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Microbial and Metabolites Dynamics during the Fermentation of Artisanal Drinks: Screening and Estimation from "cha'a" and "arky" Consumed in Yaoundé-Cameroon

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Authors' contributions

The work was carried out through collaboration among all authors. Conceptualization and methodology were handled by authors MAMF, PRFK and CMT. Validation and supervision were overseen by authors PRFK and CMT. Author MAMF was responsible for project administration, investigation, data curation, and formal analysis. Authors MAMF and PRFK provided the necessary resources. Visualization was carried out by authors MAMF and ODY. Original draft was written by author PRFK and ODY, while the review and editing of the manuscript were done by authors MAMF, PRFK, PDCD, CDN, ODY and CMT. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Objective: The present work aimed at contributing to the mastering of fermentation processes in agricultural products.
Methodology: It targeted the identification and enumeration of micro-organisms 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, alcoholic strength, reducing sugar concentrations, and pH values during fermentation. To achieve these 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 laboratories where analyses were conducted. Culture, isolation, identification and enumeration were done according to the standard protocols.
Results: The microbial screening revealed the presence of *Lactobacillus* spp., *Corynebacterium xerosis*, *Candida zeylanoides*, *Enterococcus faecalis,* and *Bacillus* spp. It also indicated that the populations of fermenting organisms were optimal between the 4th and the 5th days of fermentation, with an increase of alcohol degree. Meanwhile, the contents in reducing sugars decreased in the three resources, like the pH values. Optimal microbial 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.
Conclusion: Combined, these findings indicate that with minimal financial resources *Zea mays* may contribute to health benefits (presence of probiotics) and serve for the production of alcohol; but ingesting the substrates studied in the present works also represents a health risk for two reasons: the high alcohol contents and potentially infectious disease etiologies.

Keywords: Fermentation; Zea mays; micro-organisms; Corn-beer; "Cha'a"; "Arky".

1. INTRODUCTION

At specific stages along the production chain of large numbers of food items, microorganisms contribute significantly. 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 (Şanlier *et al*., 2019).

Fermentations for instance, are redox reactions that occurs in poorly oxygenated environments and causes partial degradation of substrates, generating reduced amounts of energy (compared to those that take place in properly oxygenated atmospheres) (Şanlier *et al*., 2019; Djubgang, 2009). Humans have built on these potentials of microorganisms to improve the nutritional and organoleptic qualities of their foods (Şanlier *et al*., 2019). Throughout the world, many companies use microorganisms to produce drinks (Djubgang, 2009). 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 population needs, their living standard and raw material availability. Namely, banana, sorghum, millet, cassava, and corn for instance, can be used. These determinants can vary over time, and reflect the inherent wealth of endogenous knowledge that is passed throughout generations (Şanlier *et al*., 2019).

During fermentation, the carbohydrates in the substrates are rapidly metabolized into ethanol and organic acids, which in turn affects the original flavor (Tiepma *et al*., 2013; Kouchade *et al*., 2017; Okafor, 1975). Microorganisms that are responsible for fermentation actually multiply and bring about changes in the physical and chemical characteristics of the drink through a series of metabolic processes. Related environmental changes cause competition amongst living microbial populations which further affects its chemical composition (Tiepma *et al*., 2013; Tapsoba *et al*., 2011; Okafor, 1975). 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, the race for energy is capital as a 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) represent an alternative foundation that future projects for fuel production should build on (Wang *et al*., 2023; Awogbemi and Kallon, 2022; Anwar *et al*., 2018; Dia *et al*. 2011).

Alongside drinks manufactured by large industrial companies, the artisanal drinks sector is also very widespread in communities (Şanlier *et al*., 2019; Djubgang, 2009). Unlike the drinks in the first group, artisanal drinks are more affordable thanks to their availability and their affordability. Their use has been known for centuries in certain countries and communities where they are firmly linked to beliefs and represent a fundamental part of local traditions (Nsaighamu *et al*., 2022).

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. 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, and football matches but also during moments of sadness like funerals (Nsaighamu *et al*., 2022; McCall, 2001).

Despite the relatively high inclination to "cha'a" and "arky" (made with *Zea mays*) consumption 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 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 an artisanal and industrial scale with a core focus on consumers' welfare. The present piece of research focused on the different metabolic processes that 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 cross-sectional survey, the sample collection was conducted in Yaoundé, the political headquarter of Cameroon. A total of 10 fermented "arky" juice specimens were 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 containers (≈ 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. Sample analysis was carried out within 24h after collection.

2.2 Physico-Chemical Analysis of the Experimental Samples

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

2.2.1 Estimation of starch and glucose

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

2.2.1.1 Starch detection using Lugol's solution

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.

2.2.1.2 Test for glucose with glucose oxidase – peroxidase

To 1 mL of glucose oxidase – peroxidase solution (GOP-POD), 10 µL of the specimen was added and mixed thoroughly. After 25 min incubation at room temperature, the color intensity of the substrate-GOP-POD mixture was measured with a spectrophotometer at 505 nm. The color intensity was proportional to the quantity of glucose in the specimen subjected. A calibration range was then designed with a standard of glucose made from distilled water and glucose.

2.2.2 Determination of pH

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.

2.2.3 Test for reducing sugars

It was conducted according to Bertrand's, 1906. 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 the laboratory, then 3, 5 and 7 days after the first test.

2.2.4 Ethanol screening (enzymatic assay)

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

2.3 Microbiological Analysis

This step was carried out immediately upon 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 (10 fold serial dilution). From the resulting 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 the 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 while the yeasts were identified with the biochemical orientation tests provided by the API 20 CAUX gallery.

2.4 Data Analysis

The data recovered were 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 a function of time and temperature.

3. RESULTS

3.1 Chemical Screening I: Starch and Glucose

Concerning starch and glucose, the collected findings were summarized and displayed as shown in Table 1 and Fig. 1.

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.

In all resources, the glucose concentrations (Fig. 1) were highest towards 150 mins 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 a qualitative test, these glucose concentrations suggest that the fresh "cha'a" was richer in starch than the other drinks and that, the fermented "arky" contained the lowest starch concentration.

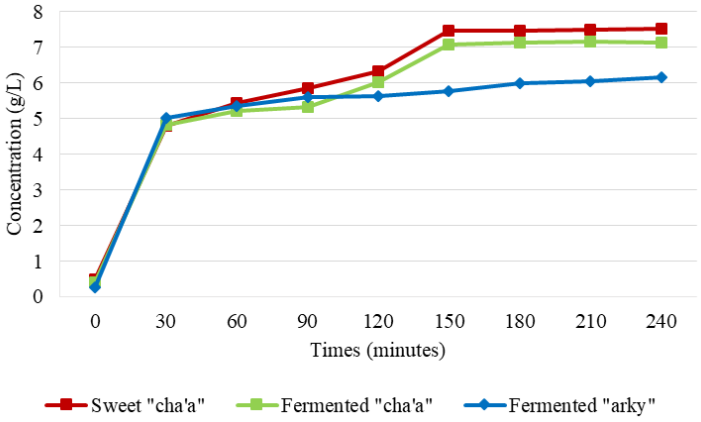

Fig. 1. Variation of glucose in samples during the acid hydrolysis

Table 1. Detection of starch and glucose

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

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

3.2 Chemical Screening II: pH Values, Alcohol Concentrations and Reducing Potential with Time

3.2.1 Evolution of pH values

Daily records of pH values (pH variation with days) from day zero through day nine were plotted and displayed in Fig. 2.

It reveals that the fresh ''cha'a'' had a neutral pH while the other drinks were relatively more acidic on day zero. These values decrease progressively until day nine (Fig. 2) when the pH was very low compared to the initial value.

3.2.2 Reducing sugar concentrations

Variations of reducing carbohydrates concentrations are presented in Fig. 3.

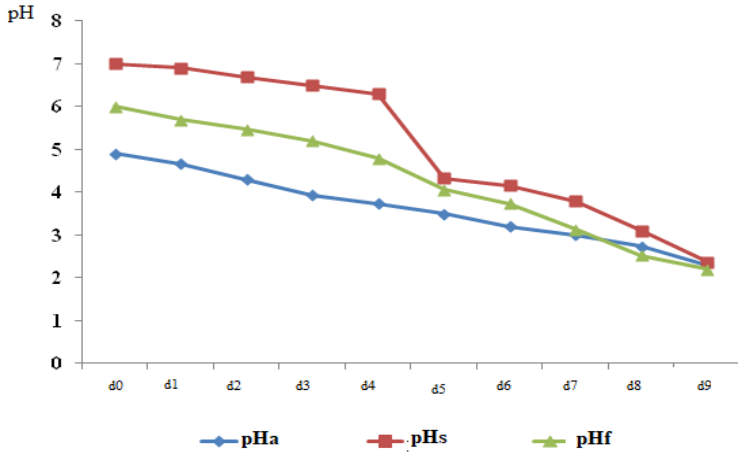

Fig. 2. Evolution of pH values with time

pHa: pH in fermented "arky" juice; pHs: pH in fresh sweet "cha'a"; pHf: pH in fermented "cha'a"

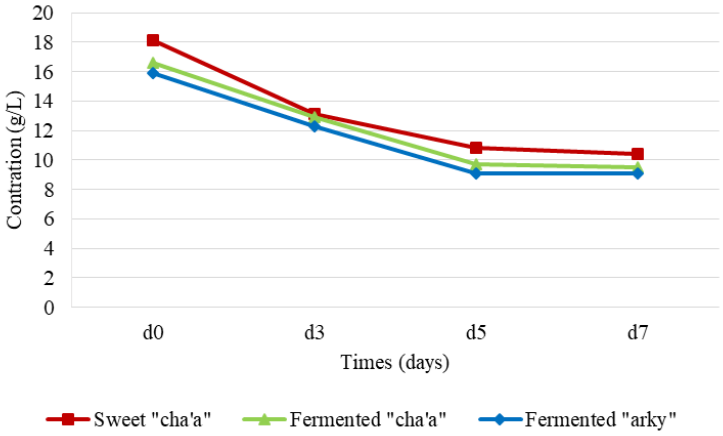

Fig. 3. Evolution of reducing carbohydrates concentrations with time

The concentrations of 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 the variation trend reduced and tended to stabilize from the fifth day.

3.2.3 Monitoring ethanol production

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

The ethanolic degree (Fig. 4) increased and tended to stabilize from the fifth day. This was mainly observed with sweet “cha’a” and fermented “arky”. In general, 30°C was recorded as the optimal temperature. 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 with what was recorded in the other substrates, the variation recorded in fermented “arky” was relatively not significant during the test period.

3.3 Microbiological Screening

3.3.1 Bacteria and fungi identified

Overall findings revealed 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, *Corynebacterium xerosis*, *Bacillus* spp., *Enterococcus faecalis* and *Candida zeylanoides*.

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

Outstanding observation is the microbial inoculums that vary with the subjected substrate; and fungi predominating in the fermented “arky”. The same populations were identified in all resources. *Candida zeylanoides* overwhelmed other population in the fermented, while *Corynebacterium xerosis* population was drastically reduced in the subjected fermented substrates. *Enterococcus faecalis* and, to some extent, *Lactobacillus* populations did not change significantly in density. Almost similar fungi populations 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, findings related to *Candida zeylanoides*, *Lactobacillus* spp., and *Enterococcus* spp., were plotted for each of the three resources. Fig. 6 displays microbial population dynamics in sweet “cha’a”.

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

microbial 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 obtained 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 dynamics in the fermented “cha’a” at varying times and temperatures

Related pieces of information were summarized in Fig. 7.

It indicates (Fig. 7) a rapid increase in *Candida zeylanoides* 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 ninth. 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 ninth 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 Fig. 8.

The *Candida zeylanoides’* population density was higher (1200 UFC/mL) compared to the one recorded in the fermented ‘’cha’a’’ between the fourth and the fifth day. A drop in the observed population density was thereafter, recorded 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 was denser than the one recorded in the fermented ‘’cha’a’’ and persisted throughout the experiment. The *Enterococcus faecalis* population was the least prolific. Like the others, 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 sugar 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. These previous studies reported the detection of bacteria from *Enterobacteriaceae* family and *Staphylococcus* genus (Tcheuffa Ngassam, 2014; Tapsoba *et al*., 2011; Ogbulie *et al*., 2007). *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 drink production. Studies conducted in Burkina Faso (Tapsoba *et al*., 2011), Cameroon (Tcheuffa Ngassam, 2014) and Nigeria (Ogbulie *et al*., 2007) on other but related resources concluded similarly regarding hygiene in the production and distribution of traditional beverages. These studies (Tcheuffa Ngassam, 2014; Tapsoba *et al*., 2011; Ogbulie *et al*., 2007) pointed out weaknesses in hygiene during the production of these artisanal drinks as they isolated indicators of fecal contamination in “Raphia” wine. Also, 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 likely occurred 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) (Nsaighamu

et al., 2022; Steinkraus, 2004), admitting that *Enterobacteriaceae* and *Staphylococcus* are susceptible to temperatures within this range. 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 (Nsaighamu *et al*., 2022). This weakness on artisanal drink production was previously reported by Nsaighamu Lawir *et al*. (2022) with corn-beer production in Cameroon, connecting poor processing, poor storage, limited market and modernization, poor preservation and packaging policies, lack of support and disunity of brewers. With regard to the consumer safety and the producer benefits, these authors suggested for good manufacturing practices (GMP) the provision of improved processing equipment, provision of cooling and storage facilities, intensification of proper regulatory policies, and training (Nsaighamu *et al*., 2022). They also observed that substantial improvement of production practices could promote these sectors, generate income, create employment with related taxes, intensify economic activities, and valorize the local culture (Nsaighamu *et al*., 2022).

According to some authors, others factors could be investigated to explain the non-detection of *Enterobacteriaceae* and *Staphylococcus*. Findings from Mensah *et al*., (1991) and Assohoun *et al*. (2013) suggested that fermented maize corn (*Zea mays*) used for "kenkey" production in Ghana could provide an important barrier to the growth of bacteria like pathogenic *Escherichia coli*, *Shigella flexneri*, *Klebsiella pneumoniae,* 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 (Djè *et al*., 2008a,b; Oyewole, 1997), although their density may vary throughout the incubation length of time. Studies carried out on lactic acid bacteria in starchy foods and beverages in West Africa (Kimmons *et al*., 1999; Mensah *et al*., 1991) and in the South-West region of Cameroon (Tatsinkou *et al*., 2016) revealed that lactic acid bacteria inhibit pathogenic bacteria growth. These include organisms belonging to the genera *Lactobacillus*, *Bacillus*, *Streptococcus*, *Leuconostoc* and *Corynebacterium*, for their role in lactic fermentation (Mensah *et al*., 1990). The presence of lactic acid bacteria, also known as probiotics, highlights the facts that these drinks can provide health benefits to consumers (Thakur *et al*., 2023; Bharti *et al*., 2020; Şanlier *et al*., 2019). In addition, 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 (Djubgang, 2009) 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. These phenomena might also be explained as a combination of factors (so far beyond current understanding) that could take into account the chemical composition of the corn variants, 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 the absence of the enzymes β-amylases in *Zea mays* malt. Consequently, an increase and uncontrollable rate of fermentation leads to a reduction in the consumption rate and shelf life of "cha'a"; so that if the consumption rate is not high enough, the drink's retailer is bound to incur financial losses (Nsaighamu *et al*., 2022). This starch abundance provides therefore, suitable environments 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 (Sharma and Kapoor, 1996). Another one showed that factors involved in yeast population decline included physical and chemical changes during carbohydrate degradation (temperature, pressure, acidity, sugar concentration, alcohol content and water availability) (Gassem and Osman, 2008).

In general, when the microorganisms were present, their density increased over the first 96 hours, after which a relatively weak abrupt decline was observed. In all cases, *Candida zeylanoides, Lactobacillus* and *Corynebacterium xerosis* populations predominated. These organisms are known to be important in the fermentation of corn beer. In some cases, the presence of one microorganism is a benefit for the others. Oyewole (1997) 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 (Osman, 2011; Mintah *et al*., 2011; Hounhouigan *et al*., 1993). These findings aligned with Tcheuffa Ngassam (2014) 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*. (2007) on one hand and, Chavan and Kadam (1989) on the other reported that the microorganisms involved in the fermentation of starchy products are *Corynebacterium*, *Lactobacillus* and *Candida* (Ben-Mahdi *et al*., 2009).

As mentioned above, pH values decrease over time. This was noted for artisanal beverages such as “Raphia” wine (Tcheuffa Ngassam, 2014). This evolution reflects the presence of by-products derived from fermentation, namely ethanol and acid products, which acidify the medium. These findings align with those reported by previous investigators (Tcheuffa Ngassam, 2014; Ohimain *et al*., 2012; Amoa-Awua *et al*., 2007; Manel *et al*., 2011) who observed a gradual decrease in pH over time. Contrasting however with what this research observed, Tapsoba *et al*. (2011) reported pH values that ranged from 4.50 through 3.60; Tiepma *et al*. (2013) recorded values ranging from 4.08 through 3.21; and Mintah *et al*. (2011) 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 was 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 stabilization for alcoholic degree and reducing sugar concentration. This stabilization infers the stop or the reduction of the fermentation process. This stationary phase coincides with the lowest concentrations in reducing sugars. In other words, when the fermentation process seems to stop (day 4 and day 5), monosaccharides are still available in the medium. “Why could the process stop while the substrate is still 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 sugar levels, one and the likely most appropriate would be in connection with the factors that are responsible for the stationary and decreased phases during typical microbial growth. Amongst these factors, there are saturation of the environment with toxic by-products, reduction of the space required for the ferments during the process, and depletion of substances that are necessary for the activity and growth of the ferments. A study by Uma and Polasa (1988) 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 (Chavan and Kadam, 1989) reported that the combination of ferments rather 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 zeylanoides*. Thus, this combination of organisms known for their role in fermentation could explain the increased yields, while also noteworthy should be that success depends on the starch source (Tatsinkou *et al*., 2009).

The most suitable temperature for microbial growth was 30°C. Findings from the present work reinforce 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 different temperatures (25 and 30°C and 37°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 (1984), Bouix and Leveau (1993) also revealed that 30°C correlates with optimal growth for most yeasts, and that this factor has a significant influence on ethanol production. Overall and for efficient use of these findings for consumer safety and better economic impact, production and distribution standards aligning with biosafety, biosecurity, good manufacturing practices (GMP) and good hygienic practices (GHP) should be put in place and sustainably (Nsaighamu *et al*., 2022).

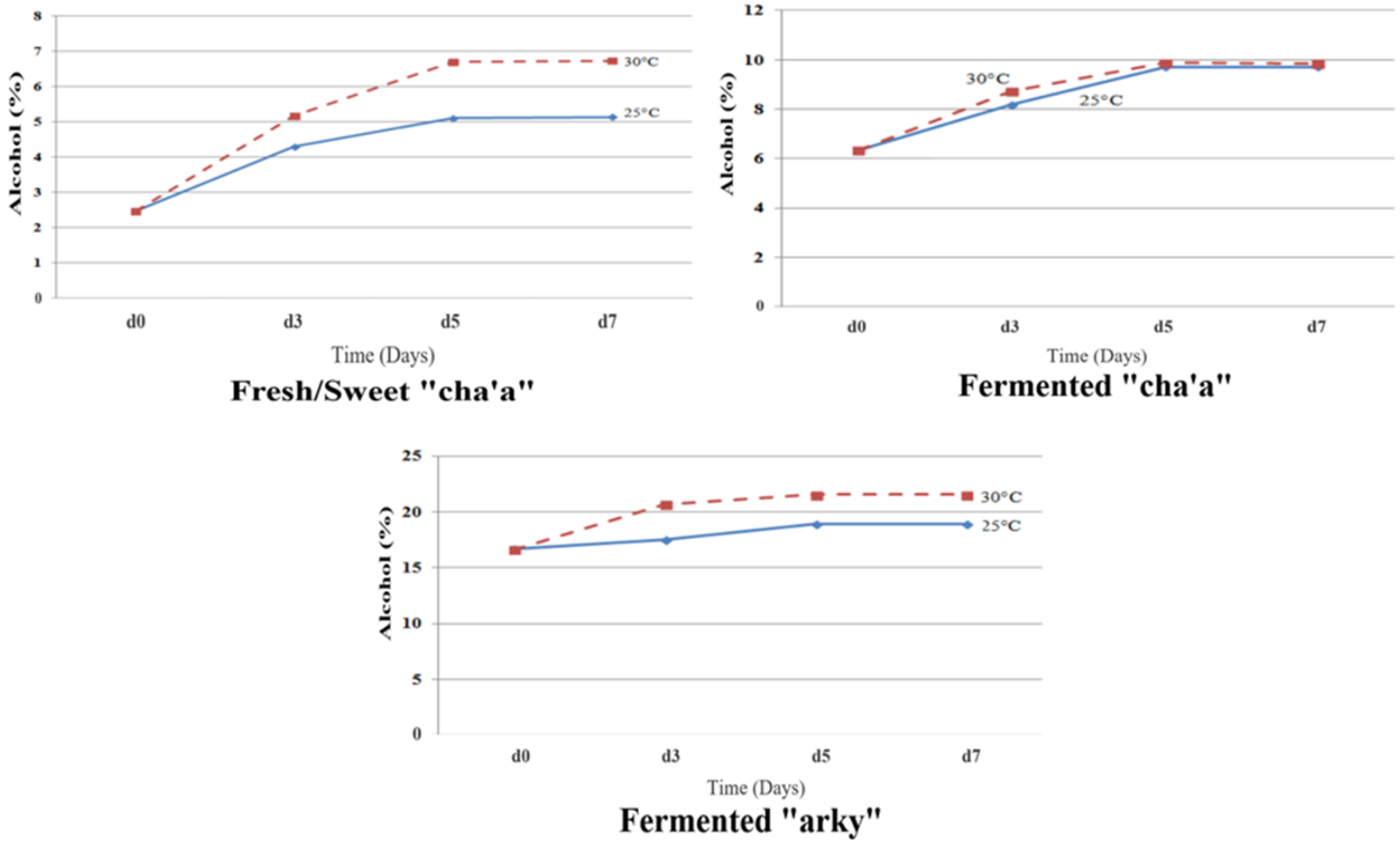

Fig. 4. Variation of ethanol degree

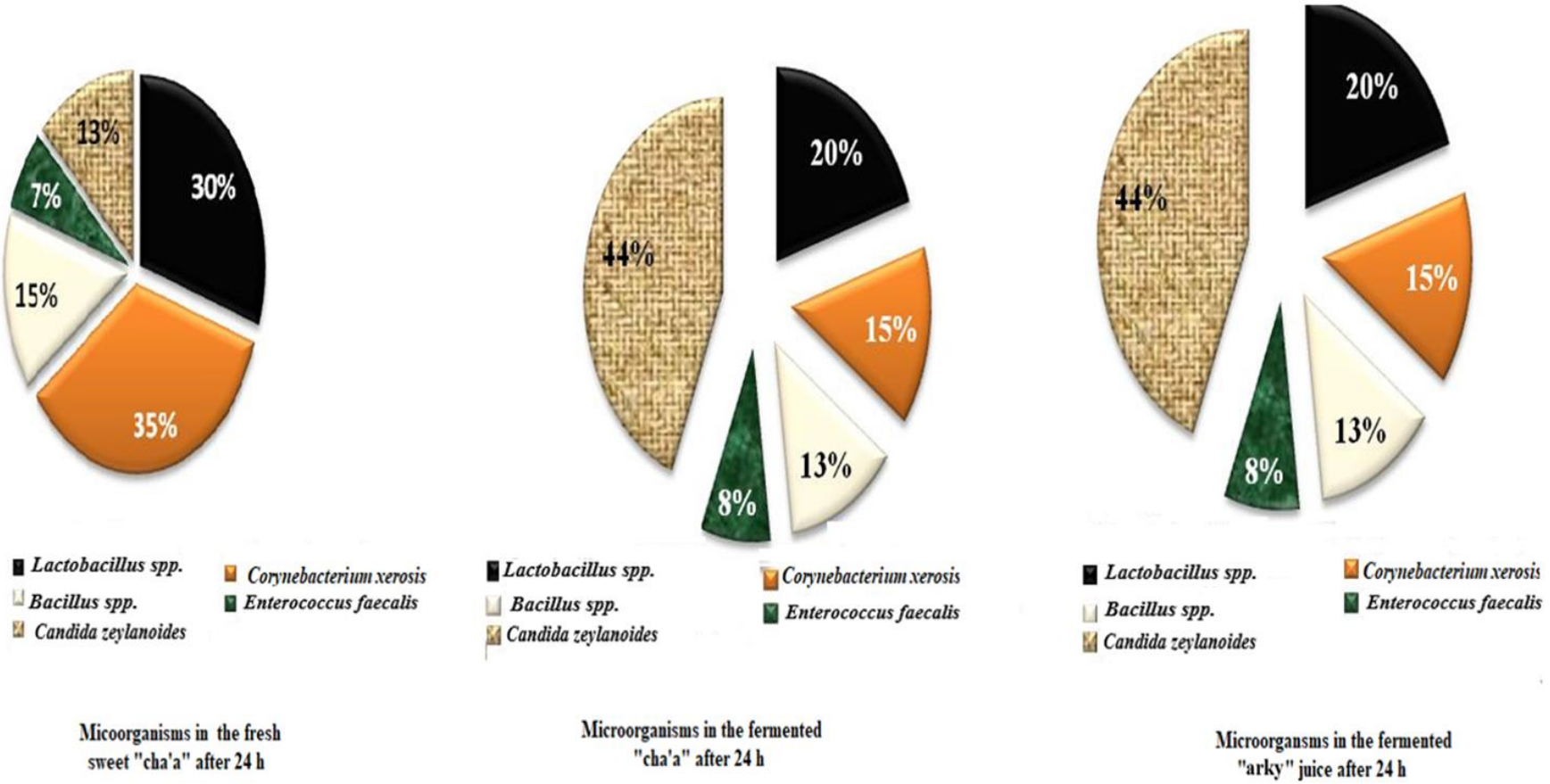

Fig. 5. Microbial populations identified and enumerated

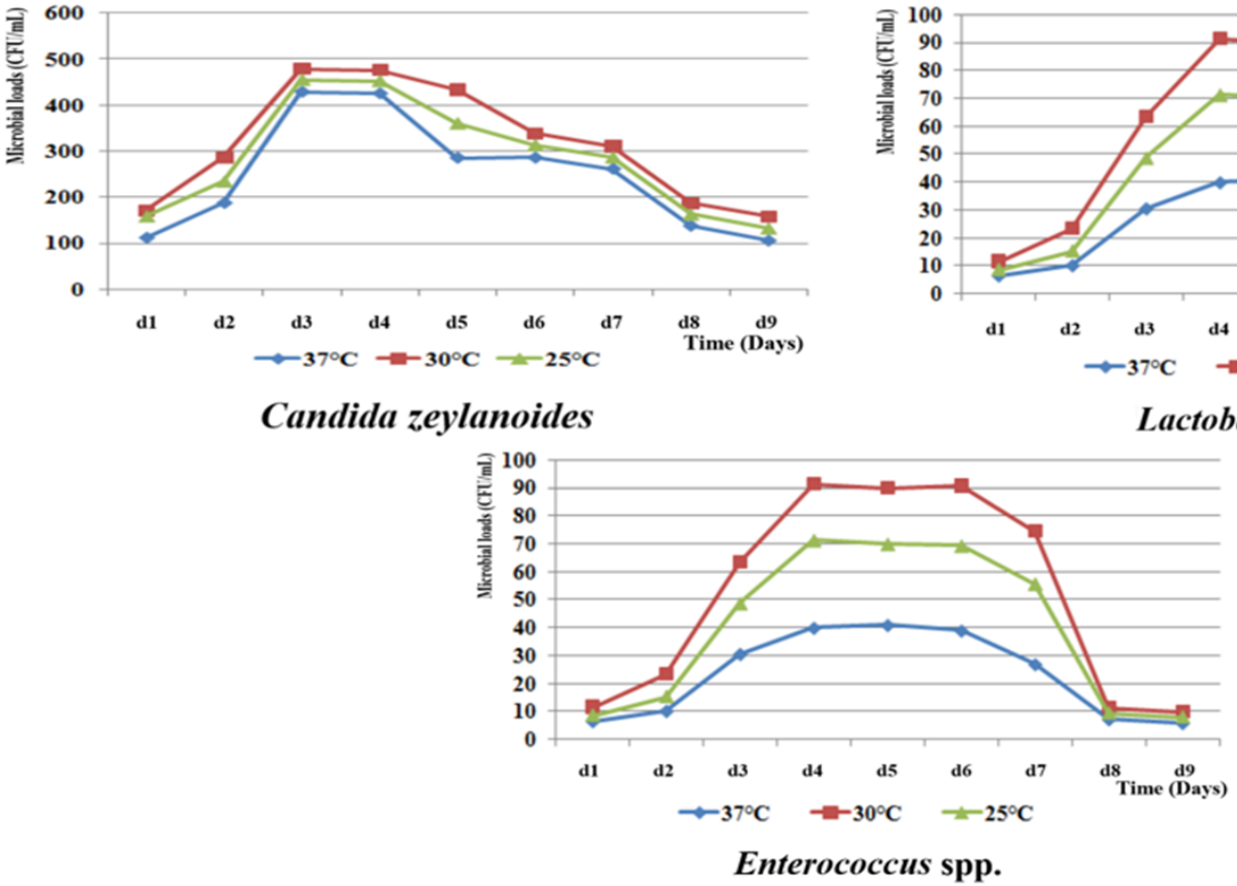

Fig. 6. Microbial population dynamics in fresh "cha'a"

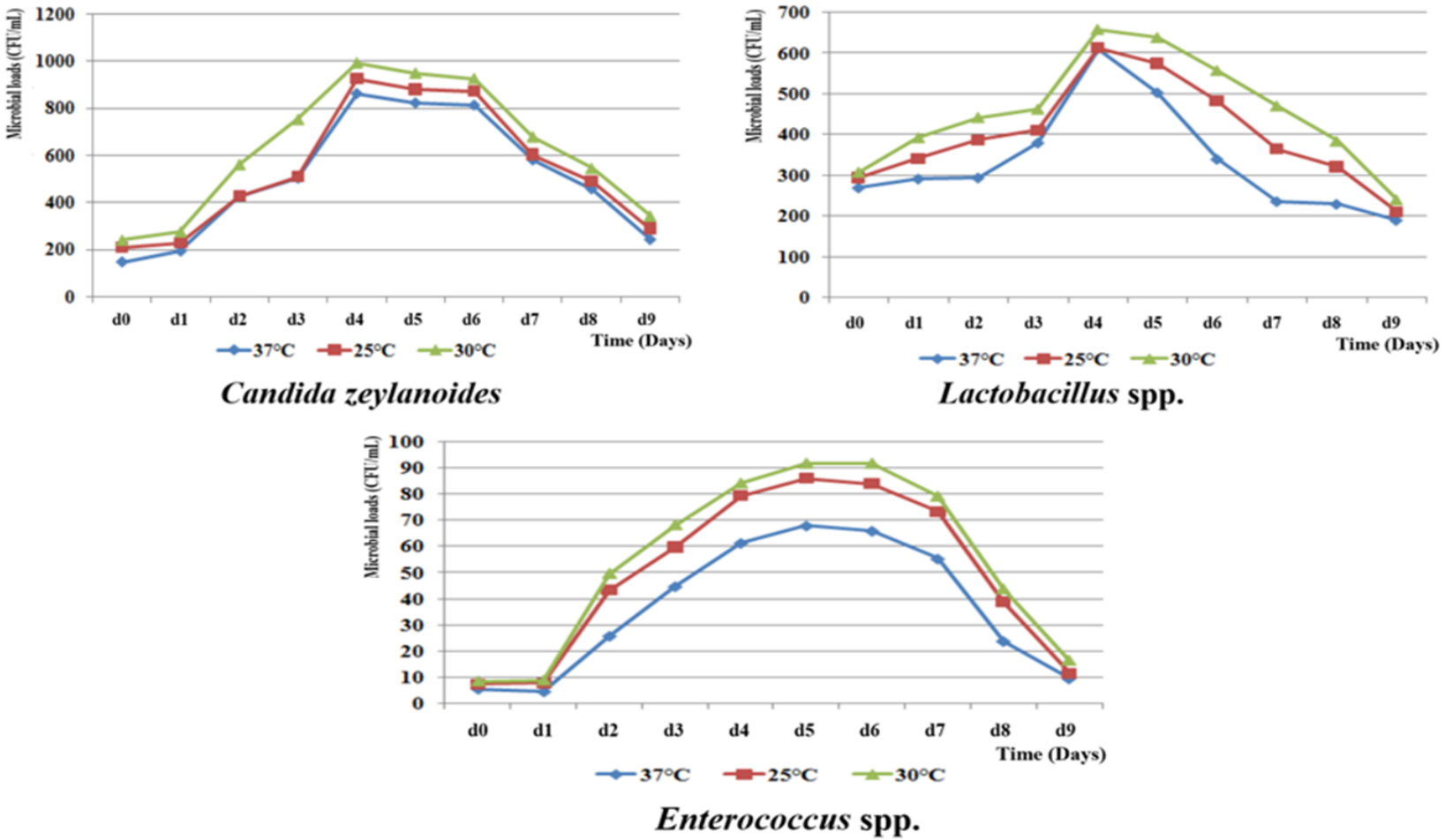

Fig. 7. Microbial population dynamics in fermented "cha'a"

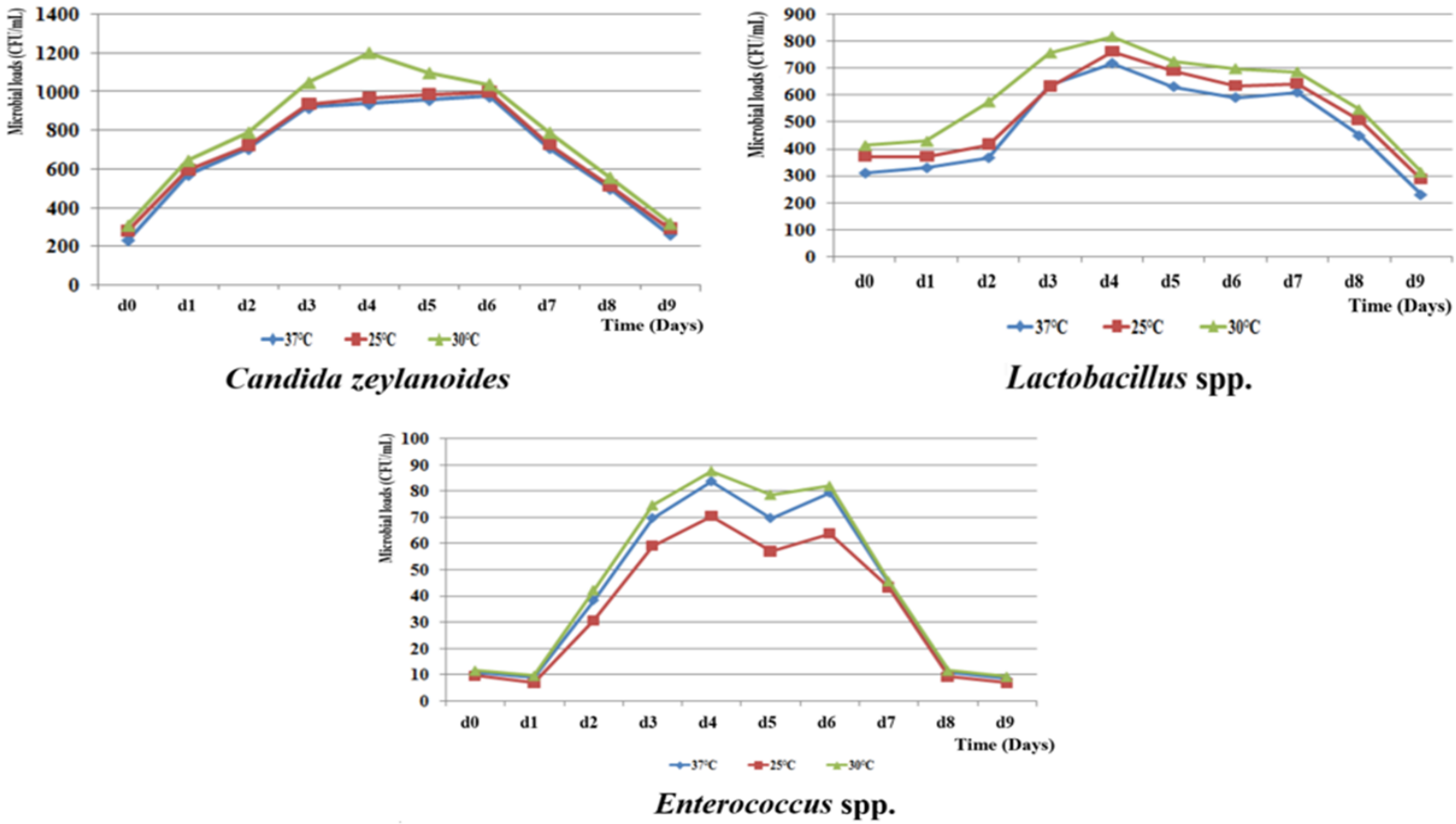

Fig. 8. Microbial population dynamics in fermented "arky"

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* was also identified as an 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” was approximately 8 times higher than the one recorded in the sweet “cha’a”. These results were evidence that the subjected resources could be used as raw materials for the production of alcohol. However, the presence of *Enterococci* indicated that they are also potential sources of microbial etiologies of infectious diseases. Therefore, specific measures should be taken by legal authorities to regulate the manufacture and distribution of these drinks for consumer safety. Overall, findings from the results of present investigation matched up with some studies or reports on artisanal drinks, highlighting that proper understanding of the microbial fermentation processes could significantly contribute to improved food safety and quality, generate income and valorize local cultural inheritance.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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