation. The "red Kapsiki" present an alcohol content of 3.85 to 4.28 (% v/v), a pH between close to 2.40, soluble extract from 6.30 to 7.29 °P, Brix from 7.0 to 7.46 °B, total sugar from 41.8 to 72.9 (g/l), conductivity from 1919 to 1960 (µS/cm), and Specific density (g/cm³) à 15°C of a 1.33. The color of the "red kapsiki" varies from a pinky brown to reddish according the variety of sorghum used. The microbial analyses indicate the presence of pathogenic *Clostridium*, *Salmonella* and *Shigella* and Yeasts and molds in the beverages which indicate the bad hygienic quality of "the red Kapsiki". Despite its importance and interesting physicochemical profile, this alcoholic beverage is of bad hygienic quality. This late potential of the beverage may plead for the improvement of its processing and hygienic quality.