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Storage behaviour and functionality of a heat stabilized starter derived from an African opaque sorghum beer

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Tchoukoutou is a traditional sorghum opaque beer produced and consumed in Benin. It is obtained from an uncontrolled fermentation of sorghum wort using a traditional starter called "*kpete-kpete*". The present study assessed the effects of storage duration on the physicochemical and microbiological characteristics as well as on the functional properties of the heat stabilized starter produced from "*kpete-kpete*". The physicochemical characteristics of the stabilized starter were not significantly affected by the storage duration. After 60.0 days of storage, the dry matter, pH and titratable acidity were stable with mean values of 92.5%, 4.76 and 1.33 g/kg, respectively. However, the microbial characteristics of the product were significantly affected by the storage duration. Yeasts, lactic acid bacteria (LAB) and total mesophilic aerobic counts were modified following three phases: a latency phase (between 0 and 10 days), an exponential growth phase (between 10 and 40 days) and the decline phase (between 40 and 60 days). As compared to the stabilized starter, the physicochemical characteristics of "*kpete-kpete*" were extensively modified after 15 days of storage. In the traditional "*kpete-kpete*", all the major groups of microorganisms, reached their maximum growth phase after 10 days of storage suggesting that they would have lost their fermentation power afterwards. The fermentation test performed using stabilized starter sampled at different growth phases, revealed that the starter of 25 days of storage possesses better fermentation capacity. In these conditions, the fermentation duration was reduced to 16 instead of 24 h.

Key words: Sorghum, opaque beer, *tchoukoutou*, starter, storage duration.

INTRODUCTION

Sorghum is an important nutrients source for millions of people in the semi-arid regions where it is a main staple (Lyumugabe et al., 2012). Cumulative to its adaptability to harsh environmental conditions, being tolerant to drought and low-input conditions, sorghum contains diverse phytochemicals with various functional properties (Shumba, 1994; Nkongoloand and Nsapato, 2003). Sorghum ranks fifth among cereals worldwide (Grubben and Partohardjono, 1996) and is directly used or transformed into various food commodities including

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traditional alcoholic beverages (Dicko et al., 2006). Commonly called traditional opaque beers, these sorghum beverages play a significant role in African people cultures (Aka et al., 2008). Tchoukoutou, a traditional opaque sorghum beer of Benin, is produced, sold and consumed as street food all over the country. Like many other opaque sorghum beers in Africa, tchoukoutou has a sour taste, relatively high dry matter content (5 to 13%) and low alcohol content (2 to 3%) which make it suitable beverage for adults and teenagers (Agu and Palmer, 1998; Briggs et al., 2004). Considering the large quantity that is daily consumed, it is regarded as a significant source of dietary nutrients. Moreover, tchoukoutou significantly contributes to the diets of millions of people and generates income for the women sellers who produce it at household level, using the traditional technology (Kayode et al., 2007). Basically, the manufacturing process consists in malting, sun drying, milling, souring, boiling, mashing and alcoholic fermentation (Odunfa, 1985; Haggblade and Holzapfel, 1989). The fermentation constitutes a major step in the process. Prior to the alcoholic fermentation, the wort is inoculated with a traditional starter called *kpete-kpete* which is harvested from the previous fermenting beer. But the preservation of such a starter is a tedious and chancy business, since it may lose its fermenting properties within three days. In order to ensure quality safety and product stability, the stabilization of *kpete-kpete* in the form of a lasting starter is necessary. The procedure to stabilize *kpete-kpete* in the form of granule starter was previously described (Kayode et al., 2012). However, the storage behaviour and the functionality of such stabilized starter is not yet investigated. The objectives of the present study are two-fold. Firstly, to assess the effects of storage conditions on the physicochemical and microbiological characteristics of the stabilized starter. Secondly, to determine the maturation period of the improved starter that warrant its efficient functionality.

MATERIALS AND METHODS

Production of stabilized granule starter

Traditional starter, locally known as *kpete-kpete*, was collected from a "tchoukoutou" producer, in one commercial processing site, Abomey-Calavi town. The samples were collected in sterile bottles, packed in ice cold box and transported to the laboratory. Four kilograms of cleaned sorghum grains were dehulled using a mini-PRL dehuller (Thiés, Sénégal) and then ground. The flour obtained was mixed with distilled water (42% w/w), inoculated with 10% (w/w) of *kpete-kpete*, after being warmed up at 70°C for 15 min. The inoculated flour was allowed to ferment in a plastic bucket with lid for 24 h before being granulated. The wet granules were oven dried for 24 h at 43°C and then conditioned.

Experimental design

In order to compare the ability of both traditional and stabilized starters to preserve their functional properties during the storage, subsamples (50 g) of each of them were prepared and stored at room temperature for a period of 40 days (traditional starter) and 60 days (improved starter). Samples of the traditional starter could not be stored beyond 40 days because after this period they were totally spoiled. Samples were withdrawn every 10 days' time and analyzed in triplicate for physicochemical and microbiological characteristics. This stage helped to identify different growth phases (latency, exponential and stationary phases) of the microorganisms. The study of the functional properties of the stabilized starters focused on their ability to ferment the sorghum wort. For this purposes, samples (50 g) of granules starters of 5 days, 25 days and 50 days of maturation corresponding to latency phase, exponential phase and stationary phase were respectively used to inoculate 1 L of sterile sorghum wort (autoclaved at 121°C for 1 h). The inoculated wort was allowed to ferment for 24 h at room temperature and samples were withdrawn at 0, 8, 16 and 24 h in triplicate for microbiological analysis.

Physico-chemical analysis

Dry matter was determined according to the AACC method (AACC, 1984). The pH was determined using a digital pH meter (HI 8418; Hanna instruments, Limena, Italy) calibrated with buffers at pH 4.0 and 7.0 (WTW, Weilheim, Germany). The titratable acidity, expressed as lactic acid, was performed by using the method described by Nout et al. (1989). The refractive index was measured using a refractometer (Sopelem 9596, France).

Microbiological analysis

Duplicate samples of starter were diluted in 90 ml sterile peptone physiological saline solution (5 g peptone, 8.5 g NaCl and 1000 ml distilled water, pH = 7.0) and homogenized with a Stomacher lab-blender (type 400, London, UK). Decimal dilutions were plated. Total counts of aerobic mesophilic (TC), lactic acid bacteria (LAB) and yeasts were enumerated as described by Hounhouigan et al. (1993). Total mesophilic aerobic bacteria counts were determined on plate count agar (PCA, oxoid, CM 325, Hampshire, England) after incubation at 30°C for 72 h. Lactic acid bacteria (LAB) were determined on Man Rogosa Sharpe Agar (MRSA, CM 361, Oxoid, Hampshire, England) with incubation in anaerobic at 30°C for 72 h. Yeasts were determined on oxytetracyclin glucose yeast extract agar (OGYA, Oxoid CM 0545, Basingstoke, Hampshire, England) containing oxytetracyclin, after incubation at 25°C for 72 h.

Data analysis

Data were analyzed with the SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Comparison of the mean values was performed applying the one-way analysis of variance (ANOVA) by using a mixed model with two factors (repetitions and type of starter).

RESULTS AND DISCUSSION

Effects of storage duration on the physicochemical characteristics of the starters

The results of ANOVA showed that the type of starter, the storage duration as well as their interactions significantly affected ($p< 0.01$) the pH, the titratable acidity and the dry matter contents of the starters (Table 1). Figure 1 showed the compared effects of storage duration on

Table 1. Results of analysis of variance on repeated measures for the traditional starter and the improved starter physicochemical and microbiological characteristics.

Sources of variation	DF	Dry matter (%)	pH	Titratable acidity (g/kg d.w)	Yeasts (log_{10}CFU ml^{-1})	LAB (log_{10}CFU ml^{-1})
Types of starter	1	0.002**	0.001**	0.003**	0.112	0.003**
Storage duration	4	0.015*	0.000***	0.000***	0.000***	0.000***
TS*SD	4	0.002*	0.000***	0.000***	0.000***	0.000***

DF= Degree of freedom *: significant : **: highly significatif ; ***: very highly significant; LAB: lactic acid bateria; TS: type of starter; SD: storage duration.

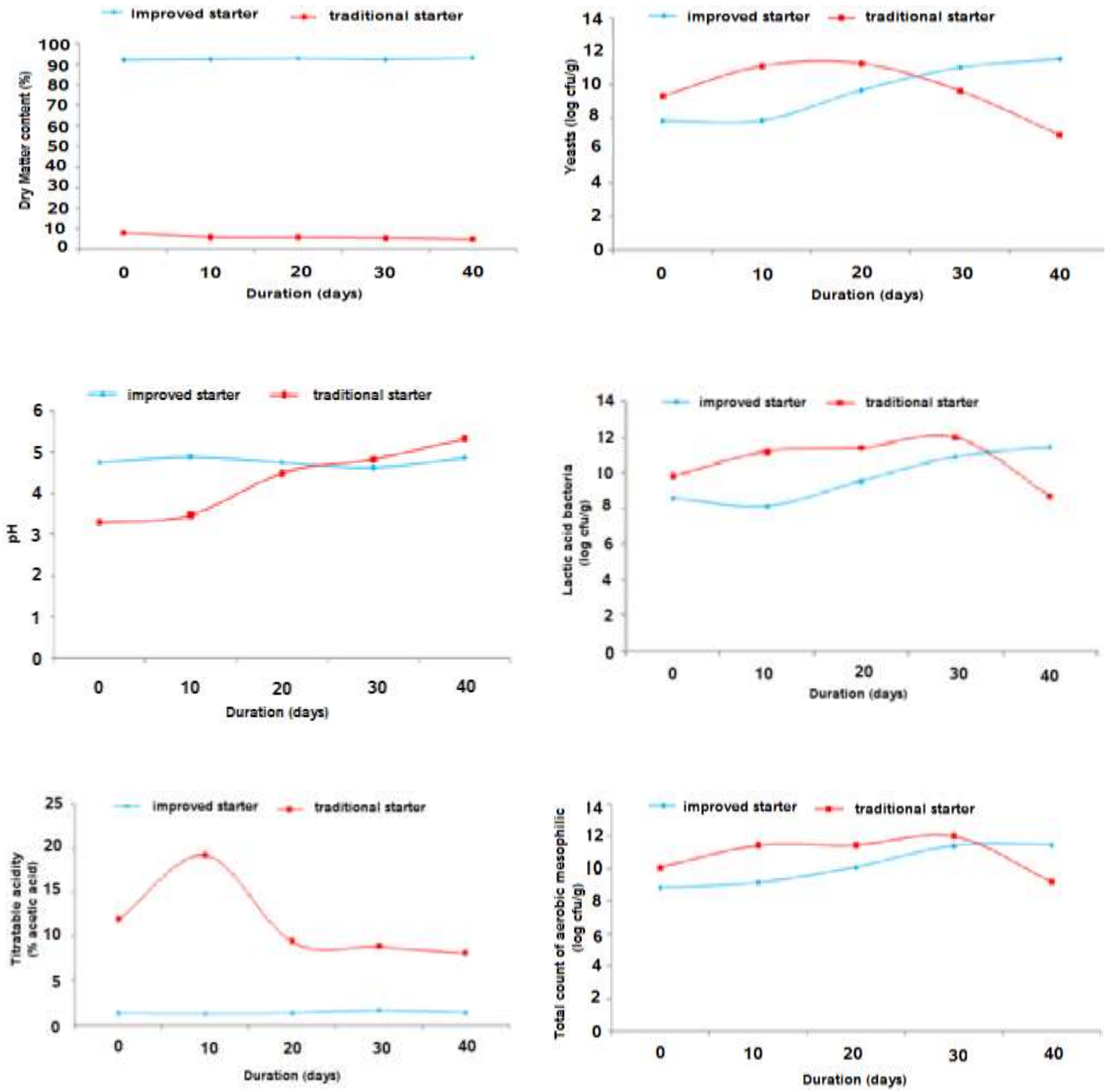

Figure 1. Changes in physicochemical and microbial properties of *kpete-kpete* (traditional) and granule starter (improved) during storage.

Table 2. Trend of Physicochemical characteristics in the improved starter over 60 days of storage

	0 day	10 days	20 days	30 days	40 days	50 days	60 days
Dry matter content (%)	92.07± 0.74[a]	92.43±0.15[a]	92.86±0.22[a]	92.35±0.07[a]	93.11±0.63[a]	92.5±0.51[a]	92.24±0.24[a]
pH	4.75±0.01[a]	4.89±0.01[b]	4.75±0.04[a]	4.62±0.05[c]	4.87±0.01[b]	4.62±0.01[c]	4.79±0.01[ab]
Titratable acidity (g/kg dw)	1.23±0.02[a]	1.18±0.01[b]	1.24±0.00[ac]	1.60±0.01[d]	1.28±0.01[be]	1.43±0.01[f]	1.32±0.01[e]

Values with the same letter are not significantly different from each other ($p < 0.05$).

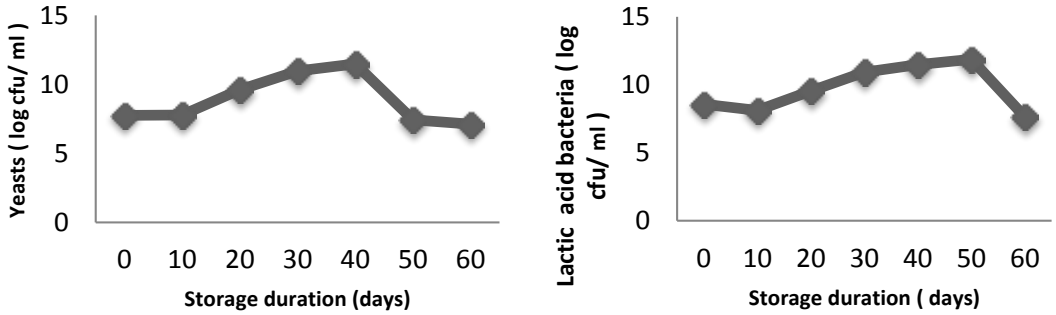

Figure 2. LAB and yeasts trend in the improved starter over 60 days of storage.

physicochemical characteristics of the traditional and improved starters. Within 10 days of storage, the dry matter content of the traditional starter significantly ($p<0.05$) decreased from 8.07 to 5.8 %. In opposite, the dry matter content of the improved starter remained stable throughout 40 days of storage. The mean value of the dry matter content of the improved starter was 92.5% which corresponds to a water content of 7.5%. Such level of humidity is desirable since it is favorable for a longer product shelf life. It was demonstrated that cereals products were preserve well with a water content < 12% (Cecil, 1992). As it shown in Figure 1, the pH value of the traditional starter increased from 3.30 to 5.30, whereas it remained quite stable in the improved starter (4.75) throughout the storage course. With respect to the titratable acidity, it also remained stable in the improved starter during the 40 days of storage, whereas it significantly increased from 12.00 to 19.30 $g.kg^{-1}$dw in the traditional starter within 10 days of storage. Thereafter, it gradually decreased from 19.30 to 8.20 $g.kg^{-1}$dw at 40 days of storage. Clearly, the results showed that the traditional starter could not be stable for more than 10 days, whereas the improved starter was stable within 40 days of storage. Moreover, the physicochemical characteristics of the improved starter were even stable up to 60 days (Table 2). Thus, the stabilized starter bears promise for a long shelf life while preserving its physicochemical properties. It had been reported that the use of stabilized starter can improve the hygienic quality and acceptability of African traditional foods (Gran et al., 2003). Such an observation was reported by Sawadogo-Lingani et al. (2008) for *dolo* in Burkina Faso, Glover et al. (2009) and Adewara et al. (2013) for *burukutu* in Nigeria, Orji et al. (2003) for *pito* in Nigeria and N'guessan et al. (2010) for *chakpalo* in Côte d'Ivoire. Thus, it could be expected that the use of the stabilized starter can contribute to reduced variations in the organoleptic and microbiological quality of African sorghum beers (Kirmaryo et al., 2002).

Effects of storage duration on the microbiological characteristics of the starters

The major groups of microorganisms involved in the fermentation of opaque sorghum beer are LAB and yeasts (Djè et al., 2009; Coulibaly et al., 2014). After the drying treatments applied, the functional microflora of the stabilized starter granule remained viable. In the improved starter stored for 60 days, yeast, LAB and total counts of aerobic mesophilic were modified following three phases: a latency phase (between 0 and 10 days), an exponential growth phase (between 10 and 40 days) and the decline phase (between 40 and 60 days) (Figure 2). The level of total counts of aerobic mesophilic, LAB, and yeasts were 8.87, 8.57 and 7.76 log cfu g^{-1}, respectively. In the traditional starter, *kpete-kpete*, as much as 10.10, 9.80 and 9.29 log cfu ml^{-1} was detected for the total counts of aerobic mesophilic, LAB and yeasts, respectively. The results of ANOVA showed that the type of starter, the storage duration as well as their interactions significantly affected ($p<0.01$) the total microbial count, the LAB and the yeasts counts of the starters (Table 1). Changes in LAB and yeasts composition of the starters during the storage process

Table 3. Results of analysis of variance showing the effects of storage duration and time of wort fermentation on yeasts and LAB contents of sorghum beer.

Source	DF	Yeasts ($log_{CFU\ ml}^{-1}$)		LAB ($log_{CFU\ ml}^{-1}$)	
		F-value	Prob. > F	F-value	Prob. > F
Storage duration (days)	2	20099.9	<0.001	5229.75	<0.001
Time of fermentation (hours)	2	157321	<0.001	294529	<0.001
Duration x time of fermentation	4	2718.20	<0.001	527.90	<0.001

DF = Degree of freedom, F-value = value Fisher's statistic, Prob.= probability of the test.

are shown in Figure 1. In the improved starter, yeasts count is stable for the first 10 days which corresponds to the latency phase. Afterwards, the yeasts level increased gradually from 7.78 log cfu g^{-1} at the 10^{th} day of storage to 11.48 log cfu g^{-1} at the 40^{th} day of storage. On the other hand, in the traditional starter, yeasts count increased from 9.29 log cfu ml^{-1} and reached its highest value of 11.48 log cfu ml^{-1} at the 15^{th} day of storage. Thereafter, it started decreasing to reach a level of 6.96 log cfu ml^{-1} at the 40^{th} day of storage. Growth trend for the LAB and total counts of aerobic mesophilic bacteria was similar to that of yeasts. Overall, the improved starter is more stable than the traditional starter in terms of physical, chemical and microbiological characteristics. The traditional starter remained stable only for 15 days while the improved starter preserved its microbiological characteristics over 60 days. Since the fermentation is the cumulative effect of the growth of yeast on wort, ultimately resulting in production of alcohol in the growth medium (Lodolo et al., 2008), the improved starter can be expected to trigger an effective fermentation process.

Effects of storage duration on the functional properties of the improved starter

Functional properties of the improved starter were assessed through its ability to ferment the sorghum wort. Thus, the effects of storage duration on the functional properties of the improved starter were evaluated using a fermentation test. In this respect, improved starter sampled of various storage durations, that is, 5, 25 and 50 days of storage, corresponding to the three growth phases were respectively used to inoculate different samples of sterile sorghum wort. The inoculated sorghum wort was allowed to ferment for 24 h. LAB and yeasts were enumerated at 0, 8, 16 and 24 h. Results of the analysis of variance showed that storage duration of the improved starter significantly affected ($p < 0.001$) the growth pattern of LAB and of yeasts during the wort fermentation. Likewise, the fermentation duration significantly affected ($p< 0.001$) the LAB and yeasts growth in the wort. Moreover, the interactive effects of starter duration x fermentation duration significantly affected ($p < 0.001$) the functional microflora concentration in the wort (Table 3). LAB and yeast were simultaneously affected by the storage duration of the improved starter, the fermentation duration and the interactive effects of starter duration x fermentation duration. A symbiotic relation between yeast and LAB in the wort was reported (Munyaja et al., 2003). LAB created an acid environment favorable to the proliferation of yeasts which produce vitamin and increase other factors such as amino-acids for the growth of LAB (Lyumugabe et al., 2010). Holzapfel (1997) reported that African opaque beers are typically characterized by a lactic fermentation followed by an alcoholic fermentation in which initially, LAB and later yeasts play the dominant role. Due to their higher growth rate, bacteria typically dominate the early stages of fermentation. Clearly, yeasts grew faster in the wort inoculated with starter of 25 days of storage than in wort inoculated with starters of 5 and 50 days of storage. At 16 h of fermentation, the maximal value of yeasts count (9.36 log cfu ml^{-1}) was reached in worts inoculated with 25 days starter; whereas in worts inoculated with days and 50 days matured starters, it took 24 h of fermentation to reach the maximum of this value (6.67 and 7.74 log cfu ml^{-1}, respectively) (Figure 3). Similar trends were observed for LAB count. At 16 h of fermentation, the count of LAB in the wort inoculated with the starter of 25 days storage (8.0 log cfu ml^{-1}) was significantly ($p<0.001$) higher than values obtained in worts inoculated with starters of 5 days (5.5 log cfu ml^{-1}) and 50 days of storage (5.5 log cfu ml^{-1}). The use of the starter of 25 days permitted to reduce the fermentation duration to 16 h instead of 24 h. Similar findings were obtained by some authors. Sawadogo-Lingani et al. (2008) tested three kinds of improved starter cultures for dolo (sorghum beer in Burkina Faso) and found a reduction of the fermentation duration. The same with N'guessan et al. (2010) for the tchakpalo (sorghum beer in Côte d'Ivoire) and Orji et al. (2003) for pito (sorghum beer in Nigeria).

Conclusion

The heat stabilized *kpete-kpete*, in the form of granules, had preserved physicochemical and microbiological characteristics and functional properties better than the

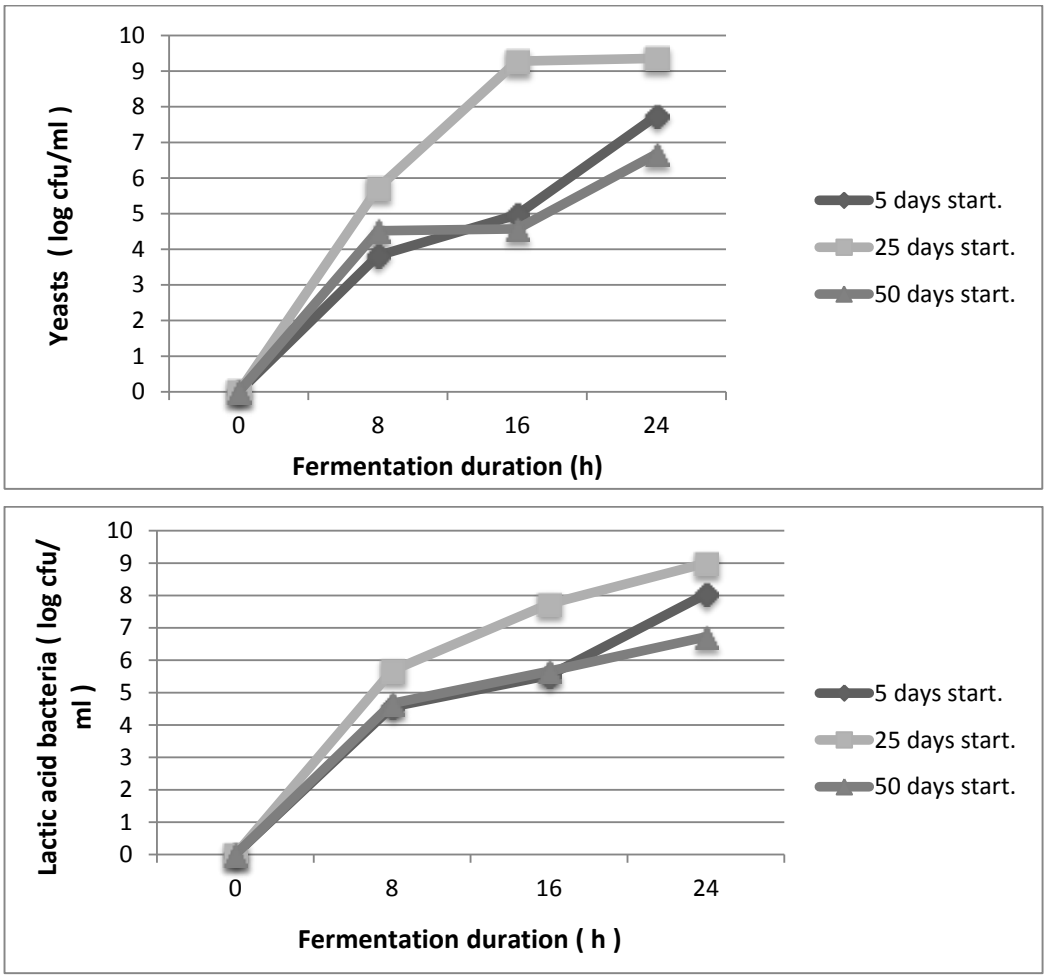

Figure 2. Fermentation efficacy of starter granules sampled at various storage times as established after inoculation in sterile sorghum wort.

traditional starter. The improved starter reached its maturation at 25 days of storage where its ability to ferment the sorghum wort was significantly better than that of starters stored for 5 or 50 days. Therefore, the 25 days stored starter could be used to produce a sorghum beer at shorter fermentation time with adequate physicochemical and microbiological properties.

Conflict of interests

The authors have not declared any conflict of interests.

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