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Effect of Traditional Starter Inoculation Rate on Sorghum Beer Quality

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Abstract: In the way to determine, the effect of inoculation rate of traditional starter on sorghum beer quality, fermentations were initiated with different inoculation rates [0% (control), 0.5, 1, 1.5 and 2% (w v^{-1})] at 35°C. Then fermentation parameters such as utilization of sugars and production of CO_2, alcohol, organic acids and yeast biomass were assayed during the process. At the beginning of the fermentation, sugar consumption rate increased with inoculation rate. But from 8 h of fermentation, all inoculated systems shown similar consumption rate and led to beers with the same sugar content. The lag phase in CO_2 production was shorter at high inoculation rate and the maximal production speed was reached at the same time for fermentation systems with 1, 1.5 and 2% (w v^{-1}) inoculum. Moreover, ethanol contents from 8 h of fermentation were not significantly different except in the control system. Maximum yeast populations were similar in the inoculated systems but they were reached at different times. Among organic acids which contribute to the organoleptic quality, only tartaric and citric acids were influenced by inoculation rate.

Key words: Inoculation rate, sorghum beer, fermentation, yeast, sugars, ethanol, organic acids

INTRODUCTION

Fermentation is one of the oldest technologies used for food production and preservation (Holzapfel, 2002; Motarjemi, 2002). Many benefits are attributed to this technology. As reported by Motarjemi (2002), it preserves and enriches food, improves digestibility and enhances the taste and flavour of foods. It is also an affordable technology and is thus accessible to all populations. Furthermore, fermentation has the potential of enhancing food safety by controlling the growth and multiplication of a number of pathogens in foods. Its importance in modern-day life is underlined by the wide spectrum of fermented foods marketed both in developing and industrialised countries (Holzapfel, 2002).

Indigenous fermented foods were developed through traditional or village-art methodologies, which were preserved over the years, in order to maintain the uniqueness and identity of these foods (Valyasevi and Rolle, 2002). Several traditional fermented products exist in different African countries and include non alcoholic beverages, alcoholic beverages, breads, pancakes, porridges, cheeses and milks. They are prepared from both plant and animal materials, using processes in which microorganisms, by virtue of their metabolic activities, play an active role in the physical, nutritional and organoleptic modification of the starting material (Aidoo, 1994). These microorganisms come from microbial populations associated with the raw materials, equipment and local environments or a residue from a previous fermentation batch.

The use of a residue from a previous fermentation batch is called back-sloping. Through this practice of back-sloping, the initial phase of the fermentation process is shortened, the risk of fermentation failure reduced and it results in the promotion of desirable changes during the fermentation process (Holzapfel, 2002).

The alcoholic fermentation of tchapalo, a traditional sorghum beer from Côte d'Ivoire, is conduced most of the time by back-sloping. Brewers use dried yeast harvested from previous brews (Yao *et al*., 1995). Drying enhances the self life of dehydrated starters. But, sundrying as reported by Holzapfel (2002) may destroy some microorganisms and thereby reduce viable numbers, while slow and insufficient airdrying during the rainy season may result in contamination and poor quality starters. Moreover, the amount of this traditional starter used in each fermentation process depends on brewer experience.

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To date, there have been no investigations published regarding the impact of dried yeast on the traditional sorghum beer quality. In this study, information was provided on the effect of different amount of dried yeast used to initiate the alcoholic fermentation on tchapalo quality. The utilization of sugars and the production of CO_2, alcohol, organic acids and yeast biomass were used as parameters.

MATERIALS AND METHODS

Fermentation experiment: The fermentation medium was made from final wort and dried yeasts. The wort was obtained from randomly identified commercial *tchapalo* brewer at Williamsville-Macaci and dried yeasts from randomly identified commercial *tchapalo* brewers at Abobo-Soghefia, Williamsville-Macaci and Cocody-Blockosso in the district of Abidjan (Southern Côte d'Ivoire).

Fermentations were performed in 500 mL Erlenmeyer flasks filled with 400 mL of final wort and covered with a cotton cap. The flasks were incubated at 35°C and the dried yeasts were inoculated at five different amounts [0% (control), 0.5, 1, 1.5 and 2% (w v^{-1})] without shaking for 12 h.

Fermentations were repeated three times and samples were taken at 0, 4, 8 and 12 h from fermenting wort for analyses.

Gas released from fermenting wort: In parallel with fermentation experiments, the volume of gas produced during fermentation was measured using an experimental device described by Pol (1996). The gas released was measured as quantity (mL) of displaced water per hour.

Determination of sugars in the fermenting wort: The Total Soluble Solids (TSS) content, expressed as °Brix value, were determined in each sample using a hand refractometer and water-soluble carbohydrates were determined by the phenol sulphuric acid method, according to Dubois *et al.* (1956).

Ethanol content: Ethanol was determined by gas-chromatographic analyses, carried out using a Shimadzu CG-14A gas chromatograph. Fermenting wort samples (2 μL) were filtered and injected directly. The temperature was set at 8°C min. Injector and detector temperatures were 200 and 250°C, respectively. Helium at 2 Kg cm^{-2} was used as the carrier gas and the external standard method was used for the quantitative determination of ethanol.

Determination of organic acids

Sample preparation for HPLC: Samples were firstly centrifuged at 3000 rpm for 20 min. Then, they were filtered through a 0.2 μm Millipore membrane filters (Sartorius AG, Goëttingen, Germany) and stored at -20°C until analyses.

HPLC equipment and operating conditions: The HPLC system (Shimadzu Corporation, Japan) was equipped with a pump (Shimadzu LC-6A Liquid Chromatograph), a detector (Shimadzu SPD-6A UV Spectrophotometric detector) and an Integrator (Shimadzu C-R 6A Chromatopac). Chromatographic separation was performed using an ion-exclusion ORH-801 column (300×6.5 mm, Interchrom, France). The eluant was 0.004 N H_2SO_4 with a flow rate of 0.8 mL min^{-1} and the detector was set at 210 nm.

A 20 μL injection volume was used for HPLC samples. Analyses were done in duplicate and mean values were used.

The organic acid standards were diluted in distilled water at concentrations ranging from 0.05-0.4 g L^{-1}. The standards were filtered and injected separately.

Components were identified and quantified by comparison of their retention times and peak areas with those of standards.

Microbiological analysis: The fermenting wort samples were directly diluted in ten-fold series in Buffered Peptoned Water (BIO-RAD, France) and aliquots (0.1 mL) were plated in duplicate on Sabouraud Chloramphenicol agar medium (BIO-RAD, France). After incubation at 30°C for 3-5 days, yeasts were enumerated on plates displaying 30-300 colonies and results were expressed as Log (cfu mL^{-1}) of sample.

Statistical analysis: The results were statistically evaluated by one way analysis of variance (ANOVA) with the software Statistica, 99 Edition. Statistical differences with $p<0.05$ were considered significant.

RESULTS

Sugars consumption: Figure 1a shows the effect of increasing inoculation rate on Total Soluble Solids (TSS) depletion. From 8 h of fermentation, all fermentation systems initiated with the traditional starter presented TSS contents which were statistically identical. It was the same way for sugar consumption rate (Fig. 1b). Values were 6.99, 8.26, 8.9 and 8.72 g /L/h at 8 h and 6.43, 6.64, 6.67 and 6.64 g/L/h at 12 h fermentation time respectively for systems with 0.5, 1, 1.5 and 2% (w v^{-1}) of inoculation rate.

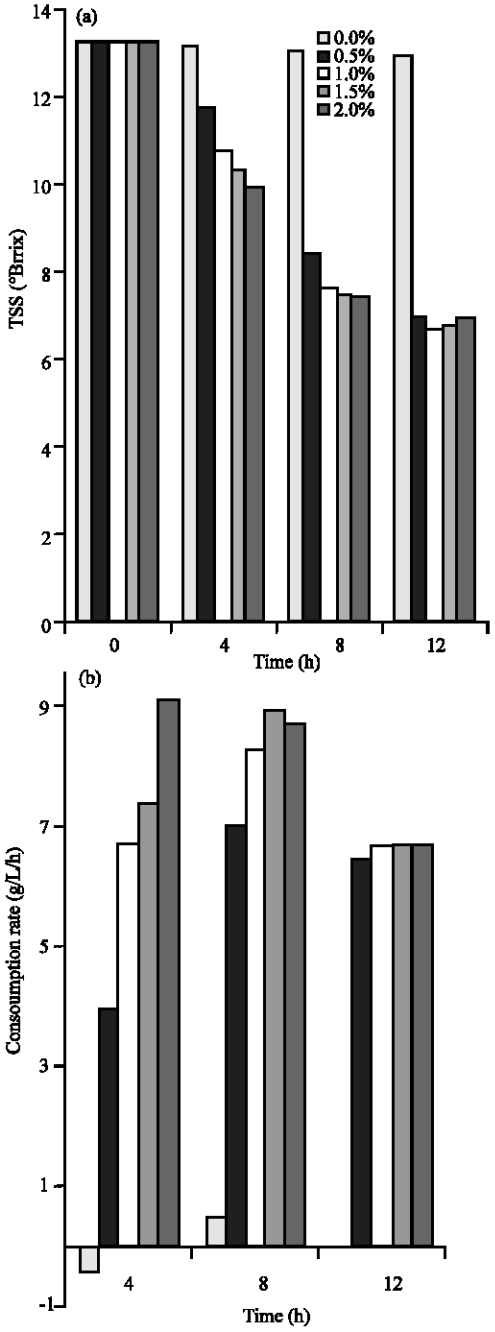

Fig. 1: Effect of increasing inoculation rate on (a) Total Soluble Solids (TSS) depletion and (b) sugar consumption rate during alcoholic fermentation of sorghum beer

We also observed a decrease of sugar consumption rate between 8 and 12 h of fermentation. The higher the inoculation rates, the higher the decrease. In sorghum beers produced after 12 h of fermentation, contents in residual total sugars were respectively 106.94, 29.40, 26.86, 26.44 and 26.81 g L^{-1} in systems of control and initiated with 0.5, 1, 1.5 and 2% (w v^{-1}) of dried yeasts.

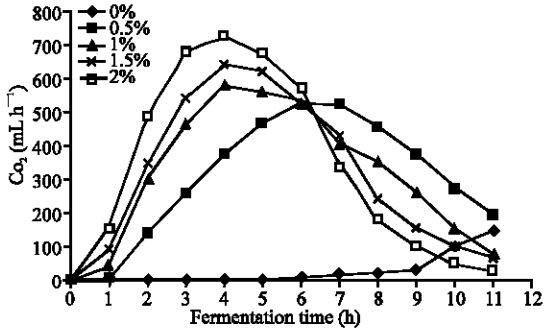

Fig. 2: Gas released during alcoholic fermentation of sorghum beer with inoculation rates at 35°C

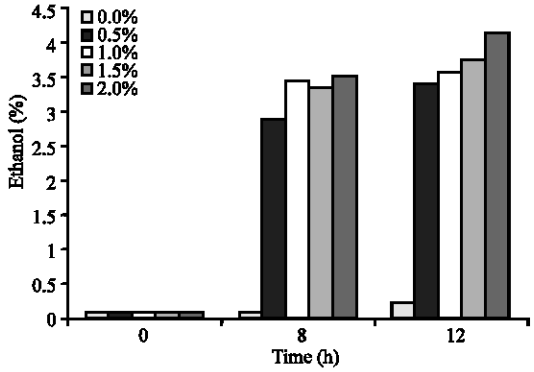

Fig. 3: Effect of increasing inoculation rate on ethanol production during alcoholic fermentation of sorghum beer

CO_2 production during alcoholic fermentation: The lag-phase in CO_2 production was shorter at a higher inoculation rate (Fig. 2). Indeed, it was about 9 h when any traditional starter was added on the sorghum wort, 1h with 0.5% of dried yeasts, <1 h with 1 and 1.5% of dried yeasts and practically inexistent with 2% of inoculum. Besides, during the exponential phase of production, CO_2 produced was more and more important when the inoculation rates used were raised more. However, the maximal production speed was reached at the same time (at 4 h) for fermentation systems with 1, 1.5 and 2% of dried yeasts. The system with 0.5% reached its maximal speed after 6 h and control had not reached the maximum at 11 h.

During the speed decline phase, CO_2 production was weaker in systems initiated with elevated inoculation rates.

After 11 h of fermentation, volumes of gas produced were respectively 0.32, 3.57, 3.72, 3.75 and 3.98 L for the control system and systems with 0.5, 1, 1.5 and 2% of inoculation rate. Except for the control system, the statistical analyses showed that these values are not significantly different ($p>0.05$).

Effect of increasing inoculation rate on ethanol production: The effect of increasing inoculation rate on ethanol production during alcoholic fermentation is shown on Fig. 3. Except in the control system, ethanol contents were not significantly different ($p>0.05$) in all systems after 8 and 12 h of fermentation. The values were 0.1, 2.88, 3.44, 3.35, 3.52% (v v^{-1}) at 8 h and 0.25, 3.43, 3.59, 3.71 and 4.15% (v v^{-1}) at 12 h fermentation time respectively for control system and systems with 0.5, 1, 1.5 and 2% of inoculation rate. We also noticed a slowing of ethanol production between 8 and 12 h fermentation time except for the control system. This slowing resulted in a decrease of ethanol production rate during the same time (not shown). Indeed, the production rate passed from 3.12-2.38, 3.66-2.41, 3.48-2.55 and 3.80-2.91 g/L/h, respectively in wort initiated with 0.5, 1, 1.5 and 2% of inoculation rate. This decrease was bigger in system with 1% inoculum.

Effect of inoculation rate on organic acids profile: Figure 4 shows the effect of increasing inoculation rate on sorghum wort organic acids profile. The production or the consumption of oxalic, malic, lactic, fumaric and propionic acids was not significantly influenced by the increase of the inoculation rate. Only tartaric and citric acids presented significant differences ($p<0.05$). Thus, at 8 h of fermentation, the tartaric acid concentration was maximal (1.95 g L^{-1}) in system with 1.5% of inoculum and minimal (0.94 g L^{-1}) in the control. After 12 h of fermentation, the system with 1% of inoculum presented the maximal content (1.81 g L^{-1}) and the control, the minimal content (1.07 g L^{-1}).

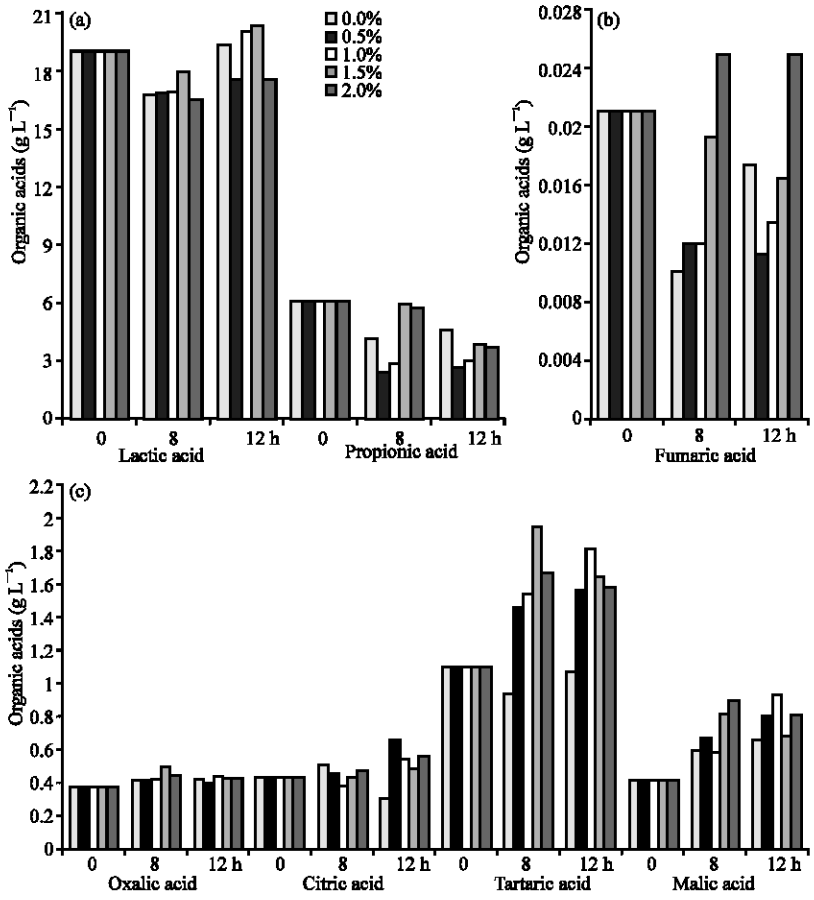

Fig. 4: Effect of increasing inoculation rate on major (a), minor (b) and intermediate (c) organic acids profile during alcoholic fermentation of sorghum beer

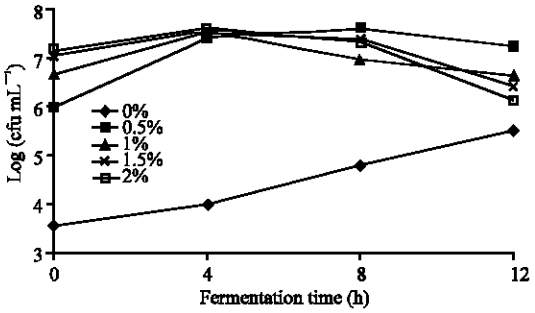

Fig. 5: Yeast growth during alcoholic fermentation of sorghum beer under different inoculation rates

Concerning citric acid, concentrations were similar at 8 h for all fermentation systems, but at 12 h of fermentation, the content was maximal with 0.5% of inoculum and minimal in the control.

Yeast growth at various inoculation rates: Inoculation rate grouped in three different profiles of growth (Fig. 5). In control system, yeast cells number increased until the end of fermentation, passing from 3.54-5.51 Log (cfu mL^{-1}). In the system with 0.5% of inoculation rate, the yeast cells number increased and reached the maximal value of 7.59 Log (cfu mL^{-1}) at 8 h of fermentation, after which the cells slightly died off, reaching 7.22 Log (cfu mL^{-1}).

In systems with 1, 1.5 and 2% of inoculation rate, the yeast cells reached a maximum cell concentration of 7.53-7.61 Log (cfu mL^{-1}) after 4 h of fermentation. Subsequently, the yeast cells slowly died off, reaching 6.12-6.63 Log (cfu mL^{-1}) after 12 h. The cells death was higher between 4 and 8 h for system with 1% of inoculum and between 8 and 12 h for systems with 1.5 and 2%. Moreover, at the end of fermentation, the concentration of yeast cells was lower for systems initiated with more elevated inoculation rates.

DISCUSSION

The alcoholic fermentation of *tchapalo*, a traditional sorghum beer produce in Côte d'Ivoire is conducted by pitching the final sorghum wort most of the time with dried yeast harvested from previous brews (Yao *et al.*, 1995). In this study, several fermentation parameters such as utilization of sugars and production of CO_2, alcohol, organic acids and yeast biomass were assayed in a way to determine the effect of inoculation rate on the beer quality.

The practice of back-sloping accelerates the initial phase of fermentation and results in the promotion of desirable changes during the fermentation process. Through this practice, the risk of fermentation failure is also reduced (Holzapfel, 2002). In our study, the initial phase was reduced from 9-1 h or less when we used dried yeast as starter. The inoculum contained large numbers of desirable microorganisms in active state which were adapted to the substrate. Increasing inoculation rate increased also the number of these microorganisms and resulted into the reduction of the initial phase time. This is in accordance with the studies by several authors in which the duration of the lag phase depends inversely on the size of the inoculation (Augustin *et al.*, 2000). Although, the lag times were different considering the inoculation rate, fermentation systems with 1, 1.5 and 2% reached their maxima speed of CO_2 production at the same time (after 4 h of fermentation). This result was closely related to the growth of yeast which shown that in these fermentation systems, the yeast cells reached a maximum cell concentration after 4 h fermentation time. Furthermore, until 6 h fermentation time, CO_2 production rate was more elevated at highest inoculation rates. The most intensive fermentation rates due to the load and the faster microflora development at highest rates might explain this observation (Gotcheva *et al.*, 2001).

Sugars were utilized as carbon and energy sources by yeasts during the fermentation. So, sugars consumption rate is related to yeast cells concentration. During the first hours of fermentation, fermenting worts did not contain the same yeast concentration as fermentations were initiated with different rates of inoculum. Thus, sugars consumption rate increased with inoculation rate. But after 8 h of fermentation and above, we found no significant effect of the inoculation rate on the sugars consumption rate for all systems inoculated although they did not contain the same yeast cells concentration. This finding may indicate that there were factors which influenced sugars uptake after 8 h of fermentation and above. The main incriminated factors are nitrogen limitation and ethanol inhibitory effect (Barre *et al.*, 1998; Manginot *et al.*, 1998). It was reported that the amount of assimilable nitrogen influences the synthesis of sugar transporters (Bisson, 1999), while ethanol influences sugar transport yield and exercises direct inhibition (Leao and Van Uden, 1985; Walker, 1998). Our results demonstrated clearly that sugar contents of sorghum beer were not affected by inoculation rate over 0.5% after 8 h of fermentation and above. This observation was closely related to the

ethanol content. Moreover, the decrease of ethanol production rate observed between 8 and 12 h fermentation time might be due to the decrease of yeast cells concentration during the same time.

Maximal yeast populations were similar for all the inoculated systems although they were not reached at the same time. It was thought to be due to nitrogen limitation Bely *et al*., 1990). Manginot *et al*. (1998) reported that nitrogen depletion in the medium is one of the main factors stalling cell division. Thus, inoculation rate did not affect the maximal yeast population attained but affect the time necessary to reach it.

The presence of weak organic acids mainly determines the acidity which is one of the most important organoleptic parameters in *tchapalo*. As mentioned by Herrero *et al*. (1999) for the wine, the composition and concentration of each acid is essential for the quality of the final product. They also act as a buffer and thus the cytosolic pH of yeast and its metabolism during fermentation can not be affected (Torija *et al*., 2003). Most of organic acids present in the final wort were not consumed or produce sufficiently during the alcoholic fermentation. Meanwhile, we observed slight fluctuations. On contrary to Aka *et al*. (2008), wort contained tartaric acid which content was influenced by the increase of the inoculation rate as citric acid.

The definitive conclusion as inoculation rate of traditional starter affects sorghum beer quality requires further study. However, the present study is the first to demonstrate clearly that at fermentation time uses in the traditional process (at least 8 h), the inoculation rate affects only tartaric and citric acids contents.

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