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RESEARCH ARTICLE

EVALUATION OF THE CHARACTERISTICS OF YEAST SPECIES ISOLATED FROM THE TERMITE *MACROTERMES SUBHYALINUS* AND THEIR POTENTIAL TO PRODUCE SORGHUM BEER.

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Abstract

In the way to found the best starter cultures for sorghum beer production, three yeast strains [one *Issatchenkia orientalis* (L1) and two *Candida tropicalis* (L2 and L6)] isolated from the digestive tract of termite *Macrotermes subhyalinus* were tested in pure culture and co-culture (L1-L2, L1-L6, L2-L6 and L1-L2-L6) in the production of sorghum beer. The effect of initial pH on the growth and acidifying power, the ability to grow in the presence of ethanol, the evolution of biomass and the physicochemical properties of the beers produced were determined. The results showed that all the starters had the ability to grow at acid (3; 4; 5.5), neutral (7) as basic (9) pH conditions but the highest growth varied with the culture medium initial pH. They had an acidifying capacity which was better expressed when the initial pH was greater than 5. The starter L1 was the best acidifying starter at all initial pH with values of 0.4, 1.3, 2.6 and 4.1, respectively at pH 4, 5.5, 7 and 9. Their growth was not completely inhibited by the ethanol up to 7.5% but the starter which better face to ethanol toxicity varied according to incubation time. These starters had the capacity to grow in sorghum sweet wort and fermented it at temperatures of 30°C, 35°C and 40°C. The principal component analysis revealed that the beers obtained at 30°C and 35°C with starters L2-L6, L1-L2-L6 on one hand and L1-L2, L1-L6, L2 on the other hand had similar characteristics.

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Introduction:-

Fermented food and beverages play a major role in the diet of African populations. Fermentation is the key process in the formulation of these products. It has been shown to improve their organoleptic and nutritional qualities. This biochemical reaction is mainly due to the action of microorganisms.

The production of sorghum beers tchapalo in particular involves lactic acid bacteria which initiate the process by acidifying the environment, thus promoting the activity of the yeasts responsible for alcoholic fermentation (Yao et al., 2009).

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The marketing of this sorghum beer constitutes an important source of income for brewers who represent a considerable segment of the population. These traditional brewers, however, encounter enormous difficulties such as the very short shelf life, a very unstable final product, organoleptic qualities very variable. These multiple problems generate overall low profitability (Maoura et al., 2006). The majority of these difficulties are very often due to the nature of the ferment used. Indeed, this one generally comes from a residue of previous fermentation. So it is a ferment of unknown composition and consequently uncontrollable.

Its use often leads to a final product of poor quality, which inevitably undergoes a rapid microbial alteration, therefore marketable only over a short period of time resulting in loss of income. Moreover, apart from the fermenting germs that this type of ferment provides, there are pathogens which confer to these drinks a character that is dangerous for the health of consumers. Today, the agri-food sector, and particularly the handicraft sector, is under severe constraints due to the socio-cultural and economic parameters. Traditional brewers include in this great ensemble do not escape this reality. They must face to the unavoidable requirements that are mainly the diversity of products, the price-quality ratio and the security of the consumer.

In order to find solutions, several investigations were carried out. Some of them described the production processes (Chevassus-Agnes et al., 1976, Haggblade and Holzapfel, 1989, N'Da and Coulibaly, 1996, Maoura et al., 2006). Others have identified and characterized the microorganisms involved in this process (Sefa-Dedeh et al., 1999, Van der Aa kühle et al., 2001, Glover et al., 2005, Maoura et al., 2005, Sawadogo-Lingani et al., 2007).

In this same vision, the use of starter cultures has been suggested as the best strategy to solve these problems and provide the necessary tools for standardization of the product (Holzapfel, 2002, Coulibaly, 2016).

Adhering to this proposal, researchers are increasingly focusing on the identification of fermenter strains and the formulation of starters cultures (Maoura et al., 2005, Sawadogo-Lingani et al., 2008; Glover et al., 2009; N'guessan et al., 2010; Adewara et al., 2013).

The problem is that most of these work is focused on the only microorganisms involved in the beers making process studied. In fact, strains from either the traditional ferment or the sorghum beer were the subject of these studies. Yet, the stage of alcoholic fermentation, which is one of the crucial steps in the production of traditional beers, requires starter cultures made up of yeasts possessing physiological and technological properties for its smooth running. All strains of yeast which possess technological properties for the production of sorghum beer are to be found, including strains isolated from this beer and those of various origins. Under these conditions, the isolated yeast of the digestive tract of the termite *Macrotermes subhyalinus* could be an alternative. In this work, we propose to evaluate the fermentation potential of yeasts isolated from the digestive tract of the termite worker *Macrotermes subhyalinus* in the production of sorghum beer.

Material and methods:-

2-1- Material

Three yeasts [one strain of *Issatchenkia orientalis* (L1) and two strains of *Candida tropicalis* (L2 and L6)] and their combination which were used as starter in this study were belonged to the culture collection of the Department of Food science and Technology (University of Nangui Abrogoua). They were isolated from the digestive tract of termite *Macrotermes subhyalinus* workers. They were identified by PCR-RFLP of the ITS region and sequencing of D1/D2 domains.

2-2- Study of the interactions between the different yeast strains on agar medium

The yeast strains were individually pre-cultured in 10 ml of Sabouraud chloramphenicol broth and incubated at 30°C for 24 h with constant stirring (150 rpm). A volume of 200 µL of the suspension of each strain was inoculated in the mass of Sabouraud chloramphenicol agar. Subsequently, the strain (L1, L2 or L6) was seeded on the surface of the agar which does not contain it in two places of the same Petri dish using a circular inoculator (Dutscher, France). Thus, we have the advantage of having a closed inner zone, so that any substance diffused by the strain seeded in the mass will be accumulated at this place, while outside it will be able to diffuse freely. A concentration effect was thus obtained inside the yeast rings. The Petri dishes were thus incubated at 30°C for 48 h. The observation of a contact zone between the two strains makes it possible to define the type of interaction. It would be an inhibition if the contact zone is more clear or transparent than the bottom of the yeast culture. On the other hand, if it is more turbid,

it would be a stimulation. Finally when there is no difference with the rest of the box, we could say that there is no interaction.

2-3- Physiological characterization of yeast strains

2-3-1-Growth at different pH

The yeast strains and their combinations were subjected to the effect of five different pH, namely pH 3; 4; 5.5; 7 and 9. The strains were previously cultured at 30°C for 48 h on Sabouraud chloramphenicol agar. Then, a colony of each strain was used for pre-culture in 2 mL of Sabouraud chloramphenicol broth. This pre-culture is then used with an initial charge of 0.1 optical density (OD) to inoculate 10 mL of Sabouraud chloramphenicol broth which pH was adjusted with 1 M phosphoric acid or with 1 M NaOH. The tubes were incubated in triplicate at 37°C with constant stirring at 150 rpm. After 36 h, the color and turbidity of each tube were noted as a simple indication of growth or not. The optical densities at 600 nm and the final pH values were read.

2-3-2- Resistance to ethanol

Growth assays in the presence of ethanol were adapted from Carrasco et al. (2001). Pre-cultures of the yeast strains to be tested were carried out in 10 mL of Sabouraud chloramphenicol broth and incubated at 30°C for 14 h. After adjusting its OD to 0.5, this pre-culture was used to inoculate 50 mL of Sabouraud chloramphenicol broth supplemented with 0%, 2.5%, 5% and 7.5% ethanol (v / v). The cultures were incubated at 30°C for 72 h with a stirring of 150 rpm. Samples were taken at 0 h, 4 h, 8 h, 12 h, 24 h, 36 h, 48 h and 72 h for the determination of the biomass at 600 nm.

2-4- Evaluation of fermentation potential of yeasts

2-4-1- Preparation of singular and multiple starters

In the case of singular starters, strains L1, L2 and L6 were inoculated in the Sabouraud chloramphenicol broth and incubated at 30°C for 24 h. From this broth, the Sabouraud chloramphenicol agar was inoculated and incubated at 30°C for 48 h. After incubation, a colony of each strain (L1, L2 or L6) was pre-cultured in 4 mL of Sabouraud chloramphenicol broth and incubated for 24 h. The optical densities (OD) of these pre-cultures were adjusted to 0.3 and then transferred individually into 25 mL of sterile sorghum sweet wort and incubated for 24 h to constitute the singular starters.

Multiple starters (L1-L2, L1-L6, L2-L6 and L1-L2-L6) were also formed by individual yeast strains in 4 mL of Sabouraud chloramphenicol broth and were incubated for 24 h.

In the case of combination of two strains, the OD of these different pre-cultures were adjusted to 0.15 and 2 mL of pre-culture was used per strain to induce the fermentation of 25 mL of sweet wort and incubated for 24 h. For the combination of three strains, 2 mL of pre-cultures at the concentration of 0.1 OD per strain were used and incubated for 24 h. The OD of this mixture was subsequently adjusted to 0.3 then 4 mL were used to inoculate 25 mL of the sorghum sweet wort and incubated for 24 h. Each of these different co-cultures then gave the multiple starters. The OD of these different starters were stabilized at 1.8 then 20 mL were used for the rest of the work.

Twenty (20) mL of each of these different starters was therefore used to induce the fermentation of 200 mL of sterile sweet wort contained in 500 mL. Erlenmeyer flasks were incubated at temperatures ranging from 30 to 40°C for 72 h and then aliquots of the wort in fermentation were taken at 0 h, 24 h, 48 h and 72 h for the physicochemical and microbiological analyzes

2-4-2- Physico-chemical and microbiological analyzes

The biomass was measured by the opacimetric method. Indeed, the disorder of a medium or its optical density was directly proportional to the cell concentration in the medium. Thus, the OD of the different samples was read at 600 nm. The measurements were made in duplicate and the averages were the different values considered.

Titratable acidity, expressed as a percentage of lactic acid, was determined by titrating the samples with 0.1 N NaOH to the phenolphthalein end point. Total Soluble Solids (TSS) content, expressed as °Brix value, was determined in each sample using a hand refractometer. Reducing sugars were determined by the method of Bernfeld (1955) using 3,5-Dinitrosalycilic acid (DNS) and water-soluble carbohydrates quantified by using the phenol sulphuric acid method according to Dubois et al. (1956). Based on the content of these two types of sugars, the saccharification rate was determined according to the following expression:

$$Tx.sacch = \frac{Tx.SR}{Tx.ST} * 100 \quad (1)$$

with
Tx.sacch = saccharification rate
Tx.SR = reducing sugar content
Tx.ST = total sugar content

2-5- Statistical analysis
Principal component analysis (PCA) was used to compare the starter cultures studied on the basis of their ability to reduce sugar. The PCA allow to group the measured variables into new variables called "components" or "factors". This grouping is based on the correlation of the variables. The XLSTAT software (Addinsoft Inc.) was used to perform the principal component analysis. The analysis of the Pearson correlation revealing the nature of the correlation between the studied parameters was carried out with the XLSTAT software (Addinsoft Inc.). Calculations and figures were made using EXCEL 2010 (XP - Microsoft Corp).

Results:-

3-1- Interaction between the strains
The results of the study of the interaction between the strains L1 and L2, L2 and L6 and then L6 and L1 illustrate by figure 1, showed a homogeneous growth of strains L1, L2 and L6 inside and outside the rings of L2, L6 and L1. These results indicate that there is no interaction between these different strains. None of them inhibited the growth of the other.

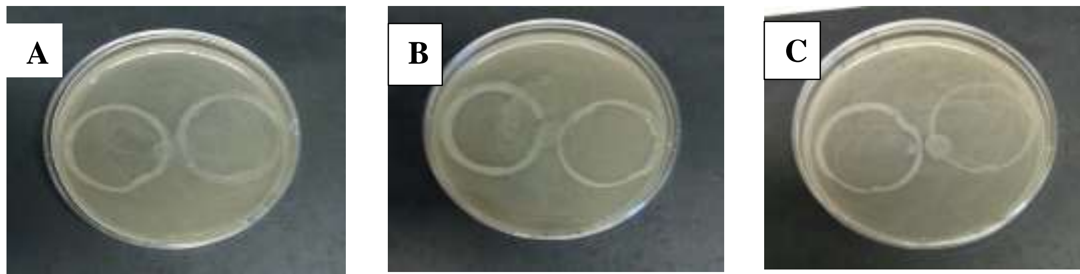

Figure 1:- Interaction between strains A: L2 (in rings) and L1 (in the mass); B: L6 (in rings) and L2 (in the mass); C: L1 (in rings) and L6 (in the mass)
L1**:** *Issatchenkia orientalis***,** L2 and L6**:** *Candida tropicalis*

3-2- Effect of initial pH (pHi) on yeast growth
Table 1 shows the growth rate of yeast starters. In general, the population of multiple starters was high for the pHi 3. The starter culture L1-L2-L6 exhibited the highest biomass with a value of 5.2 while L6 had the lowest biomass value of 3.5. At pH 4 singular starters showed an increase in their population while that of the multiple starters decreased overall. However it was the combination of strains L2-L6 that had the highest biomass with a value of 4.7 in contrast to L1-L2-L6 whose biomass of 3.5 was the smallest. At pH 5.5 the starter L2 had the highest biomass value of 4.5 and the smallest biomass was given by the starter L1-L2. At pH 7 and 9, the starter L1 had the largest biomass with values of 4.5 and 4.7, respectively, whilst L1-L2 had the smallest biomass. All the starters studied had the ability to grow at each of these different pH. But the starter L1 had the best behavior while the rising starter L1-L2 was slowed down by most of studied pH.

Table 1:- Growth rate of yeast starters at different initial pH

Starter culture	pH 3	pH 4	pH 5.5	pH 7	pH 9
L1	4.0 ± 0.3c	4.3 ± 0.1c	4.4 ± 0.2ab	4.5 ± 0.5a	4.7 ± 0.4a
L2	4.3 ± 0.01b	4.5 ± 0.0b	4.5 ± 0.0a	4.4 ± 0.0a	4.1 ± 0.0 c
L6	3.5 ± 0.0d	4.1 ± 0.0d	4.2 ± 0.0cd	3.8 ± 0.0bc	4.1 ± 0.0 c
L1-L2	4.5 ± 0.0b	4.1 ± 0.0d	3.6 ± 0.0e	3.6 ± 0.0 c	3.6 ± 0.0d
L1-L6	4.3 ± 0.0b	3.9 ± 0.0e	4.2 ± 0.0 c	4.1 ±0.0ab	4.1 ± 0.0 c
L2-L6	4.3 ± 0.0b	4.7 ± 0.0a	4.3 ± 0.0bc	4.4 ± 0.0a	4.2 ± 0.0b c
L1-L2-L6	5.2 ± 0.0a	3.5 ± 0.0f	4.1 ± 0.0d	3.9 ± 0.0c	4.5 ± 0.0ab

The values expressed are the means of three measurements. In each column, the mean values with the same letter are not significantly different ($p > 0.05$). L1**:** *Issatchenkia orientalis***;** L2 and L6**:** *Candida tropicalis;* L1-L2, L1-L6, L2-L6 and L1-L2-L6 are their combination

3-3- Effect of starter cultures on pH during their growth

The statistical analysis of the pH variation during the growth of the starters showed that those one had statistically different effects ($p <0.05$). However, we observed a reduction in pH by all our starters during their growth (figure 2). This effect is not significant when the pHi was 3. After this value, the pH-reducing rate increased progressively according to the initial pH values. The starter L1 differed from the others by the highest variations at all initial pH with values of 0.4; 1.3; 2.6; 4.1, respectively at pH 4; 5.5; 7 and 9. The variation in pH gradually increased for all other starters but with values almost identical and lower than that of the starter L1.

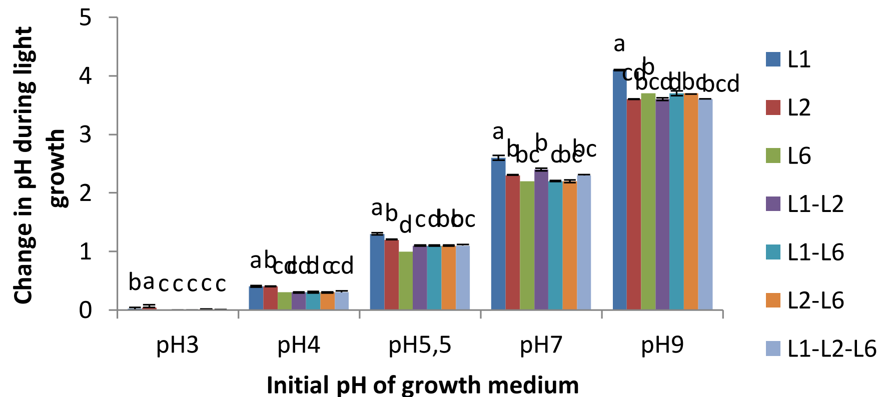

Figure 2:- Evolution of pH during the growth of yeast starters
L1**:** *Issatchenkia orientalis***,** L2 and L6**:** *Candida tropicalis*

3- 4- Growth in the presence of ethanol

The results showed that the inhibitory effect of ethanol manifested itself differently as a function of the ethanol content, the incubation time and the starter culture considered (Figure 3). Thus, during the first twelve hours, growth was accelerated in the presence of ethanol between 0 and 5%. At 7.5%, growth was strongly inhibited, so that the variation in biomass remained very low for all starters. Nevertheless, L1 yield the highest variability of the growth rate with a value of 0.16 while the lowest rate was observed in the L2 starter with a value of 0.03. Between 12 h and 24 h of incubation, a sudden drop in the variation of the growth rate was generally observed for ethanol concentrations between 0 and 5%, while rapid growth was observed in most starters at the rate 7.5%. At the same concentration of ethanol, the optimum biomass variation of a value of 0.52 was observed with the starter L1-L6 while L2-L6 gave the smallest value of the variation.

Very little growth was noticed between 24 and 36 hours of incubation both in presence and absence of alcohol. However, the starter L1-L2-L6 showed the highest variation in the presence of 7.5% alcohol.

During the next 24 hours of incubation, there was a multiplication of the population of some of the starters only in the presence of ethanol. Thus, L2-L6 showed the maximum variation with a value of 0.7 in the presence of 7.5% ethanol. In contrast, the starter L2 gave a variation of 0.05 at the same ethanol level. Above 48 hours of incubation, the population of all our starters decreased considerably in the medium containing ethanol whereas a low growth was observed in the absence of alcohol.

3-5- Evolution of the yeast biomass during alcoholic fermentation

The capacity of the yeasts to multiply in a given culture medium is a very important criterion for carrying out the fermentation. Thus, the growth of singular and multiple starter cultures was evaluated during alcoholic fermentation at temperatures of 30°C; 35°C and 40°C. Figure 4 represents the results obtained. It appears that the temperatures studied favored the growth of our starters. The growth rate was almost identical for all starters between 0 and 24 hours of fermentation, whatever the temperature and the values were between 0.71 and 0.76. At 24 h, the biomass of each of the starters increased abruptly. However, a low growth rate was recorded for the starter L6 compared to those of the others at 30°C and 35°C. On the other hand, we observed rapid and constant multiplication of the L1-L6

starter population at the three temperatures studied during all the 72 hours of fermentation with a growth rate going from 0.75 to 8 respectively at the time T0 and T72. The results also showed a good growth rate for the rest of the starters beyond 24 hours at all temperatures.

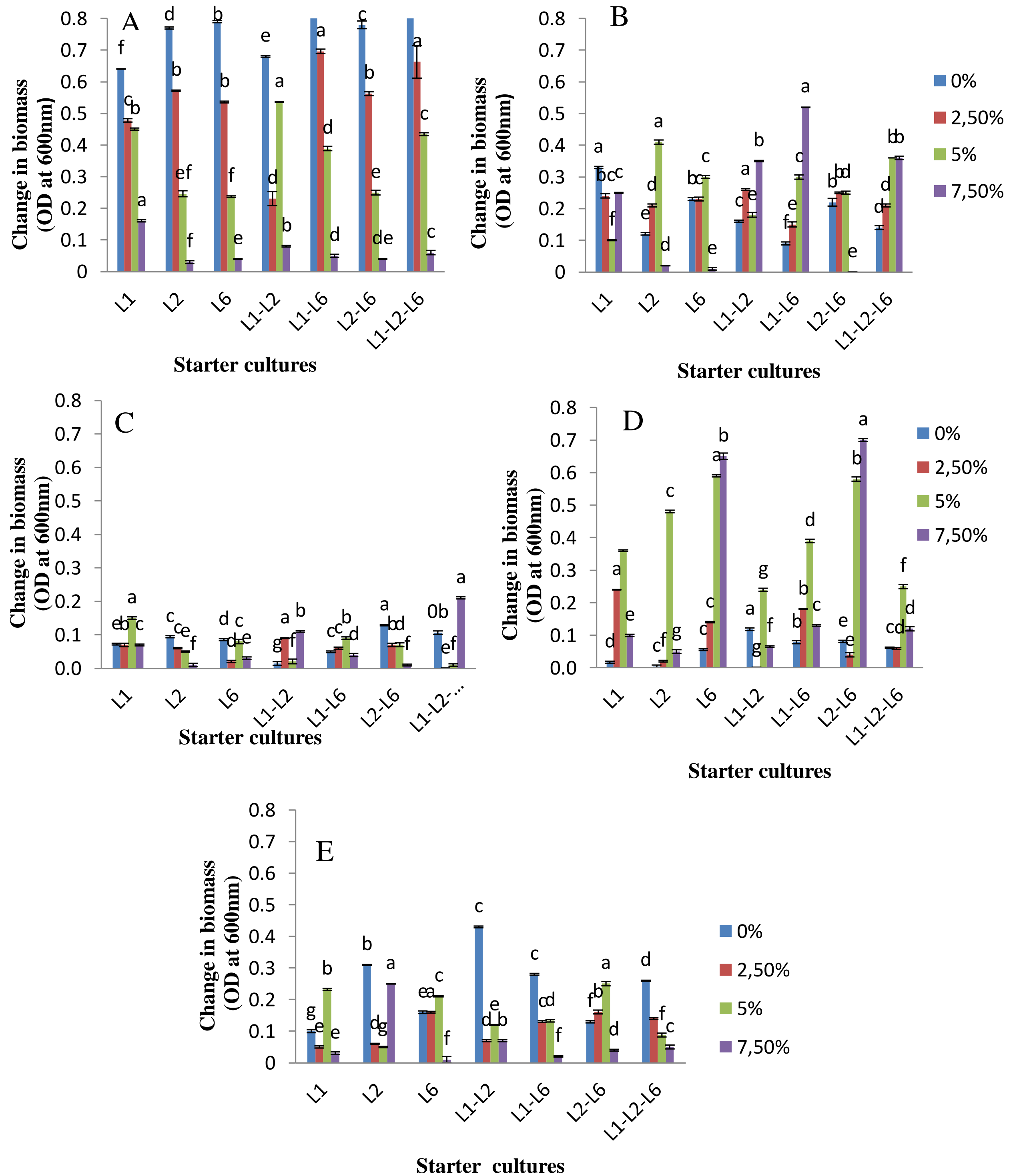

Figure 3:- Growth of starter cultures in the presence of ethanol after different incubation time (A): 0-12 h; (B): 12-24 h; (C): 24-36 h; (D): 36-48 h and (E): 48-72 h

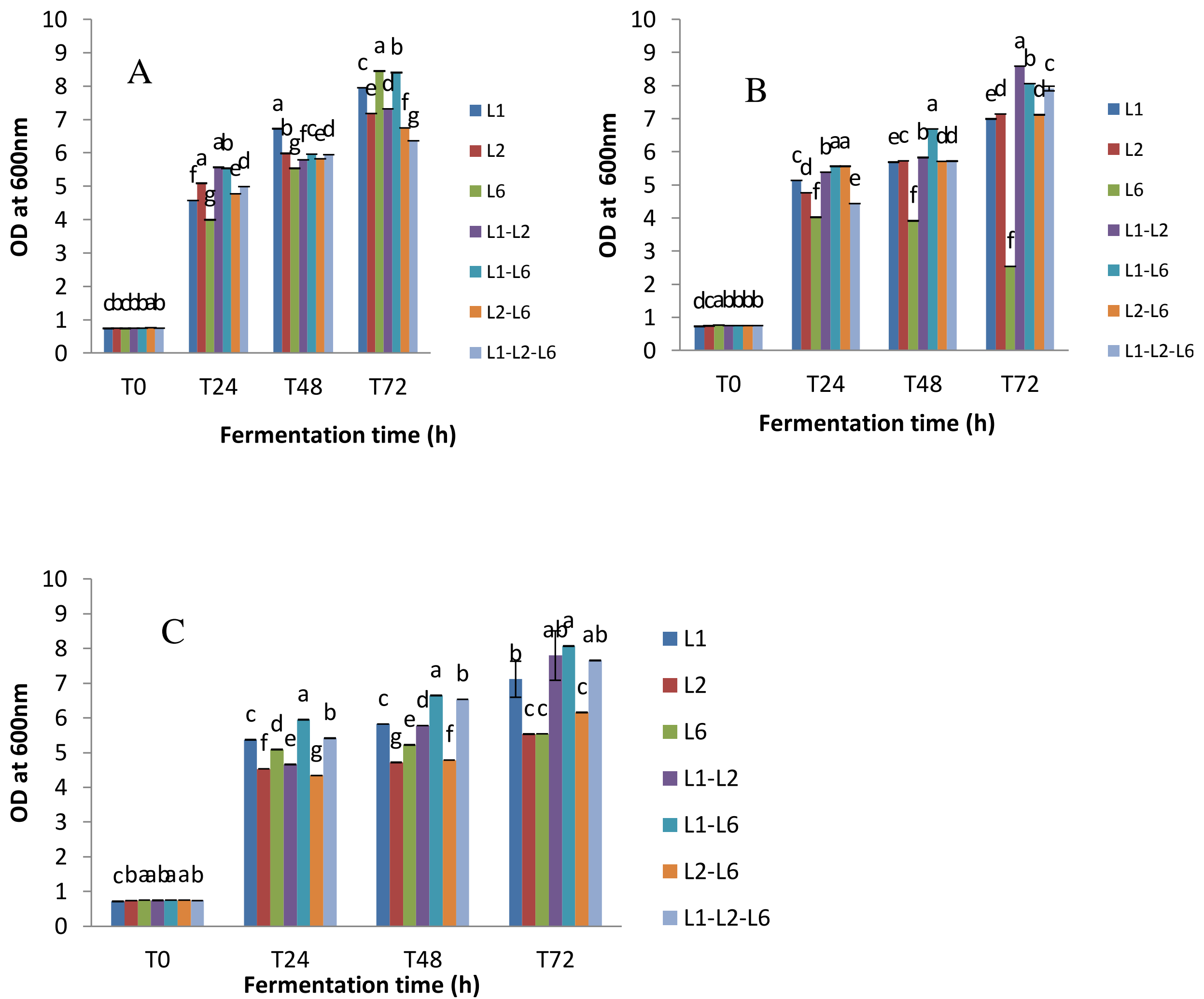

Figure 4:- Evolution of yeast biomass during alcoholic fermentation at 30°C (A), 35°C (B) and 40°C (C).
L1**:** *Issatchenkia orientalis***,** L2 and L6**:** *Candida tropicalis* L1-L2 L1-L6, L2-L6 and L1-L2-L6 are their combination

3-6- Physicochemical characteristics of the sweet wort and the beers produced

3-6-1- Titratable acidity of beers

The results indicated that the beers produced with the studied starters had statistically different titratable acidity values irrespective of the temperature. Two of them (L2 and L2-L6) were distinguished from the others by the production of beers with high titratable acidity. Concretely, at 30°C, when the fermentation times were less than 72 hours, the beer obtained with the starter L2 displayed the highest titratable acidities with values of 1.2; 1.04 and 1.01 respectively at T0, T24 and T48. At the end of the 72 hours of fermentation, the beer obtained with L2-L6 stand out with an acidity of 1.08. At the other two temperatures, the fermented product obtained with these same starters simultaneously have high titratable acidity values other than those of the others.

3-6-2- The total soluble solid contents

Figure 5 shows the evolution of the total soluble solids (TTS) during the sorghum beer production. It reveal a gradual decrease in TTS during fermentation at all temperatures studied. These decreases were significant at the first two temperatures where the TTS decreased from 14 to 5°Brix (30°C) in L2 beers and then from 14 to 5.95 (35°C)

for L6 beers. On the other hand, the decrease was low at 40°C where we recorded 11°Brix as the lowest value with the starter L2-L6 after 72 h of fermentation.

Table 2:- Titratable acidity of beers produced at 30°C, 35°C and 40°C

Temperature	Starter cultures	0 h	24 h	48 h	72 h
30°C	L1	1.10 ± 0.03ab	0.88 ± 0.12c	0.78 ±0.02bc	0.12 ± 0.02e
	L2	1.20± 0.02a	1.04 ± 0.03a	1.01 ± 0.03a	0.42 ± 0.02d
	L6	1.00 ± 0.03bc	0.08 ± 0.03d	0.92 ± 0.03ab	0.63 ± 0c
	L1-L2	0.90 ± 0.02de	0.96 ± 0.03b	0.98 ± 0.02a	0.72 ± 0b
	L1-L6	1.00 ± 0.03cd	0.91 ± 0.02bc	0.75 ± 0.05c	0.36 ± 0d
	L2-L6	0.50 ± 0.02f	0.65 ± 0.02e	0.90 ± 0.16abc	1.08 ± 0.06a
	L1-L2-L6	0.90 ± 0.06e	0.95 ± 0.03b	0.93 ± 0.02ab	0.59 ± 0.03c
35°C	L1	1.07 ± 0.02ab	1.09 ± 0.05ab	0.99 ± 0.13c	0.09 ± 0f
	L2	1.14 ± 0.05a	1.07 ± 0.02ab	1.13 ± 0b	0.90 ± 0b
	L6	0.73 ± 0.02e	0.68 ± 0.06c	0.80 ± 0.02d	0.36 ± 0d
	L1-L2	0.98 ± 0.02c	0.90 ± 0bc	0.84 ± 0.02d	0.36 ± 0.03d
	L1-L6	1.00 ± 0.05bc	0.68 ± 0.19c	0.79 ± 0d	0.27 ± 0.03e
	L2-L6	1.09 ± 0.05a	1.26 ± 0.19a	1.35 ± 0a	1.25 ± 0.08a
	L1-L2-L6	0.86 ± 0d	0.82 ± 0.05bc	0.87 ± 0.05d	0.66 ± 0.02c
40°C	L1	0.90 ± 0bc	0.78 ± 0.11bc	0.81 ± 0c	0.38 ± 0c
	L2	0.98 ± 0.02ab	0.96 ± 0.02a	1.01 ± 0.03b	0.75 ± 0.11b
	L6	0.91 ± 0.02bc	0.78 ± 0.02bc	0.80 ± 0.02c	0.75 ± 0.02b
	L1-L2	0.95 ± 0ab	0.90 ± 0ab	0.71 ± 0.02de	0.36 ± 0.03c
	L1-L6	0.82 ± 0.05c	0.73 ± 0.08c	0.65 ± 0.03e	0.18 ± 0d
	L2-L6	1.05 ± 0.11a	0.98 ± 0.02a	1.09 ± 0.05a	0.91 ± 0.02a
	L1-L2-L6	0.92 ± 0.03bc	0.74 ± 0.03c	0.75 ± 0.02cd	0.39 ± 0.02c

The values expressed are the means of two measurements. In each column, the mean values with the same letter are not significantly different ($p > 0.05$). L1**:** *Issatchenkia orientalis***,** (L2 and L6)**:** *Candida tropicalis*

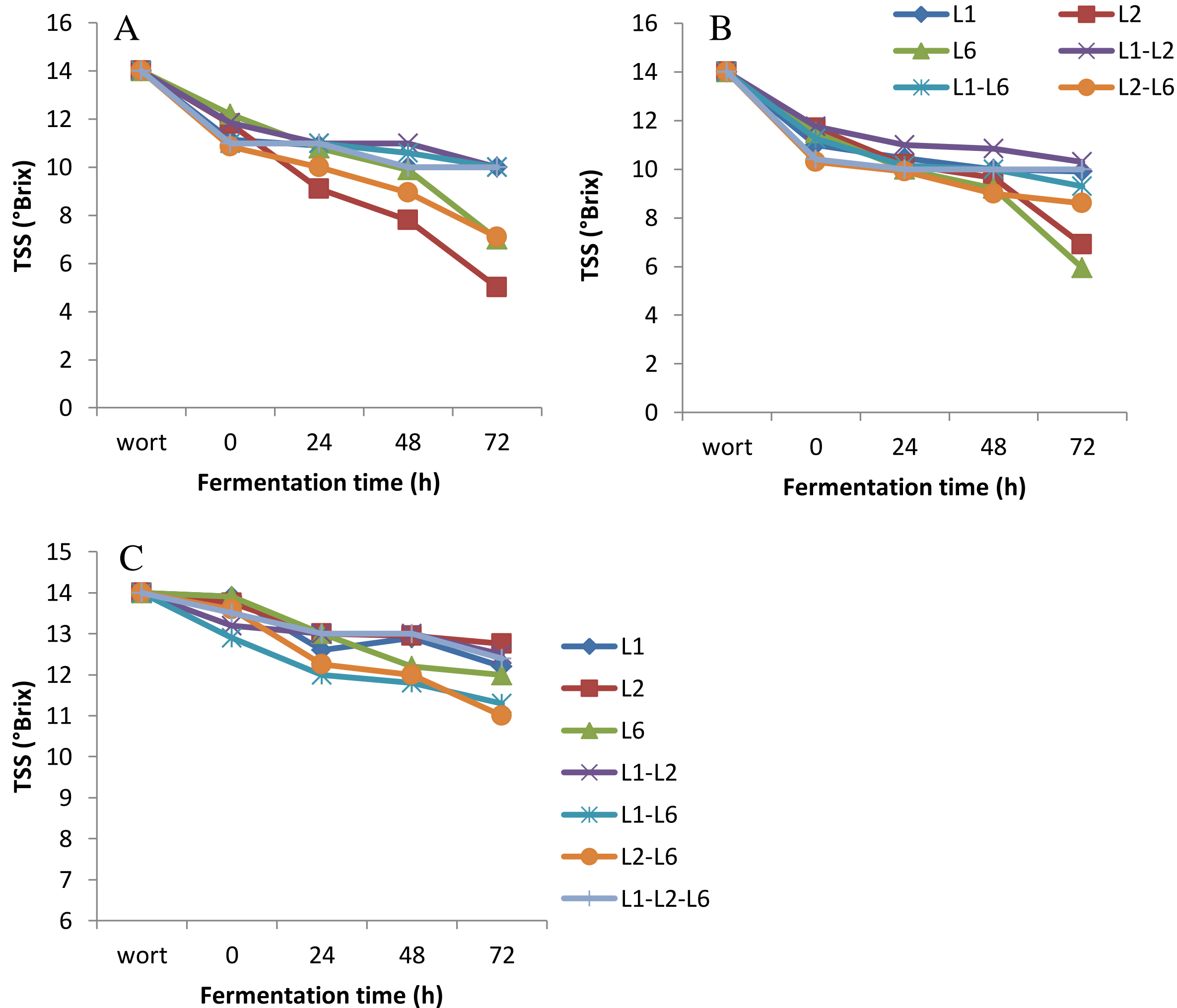

Figure 5:- Evolution of the total soluble solids of the various beers obtained with the starters at different temperatures: (A): 30°C; (B): 35°C and (C): 40°C

L1**:** *Issatchenkia orientalis***,** L2 and L6**:** *Candida tropicalis* L1-L2 L1-L6, L2-L6 and L1-L2-L6 are their combination

3-6-3- Evolution of total and reducing sugars

There was a general decline in sugar contents during alcoholic fermentation (Table 3 and 4). But at 40°C all the starters fairly influenced sugar contents of the fermenting. Thus, total sugar contents after 72 h of fermentation were between 13.77-31.60 g/L at 30°C, 16.01-32.59 g/L at 35°C and 30.45-39.24 g/L at 40°C. In the same way, reducing sugar contents after 72 h of fermentation at 30, 35 and 40°C were 10.77-21.90 g/L, 8.07-13.24 g/L and 19.28-23.58 g/L respectively. When we considered each fermentation temperature, statistical analyses showed that values varied significantly from one starter to another.

3-6-4- Principal component analysis of the produced beers

As shown in table 5 and Figure 6, the PCA using variation in the saccharification rates and the starter cultures for each tchapalo produced grouped the variables into two main components (F1 and F2). These components accounted for 86.71%, 88.67% and 96.99% of the total variation at 30°C; 35°C and 40°C respectively. At 30°C, the variables ΔTsacch48 and ΔTsacch72 contributed positively to the first main component while Δtsacch24 contributed positively to the second component. The same trend was observed at 35°C but not at 40°C. Thus the beers produced at 30°C and 35°C showed similar distribution when they plotted on the space created by the two dimensions. The beers obtained with starters L2-L6, L1-L2-L6 on one hand and L1-L2, L1-L6, L2 on the other hand grouped together while those produced with starters L1 and L6 stand alone.

Table 3:- Evolution of total sugar content during sorghum beer production at different temperatures (g/L)

Temperature	Starter cultures	0 h	24 h	48 h	72 h
30°C	L1	50.35 ± 0.47b	30.90 ± 0.02c	27.70 ± 0.18d	24.70 ± 0.20c
	L2	31.06 ± 0.14g	21.62 ± 0.07f	14.86 ± 0.20f	13.77 ± 0.02g
	L6	33.53 ± 0.02f	28.77 ± 0.16e	19.20 ± 0.03e	16.65 ± 0.7e
	L1-L2	36.35 ± 0.05e	29.52 ± 0.23d	29.35 ± 0.02b	24.23 ± 0.05d
	L1-L6	43.87 ± 0.25c	29.00 ± 0.07e	28.18 ± 0.09c	27.82 ± 0.07b
	L2-L6	53.38 ± 0.07a	37.17 ± 0.07b	27.93 ± 0.09cd	15.70 ± 0.09f
	L1-L2-L6	39.36 ± 0.14d	44.41 ± 0.11a	41.02 ± 0.05a	31.60 ± 0.09a
35°C	L1	39.42 ± 2.03b	34.54 ± 0.09d	33.64 ± 0.05b	27.24 ± 0.16c
	L2	32.33 ± 0.23c	25.30 ± 0.16e	24.74 ± 0.05d	23.27 ± 0.09d
	L6	30.91 ± 0.11c	23.41 ± 0.16g	22.81 ± 0.16e	19.04 ± 0.16e
	L1-L2	27.75 ± 0.25d	23.92 ± 0.07f	18.99 ± 0.23f	16.01 ± 0.20f
	L1-L6	48.82 ± 0.11a	36.34 ± 0.02c	29.67 ± 0.20c	27.29 ± 0.14c
	L2-L6	47.75 ± 0.23a	39.40 ± 0.02b	19.53 ± 0.05f	30.18 ± 0.02b
	L1-L2-L6	47.27 ± 0.05a	40.65 ± 0.07a	36.47 ± 0.11a	32.59 ± 0.09a
40°C	L1	56.43 ± 0.14c	42.99 ± 0.05e	40.11 ± 0.11c	33.51 ± 1.35c
	L2	58.83 ± 0.14a	48.64 ± 0.23b	43.11 ± 0.25a	39.24 ± 0.07a
	L6	54.23 ± 0.05f	43.06 ± 0.23e	38.83 ± 0.07e	33.00 ± 0.18c
	L1-L2	54.47 ± 0.07e	47.16 ± 0.02c	37.52 ± 0.02f	31.20 ± 0.03d
	L1-L6	55.39 ± 0.11d	44.02 ± 0.36d	40.98 ± 0.09b	37.93 ± 0.02b
	L2-L6	56.86 ± 0.07b	44.37 ± 0.09d	31.62 ± 0.07g	30.43 ± 0.11d
	L1-L2-L6	56.32 ± 0.11c	50.19 ± 0.11a	39.77 ± 0.14d	37.25 ± 0.14b

The values expressed are the means of three measurements. In each column, the mean values with the same letter are not significantly different ($p > 0.05$).

L1**:** *Issatchenkia orientalis***,** L2 and L6**:** *Candida tropicalis* L1-L2 L1-L6, L2-L6 and L1-L2-L6 are their combination

Table 4:- Evolution of reducing sugar content during sorghum beer production at different temperatures (g/L)

Temperature	Starter cultures	0 h	24 h	48 h	72 h
30°C	L1	37.50± 0.07a	26.59 ± 0.04b	25.27 ± 0.04a	21.90 ± 0a
	L2	25.36± 0.06d	19.05 ± 0.01f	11.63 ± 0.04f	10.96 ± 0.03f
	L6	23.96 ± 0.01f	24.02 ± 0.03c	20.32 ± 0.06d	15.01 ± 0.01e
	L1-L2	26.77 ± 0.02c	21.65 ± 0.07e	24.72 ± 0.06b	20.90 ± 0c
	L1-L6	32.53 ± 0.01b	22.05 ± 0.04d	21.74 ± 0.06c	21.52 ± 0.03b
	L2-L6	24.31 ± 0.01e	18.03 ± 0.01g	15.41 ± 0.01e	10.77 ± 0.04g
	L1-L2-L6	22.64 ± 0.01g	27.50 ± 0.10a	24.59 ± 0.13b	20.30 ±0.07d
35°C	L1	16.02 ± 0.03a	14.75 ± 0.01a	14.26 ± 0.08a	12.65 ± 0.07b
	L2	15.74 ± 0.06b	12.93 ± 0.04d	12.14 ± 0.03c	10.41 ± 0.04d
	L6	14.56 ± 0.03d	11.21 ± 0.04f	9.94 ± 0.03e	8.07 ± 0.04g
	L1-L2	15.4 ± 0.03c	13.67 ± 0.04c	11.69 ± 0.04d	9.69 ± 0.04e
	L1-L6	13.96 ± 0.03e	13.61 ± 0.01c	12.58 ± 0.03b	11.30 ± 0.03c
	L2-L6	14.61 ± 0.07d	11.80 ± 0.07e	8.77 ± 0.04f	8.82 ± 0.03f
	L1-L2-L6	13.29 ± 0.06f	14.04 ±0b	12.21 ± 0.03c	13.24 ± 0.03a
40°C	L1	36.12 ± 0.03a	14.75 ±0.07a	27.38 ±0.03a	22.69 ±0.01c
	L2	30.74 ± 0.03e	12.93 ±0.01d	24.57 ±0.04c	22.81 ±0.01b
	L6	30.59 ± 0.01f	11.21 ±0.01f	21.83 ±0.01f	19.28 ±0.03g
	L1-L2	31.81 ± 0.01c	13.67 ±0.04c	23.79 ±0.01d	20.49 ±0.01e
	L1-L6	32.89 ± 0.01b	13.61 ±0.01c	25.89 ±0.01b	23.58 ±0.03a
	L2-L6	31.74 ± 0.03d	11.80 ±0.03e	20.19 ±0.01g	19.37 ±0.04f
	L1-L2-L6	28.73 ± 0.04g	14.02 ±0.03b	22.58 ±0.03e	21.91 ±0.04d

The values expressed are the means of three measurements. In each column, the mean values with the same letter are not significantly different ($p > 0.05$).

L1**:** *Issatchenkia orientalis***,** L2 and L6**:** *Candida tropicalis* L1-L2 L1-L6, L2-L6 and L1-L2-L6 are their combination

Table 5:- Correlation of variables with factors of main component analysis after varimax rotation (A): 30°C, (B): 35°C, (C): 40°C

A			B			C		
	F1	F2		F1	F2		F1	F2
ΔTSacch24	-0,528	0,820	ΔTsacch24	-0,602	0,793	ΔTsacch24	0,974	0,063
ΔTSacch48	0,887	0,039	ΔTsacch24	0,885	0,173	ΔTsacch24	-0,409	0,911
ΔTSacch72	0,763	0,522	ΔTsacch24	0,836	0,388	ΔTsacch24	-0,918	-0,338

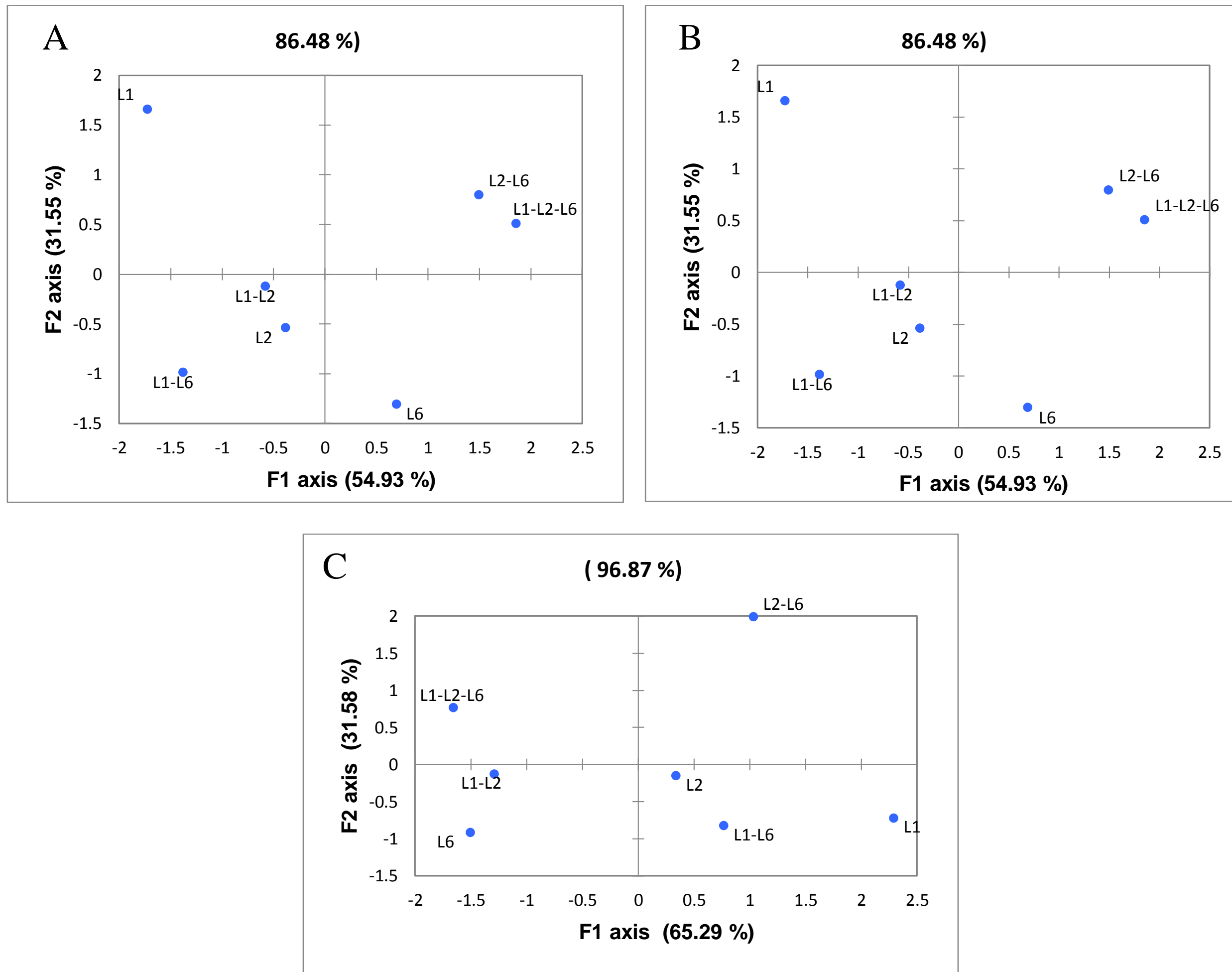

Figure 6:- Diagram of distribution of beers produced with different starter cultures according to the principal components F1 and F2 of the PCA (A): 30°C, (B): 35°C, (C): 40°C

*L1: Issatchenkia orientalis***,** L2 and L6**:** *Candida tropicalis* L1-L2 L1-L6, L2-L6 and L1-L2-L6 are their combination

Discussion:-

In general, the selection of the appropriate starter should take into account the interactions of strains in mixed culture with consideration of the behavior of these strains under defined conditions and in the food substrate (Holzapfel, 2002). According to Strehaiano et al. (2008), one of the general criteria for classifying interactions between microorganisms is the presence or absence of physical contact. Thus the result of the study of the interaction between our yeasts can be described as either neutralism or symbiosis. Indeed, their simultaneous growth could be

explained either by a lack of interaction or by the fact that the two populations derive a reciprocal benefit from the interaction (Quoc et al., 2010).

None of the pH tested, inhibited the growth of our starter cultures. Indeed, some of these pH values are included in the optimum pH zone of yeasts growth (4.6 to 6.6) and the others are within the wide pH range that yeasts tolerate (2.8 to 3 and From 8 to 8.5).

It is known that acidification power indicates glycolytic activity and endogenous reserves of the yeast cell to maintain a fixed ratio between intracellular and extracellular hydrogen ion concentrations (Opekarova et al., 1982). It is indeed related to viability and fermentation performance of yeast strains (White et al., 2003; Gabriel et al., 2008). The results of acidification power test showed that mains of starters have high acidification power. According to Karime et al. (2008), yeasts acidify the medium by metabolic activities.

The inhibitory effect of ethanol on the growth and fermentation of certain yeasts has been known for several decades (Jones et al., 1987). Also, the plasma membrane composition was identified as being central to the ethanol tolerance of yeast strains, with yeast responding to increased ethanol concentration, in a dose-dependent manner, by increasing the unsaturation index, and hence fluidity of their membranes (Odumeru et al., 1993. Alexandre et al., 1994).

None of the different levels of ethanol tested completely inhibited the growth of tested starters. These results could be explained by the fact that all the concentrations of ethanol studied are lower than the limit concentration at which yeast growth stops completely. In fact, according to Casey et al. (1986), there are two limiting concentrations of ethanol in its mechanism of toxicity. This is the threshold concentration (2 to 4%) at which the inhibitory effect of ethanol can be observed and the second (7 to 11%) which causes a complete stop of the growth of the yeasts.

Temperature is a major factor in the survival and growth of starters. In fact, it is one of the parameters that controls the growth rate of microorganisms. Moreover, Aldiguier (2006), emphasizes that it is fundamental in the conduct of alcoholic fermentation because it influences growth, ethanol production, conversion efficiencies and viability.
Study of the growth of these yeasts and their combinations at different temperatures showed that two of these temperatures (30°C and 35°C) allowed optimal growth. These two values belong to the temperature zone favorable to yeast growth. Indeed, according to Karime et al. (2008), the current yeast culture temperature is between 25°C and 30°C while that which allows maximum growth is between 35°C and 45°C. N'Guessan et al. (2010) also reported strong yeast growth at 35°C.

The assay of titratable acidity indicates that the beers produced at 40°C had a low titratable acidity rate compared to those of the beers obtained at the other temperatures. This result indicates that the zone of maximum growth of the yeasts corresponds to the zone where the titratable acidity is high. This fact can be explained by the fact that acids are one of the secondary metabolites derived from the metabolism of sugars that yeasts use during their growth.
Known as the soluble dry matter present in the wort and correlated with the sugar content, the TTS decreased progressively during the alcoholic fermentation of the wort. This result may be related to the fermentation process. In fact according to Zangué et al. (2012) fermentation would lead to the production of alcohols and other constituents such as carbon dioxide which would solitize to give carbonic acid. The PCA showed that the starter cultures studied did not have the same capacity to reduce the sugar content in the sweet wort during fermentation.

Conclusion:-

This study showed that the yeasts resulting from the digestive tract of the *Macrotermes subhyalinus* worker and their combination could be used to ferment the sweet wort of sorghum. The optimum conditions for the production of sorghum beer (tchapalo) with these starter cultures were determined. The beers produced had interesting physicochemical characteristics. These starters have the ability to grow at acid (3; 4; 5.5), neutral (7) as basic (9) pH. These cultures have an acidifying capacity which is better expressed when the initial pH is greater than 5. Their growth is not completely inhibited by the ethanol levels tested (2.5% to 7.5%).

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