Study of newly isolated sorghum strains and their application for beer production

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Abstract. Twenty yeast isolates obtained from sorghum grains were studied. Their morphological, cultural and physiological characteristics were studied. The ability of three isolates to ferment carbohydrates was evaluated by paper chromatography, and the growth capacity and fermentation activity in grape juice were determined. Biochemical identification was conducted according to Kurtzman et al. (2011). The three isolates can be assigned to the genus Saccharomyces cerevisiae. It was found that the three isolates do not ferment maltose. The possibility of using the three newly isolated sorghum strains for the production of sorghum beer was investigated. The beer produced with *Saccharomyces cerevisiae* strain 2.3 was characterized by a harmonious fresh taste, an intense aroma dominated by cloves and tropical fruit, and a spicy residual bitterness. The obtained results demonstrate that sorghum-isolated yeast strains are suitable for the production of non-alcoholic and low-alcohol sorghum beer.

1 Introduction

Sorghum is a drought-resistant crop, hardy and well adapted to growing in regions with harsh climatic conditions. It is widely distributed in the tropical regions of the American, African and Asian continents. Its annual production amounts to over 64 million tones, making it the fifth largest cereal crop [1, 2, 3]. Sweet sorghum is a promising raw material for the production of biofuels and bioproducts [4].

In recent years, research has focused on the potential use of sorghum in the food and brewing industry, especially as a sustainable cereal alternative in the face of climate change [5]. Producing beer from this grain is a practical alternative in a time when climate change is becoming increasingly noticeable, and at the same time it presents a market novelty [6, 7]. A study on the characterization and identification of yeasts isolated from Rablilé showed that *Saccharomyces cerevisiae* was the predominant yeast in this traditional starter used to produce "dolo" – a traditional sorghum beer in Burkina Faso [8]. Studies on *Saccharomyces* yeasts highlighting the aromatic potential of sorghum wort are limited. The use of industrial ale yeasts for sorghum wort fermentation does not seem to be an optimal choice due to the amino acid requirements of *Saccharomyces* strains [9].

Various countries around the world have been demonstrating production of various malts and beers from sorghum. The production of lager beers from unmalted sorghum requires the use of high or low temperature mashing and exogenous enzymes to ensure starch degradation [10]. It was found that malted sorghum and beers made from it are free of pathogens and mycotoxins [5].

With the growing interest in the topics of health and body mass, and the concerns regarding alcohol abuse, especially while driving, consumer preferences for low-alcohol and non-alcoholic beer are increasing [11], and sorghum presents opportunities for the production of such beers.

Therefore, the aim of the present study was to investigate the characteristics of yeast isolates obtained from sorghum grain batches and the possibilities of using some of them to produce non-alcoholic and low-alcohol beers from sorghum.

2 Materials and Methods

2.1. Materials

Twenty yeast isolates obtained from five sorghum grain samples were subject of this study (Table 1).

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An averaged batch of sorghum with a starch content of 73.5 ± 2.1 % (w/w) was used for wort preparation.

Dry brewer's yeast BRY-97 (*Lallemand, Austria*) was used as a control starter culture.

The following commercial enzyme products were used in the sorghum mashing process: Saczyme Plus (an exo-1,4-alpha-D-glucosidase–glucoamylase - EC 3.2.1.3) (*Novozymes, Switzerland*) and LpHera (a thermostable alpha-amylase) (*Novozymes, Denmark*).

Table 1. Yeast isolates from sorghum grain

Source	Yeast isolates indexes
Sorghum batch № 2	2.1÷2.4
Sorghum batch № 3	3.1÷3.4
Sorghum batch № 4	4.1÷4.4
Sorghum batch № 5	5.1÷5.4
Sorghum batch № 6	6.1÷6.4

Hops Saphir, *BarthHaas, Germany* was used in the beer preparation process.

2.2. Experimental design

2.2.1. Morphological and cultural characteristics of isolates

The studied isolates were morphologically characterized by microscopy of a 72-hour liquid culture. Culture characterization in liquid and on solid nutrient media was performed in sterile wort and on MEA (malt extract-agar) after thermostating at 27°C [12].

2.2.2. Fermentation activity of isolates

The fermentation activity of all isolates was studied in sterile wort in 10 cm^3 tubes (Koch steam sterilizer, 20 minutes) with an initial apparent extract of 8.50 % (w/w). The tubes were icubated at 27°C and the apparent extract was determined daily [13].

2.2.3. Experiments with selected isolates

Three isolates (2.3, 3.3 and 6.2) were selected for testing their ability to grow in sterile wort in 10 cm^3 tubes. The samples were incubated in a thermostat at 27°C, and the concentration of yeast cells was determined daily with a Bűrcker chamber [14]. Paper chromatography was applied to assess the ability of the three isolates to ferment carbohydrates [14]. A repeated study of the fermentation activity in sterile grape juice (Koch steam sterilizer, 20 minutes) with sugar content of 204 ± 6 $g.dm^{-3}$ was performed in bottles with fermentation caps by the gravimetric method. The bottles were incubated at 27°C, weighed daily, and the amount of CO^2 released was calculated based on the mass change of the samples [13]. Based on the obtained results, an approximate identification according to Kurtzman [15] was performed.

2.2.4. Beer production

The three yeast strains (2.3, 3.3 and 6.2) were used to investigate the possibilities for the production of non-alcoholic and low-alcohol beer. The strains were first activated in test tubes with sterile wort for 48 hours at 27°C, after which the wort volume was increased to 250 cm^3.

Wort was previously obtained in a 25 dm^3 "Braumaister" brewing plant. A hydromodule of 1: 6.25 (ground sorghum:water) was used. Infusion mashing was carried out with the addition of enzymes – thermostable α-amylase and glucoamylase. At the hot wort stage of the brewing process, hops of the Saphir variety were added, in an amount introducing an average degree of bitterness of about 110 $mg.dm^{-3}$ α-bitter acids,. The finished wort, with an extract of 4% (w/w) and a volume of 5 $dm^3$for each variant, was inoculated with an active seed culture of the three strains in an amount of 5% (v/v). The control strain was inoculated directly into the wort. The fermentation was carried out at 25°C for 6 days in four previously washed and disinfected "Kornelius keg" type fermenters with a volume of 10 dm^3. After establishing constant values of the residual extract, the resulting experimental batches of beer were forcibly saturated to a pressure of 1.5 bar.

2.3. Analytical methods

During the brewing process Anton Paar DMA 4500, the EBC Analytica Method 9.2.1 [16] was used to determine the alcohol content and initial, apparent and actual extract. The fermentation activity of the isolates in beer wort was determined refractometrically, and in the grape juice – by the gravimetric method [14]. The content of reducing sugars was determined according to the OIV methods [12], and the starch content in sorghum – by the Evers method [16].

The organoleptic characterization of the finished beers was performed using the basic characteristics method by a 5-member committee [16, 17, 18].

2.4. Statistical analysis.

The results are presented as the mean values of three determinations, and the coefficients of variation expressed as the percentage ratios between the standard deviations and the mean values were found to be <5 % in all cases. The means were calculated using Microsoft Excel™ at a 95 % confidence level [19].

3 Results and Discussion

3.1. Morphological and cultural characteristics of isolates

The morphological characterization of all isolates showed relatively homogeneous protoplasm and a thin cell wall. The shape of the cells was similar or identical - spherical to slightly elliptical or ovoid. The location in the nutrient medium was single or in small groups of 3-4 cells. Larger groups of clustered cells were observed only for isolate 2.4. Vegetative reproduction is unilaterally polar, typical of yeasts of the genus *Saccharomyces*. The cells, although similar in shape, differed in size, which was more pronounced in isolates 4.1 and 5.2. The sizes of the cells varied and were in the range of 4-6 µm to 7-9 µm. Fig. 1 presents a microscopic photograph of isolate 2.3.

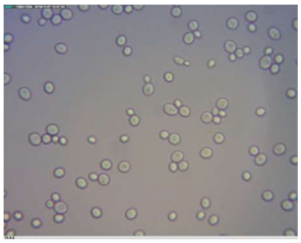

Fig.1. Photomicrograph of yeast isolate 2.3.

During the development of the isolates in liquid culture medium, characteristic signs of active fermentation were observed, starting with a slight opalescence, and subsequently a distinct clouding of the medium, varying in degree for individual isolates. The release of gas bubbles was most intense between the 36th and 96th hours of inoculation. A medium amount of bubbly foam and sediment formed on the surface of the liquid in the tube. No formation of a complete or partial ring was observed on the wall of the tube. At the 120th hour of inoculation, the process had ended in all tubes, since the liquid had clarified, the release of gas had ended, and the sediment was relatively compact.

Growth of the isolates on solid culture medium in Petri dishes was observed at the 48th hour of inoculation, with the colonies being small (d ~1-1.5 mm), white to slightly cream-colored with varying density. The colonies continued to grow during the next 120-144 hours, reaching a diameter of 2-3 mm, with smooth, semi-glossy, light cream, dome-shaped appearance, and paste-like consistency.

3.2. Fermentation activity of isolates

Fig. 2 presents the results of the fermentation activity of the studied new yeast isolates from sorghum.

It was noted that in all studied isolates the alcoholic fermentation process ended at high values of the residual extract – 3.50 – 6.50 % Brix. This can be explained by the low fermentation activity of the isolates, as well as the difficulty or impossibility of the isolates to metabolizing the available carbohydrate sources. In general, the studied yeast isolates can be divided into three groups: Group I – with an active fast start and dynamic course of the fermentation process (isolates from series 2 - 2.1, 2.2, 2.3), Group II – slower start and acceleration of fermentation in the following stages (isolates from series 3 - 3.2, 3.3), and Group III – very slow start, slow kinetics of the process and significantly early termination of fermentation (isolates from series 4, 5 and 6 – 4.3, 5.1, 6.2). Based on the obtained results, 3 yeast isolates (one from each group - 2.3, 3.3 and 6.2) were selected for further studies.

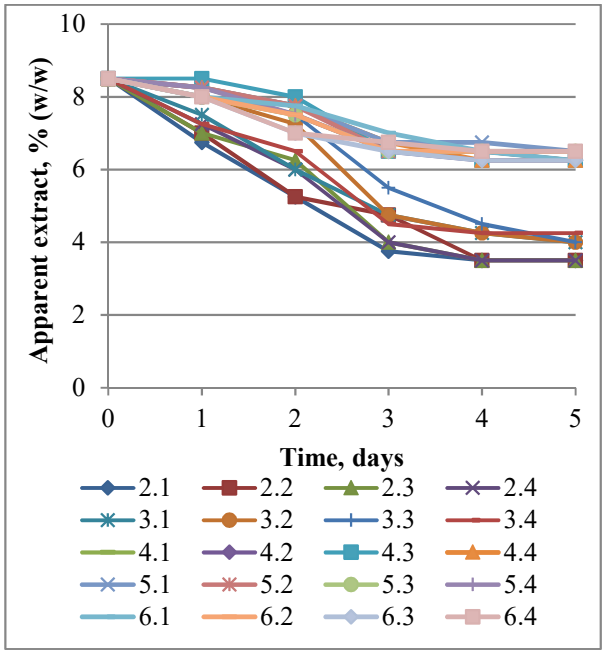

Fig.2. Fermentation activity of the yeast isolates

3.5. Characterisation of the selected yeast isolates

Fig. 3 presents the results of the study of the reproductive ability of the three experimental isolates.

The growth curves show similar dynamics of cell accumulation, with the highest concentration of 163.8 ± 4.4 x10^{6}.cm^{-3} achieved for isolate 2.3, and the lowest of 120 ± 3.55 x10^{6}.cm^{-3} was observed for isolate 6.2. These values reflect the established fermentation activity of the isolates, as it is well known that the cell concertation affects the rate of the fermentation process.

The characterization of the selected isolates continued with determination of their ability to ferment carbohydrates, which was performed by paper chromatography. Glucose, galactose, sucrose, maltose and raffinose were included in the chromatography medium. It was found, that all three isolates ferment glucose, fructose, galactose, sucrose and 1/3 raffinose. All three isolates did not ferment maltose. This result agrees well with the relatively low fermentation degree achieved by all isolates in the previous stages of the study. The inability to assimilate maltose offers a prospect application of the studied isolates in obtaining low-alcohol and non-alcoholic sorghum beers. For the production of beers with normal alcohol content with the use of the studied isolates, it is necessary to select a more

specific mashing regime for the sorghum mash in order to obtain higher glucose content in the must.

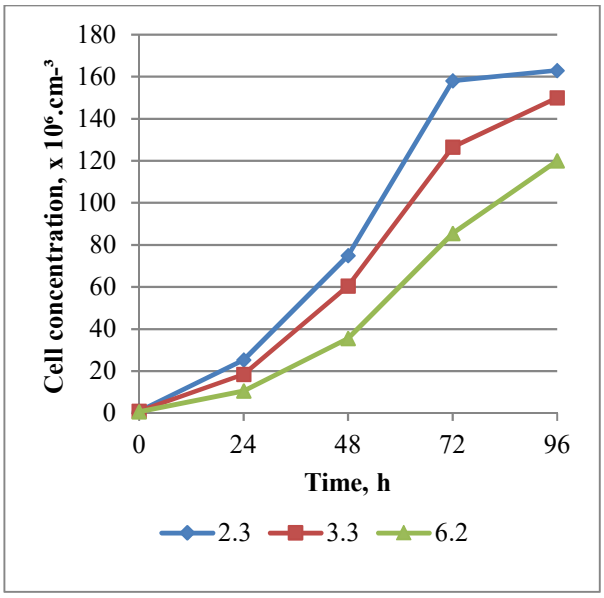

Fig. 3. Growth of the selected yeast isolates

To further investigate the fermentation activity of the three selected isolates and in view of their inability to ferment maltose, an experiment in grape juice was further conducted.

The fermentation curves of the three isolates are presented in Figure 4. The isolates demonstrate excellent fermentation ability, active assimilation of the substrate and release of carbon dioxide corresponds to a high degree of utilization of the available carbohydrates.

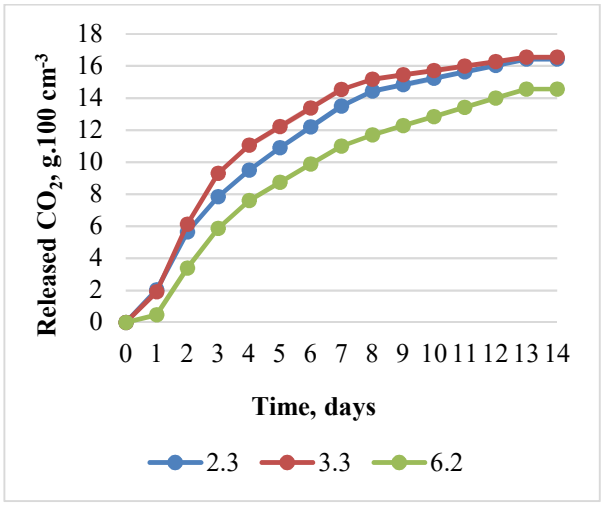

Fig.4. Fermentation curves of the selected isolates

Table 2 presents the concentrations of alcohol and residual reducing sugars in the experimental batches after completion of alcoholic fermentation. In all three samples, the alcohol reached is significant and corresponds to the amount of CO_2 released. This observation demonstrates the high fermentation activity of the selected isolates at the availability of a digestible carbon source – glucose and fructose. The residual sugars were in the range of 13-27 g.dm^{-3}, which does not change the positive assessment of the fermentation capacity of the studied yeasts in view of the direction of their application. The effect of anaerobiosis which was emphasized in the weight method was also taken into account.

Table 2. Fermentation parameters of the yeast isolates

Yeast isolates	Alcohol content, % vol	Reducing sugars, g.dm^{-3}
2.3	11,50 ± 0,20	13,20 ± 1,30
3.3	11,35 ± 0,22	14,30 ± 1,46
6.2	10,55 ± 0,20	27,70 ± 2,11

The results are expressed in % vol and g.dm^{-3} ± SD of three replicates

Analysis of the results from studying the different stages of the fermentation activity of the three isolates indicates their good potential for producing low-alcohol beers from sorghum.

Based on the obtained results of the morphological, cultural and physiological characteristics, the ability to ferment carbon sources and the observed fermentation activity, yeast isolates 2.2, 3.3 and 6.2 can be attributed to the species *Saccharomyces cerevisiae* according to Kurtzman [15].

3.6. Beer production

Fermentation in sorghum wort with an initial extract of 4 °P was carried out in order to explore the possibilities of obtaining low-alcohol and non-alcoholic beers from sorghum with the studied strains *Saccharomyces cerevisiae* 2.3, 3.3 and 6.2.

Table 3. Indicators of experimental sorghum beer

Parameters	Yeast strains			
	2.3	**3.3**	**6.2**	**BRY-97**
Alcohol content, % vol.	0,20 ± 0,02	0,18 ± 0,01	0,08 ± 0,01	0,17 ± 0,02
Initial extract, % (w/w)	4,00 ± 0,41	4,00 ± 0,41	4,00 ± 0,41	4,00 ± 0,41
Apparent extract, % (w/w)	3,72 ± 0,34	3,80 ± 0,33	3,41 ± 0,29	3,69 ± 0,34
Actual extract, % (w/w)	3,80 ± 0,35	3,87 ± 0,34	3,46 ± 0,30	3,76 ± 0,33

The values of some indicators of the obtained low-alcohol beer are in Table 3. The change in the apparent extract and the established final alcohol contents correspond to the levels for non-alcoholic beers.

Fermentation activity data are presented in Fig. 5. The kinetics of the fermentation process is similar in all samples, the change in extract content is slow and weak, but, taking into account the reported alcohol contents, it

is completely suitable for producing low-alcohol and non-alcoholic beers.

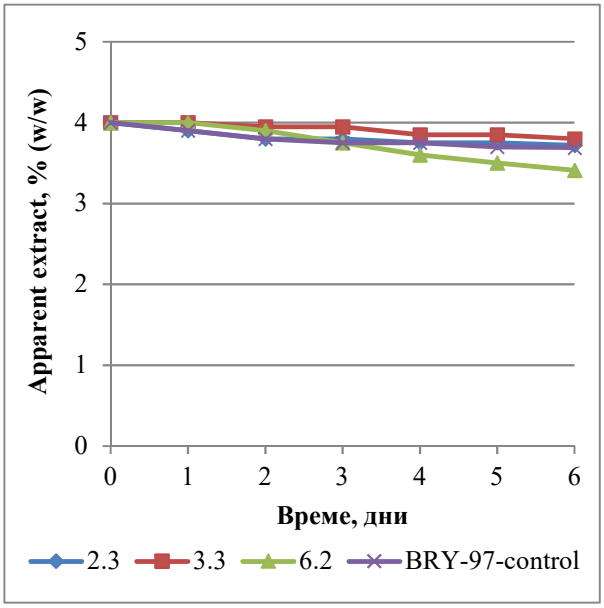

Fig. 5. Fermentation activity of experimental isolates in beer wort

The sensory profiles of the experimental beers are presented in Fig. 6. All beers showed low extractability, which corresponds to the initial and actual extract.

The aroma was relatively pure in all variants, slight lactic acid nuances detected only with strain 3.3. The spicy tone with strain 2.3 was particularly interesting, in which pronounced nuances of cloves, coriander and light hop notes were detected. The taste in all variants was fresh, sharp and with a spicy residual bitterness. Distinctive preference of the experimental non-alcoholic beer obtained with strain 2.3 was demonstrated. It was found particularly suitable in terms of aroma, freshness and sharpness and fit perfectly into the profile of a non-alcoholic sorghum beer.

4 Conclusions

Based on the experiments conducted, it can be concluded that the yeast strains isolated from sorghum grain possess the characteristics of *Saccharomyces* cerevisiae and exhibit good fermentation activity if there are enough digestible sugars in the wort. It was found that the three selected isolates 2.3, 3.3 and 6.2 could not ferment maltose, which deemed them suitable candidates for the production of low-alcohol and non-alcoholic beers from sorghum.

The sorghum beers obtained with the selected strains *Saccharomyces cerevisiae* 2.3, 3.3 and 6.2 had a clean aroma and a fresh sharp taste. Their sensory profile corresponded to non-alcoholic beers. The experimental beer obtained with strain 2.3 had the most suitable sensory profile, yielding an aroma with pronounced nuances of cloves, coriander and light hop notes.

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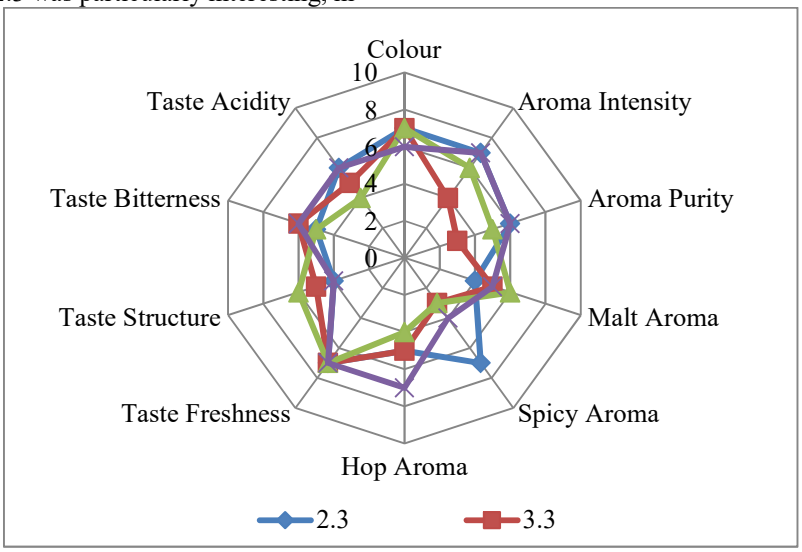

Fig. 6. Sensory profiles of non-alcoholic beers from sorghum

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