Food Microbiology 83 (2019) 181–186

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Selection of a new *Saccharomyces* yeast to enhance relevant sorghum beer aroma components, higher alcohols and esters

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ARTICLE INFO

Keywords:
Saccharomyces
aromagenesis
Higher alcohols
Esters
Vicinal diketones

ABSTRACT

The selection of *Saccharomyces* yeast which is able to produce high amounts of relevant aroma components from sorghum wort has been scarcely explored. Furthermore, generating sorghum beer with low levels of vicinal diketones (VDKs) is a permanent concern of brewers because of the wort's low amino acid content. To address these challenges, a performant *Saccharomyces cerevisiae* yeast was selected based on the higher alcohols and esters it synthesizes, and conditions influencing the young beer's VDK content were studied. Our results confirm that the low amino acid content limits aromagenesis by industrial ale yeast. By contrast, the selected yeast is able to efficiently produce higher alcohols and esters and is well adapted to tropical temperatures (27 °C). The young beer obtained from 13 °P sorghum wort using the isolated strain is characterized by a higher amount of aroma-active components such as isoamyl alcohol (116.25 ± 0.38 mg/L), isoamyl acetate (1.81 ± 0.02 mg/L) and ethyl acetate (22.07 ± 0.74 mg/L). The VDK content, however, is not only yeast strain dependent; it is strongly reduced by high fermentation temperature (27 °C) and increased wort gravity.

1. Introduction

The main yeast strains employed for worldwide beer production are classified into the categories of ale or top-fermenting yeast (*Saccharomyces cerevisiae*) and lager or bottom-fermenting yeast (*Saccharomyces pastorianus*). Endeavors to obtain beers with more complex sensory characteristics have led experts to prospect for non-conventional yeasts. Besides the widespread use of *Brettanomyces* for the production of sour beer, *Wickerhamomyces anomalus*, *Torulaspora delbrueckii*, *Hanseniaspora uvarum* and *Pichia kluyveri* have been successfully selected to increase fruity esters or to produce low-alcohol beer with full flavor (Basso et al., 2016; Canonico et al., 2016; Bokulich and Bamforth, 2013; Holt et al., 2018; Steensels et al., 2015). Despite this increased variety of yeast strains used in the brewery, their performance depends on their ability to synthesize specific aroma compounds. All brewing yeasts produce glycerol, vicinal diketones (VDKs), short-chain fatty acids, organic acids, and sulfur-containing substances as well as relevant aroma components, i.e. higher alcohols and esters (Bokulich and Bamforth, 2013; Dzialo et al., 2017). The levels of each of these components found in beer depends not only on the fermentation conditions but also on the yeast strain. With the advances in beer production worldwide, new challenges arise each year in the search for novel approaches to developing distinctive beverages with attractive sensory and nutritional characteristics. To obtain beer of higher nutritional value, an appropriate yeast and cereal grain should be selected. Among the raw materials used in brewery, the grain of *Sorghum bicolor* is now receiving more interest with regard to functional food and beverage production. This gluten-free raw material contains large amounts of anthocyanins such as luteolinidin and apigenidin (Awika and Rooney, 2004), which are well known for their functional, health-promoting properties (Althwab et al., 2015; Taylor et al., 2006). However, when mashing with sorghum malt, protein digestion during saccharification is limited (Duodu et al., 2003; Tokpohozin et al., 2018). This is due to a low protease content combined with overall poorly digestible protein, which is characterized by intermolecular disulfide bonds (Ng'Andwe et al., 2008) in the poly- and oligomers of kafirin – prolamins that comprise 93% of sorghum protein (Espinosa-ramírez and Serna-saldívar, 2016; Chibber et al., 1980); phytate-protein complexes (Elkhalil et al., 2001); or polypeptide-carbohydrate linkage (Fry, 1982) that forms during mashing limits protein hydrolysis. Altogether, this compromises the wort's amino acid content. However, amino acids are precursors for several relevant aroma-active higher alcohols and esters (Bolat et al., 2013; Ehrlich, 1907; Hazelwood et al., 2008; Kispal et al., 1996; Pires et al., 2014; Sentheshanmuganathan, 1960), and the expression and regulation of different genes encoding enzymes that catalyze their synthesis are influenced by amino acids

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https://doi.org/10.1016/j.fm.2019.05.014
Received 10 September 2018; Received in revised form 24 May 2019; Accepted 25 May 2019
Available online 27 May 2019

(Hazelwood et al., 2008b; Procopio et al., 2015). It is thus evident that the low sorghum wort amino acid content will compromise aromagenesis by yeast during fermentation. Furthermore, as Dafour and Melotte (1992) have pointed out, it impairs the ability of industrial *Saccharomyces* yeast to take up VDKs synthesized during fermentation of sorghum wort. Not least, sorghum wort, in comparison to barley wort, contains much polyphenol and – whatever their health-promoting and antioxidant properties – some of these may inhibit yeast growth and enzyme activities during fermentation (Daiber, 1975). Considering all these inhibition factors, an appropriate yeast well adapted to sorghum wort composition should be selected to cope with them. In the prospect of achieving these goals, we selected a performant autochthonous *Saccharomyces* yeast from traditional African sorghum beer starter, which is able to generate a large amount of higher alcohols and esters from sorghum wort. Conditions influencing the young sorghum beer's diacetyl and pentanedione (VDKs) content were also evaluated.

2. Material and methods

2.1. Raw material collection and malting conditions

The traditional brown sorghum grains used for traditional sorghum beer production was provided from a research and experimentation site of Alafiarou (Borgou/Benin). Sorghum was malted using the optimal malting conditions proposed by Tokpohozin et al. (2019). Five kilograms of brown sorghum grain were therefore steeped to 42% and germinated at 27 °C for a total of 5 days. The obtained green malts were maintained at 50 °C for one day and shoots and rootlets were removed.

2.2. Computer-assisted sorghum mashing by decantation

The standard sorghum mashing by decantation procedure including mash pre-heating proposed by Tokpohozin et al. (2018) was used. One kilogram of brown sorghum grains was milled, 6 L of distilled water was added, and the mixture was homogenised. The mashing was conducted with computer assistance using the 12 L capacity in-house brewing equipment (Joh Albrecht-Brautechnik, Germany). The mash was filtered, the wort gravity adjusted from 10 °P to 11 °P and 13 °P by evaporation, and then sterilized at 115 °C for 10 min.

2.3. Yeast propagation, inoculation and dynamism of fermentation

The yeasts used in this study (TO-37, BE-98, DA-132, GL-198, PA-286) were isolated from the traditional Beninese sorghum beer (*Tchoukoutou*) starter as *S. cerevisiae*. These yeast strains were selected on the basis of their phenotypic characteristics: glucose positive (Glu^+), maltose positive (Mal^+), sucrose positive (Suc^+) and fructose positive (Fru^+) (Tokpohozin et al., 2016). The ale yeast TUM68 was used as a reference. Yeasts were propagated overnight and the yeast cell count was computed using the Thomas counting method. Eleven and 13 °P brown sorghum wort was inoculated with each of the young viable yeast cell cultures to log(UFC/mL) = 7. After inoculation, the fermentation of 11 °P sorghum wort was conducted at 20 °C and 27 °C. The second fermentation of 13 °P brown sorghum wort was conducted at 27 °C with the isolated *Saccharomyces* yeasts (TO-37, DA-132, GL-198) and at 20 °C for the ale yeast TUM68.

2.4. Determination of sorghum wort nutrient composition

2.4.1. Determination of fermentable sugars in sorghum worts

The glucose, maltose, maltotriose, fructose and sucrose contents of 13 °P of the obtained sorghum worts were determined using high-performance anion-exchange chromatography with pulsed amperometric detection and Dionex ICS 500 column (Dionex CarboPack PA10 analytical; 2 × 250 mm). The internal standard solution was prepared by disolving 1 g of 2-deoxy-D-glucose in 1 L of HPLC-water. The worts were diluted to 1:400 with double distilled water, filtered through a 0.45-μm filter. The measurement was performed in duplicate. After measurement, Cromeleon software, version 6.0, was used for data evaluation.

2.4.2. Determination of wort extract and free amino nitrogen content

The density meter alcolyser (Anton Paar, Austria) was used to analyze wort extract. The free amino nitrogen of the different samples was measured following the EBC ninhydrin method (Lie, 1973). This test was performed in triplicate.

2.4.3. Determination of amino acids in sorghum worts

The amino acids were separated by hydrophilic interaction liquid chromatography (HILIC) and quantified by mass spectrometry (MS) in multiple reaction monitoring (MRM) mode. The standard amino acid solutions were prepared by weighing in equimolar amounts of different pure amino acids in a 100 mL volumetric flask. The pure amino acids were dissolved in 100 mL of 70% acetonitrile and diluted 1:20 with acetonitrile to obtain the standard solution (S1). A twenty millimolar mixture of labeled amino acids was diluted 1:100 to obtain the internal standard solution (SI). Seven different concentrations were prepared for the calibration curves by pipetting 0.1, 0.2, 0.5, 1, 2, 3 and 5 mL of S1, adding 0.2 mL of SI, and filling to 10 mL with 70% acetonitrile solution. The solutions were distributed in 1 mL reaction tubes, centrifuged for 5 min at 13,000 rpm, filtrated over 0.2 μm filters. This was performed in duplicate. The wort samples were centrifuged and diluted with 70% acetonitrile until the concentration of amino acids was within the calibration curves. The wort was diluted 1:40. The sorghum samples were prepared similarly.

2.5. Determination of higher alcohols, esters and vicinal diketone

Headspace gas chromatography coupled with flame ionization detection (HS-GC-FID) was used for young sorghum beer higher alcohols and esters measurement. Sample (5 mL) were distributed in 15 mL precooled glass tubes in duplicate and immediately closed. The samples were analyzed with a calibrated Hewlett-Packard 6890 gas chromatography equiped with a headspace sampler (HP 7694) and an HP-5 column. For vicinal diketone measurement, samples were first heated 65 °C for 90 min and then analyzed by headspace gas chromatography coupled with electron capture detector (HS-GC-ECD). The determination of aroma components in young sorghum beer was performed in duplicate. The results were analyzed with Agilent Technologies Chemstation Rev. A.10.01 software.

2.6. Statistical analysis

Data were analyzed using analysis of variance, and means were compared with the Turkey test. A value of $p \leq 0.05$ were considered statistically significant.

3. Results

3.1. Sorghum wort nutrient content

The free amino nitrogen content was found to be 166.6 mg/L, with the 6 most abundant amino acids being proline followed by glutamine, asparagine, arginine, alanine and glutamic acid (Fig. 1a). Three important fermentable sugars in the wort were maltose, maltotriose and glucose (Fig. 1b).

3.2. Fermentative aptitude of isolated Saccharomyces and industrial ale yeasts

3.2.1. Influence of fermentation temperature and wort gravity on higher alcohol and ester content in young beer

The yeast activity was evaluated during fermentation, and the

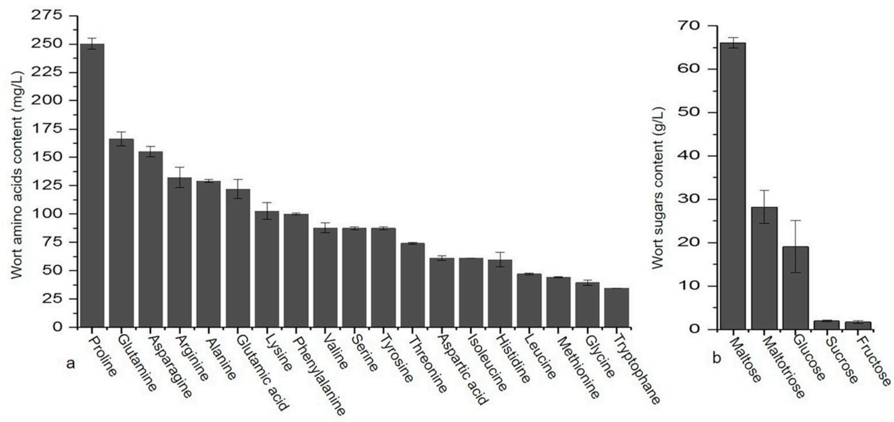

Fig. 1. Composition of 13 °P brown sorghum wort regarding amino acids (a) and fermentable sugars (b). Proline followed by glutamine and asparagine are three abundant amino acids of the obtained sorghum wort. The relevant beer aroma component precursors valine, leucine and isoleucine (branched amino acids) are relatives low in sorghum wort. This was performed in duplicate.

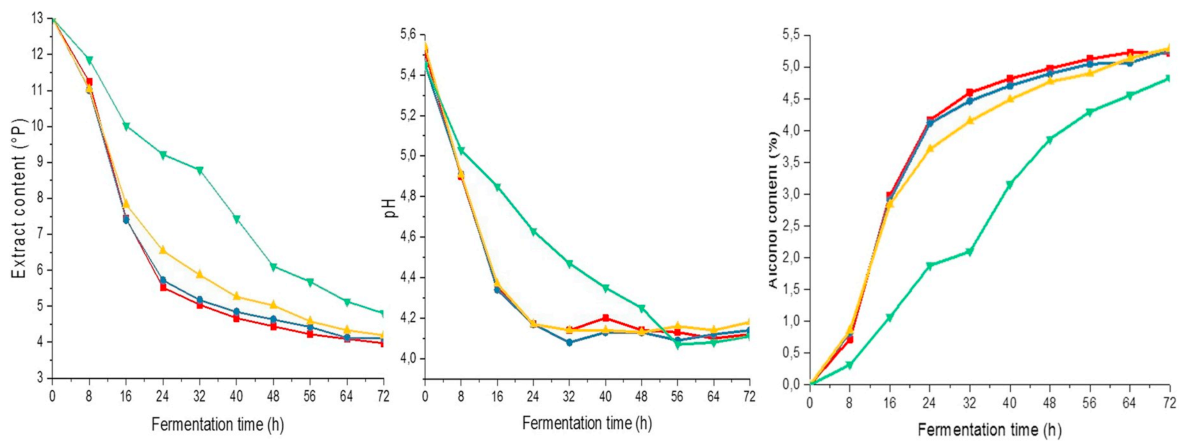

Fig. 2. Decrease of extract (a) and pH (b), and an increase of alcohol (c) during the fermentation of 13 °P sorghum wort with different *Saccharomyces* yeasts including the TUM68 ale yeast (green graph) and TO-37 (red graph), DA-132 (blue graph) and GL-198 (yellow graph) isolated from traditional sorghum beer starter. The fermentation was conducted in duplicate. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

aroma-active higher alcohols and esters synthesized from sorghum wort after 72 h of fermentation at two different temperatures (20 °C and 27 °C) were measured. The isolated yeasts' uptake of nutrients during fermentation at 27 °C was rapid compared to that of ale yeast at 20 °C (Fig. 2). Our results also confirmed that 20 °C is the appropriate fermentation temperature allowing the TUM68 industrial yeast to efficiently produce higher alcohols and esters (Table 1). By contrast, the isolated strains, especially TO-37, BE-98, DA-132 and GL-198, produced higher amounts of these compounds at 27 °C than at 20 °C. TO-37 and DA-132 were the two most remarkable strains when considering the young beer's total content of both higher alcohols and esters simultaneously. GL-198 was notable for synthesizing the highest amount of higher alcohols during fermentation (Table 1).

To supply nutrients sufficient for such aroma compound synthesis, the wort gravity was increased to from 11 °P to 13 °P. The fermentation was carried out at optimal temperature, 20 °C for the industrial ale yeast TUM68 and 27 °C for TO-37, DA-132 and GL-198. This increased of the wort gravity considerably improved the young sorghum beer's higher alcohol and ester content (Table 2). Moreover, TO-37, DA-132 and GL-198 exhibited particular characteristics absent from the ale yeast TUM68: The young beers obtained with the former at 27 °C were characterized by high amounts of amyl alcohol (26.40 ± 0.71 to 32.27 ± 0.28 mg/L), isoamyl alcohol (105.05 ± 2.12 to 116.25 ± 0.38 mg/L) and its corresponding ester isoamyl acetate (1.36 ± 0.01 to 1.81 ± 0.02 mg/L) as well as ethyl acetate (18.01 ± 0.23 to 22.07 ± 0.74 mg/L) compared to industrial ale yeast (Table 2). Conversely, fermentation at 20 °C with ale yeast TUM68 resulted in a high amount of isobutanol (59.73 ± 1.20 mg/L) compared to *Tchoukoutou* starter yeasts.

3.2.2. Impact of fermentation conditions on young beer vicinal diketone content

To assess the stress caused by the low amino acid content, especially regarding branched amino acids (valine, leucine and isoleucine), on yeast fermentability, diacetyl and pentanedione (VDKs) were quantified in the fermenting wort and young beer. The young beer's VDK content proved to be yeast strain dependent, but external factors played a role as well. As such, increased fermentation temperature lowered VDK content (Fig. 3), as did elevated wort gravity – especially in sorghum beer obtained with ale yeast S81, but not with strain GL-198. The highest peak value of VDKs during fermentation was obtained with TUM68 (Fig. 4).

4. Discussion

Brewing from sorghum wort without external enzymes is challenging. Due to the low malt hydrolase content and the high starch gelatinization temperature in comparison to barley, the decantation procedure was used to improve saccharification. Fermentable sugar content seems not to be the limiting factor for yeast fermentation even when diluted to 12 °P. In addition to sugar content, free amino nitrogen – which includes peptides and amino acids – exerts a decisive influence on fermentation. O'Connor-cox & Ingledew (1989) reported that 130–150 mg/L of free amino nitrogen in wort is the minimum necessary to support optimal yeast growth and fermentation efficiency. The free amino nitrogen content of the obtained 13 °P sorghum wort was

Table 1
Influence of temperature on aroma-active components acetaldehyde, higher alcohols and esters of the young sorghum beers obtained from 11 °P sorghum wort fermented with different *Saccharomyces cerevisiae* yeasts.

Aroma components (mg/L)	FT (°C)	TO-37	BE-98	DA-132	GL-198	PA-286	TUM 68
Acetaldehyde	20	51.353 ± 0.488	50.301 ± 4.384	45.067 ± 0.118	29.345 ± 0.031	18.732 ± 0.214	21.522 ± 2.26
	27	106.345 ± 5.721	39.045 ± 2.603	79.753 ± 1.525	25.991 ± 4.001	15.35 ± 1.205	17.191 ± 2.892
Higher alcohol							
Isoamyl alcohol	20	30.741 ± 0.38	37.311 ± 2.125	32.921 ± 0.752	57.721 ± 1.749	33.444 ± 0.691	43.473 ± 1243
	27	51.996 ± 6.297	44.282 ± 2.803	53.765 ± 4.251	63.181 ± 2.561	30.288 ± 3.184	41.137 ± 0.293
2-methyl propanol	20	14.183 ± 0.093	14.136 ± 0.355	11.691 ± 0.058	21.664 ± 0.266	13.121 ± 0.151	42.111 ± 4.967
	27	28.933 ± 1.155	21.478 ± 1.022	26.341 ± 0.159	33.189 ± 6.061	17.714 ± 1.198	29.713 ± 3.641
2-methylbutanol	20	10.967 ± 0.091	11.819 ± 0.226	10.339 ± 0.096	21.839 ± 0.065	11.144 ± 0.057	17.131 ± 0.921
	27	21.322 ± 1.111	16.84 ± 0.466	19.475 ± 0.972	25.975 ± 0.315	18.622 ± 0.586	9.996 ± 1.886
Propanol	20	11.132 ± 0.101	10.145 ± 0.014	9.371 ± 0.023	20.334 ± 0.025	13.811 ± 0.100	17.121 ± 0.031
	27	19.939 ± 1.539	15.320 ± 0.055	19.168 ± 0.162	27.637 ± 1.377	20.584 ± 0.917	8.511 ± 0.884
Total HA	20	67.023 ± 0.665	73.411 ± 2.72	64.322 ± 0.93	121.558 ± 2.105	71.52 ± 0.999	119.836 ± 7.162
	27	122.19 ± 10.102	97.92 ± 4.346	118,759 ± 5.544	149.982 ± 10.314	87.208 ± 5.885	89.357 ± 6.704
Esters							
Isoamyl acetate	20	0.185 ± 0.004	0.171 ± 0.0	0.186 ± 0.002	0.243 ± 0.0	0.245 ± 0.0	0.964 ± 0.06
	27	0.168 ± 0.0	0.252 ± 0.002	0.37 ± 0.0	0.169 ± 0.0	0.215 ± 0.003	0.147 ± 0.002
Ethyl acetate	20	7.224 ± 0.051	7.658 ± 0.329	7.375 ± 0.185	8.940 ± 0.267	11.233 ± 0.442	17.121 ± 0.031
	27	18.23 ± 1.872	12.97 ± 1.948	16.557 ± 0.388	8.799 ± 2.196	13.739 ± 1.54	7.584 ± 1.055
2-methyl propyl acetate	20	0.022 ± 0.0	0.018 ± 0.0	0.015 ± 0.0	0.021 ± 0.0	0.026 ± 0.0	0.027 ± 0.0
	27	0.054 ± 0.0	0.031 ± 0.0	0.038 ± 0.0	0.021 ± 0.0	0.023 ± 0.0	0.025 ± 0.0
2-methyl butyl acetate	20	0.055 ± 0.001	0.031 ± 0.0	0.036 ± 0.0	0.046 ± 0.0	0.04 ± 0.0	0.072 ± 0.002
	27	0.074 ± 0.000	0.040 ± 0.0	0.053 ± 0.0	0.024 ± 0.0	0.047 ± 0.001	0.029 ± 0.0
Ethyl hexanoate	20	0.11 ± 0.001	0.067 ± 0.0	0.081 ± 0.0	0.064 ± 0.0	0.097 ± 0.0	0.057 ± 0.0
	27	0.092 ± 0.0	0.08 ± 0.0	0.084 ± 0.0	0.07 ± 0.0	0.091 ± 0.0	0.087 ± 0.001
Ethyl butanoate	20	0.071 ± 0.001	0.037 ± 0.0	0.042 ± 0.0	0.034 ± 0.0	0.042 ± 0.0	0.028 ± 0.0
	27	0.024 ± 0.0	0.05 ± 0.0	0.049 ± 0.0	0.021 ± 0.0	0.041 ± 0.0	0.023 ± 0.0
Total esters	20	7.667 ± 0.058	7.98 ± 0.329	7.737 ± 0.187	9.35 ± 0.267	11.684 ± 0.442	18.27 ± 0.09
	27	18.644 ± 1.873	13.424 ± 1.951	16.782 ± 0.388	9.105 ± 2.197	14.158 ± 1.544	7.896 ± 1.058

FT = Fermentation temperature. The fermentation was conducted in duplicate.

166.6 mg/L. Jones and Pierce (1964) categorized the amino acids on the basis of their assimilation by yeast during fermentation, where glutamic acid, aspartic acid, asparagine, glutamine, serine, threonine, lysine and arginine were determined to be absorbed quickly. Most of these important amino acids are present in high concentrations in sorghum wort, though still less than in a standard 12 °P barley wort amino acid composition. The wort of our West African sorghum, by virtue of its high proline content, possesses an amino acid profile different from that reported by Taylor (1982) in South African sorghum wort, where asparagine and glutamine are the two most abundant amino acids. This prevalence of proline is quite intriguing. Note that, though proline itself is not converted to higher alcohols following the Ehrlich pathway, it can be transformed into the highly assimilable amino acid glutamate. As such, Procopio et al. (2013) also enhanced higher alcohols and esters by increasing the wort proline content.

In contrast to the carbohydrate content, the low amino acid concentration is a potential limiting factor for efficient fermentation and aroma-active component synthesis: The relevant aroma-active higher alcohols isoamyl alcohol, amyl alcohol, and isobutanol result from the metabolism of leucine, isoleucine, and valine, respectively (Ehrlich, 1907). Still, the isolated yeast strains produced high amounts of amyl alcohol, isoamyl alcohol, isoamyl acetate, and ethyl acetate when the fermentation was conducted at 27 °C as opposed to industrial ale yeast TUM68 (fermented at 20 °C). These new *S. cerevisiae* yeasts are therefore well adapted to West African tropical temperature and the lower amino acid content of sorghum wort. By contrast, the TUM68 yeast generally requires higher amounts of leucine, isoleucine, valine, histidine, glutamine and proline to effectively synthesize aroma-active compounds (Procopio et al., 2013). Thus, the limited synthesis of isoamyl alcohol and amyl alcohol by industrial ale yeast is probably due to

Table 2
Young sorghum beer flavour active components acetaldehyde, higher alcohols and esters obtained from 13 °P sorghum wort.

Aroma components	TO-37	DA-132	GL-198	TUM 68
Acetaldehyde (mg/L)	8.176 ± 0.692 a	11.962 ± 0.097 b	7.313 ± 1.081 a	9.191 ± 2.478 ab
Higher alcohol (mg/L)				
Isoamyl alcohol	116.259 ± 0.385 a	105.057 ± 2.123 b	109,441 ± 0.711 c	74.681 ± 1.242 d
2-methyl propanol	37.128 ± 0.091 a	33.827 ± 0.35 b	30.649 ± 0.06 c	59.728 ± 1.209 d
2-methylbutanol	30.763 ± 0.328 a	26.409 ± 0.71 b	32.273 ± 0.287 a	25.417 ± 1.077 b
Propanol	19,822 ± 0.107 a	19.442 ± 0.017 a	18.699 ± 0.026 a	25.094 ± 0.03 b
Total	203.972 ± 0.911	184.737 ± 3.201	191.065 ± 1.086	184.922 ± 3.558
Esters (mg/L)				
Isoamyl acetate	1.836 ± 0.028 a	1.405 ± 0.021 b	1.322 ± 0.017 bc	1.238 ± 0.053 c
Ethyl acetate	22.067 ± 0.746 a	20.35 ± 0.36 b	18.012 ± 0.239 c	15.671 ± 0.44 d
2-methyl propyl acetate	0.1 ± 0.001 ab	0.132 ± 0.003 a	0.063 ± 0.04 ab	0.144 ± 0.001 b
2-methyl butyl acetate	0.19 ± 0.003 a	0.141 ± 0.001 b	0. 833 ± 0.003 c	0.158 ± 0.003 d
Ethyl hexanoate	0.14 ± 0.004 a	0. 144 ± 0.002 a	0.12 ± 0.001 b	0.051 ± 0.007 c
Ethyl butanoate	0.14 ± 0.001 a	0.113 ± 0.004 b	0.07 ± 0.002 c	0.071 ± 0.008 b
Total	24.336 ± 0.781	22.288 ± 0,393	20.422 ± 0.306	17.336 ± 0.514

The beer aroma component values affected with different letters a, b, c and d in the same row indicate a significant different ($p < 0.05$). The measurement was performed in duplicate.

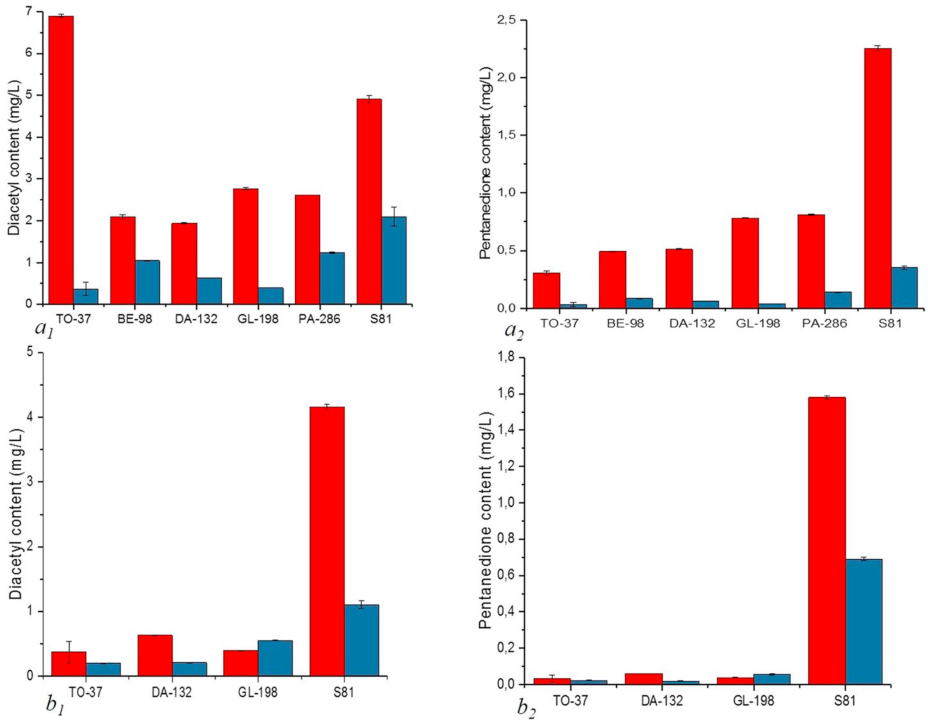

Fig. 3. Influence of fermentation temperature and wort gravity on the young beer's vicinal diketone content. Red and blue graphs correspond to VDKs obtained at 20 °C and 27 °C, respectively, for a_1 and a_2, and for 11 °P and 13 °P sorghum wort, respectively, for b_1 and b_2. The reported flavour threshold values of diacetyl and 2,3-pentanedione in ale beer are around 0.1–0.4 mg/L and 0.9–1.0 mg/L, respectively. The measurement of VDK was performed in duplicate. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

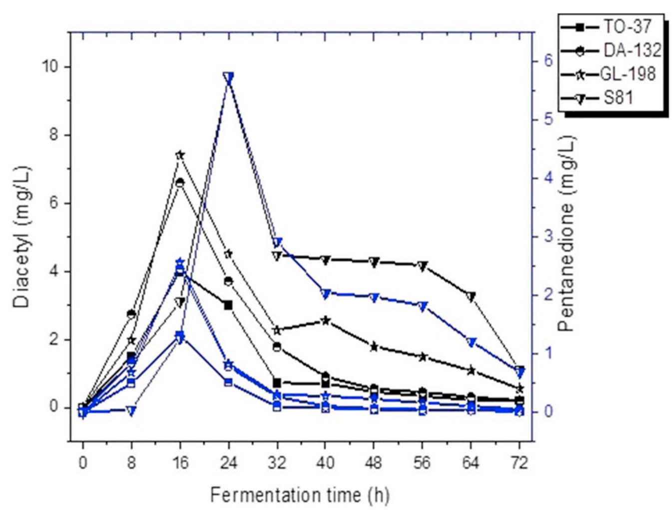

Fig. 4. Diacetyl and pentanedione production and their uptake during fermentation of sorghum wort by different *S. cerevisiae* yeast strains. The fermentation was conducted at 27 °C with the isolated yeasts TO-37, DA-132 and GL-198 and at 20 °C with industrial TUM68 yeast S81. The measurement of VDK was performed in duplicate.

sorghum wort's typically low content of branched amino acids, particularly leucine and isoleucine. The high peak value of VDKs obtained during the fermentation of sorghum wort with industrial ale yeast TUM68 (Fig. 4) supports this hypothesis and shows that the ale yeast is more sensitive to low amino acid content than the isolated *Saccharomyces* yeasts; the lack of branched amino acids thus exacerbates VDK synthesis which unfortunately leads to high concentrations of diacetyl and pentanedione in beer. However, this is manageable as the VDK content is not only yeast strain dependent, but also reduced by an increase of wort gravity from 11 °P to 13 °P and of the fermentation temperature from 20 °C to 27 °C (Fig. 3). The influence of strain may arise from varying levels of enzyme expression implicated in catalyzing conversion of 2,3-butanedione and 2,3-pentanedione to the corresponding alcohols. The high fermentation temperature and an increase of wort gravity likely increased the yeast population, thereby improving uptake of excreted VDKs. Elevated wort gravity moreover increases the amino acid content, including that of branched amino acids, thus further limiting VDK synthesis. For instance, improved valine content inhibits enzymes controlling the formation of diacetyl precursor α-acetolactate; as such, Krogerus and Gibson (2013) observed a decrease of beer VDK levels when increasing vaine content.

TUM68 scavenges much of the accumulated VDKs during fermentation, but in young beer obtained with this ale yeast, VDK levels still exceed the lower threshold value of diacetyl (0.1–0.4 mg/L). Additionally, reducing VDK content by increased fermentation temperature negatively affected aromagenesis by industrial *S. cerevisiae* yeast and is thus unfavorable. Therefore, industrial *Saccharomyces* yeast is suboptimal for sorghum wort fermentation overall, seeming to be well adapted to barley wort and being exigent regarding amino acids for efficient growth and aroma component synthesis. By contrast, the *Saccharomyces* yeasts isolated from African sorghum beer starter seem well adapted to sorghum wort composition. Applied to sorghum wort fermentation, they may help generate beer with large amounts of higher alcohols and esters. Furthermore, an increase of fermentation temperature from 20 °C to the tropical West African agro-ecological temperature (27 °C) improves the synthesis of aroma components and reduces VDK content. Altogether, while the beer's sensory characteristics result from the entire aroma bouquet – not only synthesized by yeast but also generated by hopping – and aromagenesis by yeast during fermentation could be affected by serial repitching, the efficient production of higher alcohols and esters is an exciting prospect for designing appropriate yeasts for sorghum wort fermentation.

5. Conclusion

The application of industrial ale yeast to sorghum wort fermentation seems not to be an optimal choice due to the amino acid requirements of this *Saccharomyces* yeast. However, brewing from 100% sorghum grain without external enzymes is indeed possible. To circumvent the need for additional enzymes, however, it is necessary to select appropriate *Saccharomyces cerevisiae* yeasts well adapted to sorghum wort composition after optimizing sorghum malt hydrolase content and using the decantation mashing procedure. The isolated yeasts DA-132, GL-198 and especially TO-37 are performant *S. cerevisiae* strains which can be used for sorghum beer production. The fermentation temperature of 27 °C allows these potent yeasts to efficiently express aroma-active components, i.e. higher alcohols and esters. The vicinal diketone content is influenced not only by yeast strain but also the time and temperature of fermentation and the wort sugar and amino acid content. These novel yeast strains provide the opportunity to produce sorghum beer with high levels of aroma-active compounds.

Acknowledgements

The authors thank Deutscher Akademischer Austauschdienst (DAAD) for providing scholarship under the grant number 57076385.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fm.2019.05.014.

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