Study on Fermentation Technology and Flavor Substances of Strong Ale Beer

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Abstract. In this study, we selected three kinds of strong ale beer yeast, which were SafAle HA-18, Lallemand Saison and SafAle BE-134, for comparative brewing experiments. The comprehensive sensory evaluations were made by testing the physical and chemical indicators, flavor substances and antioxidant capacities, according to which a yeast suitable for the brewing of ale beer with high alcohol content was selected. We showed that there were some differences between the three kinds of yeast on fermentation performance. HA-18 performed the best on sugar lowering speed, yeast sedimentation, tolerance to alcohol and production of alcohol. Saison was outstanding on reproductive capacity and reduction speed of diacetyl. Although the fermentation speed was relatively close to HA-18, the sedimentation of Saison was poor. BE-134 had better yeast sedimentation, but the overall fermentation performance was average and the sensory quality of the finished beer was poor.

Keywords: Ale Beer; Flavor Substance; Sensory Evaluation.

1. Introduction

Beer fermentation refers to the process in which wort produces alcohol, CO2, and numerous by-products through the respiration of brewing yeast. Different types of yeast product different styles of beer. Beer yeast is mainly divided into two types: Ale yeast of the upper fermentation type and Lager yeast of the lower fermentation type. Lager beer often has lower fermentation temperature and wort concentration than Ale beer, resulting in less esters which produced by yeast respiration metabolism. With the popularity of craft beer and the improvement of people's living standards, beer products with a single style cannot meet people's consumption needs. Therefore, beer fermented with Ale's yeast with rich aroma, diverse taste, and multiple styles has been chosen more.

A suitable yeast with excellent fermentation performance can often directly determine the quality of beer. The products of yeast respiration metabolism are one of the sources of beer flavor, and these substances are one of the "flavor labels" of beer. However, if the content of these substances exceeds a certain threshold, it can cause beer to produce defective flavors [1]. Hops are also one of the sources of aroma substances which mainly come from hop essential oil in beer [2]. These flavor substances are usually extracted by headspace solid-phase microextraction (HS-SPME) and detected by gas chromatography-mass spectrometry (GC-MS) [3].

This study mainly used three commercially available dry powder Ale yeast strains for comparative brewing experiments, which were SafAle HA-18, Lallemand Saison, and SafAle BE-134. We studied the fermentation performance of these three types of yeast: growth and reproduction performance, sugar consumption performance, alcohol production performance, alcohol tolerance performance, and reduction performance against diacetyl. By analyzing and testing the physical and chemical indicators, flavor substances, and antioxidant capacity of the finished beer brewed with three types of yeast, and comprehensively evaluating the fermentation performance of the three selected commercially available yeast through sensory evaluation, the most suitable one was selected.

2. Materials and Methods

2.1 Materials

Table 1. Specifications and manufacturers of main test reagents

Reagent name	Specification	Manufacturer
o-phenylenediamine	AR	TaKaRa Bio
isooctane	AR	TaKaRa Bio
absolute ethanol	AR	Sangon Biotech
DPPH	AR	Sinopharm Chemical Reagent
thiobarbituric acid	AR	Sinopharm Chemical Reagent
sodium hydroxide	AR	TaKaRa Bio
4-Methyl-2-pentenal	HPLC	Sinopharm Chemical Reagent

2.2 Experimental Equipment

Table 2. Specifications and manufacturers of main test equipment

Equipment name	Manufacturer
roller crusher	Shandong Xinwei machinery LTD
vertical automatic control fermentor	Jinan Zhengmai machinery equipment LTD
refractometer	Shanghai Lichen LTD
autoclave	Shanghai Shenan medical equipment LTD
colorimeter SD9012B EBC	Shanghai Xinrui machinery instrument LTD
uv-vis	Beijing Junyidongfang electronic technology LTD
pH meter PHS-25	Shanghai Yueping instrument LTD
turbidimeter WGZ-1A	Shanghai Xinrui machinery instrument LTD
diacetyl distiller	Shanghai Qiujing biochemical reagent instrument LTD
gc-ms	Agilent
headspace sampler	Shanghai Qiujing biochemical reagent instrument LTD

2.3 Fermentation Process of Strong Ale Beer

Malt crushing→saccharification→wort filtration→wort boiling→cyclotron precipitation→wort cooling→wort fermentation→fermentation broth cooling→finished liquor

2.4 Determination of physical and chemical indicators

Sampling: clean and dry the sampling bottle, take an appropriate amount of the tested liquid to simply rinse the sampling bottle. Afterwards, take enough of the test solution and seal it in a rinsed sampling bottle and let it stand still for treatment. Leave an appropriate amount of the test solution to measure turbidity, and the determination of other indicators requires removal of carbon dioxide and filtration.

Removal of carbon dioxide: add the treated liquid to be tested and the magnetic rotor into the beaker, and then place the beaker on the magnetic stirrer to remove carbon dioxide.

Filtration: shake the test solution to remove carbon dioxide evenly and filter the test solution using sterile filter paper. The test solution should be sealed, and the analysis of the test solution should be completed within 24 hours.

(1) Determination of yeast quantity in fermentation broth

Count the yeast in the fermentation broth using a blood cell counting board (25×16). The calculation method is as: number of yeast cells in the fermentation broth (mL-1) = 400×104×dilution ratio× (total number of cells /80)

(2) Determination of apparent sugar content

A handheld sugar meter was used to directly measure the appearance sugar content of fermentation broth.

(3) Determination of the alcohol content

The density bottle specific gravity method was used to determine the alcohol content of the sample, which was as: the relative density of the sample at 20℃ = (m2-m)/(m1-m)

m: mass of density bottle

m1: mass of density bottle with distilled water

m2: mass of density bottle with sample

Check the table to obtain the alcohol content of the beer sample.

(4) Determination of diacetyl content

A diacetyl distillation device was used to evaporate diacetyl, and then measure the absorbance value of the distillate to determine the diacetyl content in beer. The calculation method is as: the diacetyl content = A335×2.4

A335: The absorbance value of the sample at a wavelength of 335 nm

2.5 Detection of Flavor Substances in Finished Beer

The headspace solid-phase microextraction was used to extract the sample, and then the gas chromatography-mass spectrometry was used to determine the flavor substances in finished beer.

2.6 Determination of DPPH free radical clearance rate

20 mg of DPPH was dissolved in 250 milliliters of anhydrous ethanol. Then add 2 mL of DPPH solution to 2 mL of aged and carbon dioxide removed sample, mix well, and let stand for half an hour. Using anhydrous ethanol with DPPH as a blank control, determine the absorbance values of both solutions at a wavelength of 517 nm. The DPPH free radical clearance rate of the samples were calculated.

3. Results and Discussion

3.1 Changes in yeast quantity during fermentation process

The changes in yeast quantity during the fermentation process of the three selected yeasts are shown in Figure 1.

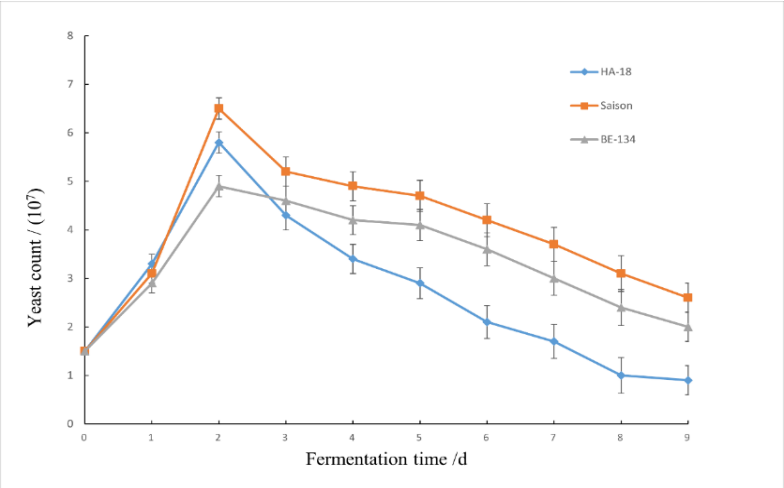

Figure 1 Changes in yeast quantity during fermentation process of three selected yeasts

We can evaluate the growth rate and sedimentation of yeast by analyzing changes in yeast count. The number of yeasts in the fermentation broth of the three yeast strains reached its peak on the second day, with Saison yeast having the highest peak, indicating that Saison yeast has the strongest reproductive ability among the three selected yeast strains. The reproductive capacity of BE-134 yeast is the weakest among the three selected yeasts, with a peak of only 4.9 × 107 mL-1. According to the slope of the line showing the decrease in yeast count in the fermentation broth and the final yeast

count, it can be inferred that HA-18 yeast has the best settling ability. Saison yeast has the weakest sedimentation ability among the three types of yeast.

3.2 Changes in appearance sugar content during fermentation process

The changes in the appearance sugar content of the fermentation broth of the three selected yeasts are shown in Figure 2. We can directly determine the fermentation speed of yeast by the changes in the appearance sugar content of the fermentation broth.

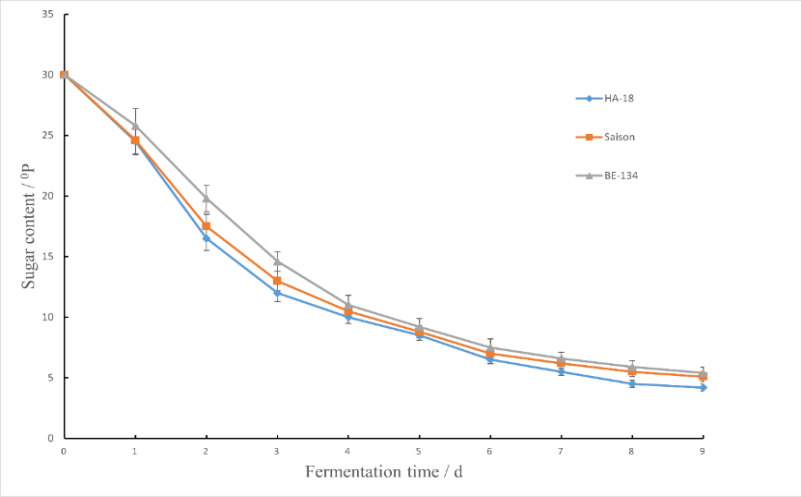

Figure 2 Changes in appearance sugar content of fermentation broth

By comparing and analyzing the changes in sugar lowering rates of the three yeast strains in the first three days, we found that the fermentation rates of HA-18 and Saison yeast strains were faster than those of BE-134 yeast strains, with HA-18 yeast strain having the fastest fermentation rate among the three selected yeast strains. After fermentation, the final appearance sugar content of the fermentation broth with HA-18 yeast was 4.2 °P, which is the lowest among the three yeast strains. This indicates that HA-18 yeast has the strongest fermentation ability among the three yeast strains selected in the experiment, and its tolerance to alcohol is also the highest among the three.

3.3 Changes in alcohol content during fermentation process

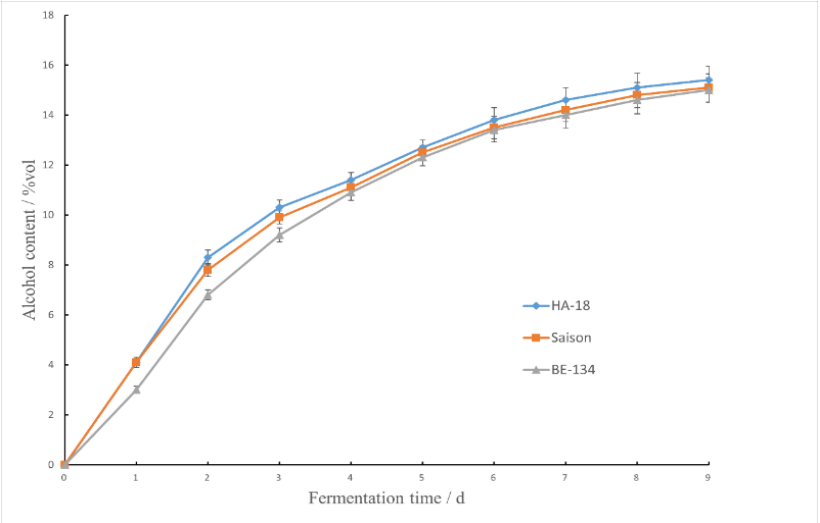

Figure 3 Changes in alcohol content of fermentation broth

As shown in the figure 2 and figure 3, in the first 3 days the sugar content and alcohol content of the fermentation broth of the three yeast strains changed relatively quickly. The alcohol content gradually stabilizes during the later stage of fermentation.

3.4 Changes in diacetyl content during fermentation

The changes in diacetyl content in the fermentation broth of the three selected yeasts are shown in Figure 4. When the diacetyl content in beer is less than 0.10 mg/L, it indicates that the beer has matured. We need to choose yeast with low diacetyl production and strong ability to reduce diacetyl for beer brewing. According to Figure 4, the diacetyl content in the fermentation broth of HA-18 and Saison yeast reached its highest value on the second day, while BE-134 yeast did not reach its highest value until the third day. After the highest content of diacetyl is reached, the fermentation process enters the diacetyl reduction stage where beer gradually matures, and the content of diacetyl in the fermentation broth begins to decrease. According to the slope of the line, it can be seen that Saison yeast had the fastest reduction rate of diacetyl among the three types of yeast. On the 7th day, the content of diacetyl in the fermentation broth decreased to 0.10 mg/L. HA-18 yeast only lowered the diacetyl content below the threshold on the 8th day; The diacetyl reduction rate of BE-134 yeast was the slowest among the three yeast strains, and the diacetyl content was only reduced to below 0.10 mg/L on the 9th day. Through comprehensive comparison, we found that HA-18 yeast has the best ability to handle diacetyl among the three types of yeast.

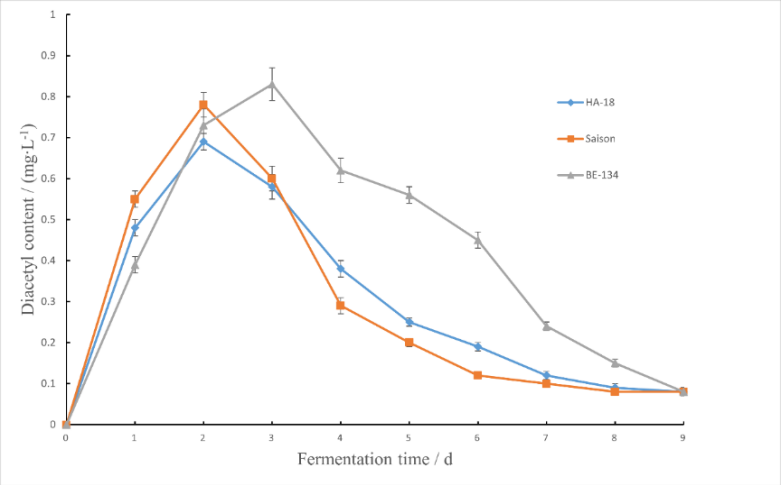

Figure 4 Changes of diacetyl content of fermentation broth

3.5 The effect of different yeasts on the flavor substances

Under the premise of the same raw material ratio and fermentation process parameters, three types of yeast, SafAle BE-134, Lallemand Saison, and SafAle HA-18, were used to brew strong Al beer. After being stored at 0 ℃ for 7 days, samples of each type of beer were taken. GC-MS was used to detect three types of beer samples, and then the various flavor substances detected were analyzed. The types and contents of flavor substances in the three finished strong Al beers are shown in Table 3.

Table 3 The main flavor substances and contents using different yeasts brewing

flavor substances (mg·L-1)	HA-18	Saison	BE-134
n-Propanol	39.322±0.081	45.314±0.215	20.205±0.073
isopropanol	34.045±0.343	27.051±0.083	44.823±0.671
isoamyl alcohol	125.738±1.309	112.414±2.196	90.890±1.316
phenylethanol	27.108±0.138	23.934±0.316	36.236±0.951
ethyl acetate	49.982±0.963	44.205±0.346	28.374±0.321
isobutyl acetate	0.474±0.042	0.135±0.018	0.121±0.035
isoamyl acetate	10.061±0.357	5.118±0.375	8.037±0.576
ethyl caproate	0.124±0.019	0.115±0.037	0.197±0.039
ethyl octanoate	0.298±0.045	0.276±0.038	0.488±0.043
ethyl decanoate	0.016±0.012	0.028±0.012	0.096±0.012
phenylethyl acetate	3.235±0.081	1.438±0.029	0.231±0.012

internal standard	5.041	5.041	5.041
acetic acid	60.927±0.636	72.618±0.940	47.528±1.258
isovaleric acid	4.193±0.054	6.305±0.024	5.302±0.051
hexanoic acid	3.991±0.018	2.981±0.043	7.136±0.017
octoic acid	3.343±0.034	1.792±0.023	6.211±0.057
capric acid	0.217±0.024	0.058±0.012	0.269±0.009
acetaldehyde	3.036±0.027	3.209±0.038	4.175±0.229

3.6 Changes in DPPH free radical scavenging rate of strong beer

As shown in Figure 5, the DPPH free radical clearance rates of three types of strong Al beer samples gradually decreased. The DPPH free radical clearance rate of HA-18 has always been the highest among the three types of samples, so the antioxidant activity of the strong Al beer brewed with HA-18 yeast is better than that of the other two types of yeast.

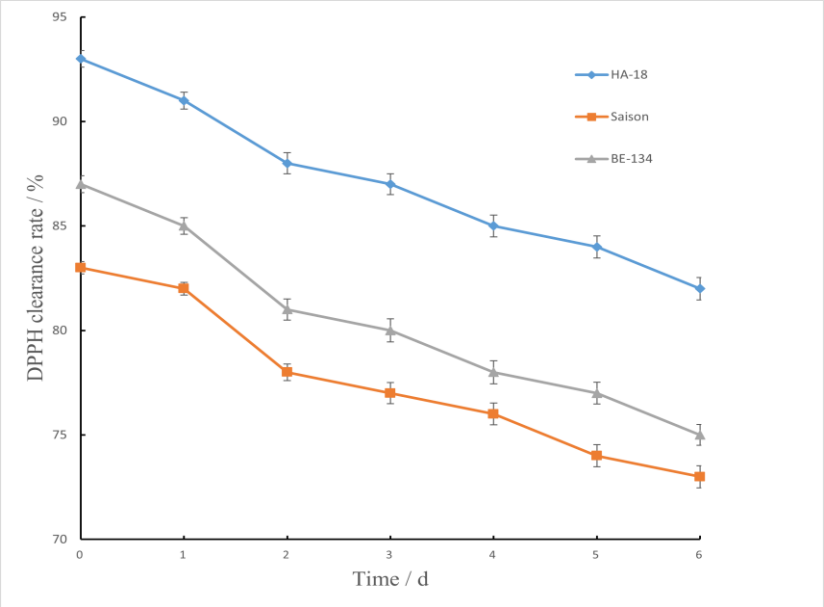

Figure 5 Change trend of DPPH free radical clearance rate

4. Summary

This article selected three commercially available Al yeasts, SafAle HA-18, Lallemand Saison, and SafAle BE-134, and selected the most suitable yeast for brewing strong Al beer through comparative brewing experiments. The results show significant differences in the comprehensive performance of the three yeast strains. Among them, HA-18 yeast's alcohol production ability, tolerance to alcohol, sugar lowering speed, and settling ability are the most excellent among the three selected yeasts. At the same time, the other performance of HA-18 yeast is also at a moderate level among the three selected yeasts in the experiment, and there are no obvious shortcomings; Saison yeast has the best reproductive ability and the fastest rate of diacetyl reduction among the three selected yeasts in the experiment, and is also close to HA-18 yeast in terms of sugar reduction rate. However, Saison yeast has poor settling ability, and the finished strong Al beer brewed with it will be relatively cloudy; BE-134 yeast has good settling ability, but its overall fermentation performance ranks last among the three selected yeasts.

The flavor substances of three finished strong Al beers obtained through GC-MS showed that the content of fusel oils in the strong Al beers brewed by HA-18, Saison, and BE-134 yeast was relatively high. The strong Al beer brewed with HA-18 yeast has the highest content of ethyl acetate, approximately twice that of BE-134 yeast. The content of acetic acid and acetaldehyde in the three types of strong Al beer samples is within the standard range of beer content, and there is no obvious adverse flavor.

The DPPH free radical clearance rate of three types of strong Al beer samples was measured, which gradually decreased and was faster in the early stage than in the later stage. The antioxidant properties of the strong Al beer brewed with HA-18 yeast are better than those of the other two yeast brewed strong Al beer.

In summary, HA-18 yeast is the most suitable yeast among the three commercially available dry powder Al yeast strains for brewing strong Al beer, and these comparative experiments also provide a theoretical and practical basis for the study of the brewing process of strong Al beer.

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