Cropping Methods and Generation Numbers of Top- Fermenting Yeasts and the Effects on the Physical and Chemical Index of Wheat Beer

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Abstract: The continuous repitching of Saccharomyces cerevisiae is an important operation in the beer brewing industry, because it takes a long time to carry out yeast propagation and increase the cost. Therefore, it is of great significance for breweries to adopt an efficient way to recycle yeast. In this article, we have studied the effects of two different methods of yeast recovery on yeast cell morphology and the quality of final wheat beer, including yeast recovery from the sampling valve during the main fermentation period and yeast recovery from the cone bottom of the cylindro-conical vessel (CCV) after cooling. Three parallel tests were performed in three 100 L conical fermentors. In addition, the morphology of the yeast cells was observed by scanning electron microscope (SEM). The entire sampling time was 15 days, including the fermentation and maturation process. The study found that in terms of physical and chemical indicators of final wheat beer, the number of cells suspended in the final wheat beer brewed from the yeast recovered from the bottom of the CCV was more, lactic acid, total acid, turbidity, fermentable sugar and alcohol content were relatively high.

1. Introduction

With the improvement of people's living standards and the change of consumption concept, wheat beer is characterized by its unique ester flavor (ethyl acetate and isoamyl acetate) and phenol flavor (4-vinylguaiacol and 4-vinyl Phenol) and loved by consumers. In Germany, wheat beer is considered to be a very important fermented specialty beer. In particular, German wheat beer must be fermented using the top-fermenting yeast, which is not filtered and clarified at the end of fermentation. Top-fermenting and bottom-fermenting yeasts are also called Ale and Lager yeasts, respectively, and are the two main brewing yeasts used in breweries [1].

For the brewery, yeast recovery is very necessary, which is conducive to stabilizing the fermentation process and saving costs. Nowadays, the research on the recovery of yeast mostly focuses on the determination of the time of recovery of yeast, the comprehensive utilization of yeast mud recovery, and the research on the storage conditions of yeast mud. For example, lack of nutrition, high concentration of ethanol and CO_2, and the presence of various static pressures, continuous use of yeast mud just cooled to 0 ℃ will cause yeast degradation and autolysis, resulting in “yeast taste” in beer. According to the research of Kucharczyk et al. [2], yeast cells were recovered on the 1st, 4th and 6th days of beer fermentation. The best yeast recovery time is to recover the yeast immediately after the fermentation and before the maturation process begins. Many previous studies have confirmed that continuous inoculation can affect the metabolic activity of yeast cells and destroy the flavor stability of the finished beer. In addition, yeast cells have a limited replication life span [3]. At the end of fermentation, the vitality and vitality of the collected yeast cells is very important, because it is necessary to ensure that a certain number of high-quality yeast cells are relocated to the next batch of fermentation [4]. Generally, the yeast cell death rate of industrial breweries should not exceed 5% [5].

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2. Mrterial and Methods

2.1 Brewing Raw Materials and Experiment Reagent

Brewing raw materials: brewing water after reverse osmosis treatment; A top-fermenting yeast strain DM303 preserved at the China-Germany Brewing Technical Center, Qilu University of Technology; Malt contains wheat malt, barley maltand caramel malt; Hops contains Qingdao Dahua and Saaz hops.

Experiment reagent: methylene blue, sodium hydrogen phosphate, monosodium Phosphate, 50% glutaraldehyde, absolute ethyl alcohol, tert-butyl alcohol, antifoaming agents, glycereth, o-Phenylenediamine, HCl, 95% ethanol, phenothalin, NaOH.

2.2 Yeast Propagation and Yeast Recovery Methods

Before industrial brewing, it is necessary to carry out yeast expansion and multiply to obtain a certain number of yeast cells. The whole yeast expansion operation is carried out on the ultra-clean workbench. Use the inoculation needle to pick the yeast deposited on the slope of wort agar, transfer to 10mL wort medium, incubate at 26 ℃ for 18-20 h, and then transfer to 100mL The wort culture medium is expanded ten times. During the cultivation period, the culture medium should be protected from bacteria.

The recovery operations adopted in this experiment are mainly divided into the following three types:(1) 0^{th} generation yeast is recorded as G0 yeast: during the main fermentation period of the yeast, transfer the fermentation liquid from the sampling valve of the 2-ton fermentation tank to the 100L fermentation tank, filled with wort and oxygenated for fermentation; (2) 1^{st} generation yeast is recorded as G1 yeast: after the temperature of the 2-ton fermentation tank was reduced to 0 ℃, the yeast was settling to the CCV bottom, and after the dead cells and impurities of the bottom layer yeast were discharged, the intermediate yeast was transferred to the 100L fermentation tank and oxygenate the wort; (3) 2^{nd} generation yeast is recorded as G2 yeast: After the G1 yeast was cooled down, draining the dead yeast cells and impurities from the bottom, the wort is re-entered and fermented by oxygenation.

2.3 Scanning Electron Microscope

Centrifuge the fermentation liquor or yeast slurry with yeast in a centrifuge at 8000r for 3-5min, wash the yeast cells with 0.1 mol/L pH 7.2 phosphate buffer solution, change the solution 4 times, add 2.5% glutaraldehyde solution, After fixing in 4℃ environment for 4h, the yeast cells were dehydrated with 30%, 50%, 70%, 80%, and 90% ethanol, and 100% tert-butanol and absolute ethanol were used to replace ethanol at a ratio of 1: 1. The yeast cells were dried in a vacuum freeze dryer for 7-8 h. At this point, the pretreatment of the yeast cells is complete and scanning electron microscope observation can be performed.

2.4 Physical and Chemical Indexes

Within 15 days, take samples every day to monitor the physiological state of yeast, diacetyl and alcohol content. Yeast growth status, including: number of yeast cells suspended in fermentation broth, yeast cell diameter, yeast clumping rate, and yeast viability. Yeast cells were stained with 0.01% methylene blue staining solution to identify yeast cell viability [6]. Diacetyl was measured by distillation, and the color reaction between distillate and o-phenylenediamine occurred. At 335nm, the 2, 3-dimethylquinoxaline produced by the color reaction had the largest absorption peak. The content of diacetyl meets Lambert-Beer law. Use the one-time pre-filled kit for the automatic beer analyzer to detect the alcohol content in the sample.

After 15 days of fermentation, the lactic acid, bitterness, color, fermentable sugar, CO2 content, total acid and turbidity in the finished beer brewed by different yeast recovery methods are detected, in which the lactic acid and bitterness are detected by the automatic beer analyzer , Color and fermentable sugar, use carbon dioxide analyzer to detect carbon dioxide, use phenolphthalein

indicator method for acid-base neutralization titration to detect total acid, and use turbidity meter to detect turbidity.

2.5 Statistical Analysis

The results presented in this study are the average of three independent experiments, and the data line represents the standard deviation. The SPSS software (V16.0, SPSS) was used to conduct a one-way analysis of variance on the experimental data to analyze the effect of different yeast recovery methods on the physical and chemical indicators of beer. Once the analysis of variance determines that there is a significant difference between the means of a certain indicator, use the Duncan test ($P < 0.05$) to confirm.

3. Results

3.1 Scanning Electron Microscope to Observe Yeast Cells

As the number of successive re-inoculations of yeast increases, yeast cells gradually age, and some cells die or cell autolysis occurs. Scanning electron microscope observations showed that the surface of G0 yeast cells in the main fermentation period was round, full, smooth and without wrinkles, and showed a complete oval shape (Figure a). After 5 days of cold storage at low temperature, due to pressure factors such as pressure, hydraulic pressure, and low temperature of the fermenter, the surface of the G0 yeast cells that settled to the bottom of the cone of the fermenter sag downward and began to wrinkle (Figure b). Compared with G0 yeast, G1 yeast cells after one generation of fermentation and G2 yeast cells after two generations of fermentation aggregated significantly, the cell wall surface folds increased, and the adhesion between cells was easier (Figure c and Figure d). With the increase of yeast generation, the number of bud marks on the surface of the mother cell increased.

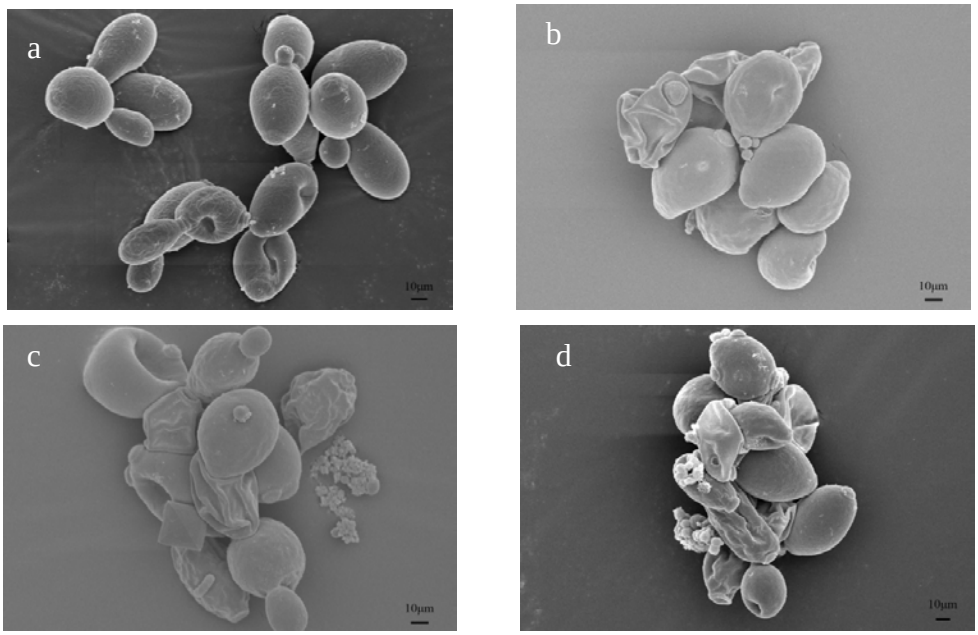

Fig.1 The Sem Images of Dm303 Yeast Cells of Different Cropping Methods and Generations a-G0 yeast cells at yeast pitching, b-G0 yeast cells at day 5 of cold storage, c-G1 yeast cells at day 5 of cold storage, df-G2 yeast cells at day 5 of cold storage.

3.2 The Change of Total Yeast Cells Numbers during Fermentation

The amount of yeast inoculation in each fermentor is controlled within the range of 1-1.5×10^7cells/mL. In Figure 2, the yeast just inoculated into the wort, G0 yeast cells proliferated fastest, and reached the peak of the cell number first, the peak proliferation of G0, G1 and G2 yeast cells were 11.7×10^7cells / mL, 7.3×10^7cells/mL and 8.47×10^7cells/mL, which is consistent with the study of Sigler et al. [7]. As the number of inoculation increases, the maximum number of suspended

cells in the fermentation broth gradually decreases. After the main fermentation is completed, the exhaust valve of the fermenter is closed, the yeast cells are affected by the pressure of the fermenter, and the cells begin to settle to the bottom of the fermenter, so the number of cells in the fermentation liquid begins to decrease.

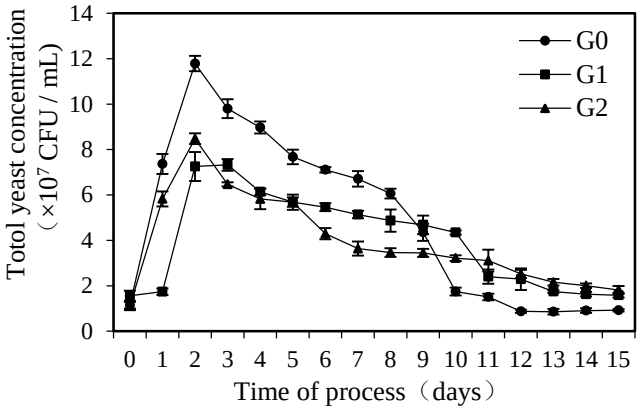

Fig.2 Evolution of Total Yeast Cell Counts ($\times 10^7$cfu/Ml) of G0, G1 and G2 during 15 Days, Values Are Means ± Sd (n = 3)

3.3 The Change of Diacetyl Content during Fermentation

As shown in Figure 3, 24 h after the G0 yeast was inoculated into the wort, the fermentation broth first reached the peak of 1.3056 mg/L of diacetyl. After fermentation of G1 yeast and G2 yeast for 48h, the corresponding peaks of diacetyl in the fermentation broth were 1.1736 mg/L and 1.253 mg/L, respectively. When the exhaust valve is closed, the enzyme produced by yeast begins to reduce diacetyl, and the content of diacetyl in the fermentation broth decreases. After 6-7 days of canister reduction, the diacetyls all fell below 0.1 mg / L, in line with national standards. G0 yeast enters the diacetyl reduction stage first. On the 5th day, the diacetyl in the fermentation broth corresponding to G0 yeast and G2 yeast is reduced to the qualified standard, but on the 6th day, the diacetyl in the fermentation broth corresponding to G2 yeast is reduced to qualified standard. At the end of fermentation, the diacetyl content in finished beer brewed by G0 yeast, G1 yeast and G2 yeast was 0.0374 mg / L, 0.0431 mg / L and 0.0336 mg / L, respectively.

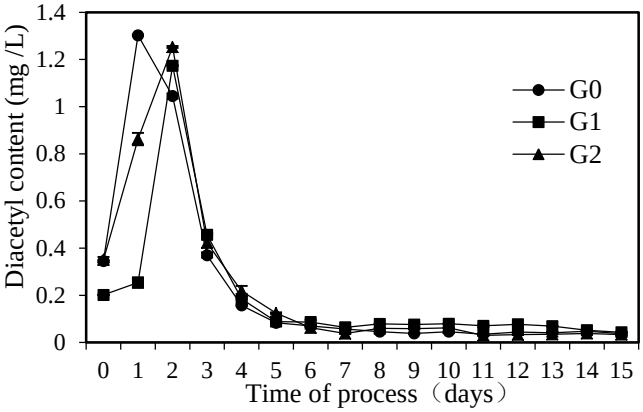

Fig.3 Diacetyl Curve of G0, G1 and G2 Yeasts during Fermentation. Values Are Means ± Sd (n=3)

3.4 The Change of Alcohol Content during Fermentation

It can be seen from Fig. 3 that G1 and G2 yeast cells have undergone re-inoculation and are more adaptable to the fermentation environment. During the entire fermentation process, the alcohol content in the fermentation broth was higher than that of the G0 yeast cells. After 15 days, G1 and G2 yeast had higher alcohol content than G0 yeast. The alcohol content of finished beer brewed by G0 yeast, G1 yeast and G2 yeast was 4.2% vol, 5.1% vol and 5.1% vol respectively.

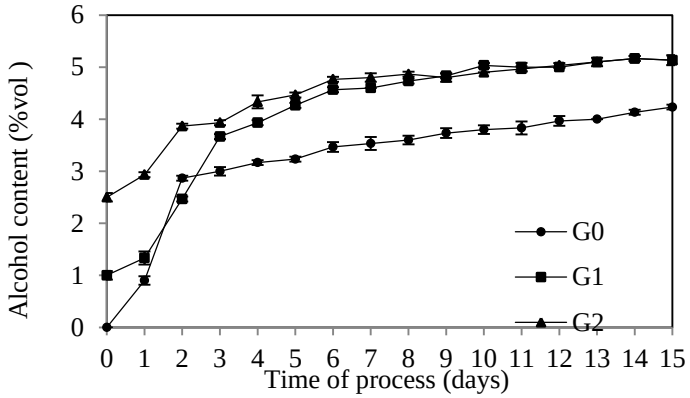

Fig.4 Variations of Alcohol Contents in Top-Fermented Wheat Beers Brewed with Different Generation Yeasts. Values Are Means ± Sd (n = 3)

3.5 Physical and Chemical Indexes in Final Wheat Beer

Table 1 lists the values of physical and chemical indicators in finished wheat beer. Compared with the finished beer brewed by the sampling valve recovery yeast, the turbidity, D (-) and L in the finished beer brewed by the cone bottom recovery yeast for re-inoculation (+) Yeast cells and cell diameters suspended in lactic acid, total acid, fermentable sugar, alcohol, and liquor increased significantly, pH and yeast agglomeration rate decreased, and there was no significant difference in beer color.

Table.1. Impact of Moments of Yeast Recoveryed from the Ccv on the Physiological Indicators of Yeast and Final Physical Index of Wheat Beer

Physical and chemical indexes	Yeast recovery methods[y]			significance
	recovery from sampling valve	recovery from conical bottom of CCV		
	G0	G1	G2	
total yeast cells (×10^7CFU/mL)	0.93[a]	1.6[b]	1.8[b]	*
D(-) and L(+) Lactic acid (mg/L)	354[a]	564[b]	998[c]	*
total acid (mL/100mL)	1.895[a]	3.1333[b]	3.4375[c]	*
bitterness (IBU)	19.4[a]	20.7[b]	20.5[c]	*
turbitity (EBC)	79.4[a]	84.1[b]	95.3[c]	*
fermentable sugars (g/L)	1.6[a]	1.8[b]	2.0[c]	*
color (EBC)	12[a]	12[a]	12[a]	ns

*signifcance at 5%; ns – not signifcant; [y]according to the Duncan's test means within columns followed by the same letter are not signifcantly different

4. Conclusion

In Germany, the brewing environment is clean. The traditional equipment for brewing wheat beer is an open fermentation tank. Following the brewing characteristics of the above yeast, the upper yeast is recovered from the top of the open fermentation tank. However, in China, due to the impact of environmental quality, wheat beer is generally brewed in large closed conical fermentation tanks. Due to the large hydrodynamic pressure, the size of the CO2 bubbles wrapped by the yeast flocs is limited, and more yeast is recovered. After the temperature is lowered, the yeast sinks to the bottom of the conical fermentation tank and is then recovered. Due to the influence of the fermentation broth height and fermentation pressure in the fermentor, the fermentation yeast deposited on the cone bottom is subjected to extreme pressure (sometimes up to 0.3-0.5MPa, $p = \rho gh$, $F = ps$), which seriously affects the yeast morphology and Physiological characteristics, thus affecting the inherent typical flavor of fermented wheat beer above. As the number of inoculations increased, the cell morphology changed, and the degree of folds and depressions on the cell wall surface increased. This

study found that different yeast recovery methods have a greater impact on the physical and chemical indicators of finished wheat beer. Compared with G0 yeast brewed beer, G1 and G2 yeast The brewed beer has a cloudy appearance, obvious beer acidity, and headache after drinking.

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