Original Research Article

Environmental microorganisms involved in fermentation of common natural substrates: screening from “cha’a” and “arky”, two artisanal drinks consumedin Yaoundé-Cameroon

ABSTRACT

The present work aimed at contributing to the mastering of fermentation processes in agricultural products. It targeted identification and enumeration of micro-organisms that are present in three corn-based substrates namely sweet and fermented ‘‘cha'a’’, and the fermented original juice for ‘‘arky’’. Related investigations also focused on microbial population dynamics, the alcoholic strength, reducing sugar concentrations and pH values during the process of fermentation. To achieve this goals, 10 specimens of fermented ‘‘arky’’ juice, 10 of sweetened ‘‘cha'a’’ and 10 of fermented ‘‘cha’a’’ were collected in some districts of the Yaoundé neighborhood and conveyed in refrigerated containers (≈2°C) to the Université des Montagnes teaching hospital Laboratories of Microbiology where analyses were conducted. Culture, isolation, identification and enumeration were done according to the standard protocols. Data recorded were presented as tables and graphs. At 1/10-dilution, they revealed the presence of *Lactobacillus* spp., *Corynebacteriumxerosis*, *Candidazeylonoides*, *Enterococcus feacalis* and *Bacillus* spp. They also indicated that the populations of fermenting organisms were optimal between the $4^{th}$and the 5^{th}days of fermentation, withan alcohol degree. Meanwhile, the contents in reducing sugar decreased in the three resources, like the pH values. Microbial optimal growth was observed at 30°C. All microbial populations persisted although the experiment. Identification of bacteria from the *Enterococcus* genus appeared as evidence of contamination of the substrates subjected, implying adulteration. Combined, these findings indicate that with minimal financial resources *Zea mays* may serve for the production of alcohol; but ingesting the substrates studied in the present works represent a health risk for two reasons: the high alcohol contents and potentially infectious disease etiologies.

Keywords: Fermentation, *Zea mays*micro-organisms, ‘‘cha'a’’, ‘‘arky’’

1. INTRODUCTION

At specific stages along the production chain of large numbers of food items, microorganisms contribute significantly. They are involved in several biological processes which regulate and control the life and activity of living systems [1]. In particular, microbes are known for their role in fermentation processes which ultimately leads to the production and preservation of foodstuffs like milk, cheeses, sauerkraut and alcoholic drinks.

Fermentation for instance, are redox reactions which occurs in poorly oxygenated environments and causes partial degradation of substrates, generating reduced amounts of energy (compared to those which take place in properly oxygenated environments) [2]. Humans have built on these potentials of microorganisms to improve the nutritional and organoleptic qualities of their foods [1]. Throughout the world, many companies use microorganisms to produce drinks [2]. These microorganisms partially metabolize certain substrates to produce by-products such as alcohols and organic acids which contribute to improving the quality of the final products. The types, volumes and amounts derive vary from one community to the other in connection with the needs, the living standard and raw material availability. Namely, banana, sorghum, millet, cassava, corn for instance can be used. These determinants can vary over time, and reflect the inherent wealth of endogenous knowledge which is passed throughout generations.

During fermentation, the carbohydrates in the substrates are rapidly metabolized into ethanol and organic acids, which in turn affects the original flavor [3-5]. Microorganisms that are responsible for fermentation actually multiply and bring about changing in the physical and chemical characteristics of the drink through series of metabolic processes. Related environmental changes cause competition amongst living microbial populations

which further affect its chemical composition [3,5,6]. Despite this change, it is noteworthy that anabolisms of ethanol and organic acids have paramount importance in the overall environmental equilibrium that otherwise should adjust with microbial populations. Nowadays, race for energy is capital as tool for economic growth, social development and overall welfare in large numbers of human communities. Accordingly, ethyl biofuels or bioethanol (ethanol obtained from plant's carbohydrates) [7] represent an alternative foundation that future project for fuel production should build on.

Alongside drinks manufactured by large industrial companies in Africa, the artisanal drinks sector also develops [2]. Unlike the drinks in the first group, artisanal drinks are more affordable thanks to their availability and their cost. Their use has been known for centuries in certain countries and communities where they are firmly linked to beliefs and represents a fundamental part of local traditions.

In different communities throughout Cameroon, these drinks are obtained from ingredients like millet, cassava and corn, to name a few. They are also named differently depending on communities and vernacular languages. In the Northern region it is called "bile-bile" when produced from millet. In the Center, it is called "odontol" (produced from cassava). In the West of Cameroon more precisely in Bangangté, it is called "cha'a" and produced from corn. The manufacturing process of cha'a essentially involves malting, drying, milling, boiling, mashing and alcoholic fermentation, but variations may occur depending on the method used and cultural determinants [8]. Artisanal drinks produced in Cameroon are commonly used during traditional ceremonies such as inductions and weddings; it is noteworthy that cha'a is much consumed both during moments of joy like festivals, traditional weddings, success in examinations, football matches but also during moments of sadness like funerals [9].

Despite the relatively high inclination to consumption of "cha'a" and "arky"(made with *Zea mays*), and their abundance in the rural areas of Cameroon, their use as a substrate for the production of ethanol or other derivatives is not yet developed. The hygienic quality of these drinks is not met either. In order to promote both the human and economic health in Africa and in Cameroon in particular, it would be suitable to master the fermentation mechanisms of these agricultural products so that they can scientifically be implemented at artisanal and industrial scale with core focus to consumers' welfare. The present piece of research focused on the different metabolic processes which occur during the fermentation of "cha'a", the fermented "arky" juice and on the microorganisms involved in fermentation. The main focus was on investigating the microbial flora in "cha'a", "arky" and the microbial population dynamics that develop throughout fermentation processes in connection with the hygienic quality on one hand, variation in reducing sugars and alcohol on the other.

2. MATERIAL AND METHODS

2.1 Study design

For this descriptive cross-sectional survey, the sample collection was conducted in Yaoundé, the political Headquarter of Cameroon. The total of 10 fermented "arky" juice specimens was purchased from "arky" manufacturers in the ''Briqueterie'', ''Melen'' and ''Nkolbisson'' districts. Alongside, 10 samples of fresh/sweet "cha'a" and 10 samples of fermented "cha'a" were collected in the "Obili" and "Melen" neighborhoods. All the specimens were made from *Zea mays*. Once collected, they were kept in refrigerated contains (≈ 2°C) and conveyed without delay to the Chemistry Laboratory at the Université des Montagnes and the Laboratory of Microbiology at the Université des Montagnes Teaching Hospital. Specimen analysis was launched within 24h after collection.

2.2Investigating through chemical characteristics of collected specimens

For all specimens, tests caried out consisted in detecting starch and glucose. They were followed by quantification of reducing carbohydrate, pH, and ethanol.

2.2.1Screening of starch and glucose

The screening was performed before and after acid hydrolysis. This hydrolysis was carried out by reflux heating (30°C) for 4 hours of a mixture of 50mL of the subjected sample and 50mL 6N HCl. During these 4 hours, 5 mL of the mixture was collected every 30 min. To this mixture, 5mL of 6N NaOH was added to stop the acid hydrolysis. This neutralized mixture was the one used for the tests "after hydrolysis".

2.2.1.1Test for starch with Lugol

In a test tube containing 2 mL of each specimen, 4 drops of a 2% Lugol solution were added. A blueish color that turned into blackish (depending on the starch's concentration) indicated that starch was present in the specimen subjected.

2.2.1.2Test for glucose with glucose oxidase

To 1mL of glucose oxidase, 10µL of specimen was added and mixed thoroughly. After 5 min incubation at room temperature, the color intensity of the substrate-glucose oxidase mixture was measured at 505nm.

Acknowledging that the recorded color intensity is proportional to the quantity of glucose in the specimen subjected, a calibration range was designed with a standard of glucose made from distilled water and glucose.

2.2.2pH screening

On arrival at the laboratory, the pH of each drink was measured using a pH meter. This measurement was also conducted every day for the 9 days that followed the first essay.

2.2.3Screening of reducing carbohydrate

It was conducted according to Bertrand's [10]. The quantification was performed with a calibration curve developed from the glucose calibration range with decreasing concentrations. The measure of reducing sugars was performed immediately upon arrival at laboratory, then 3, 5 and 7 days after the first test.

2.2.4Screening of ethanol

Ethanol was quantified according to Gadsen *et al*. (1986) [11]. This test was performed immediately upon arrival at laboratory, then on day 3, day 5 and day 7. For this screening, the sample used were incubated at 25°C and 30°C throughout the experiment.

2.3Microbiological analyzes: Culture, isolation, enumeration, identification of microorganisms from "cha'a" drinks and from fermented ''arky"

This step was carried out immediatelyupon arrival and each of the 9 days subsequent to the first culture.

Each specimen was plated onto six isolation culture media, namely McConkey agar (McC); Plate-count agar (PCA); Bile Esculin azide agar (BEA); DeMan Rogosa and Sharpe (MRS) agar; Sabouraud/chloramphenicol (5%) agar (SAB); and Chapman agar.

All specimens subjected were diluted (1/10). From the resulted preparation, 50 µL was plated, then aerobically incubated for 72 hours at 25°C, 30°C, and 37°C. The enumeration step was done upon completion of incubation. The results thereof were expressed in terms of Colony Forming Unit per mL (CFU/mL) of the subjected specimens. Bacteria identification steps were performed according to core principles from the Bergey's manuals for Determinative Bacteriology and the yeasts identified with the biochemical orientation tests provided by the API 20 CAUX gallery.

2.4Data analysis

The data recoveredwere bacterial type, bacterial load, pH value, ethanol degree and reducing sugar concentrations. These data were recorded and analyzed with tools provided by the Microsoft Excel 2013 Software. Bacterial load, pH value, ethanol degree and reducing sugar concentrations were presented in this paper by charts to display the evolution as function of time and temperature.

3. RESULTS

3.1 Chemical screening I: Starch and glucose

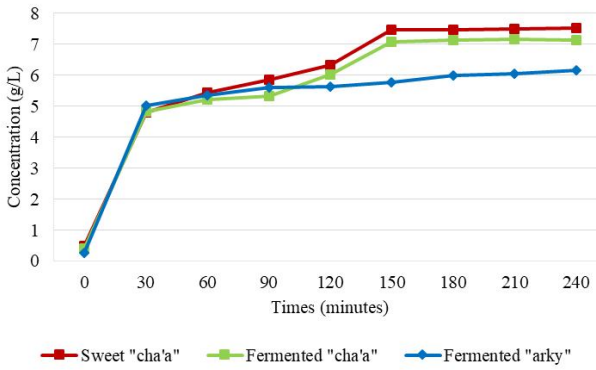
Concerning starch and glucose, the resulting findings were summarized and displayed as shown in table 1 and figure 1.

Table 1. Detection of starch and glucose

Tests	Fermented "arky"	Fermented "cha'a"	Sweet "cha'a"
Before hydrolysis			
GOD	-	-	-
Iodine	++	+++	++++
After hydrolysis			
GOD	++	+++	++++
Iodine	+	++	+++

GOD: glucose oxidase; -: negative reactivity; +: positive reactivity; ++: intense positive reactivity; +++: highly intense positive reactivity;++++: very highly intense positive reactivity

After acidic hydrolysis, positive reactivity (table 1) was recorded for glucose, and starch. During the hydrolysis,



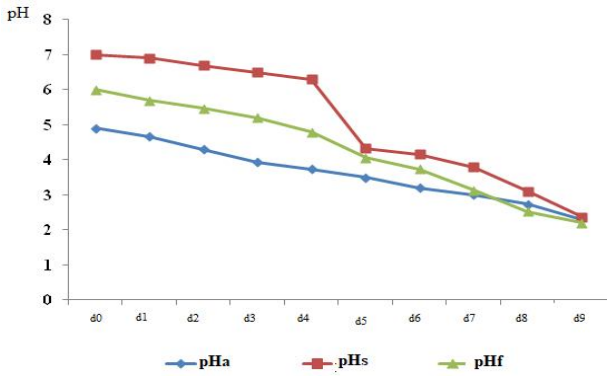
starch contents appeared to reduce with increased glucose concentration.

Figure1. Variation of glucose in specimens during the acid hydrolysis

Globallyand in all resources,the glucose concentrations (figure 1) were highest towards 150mins after an exponential increase within the early 30 mins. Concentration variations significantly reduced from the third to the fourth hours of the hydrolysis (implying a reduced hydrolytic activity with reduced carbohydrate polymers). Further, the glucose concentrations were highest in the "cha'a" drinks. The fresh (sweet) "cha'a'' appeared richer in glucose than the fermented ''cha'a''. As aqualitative test, these glucose concentrations suggest that the fresh "cha'a"was richer in starch than the others drinks and that, the fermented "arky" contained the lowest starch concentration.

3.2Chemical screening II: Evolution of pH values, alcohol concentrations and reducing potential with time

3.2.1 Evolution of pH values



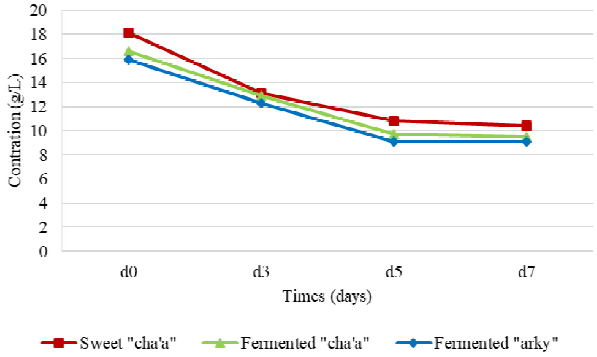
Daily records of pH values from day zero through day nine were plotted and displayed in figure 2.

Figure 2. Evolution of pH values with time

pHa: pH in fermented “arky” juice; pHs: pH in fresh sweet “cha’a”; pHf: pH in fermented “cha’a”

It reveals that the fresh ‘’cha’a’’ had a neutral pH while the other drinks were relatively more acidicon day zero. These values decrease progressively unto day nine (figure 2) when the pH was very low compared to the initial value.

3.2.2 Assessing contents in reducing carbohydrates

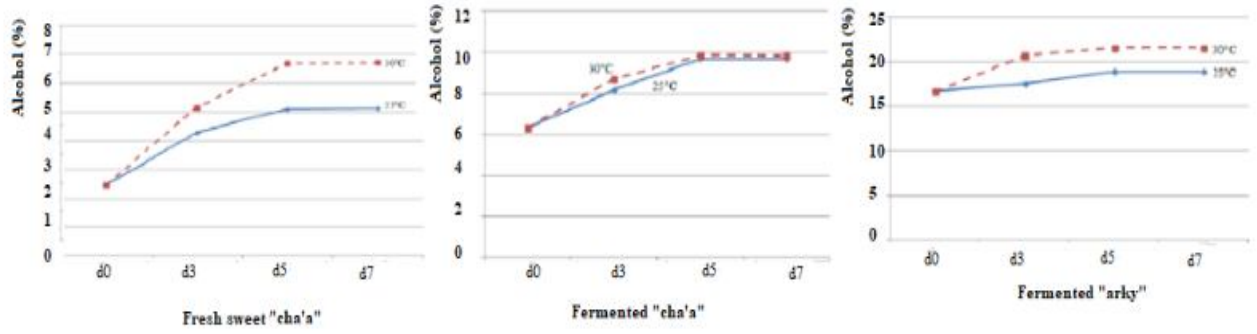


Variations of reducing carbohydrates concentrationsare presented in figure 3.

Figure 3. Evolution of reducing carbohydrates concentrations with time

The concentrations in reducing carbohydrates were higher in fresh “cha’a” than the other drinks,while the fermented “arky” contained the lowest. For all drinks, an abrupt drop in reducing carbohydrates was recorded until day five when variation trend reduced and tended to stabilize from the fifth day.

3.2.3 Ethanol degree evolution



All related pieces of information were summarized as presented in figure 4.

Figure 4. Variation of ethanol degree

The ethanolic degree (figure 4) increased and tended to stabilize from the fifth day, with 30°C as the optimal temperature in general. This was mainly observed with sweet “cha’a” and fermented “arky”. Further, incubation temperature did not significantly influence the process in the fermented ‘’cha’a’’. In the fermented ‘’arky’’, alcoholic content was high, right from day zero. Contrasting withwhat was recorded in the other substrates, the variation recorded in fermented “arky” were relativelynot significant during the test period.

3.3 Microbiological screening

3.3.1 Bacteria and fungi identified

Overall findings reveal the most prolific microbial populations on MRS, PCA and SAB for the three substrates. Fungal populations predominated in the fermented “cha’a” and the fermented “arky”, why only a few positive cultures were observed on BEA. Namely, identified microorganisms consisted of *Lactobacillus* spp., *Corynebacterium xerosis*, *Bacillus* spp., *Enterococcus feacalis* and *Candida zeylonoides*.

The number of major microbial groups was five, schematically represented in their proportion by figure 5.

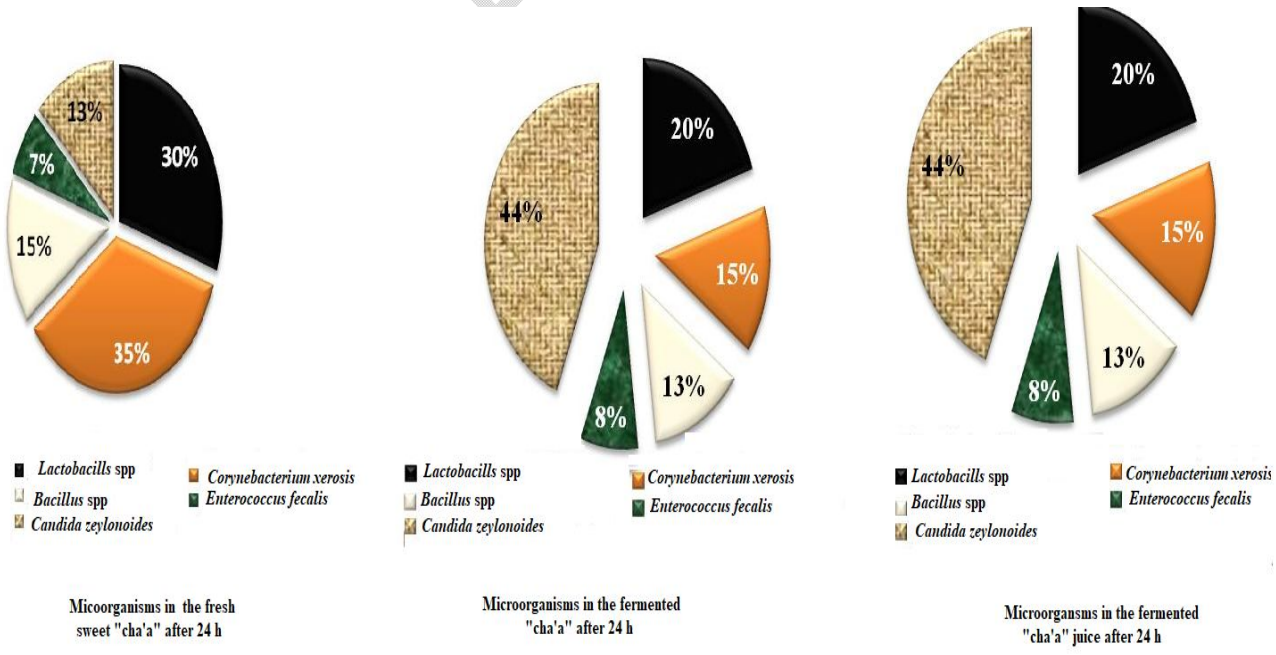


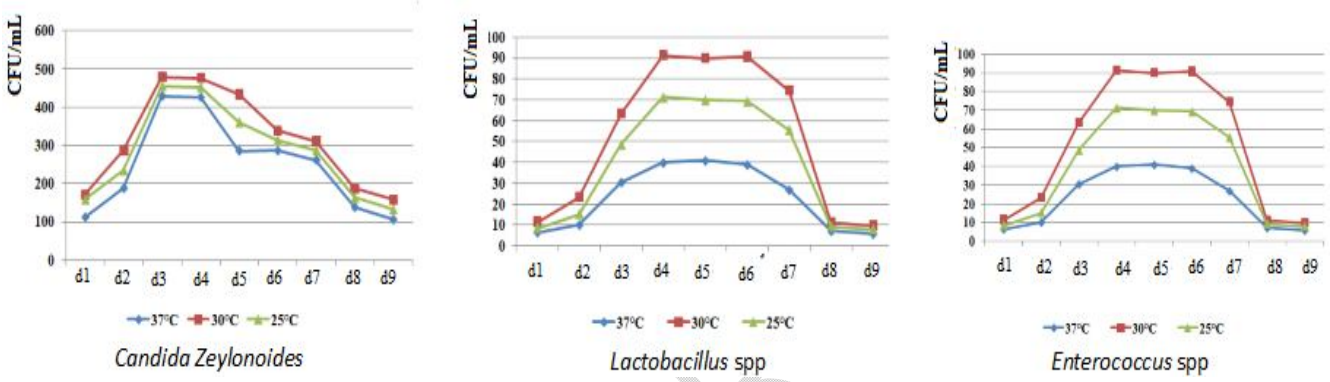
Figure 5: Microbial populations identified and enumerated

Outstanding observation is the microbial inoculums that vary with the subjected substrate; and fungi predominating in the fermented "arky". The same populations grew in all resources. *Candida zeyloides* overwhelmed other population in the fermented, while *Corynebacterium xerosis* population was drastically reduced in fermented substrates. *Enterococcus feacalis* and, to some extent, *Lactobacillus* populations did not change significantly in density. Almost similar fungi population densities were recorded in the fermented "cha'a" and the fermented "arky".

3.3.2 Microbial load evolution

3.3.2.1 Microbial growth dynamics in fresh "cha'a" with respect to time and temperature

Upon completion of incubation that was conducted for nine consecutive days at 25°C, 30°C and 37°C, pieces of information recorded for *Candida Zeylonoides*, *Lactobacillus* spp., and *Enterococcus* spp., were plotted for each



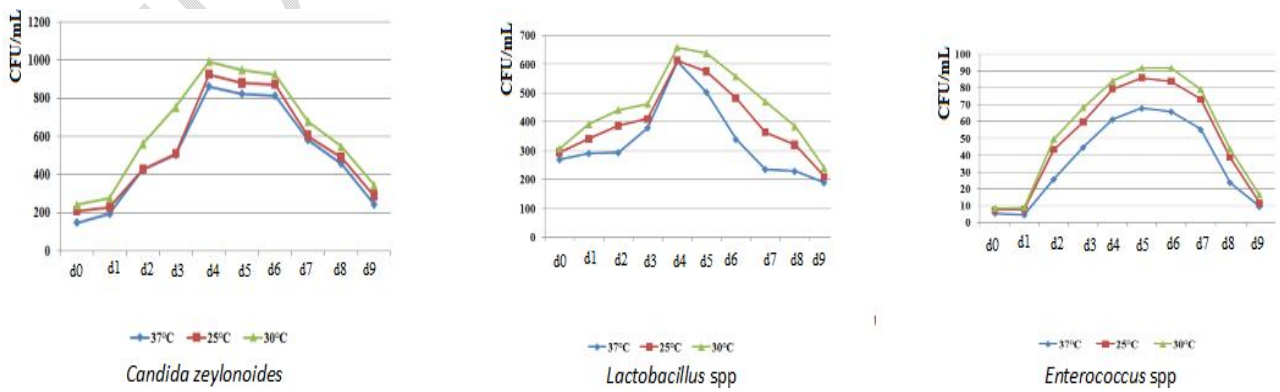
of the three resources. Figure 6 displays microbial population dynamics in sweet "cha'a".

Figure 6: Microbial population dynamics in fresh "cha'a"

Related data reveal that the *Candida zeylonoides* population density steadily increased from the first through the fourth day; then decreased progressively until the nineth day of incubation at the three experimental temperatures. However, and throughout, 30°C was observed as the most conducive for growth.

A similar trend was recorded for *Lactobacilli,* with a stationary phase of growth recorded from the fourth through the sixth day. The highest densities (91 to 98 CFU/mL) were recorded at 30°C. Also, similar growth curves' trends were recorded with *Enterococcus*. Overall, 30°C appeared as the most suitable for all microbial growth in the fresh ''cha'a''.

3.3.2.2 Microbial growth dynamic in the fermented "cha'a" at varying times and temperatures



Related pieces of information were summarized in figure 7.

Figure 7. Microbial population dynamics in fermented "cha'a"

The figure 7 indicates a rapid increase in*Candida zeylonoides* population density from the first day through the fourth (1000 CFU/mL). This increased density was followed by a slow decrease between the fourth and the sixth day of incubation. After the sixth, a rapid decline was recorded until the nineth. This trend was similar at all temperatures although, 30°C was observed as the most conducive for microbial survival and growth. In this substrate, the *Lactobacillus* population also increased steadily from the first through the fourth day. An abrupt density decline was then recorded from that point through the nineth day (200 CFU/mL). Fitness in this bacterial type is likely highest at 30°C in the fermented resources. In *Enterococcus*, a regular growth trend was observed at all temperatures. The optimal population density was also recorded at 30°C, between day four and day seven.

3.3.2.3 Microbial growth dynamics in the fermented "arky" substrate at varying times and temperatures

Related pieces of information were summarized and displayed in figure 8.

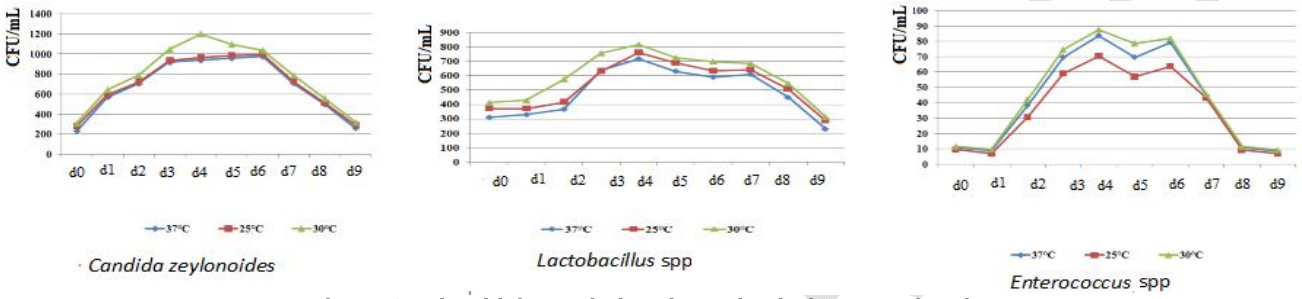


Figure 8: Microbial population dynamics in fermented «arky»

The *Candida zeylonoides'* population density was higher (1200 UFC/mL) compared to the one recorded in the fermented ''cha'a'' between the fourth and the fifthday. A drop in the population density was thereafter, observed subsequent to the stationary phase that extended to the sixth day from which a steady decline was experienced. The maximal load of *Lactobacillus* spp. in this resource was 800 CFU/mL. This population density is higher than the one recorded in the fermented ''cha'a'' and persisted throughout the experiment. *Enterococcus faecalis* population was the least prolific, though it also persisted throughout the experiment.

4. DISCUSSION

The present investigation aimed at identifying and enumerating major microorganisms that are present in fermented "arky'' juice, fermented "cha'a'', fresh (sweet) "cha'a'' and which likely influence the fermentation processes. It was also intended to describe the evolution of these microbial populations over time, and identify indicators of contamination that might make the final product unsuitable for consumption. During fermentation, pH values, reducing sugars concentrations and alcoholic degree were also measured.

The microorganisms isolated from these drinks were fundamentally Gram-positive bacteria and fungi. The overall trend was that ubiquitous environmental bacteria such as those that belong to the *Enterobacteriaceae* family and the genus *Staphylococcus* were not present in any of the substrates used, contrary to what was recorded in previous investigations carried out on other types but related substrates [1,12-14]. In addition, *Enterococcus* which is a sign of adulteration from the fecal origin was detected, likely originating from a variety of sources, more specifically humans and animals; suggesting a low level of hygiene during the drinks production. Studies conducted in Cameroon [1], Burkina Faso [6], Nigeria [12], Ghana [13] and Tunisia [15] on other but related resources concluded similarly regarding hygiene in the production and distribution of traditional beverages. These results may reflect the limits in traditional drink manufacturing which takes place in the farms that are not ideally designed for this type of production. Similar observations were reported by other [6,12,13,15] when they isolated indicators of fecal contamination in "Raphia" Wine.

The isolation of bacteria belonging to the genus *Enterococcus* in these beverages which generally infers the potential presence of pathogens in the food alongside with the absence of bacteria such as *Escherichia coli*, *Salmonella*, other *Enterobacteriaceae* or *Staphylococcus* could suggest that the contamination was done at the end of the production process. This hypothesis is reinforced by the fact that the production of these drinks includes a step of heating (50-60°C),admitting that *Enterobacteriaceae* and *Staphylococcus* are susceptible to

temperature within this range. However, according to some authors, others factors could be investigated. Findings from Mensah *et al.* [16] and Marina *et al*. [17] suggested that fermented maize corn (*Zea mays*) used for "kenkey" production in Ghana could provide an important barrier to the development of bacteria like pathogenic*Escherichia coli*, *Shigella flexneri*, *Klebsiellapneumoniae,* and *Staphylococcus aureus*. Several others observed that the organic acids produced during fermentation of "pito" in Ghana, "ben-saalga" in Burkina-Faso and "tchapalo" in Côte d'Ivoire, provide good microbial stability to the product [18-20], although their density may vary throughout the incubation time. Studies carried out on lactic acid bacteria in starchy foods and beverages in West Africa revealed that lactic acid bacteria have a pre-biotic activity that inhibits pathogenic bacteria [16,21]. These bacteria belong to the genera *Lactobacillus*, *Bacillus*, *Streptococcus*,*Leuconostoc* and *Corynebacterium*, responsible for lactic fermentation [22]. The absence of other bacteria types could therefore, also be explained, at least partially, by the presence of *Corynebacterium xerosis*. Furthermore, analyses revealed high alcohol contents in all the resources used ($2.5/d_0$ - $6.8/d_5$ for sweet "ch'a"; $6.4/d_0$-$10.2/d_5$ for fermented "cha'a"; and $16.9/d_0$-$21.3/d_5$ for fermented "arky"). A previous study [2] reported a similar alcohol content in sweet "cha'a". Alcohol is a well-known inhibitor of bacterial growth, consistent with the fact that it negatively selected these bacteria throughout the process. It may also be a combination of factors (so far beyond current understanding) which could take into account the chemical composition of the corn variant, the preparation conditions and interactions with other non-identified microorganisms and substrate additives.

"Cha'a" and "arky" fermented juice are essentially starchy substrates. There is usually a high level of sugar content in "cha'a" due to absence of the enzymes β-amylases in *Zea mays* malt. Consequently,an increase and uncontrollable rate of fermentation leads to the reduction in the consumption rate and shelf life of "cha'a"; and if the consumption rate is not high enough, the retailer is bound to incur financial losses [23]. This starch abundance provides therefore, a suitable environment for the yeasts. Yeasts first hydrolyze this polymer with amylase to produce maltose, which is then transformed into glucose that eventually enters into the glycolysis chain. One study revealed that factors involved in yeast proliferation include osmotic pressure, substrate rate, nutrient depletion, temperature and intracellular ethanol accumulation [24]. Another one showed that factors involved in yeast populations decline included physical and chemical changes during carbohydrate degradation (temperature, pressure, acidity, sugar concentration, alcohol content and water availability) [25].

In general, when the microorganisms were present, their density increased over the first 96 hours, after which a relatively less abrupt decline was observed. In all cases, *Candida zeylonoides,Lactobacillus* and *Corynebacterium xerosis* populations predominated. These organisms are known to be important in the fermentation of corn beer. In some case, the presence of one microorganisms is a benefit for the others. Oyewole [19] reported that the growth of a *L. plantarum* strain was considerably enhanced in the presence of *Candida krusei* during cassava fermentation aiming at manufacturing "fufu". Also, previous findings reported synergistic interactions between organisms from the two major groups identified [26-28]. These findings aligned with Tcheuffa Ngassam [1] in that fungi and *Lactobacillus* cohabit. However, the inhibition of *Lactobacillus* that they reported in the "Raphia" wine was not observed in the present investigation. Similarly, Amoa-Awua*et al*. [14] on one hand and Chavan and kadam [29] on the other reported that the microorganisms involved in the fermentation of starchy products are *Corynebacterium*, *Lactobacillus* and *Candida* [30].

As mentioned above, pH values decrease over time. This was noted for artisanal beverages such as "Raphia" wine [1]. This evolution reflects the presence of bioproduct derived from fermentation, namely ethanol and acid products, which acidify the medium. These findings align with those reported by previous investigators [1,13-15], who observed a gradual decrease in pH over time. Contrasting however,with what this research observed, Tapsoba *et al*. [6] reported pH values that ranged from 4.50 through 3.60; Tiepma*et al.* [3] recorded values ranging from 4.08 through 3.21; and Mintah *et al.* [28] values ranging from 4.05 through 3.94. This may be explained by the inherent properties of the substrates, which are different as observed above. In fact, these authors worked on Raphia wine, which is basically richer in sucrose than starch. Otherwise, the use of starchy substrates naturally leads to greater amounts of acid than those produced by sucrose fermentation.

During the tests, it was found that reducing sugar concentrations were inversely proportional to alcoholic strength. This is consistent with the fact that it is through the degradation of these substrates that alcohol derivatives are metabolized. The evolution of the processes also indicates a phase of alcoholic degree and reducing sugars concentration stabilization. This stabilization infers the stop or the reduction of fermentation process. This stationary phase coincides with the lowest concentrations in reducing sugars. In other words, when the fermentation process seems to stops (day 4 and day 5), monosaccharides are still available in the medium. "Whycouldthe process stop while the substrate is available?" is a major puzzle. Several answers are

possible. In fact, since the microbial exponential growth phase seems to correspond to the exponential phase observed in the measurement of alcoholic strength and reducing sugars level, one and the likely most appropriate would be in connection with the factors responsible for the stationary and decrease phases during typical microbial growth. Amongst these factors, there are saturation of the environment with toxic bio-products, reduction of the space required for the ferments to flourish during the process, depletion of substances necessary for the activity and growth of the ferments. A study by Uma and Polassa [31] revealed that ethanol production peaked during the cell growth phase. In combination, these factors would contribute more to the inhibition of the polymer hydrolysis process. A source [34] reported that the combination of ferments improved fermentation yield. In this case, albeit at varied concentrations, three categories of microorganisms responsible for fermentation were identified from the start to the end of the process: *Lactobacillus* spp., *Corynebacterium xerosis* and *Candida zeylonoides*. Thus, this combination of organisms known for their role in fermentation could explain the increased yields, while also noteworthy shouldbe that success depends on the starch source [32].

The most suitable temperature for microbial growth was 30°C. Findings from the present work reinforces the idea that fermentation proceeds best at 30°C for the subjected drinks. This was confirmed by the trend of alcohol concentrations in the preparations. Though the fermentation was carried out at two different temperatures (25 and 30°C) the higher values were recorded at 30°C, further confirming that the suitable temperature for fermentation would be 30°C. Studies conducted by Layokun [33], Bouix and Leveau [34] revealed that 30°C correlates with optimal growth for most yeasts, and that this factor has a significant influence on ethanol production.Overall, production and distribution standards aligning with biosafety, biosecurity, good manufacturing practices (GMP) and good hygienic practices (GHP) should be point in place for consumer's safety.

5. CONCLUSION

The present investigation on microorganisms involved in the fermentation of the drinks made with *Zea mays* revealed that among the isolated microorganisms those endowed with fermentative potentials are Gram-positive rod bacteria belonging to the genera *Lactobacillus* and *Corynebacterium*, and yeasts from the genus *Candida*. *Enterococcus* has also been identified as indicator of resource adulteration. If the population of yeasts remained dominant, all persisted throughout the process, including the least densely observed (*Enterococcus*). Their optimal growth was recorded at 30°C and the pH declined regularly along the process. Further details also revealed that the contents in alcohol increased steadily from day 0 through day 7 and respectively, its variation was inversely proportional to those of reducing carbohydrates. The ethanolic degree obtained with the fermented "arky" were approximately 8 times higher than the one recorded in the sweet "cha'a". These results are evidence that the subjected resources can be used as raw materials for the production of alcohol. However, the presence of *Enterococci* indicates that they are also potential sources of microbial etiologies of infectious diseases. Therefore,specific measures should be taken by legal authorities to regulatemanufacture and distribution of these drinksfor consumer's safety.

DATA AVAILABILITY

Data associated with this work were not deposited into a publicly available repository. All the data of this work are present in this paper.

REFERENCES

1. TcheuffaNgassam M. Études des microorganismes impliqués dans la fermentation du vin de « Raphia », boisson artisanale consommée dans le département du NDE, région de l'Ouest du Cameroun [thesis en Pharmacie]. Bangangté-Cameroon: Université des Montagnes. 2014; 65p. [French]
2. Djubgang R. Évaluation microbiologique et dosage des produits secondaires toxiques dans les boissons alcooliques artisanales de la région du Ndé (Cameroun). [thesis en Pharmacie]. Bangangté-Cameroon: Université des Montagnes. 2009; 102p [French]
3. Tiepma N, Zambou F, Agbor E and Tchouanguep F. Physicochemical changes of raffiasap (Raphia mambillensis) contents during spontaneous fermentation. Afr J Biotechnol. 2013; 12(41) : 6013-18.
4. Kouchade A and Awokou K. La récolte de vin de palme : procédé et effets des conditions environnementales. CIFEM. 2010;(1):1-6. [French]

5. Okafor N. Microbiology of Nigerian palm wine with particular reference to bacteria. JAppl Bact. 1975; 38(2) :81-8.

6. Tapsoba F, Savadogo A, Somda K, Zongo C, Barro N and Traore SA. Biodiversité microbienne et paramètres physicochimiques de quelques vins de ronier (*Borassus akeassii*) produits traditionnellement au Burkina Faso. Rev Microbiol Ind San Environn. 2011; 5(2) :1-22. [French]

7. Dia D, Sakho-Jimbira S, Fall CS, Ndour A and Dieye PN. Le Sénégal face à la crise énergétique mondiale : Enjeux de l'émergence de la filière des biocarburants. Sud Sciences & Technologies. 2011 ; 19-20 : 69-80. [French]

8. Steinkraus K. Industrialization of Indigenous Fermented Foods, Revised and Expanded. 2nd ed. CRC Press. 2004; 92p

9. McCall M. Brewing rural beer should be a hotter issue. Boiling Point. 2001; 47: 23-5

10. Bertrand G. Le dosage de sucres reducteurs. Bull Soc Chim. 1906; 35:1285-99. [French]

11. Gadsen RH, Taylor EH, Steindel SJ et al. Ethanol in Biological Fluids by Enzymic Analysis. In: Selected methods of emergency toxicology. Eds. Vol 11.Selected methods of Clinical Chemistry, Washington DC, AACC press, 1986; pp:63-65

12. Ogbulie TE, Ogbulie JN and Njoku HO. Comparative study on the microbiology and shelf life stability of palm wine from Elaeis guineensis and Raphia hookeri obtained from Okigwe, Nigeria. Afr J Biotechnol. 2007; 6(7): 914-22.

13. Amoa-Awua WK, Sampson E and Tano-Debrah K. Growth of yeasts, lactic and acetic acid bacteria in palm wine during tapping and fermentation from felled oil palm (Elaeis guineensis) in Ghana. J Appl Microbiol. 2006; 102(2) pp: 599-606

14. Ohimain I, Tuwon P and Ayibaebi E. Traditional Fermentation and Distillation of Raffia Palm Sap for the Production of Bioethanol in Bayelsa State, Nigeria J Technol InnovReEnerg. 2012; 1: 131-41.

15. Manel Z, Sana M, Nedia K, Moktar H and Ali F. Microbiological analysis and screening of lactic acid bacteria from Tunisian date palm sap. Afr J Microbiol Res. 2011; 5(19): 2929-35.

16. Mensah P, Tomkins AM, Drasar BS, Harrison TJ. Antimicrobial effect of fermented Ghanaian maize dough. J Appl Bacteriol. 1991; 70(3). pp: 203-10.

17. Assohoun MCN, Djeni T, Koussémon-Camara M and Brou K. Effect of Fermentation Process on Nutritional Composition and Aflatoxins Concentration of Doklu, a Fermented Maize Based Food. Food and Nutrition Sciences. 2013; 4(11): 1120-7.

18. Djè M, Djeni TN, Toka M and Aka S. Biochemichal and Microbiological changes of cassava dough fermenting in different temperature conditions. J Food Technol. 2008; 6: 114-9.

19. Oyewole OB. Lactic fermented foods in Africa and their benefits. Food Control. 1997. 8: 289-97

20. Djè KM, N'Guessan KF, Djeni NT and Dadie AT. Biochemical Changes during Alcoholic Fermentation in the Production of "Tchapalo", a Traditional Sorghum Beer. Int Journal Food Eng. 2008; 4(7): 17p.

21. Kimmons JE, Brown KH, LarteyA, Collinson E, Mensah PP and Dewey KG. The effects of fermentation and/or vacuum flask storage on the presence of coliforms in effects of complementary foods prepared for Ghanaian. Children. Int J Food Sci Nurt. 1999; 50(3): 195-201.

22. Mensah PP, Tomkins AM, Drasar BS and Harrison TJ. Fermentation of cereals for reduction of bacterial contamination of weaning foods in Ghana. Lancet. 1990; 336(8708):140-3.

23. NsaighamuLawir C, Toh Nkwain K, Fatyy Lamin KM and Agbor Agbor E. Socio-Economic Role of Sha (Corn Beer) in the Livelihood of Value Chain Actors and Consumers in Cameroon. Asian Journal of Advanced Research and Reports. 2022; 16(3): 1-11.

24. Sharma A and Kapoor AC. Levels of antinutritional factors in pearl millet as affected by processing treatments and various types of fermentation. Plant Foods Hum Nutr. 1996; 49(3): 241-52.

25. Gassem MA and Osman MA. Chemical compositions and nutritional potential of pearl millet grown in Jazan region of Saudi Arabia. Saudi Soc Agric Sci. 2008; 7:.1-22

26. Hounhouigan JD, Nout RMJ, NAGO CM, Houben JH and Rombouts FM. Composition and microbiological and physical attributes of mawè, a fermented maize dough from Benin. Int J Food Sci Techno. 1993; 28(5): 513–7.

27. Osman MA. Effect of traditional fermentation process on the nutrient and antinutrient contents of pearl millet during preparation of Lohoh. J Saudi Soc Agricul Sci. 2011; 10: 1-6.

28. Mintah BK, Eliason AE, Barimah J and Oldham JH. Development of syrup and “malt-like” drink from Raphia hookeri sap. AJFAND. 2011; 11(5) : 5203-19.

29. Chavan JK and Kadam SS. Nutritional improvement of cereals by fermentation. Crit Rev Food SciNutr. 1989; 28(5):349-400.

30. Ben-Mahdi MH, Ouslimani S. Mise en Évidence de Résidus d’Antibiotiques dans le Lait de Vache Produit dans l’Algérois. European Journal of Scientific Research. 2009 ; 36(3): 357-62

31. Uma V, Polassa H. Rapid production of ethanol by newly isolated Saccharomyces cerevisae of palm wine. J MicrobialBiotechnol. 1988; 3(2): 70-4.

32. TatsinkouFossi B, Jiwoua C, Tavea F, TatsadjieuNgoune L, Ndjouenkeu R. Production directe d’éthanol à partir de l’amidon par co-culture de deux souches de levures Schwanniomycessp et Saccharomyces cerevisiae : application à la bioconversion de l’amidon de manioc et de maïs. Syllabus Review. 2009; 1(1) : 28-35.

33. Layokun SK. Use of the palm wine cultures for ethanol production from black strap molasses with particular reference to conditions in the tropics. Process Biochem1984; 19(5).

34. Bouix M, Leveau J. Les microorganismes d'intérêt industriel. Tec & Doc Lavoisie1999; 2(4).