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Technical sheet of influence of freeze-dried yeast starter cultures on volatile compounds of tchapalo, a traditional sorghum beer from Côte d'Ivoire

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ABSTRACT

The production of the Ivorian sorghum beer known as *tchapalo* remains more or less an empirical process. The use of starter cultures was therefore suggested as the appropriate approach to alleviate the problems of variations inorganoleptic quality and microbiological stability. In this study, we evaluated the capacity of *S. cerevisiae* and *C. tropicalis* to produce sorghum beer as freeze-dried starter in mixed or pure cultures. Beers produced with mixed freeze-dried cultures of *S. cerevisiae* F12-7 and *C. tropicalis* C0-7 showed residual sugars and ethanol contents similar to beers obtained with S. cerevisiae F12-7 pure culture, but the total sum of organic acids analyzed was the highest with the mixed culture (15.71 g/L). Higher alcohols were quantitatively the largest group of volatile compounds detected in beers. Among these compounds, 2-phenyl ethanol, a higher alcohol that plays an important role in beer flavor, was highly produced with the mixed culture (10174.8 µg/L) than with the pure culture (8749.9 µg/L).

Indexing terms/Keywords

mixed starter; *Saccharomyces cerevisiae*; *Candida tropicalis*; volatile compounds; sorghum beer

Academic Discipline And Sub-Disciplines

Food and technology sciences

SUBJECT -CLASSIFICATION

Biotechnology and Microbiology

TYPE (METHOD/APPROACH)

Experimental

INTRODUCTION

Sorghum beer is a traditional fermented beverage from most of the West African countries where sorghum is produced. It is known as *tchapalo* in Côte d'Ivoire and by various other names in other African countries. The composition and sensory character of the beer is mainly determined by the metabolism of microorganisms during fermentation. Those microorganisms, by the virtue of their metabolic activities, play an active role in volatile compounds production. Like other beers, sorghum beer is one of the most delicate and labile beverages from a sensory point of view. It has a complex but moderate taste and aroma and shows poor flavor stability.

The use of starter culture was thus suggested to alleviate the problems of variations in organoleptic quality and microbiological stability [1,2]. During the brewing of sorghum beer, the alcoholic fermentation stage is performed by several yeast strains, among which *Saccharomyces cerevisiae* was the predominant strain [3–4]. Among the non-*Saccharomyces* yeasts, the predominant species varied according to geographic origin of the beer. In recent years, growth of non-*Saccharomyces* yeasts has been observed also in fermentation inoculated with *S. cerevisiae*, suggesting a greater contribution of these yeasts to the flavour and quality of alcoholic beverages than previously thought. Beers obtained from inoculation tests with these starters showed organoleptic and sensory characteristics comparable to beers produced in the traditional way. However, these starter cultures were introduced during fermentation in the form of fresh microbial suspensions, which are difficult to maintain for extended periods. To overcome this problem, the use of dried starter cultures, active and stable during storage, is the viable and sustainable solution. Furthermore, such starter preparations require the use of cheap raw materials in order to be economically feasible. An enhanced stability of microorganisms can be achieved by adding many protective compounds, such as disaccharides, polyols, monosaccharides, skim milk, and other organic molecules [5]. Recently, we showed the possibility of producing freeze-dried starter culture for the brewing purpose of *tchapalo* with sucrose and cassava flour as the best protective agent and support material, respectively [6].

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In this study, we aimed to evaluate the capacity of *S. cerevisiae* and *C. tropicalis* to produce sorghum beer as freeze-dried starter culture. Organic acids, sugars, ethanol and volatile compounds were therefore determined in the produced beers.

MATERIALS AND METHODS

Materials

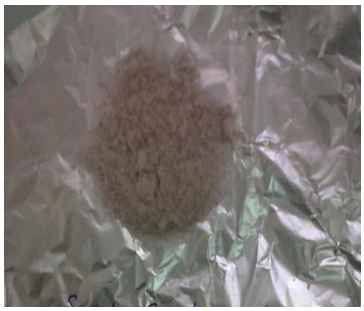


Figure 1. Yeasts freeze drying in sucrose solution and cassava flour

Figure 2. Red sorghum grains

Figure 3. Sweet wort

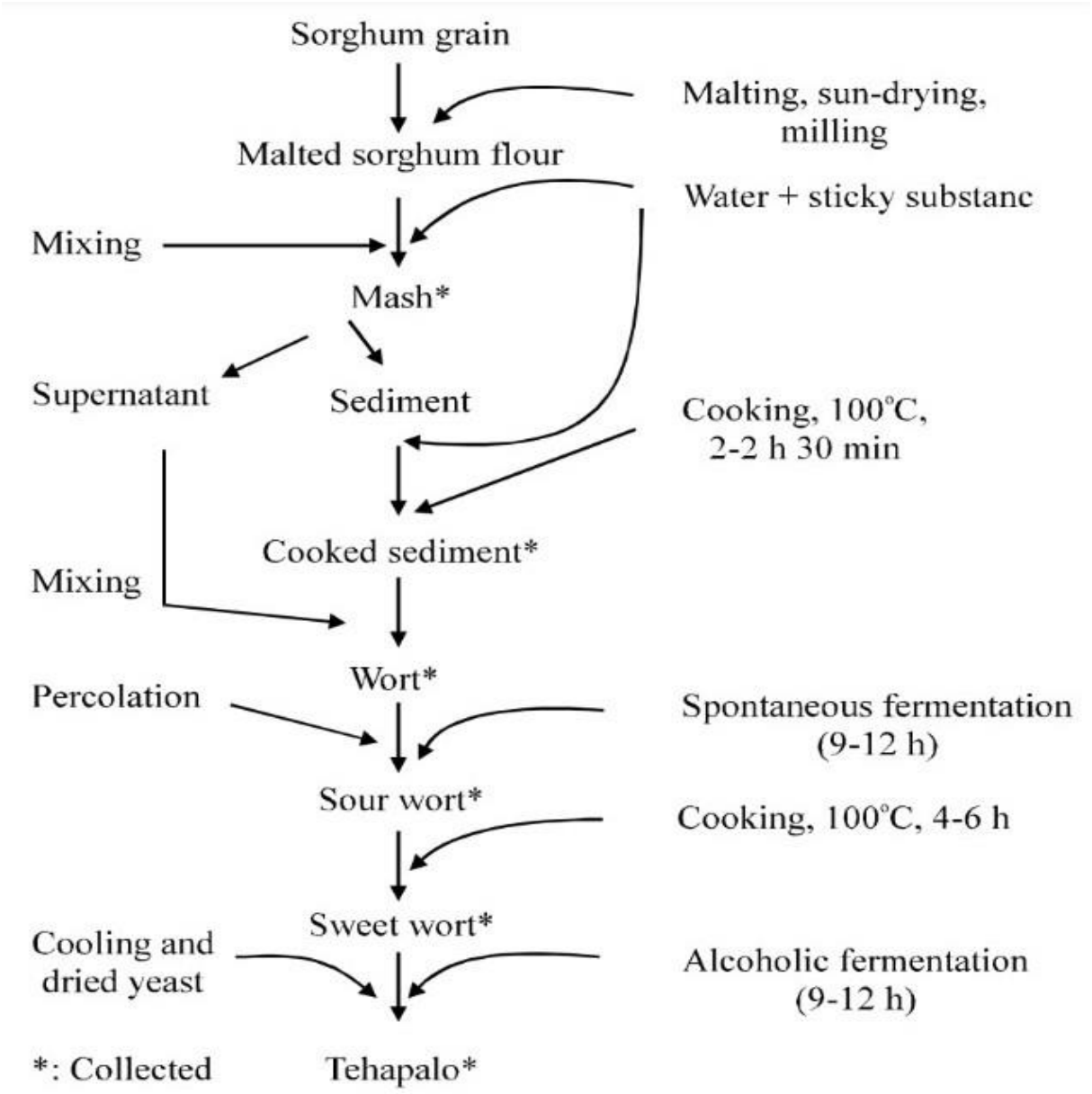
The yeasts strains of *S. cerevisiae* F12-7 and *C.tropicalis* C0-7 were freeze-drying in cassava flour and sucrose solution respectively as support and as protective agent (Figure 1). Two starters were being carried, pure culture of *S. cerevisiae* F12-7 and the mixture of *S. cerevisiae* F12-7/ *C. tropicalis* C0-7 (1:2, v/v). The traditional sorghum beers were usually prepared by the method described above (Figure 4). The red sorghum grains (Figure 2) were the main vegetable material which was transformed to sweet wort (Figure 3).

Methods

Glucose, maltose, ethanol and organic acids (lactic, malic, oxalic, propionic, tartaric, citric and acetic acids) were separated and quantified by HPLC (Agilent Technologies, 1200 series, UK) using a column Aminex HPX-87H, 300 mm × 7.8 mm (Biorad).

The volatile compounds (esters; highers alcohols; acids) were analyzed with a Hewlett Packard (Agilent Technologies) 6890 gas chromatograph equipped with a CTC Combi PAL Autosampler AOC-5000 (Shimadzu, Columbia, MD, USA) and coupled to an HP 5973 mass spectrometry detector (HP, now Agilent Technologies).

The analysis of variance (ANOVA) and Tukey tests and Levene (t-test) were performed with the STATISTICA software, 99th Edition, to compare variables analyzed the beers. Statistical differences with $p < 0.05$ were considered significant.



(A) **(B)**

Traditional bottled sorghum beers

Beer (A) from pure culture of *S. cerevisiae* F12-7 and beer (B) from the mixture of *S. cerevisiae* F12-7/ *C. tropicalis* C0-7

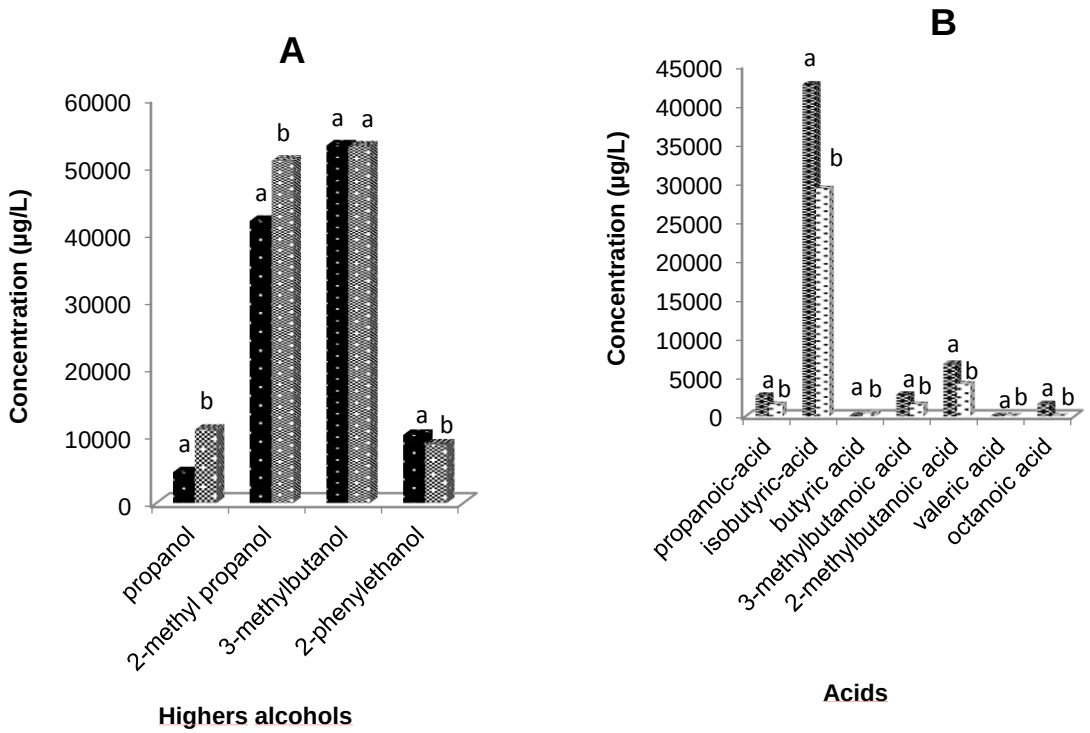
Figure 4. Diagram of traditional sorghum beer process

RESULTS AND DISCUSSION

Except for tartaric and malic acids content, all the others fermentation characteristics of the mixed culture were similar to those of the *S. cerevisiae* F12-7 pure culture. Tartaric acid was not found in beer obtained with the mixed culture, while it was about 8 mg/L in beer fermented by the pure culture. Contrary to tartaric acid, malic acid was produced during the fermentation and its content was higher in mixed culture (4.7 g/L) than pure culture (2.7 g/L). In the beers produced, the total sum of organic acids analyzed was the highest with the mixed culture (15.71 g/L). These compounds are important in several respects. Firstly, they contribute to lowering the pH during fermentation and, secondly, they influence the sourness attribute, although some acids have their own flavour and aroma characteristics [7]. This is consistent with previous results, which reported that non-*Saccharomyces* yeasts are characterized as secondary compound producers [8, 9].

The use of new fermentation technologies for optimizing beer quality and producing beers with particular flavor profiles is one of the worldwide trends in brewing [8, 10,11]. In this context, there is a growing demand for new and improved sorghum beer yeast strains to obtain beers with constant organoleptic characteristics. Different studies have shown that apiculate yeasts in pure and mixed starters produced lower amounts of higher alcohols when compared to *S. cerevisiae* [10, 12–13], which corresponded to our assays. In fact, among the beers, *S. cerevisiae* F12-7 pure culture consistently produced the highest amount of propanol (10947.8 µg/L) and 2-methylpropanol (50795 µg/L), while the mixed culture produced the highest amount of 3-methylbutanol (53054.2 µg/L) and 2-phenylethanol (10174.8 µg/L) (Figure 5A).

Acids are quantitatively the second largest group (Figure 5A). Most of the compounds in this group (propanoic, isobutyric, 3-methylbutanoic and 2-methylbutanoic acids) were highly found in beers produced with the mixed culture. The esters found in the sorghum beers are acetate and ethyl esters, with ethyl lactate and ethyl propionate forming the bulk of the esters (Figure 5C). The highest amount of these compounds was obtained with *S. cerevisiae* F12-7 pure culture. The mixed culture had lower amounts of ester compounds than the pure culture, which is consistent with others studies [14,15]. In contrast, several authors commented that the presence of apiculate yeasts in the starter did not affect ethyl esters production [13]. These different results may be due to yeast characteristics, medium composition and fermentation parameters, which could affect the production of esters. Ethyl lactate and ethyl propionate were the esters found in the highest quantities in the produced beers. Ethyl lactate, which has a mild, buttery and creamy odor with hints of fruit and coconut, can have a significant influence on the taste and character of sorghum beers. However, like other esters, it can to some extent be the basis of organoleptic defects.



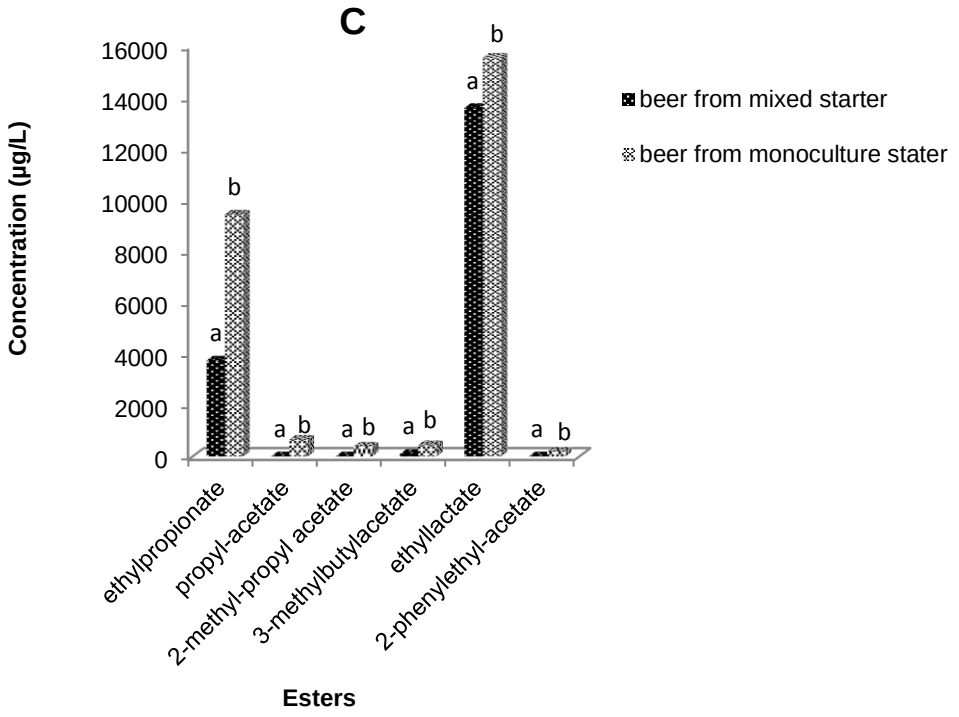


Figure 5. Volatile compounds in sorghum beers produced with mixed and pure freeze-dried starter cultures; (A): Higher alcohols; (B): Acids; (C): Esters. Histograms with the same letters are not statistically different ($p \geq 0.05$).

CONCLUSION

The mixed culture produced the highest content of organic acids, especially malic acid. Analysis of volatile compounds demonstrated that produced beers differed on their higher alcohol, acid and ester contents. The beers produced from pure culture of *S. cerevisiae* F12-7 were characterized by a relatively high concentration of esters, while those from the combination of *S. cerevisiae* and *C. tropicalis* were instead characterized by a high concentration of 2-phenylethanol, a higher alcohol that plays an important role in beer flavour. The mixed freeze-dried culture of *S. cerevisiae* F12-7 and *C. tropicalis* C0-7 is thus proposed as a promising starter for the production of sorghum beers and more studies should be conducted, especially examining the sensory qualities of the product and general acceptability by consumers compared to traditional beer.

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Author' biography

Coulibaly Wahauwouélé Hermann was born in September 1979 in Fronan in Ivory Coast. In 2009, he starts the PhD studies at Nangui Abrogoua University to Abidjan, Ivory Coast after get the grade of Master II degree in Microbiology and Biology molecular. In 2014 he go to France to Institut National Sciences Appliquées to Toulouse for PhD training at Laboratory of engineer of biological systems and process, he was working assays of fatty acids in yeast membranes and genes expression *TIF11* and *YJL144W*, and SUPAGRO Montpellier with Pr Peggy Rigou for the assays of volatiles compounds. During three years, he has participated to several activities organized by AUF (Agence Universitaire de la Francophonie) at Hammamet (Tunisia), Yaoundé (Cameroon), Bingerville (Ivory Coast). In September 2016 he has get the PhD in Biotechnology and Food Microbiology. Since January 2017, he is researcher at Laboratory of Biotechnology and Food Microbiology at Food Science and Technology department to Nangui Abrogoua University, Abidjan, Ivory Coast.

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