Application of Biological Acidification Technology in Brewing Process of Sour Beer

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Abstract: Sour beer is a high-end craft beer with high organic acid content. In this paper, Lactobacillus bulgaricus is inoculated in wort before wort boiling without hops to bioacidify for sour beer brewing. Through orthogonal test, taking the pH value of acidified wort as the evaluation index, the influence of the original gravity, acidification temperature and inoculation amount on the acidification performance of Lactobacillus bulgaricus is studied. The best acidification conditions of Lactobacillus bulgaricus are determined as follows: the original gravity is 14°P; the acidification temperature is 42 ℃; the inoculation amount is 1×107 CFU/mL, acidification 72 hours, and the final wort pH is 3.47. After acidification by Lactobacillus bulgaricus, the content of lactic acid and α-amino nitrogen in wort is up to 5.32g/L and 156.72mg/L respectively. The acidified wort made by Lactobacillus bulgaricus under the best acidification conditions is used in the production of sour beer. The finished sour beer is rich in organic acid, amber color, mellow taste, strong mouthkilling power, strong fruit flavor, outstanding ester flavor, rich taste and balanced acidity.

1. Introduction

Sour beer originated in Belgium. Lambic is one of the most typical representatives of sour beer. It is a kind of naturally fermented beer with mixed bacteria. It has a production history of more than 1500 years [1].The fermentation of Lambic mainly relies on traditional natural fermentation, and then can be stored in oak barrels to enrich the flavor and taste of sour beer. The brewing cycle can be as long as 1-3 years. This mixed fermentation mode leads to the competition between microorganisms on nutrients, which will affect the growth and reproduction of microorganisms and the production of metabolites. Therefore, this traditional mixed fermentation mode has an impact on product quality Consistency is hard to control [2-4].In recent decades, people's interest in brewing sour beer is growing.

Biological acidification technology plays an important role in the brewing process of sour beer. It can not only improve the buffering performance of mash and wort, so as to improve the fermentation performance, but also enhance the activity of various enzymes in raw materials, so as to improve the utilization rate of raw materials. There are three kinds of biological acidification methods for sour beer: in tank acidification, mixed fermentation acidification and acidification with producing acid yeast [5].*Lactobacillus bulgaricus* has a high production of lactic acid, and does not produce diacetyl, amines and other substances that affect the flavor of sour beer. However, *Lactobacillus bulgaricus* is highly sensitive to hops and can hardly grow in wort containing hops [6].Therefore, *Lactobacillus bulgaricus* was inoculated into wort without hops for biological acidification. The acidification method in the tank is simple and easy to control. The acidification process can be stopped at any time by boiling, and the fermentation cycle is shorter, which provides technical support for the development of sour beer.

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2. Materials and Methods

2.1 Preparation of Wort

Weigh the proper proportion of malt(67% Pilsner malt and 33% wheat malt), Crushed the weighed malt and mashed(1 kg of malt/3.5 L of water).The mashing process is 52℃ for 40 minutes, 65℃ for 70 minutes, 72℃ for 10 minutes, and 78℃ for iodine detection. If the iodine detection is complete, the mashing is over, and the heating rate is 1℃/min. After mash, the wort is refluxed, refluxed until the wort is clear and filtered, and the filtered wort is boiled. After boiling, the wort was cooled to a suitable temperature for biological acidification.

2.2 Production of Sour Beers

Using 100L professional pilot brewing equipment to produce sour beer. The procedure before boiling is the same 2.1.After boiling, it is cooled to the proper temperature for inoculation of *Lactobacillus bulgaricus* (SBI International Trade Co.Ltd, China), and then Lactobacillus bulgaricus is inoculated for wort acidification. When the required acidity is reached, it is boiled again to terminate acidification. Keep boiling for 70 minutes, add hops three times in the boiling process, and the proportion of hops is 1.2‰ of the weight of wort. After the second boiling, the wort was cooled to 22℃, then the wort was introduced into the fermentation tank, and top-fermentation dry yeast (Safale US-05, Fermentis, France)was inoculated in wort with 0.5g/L for primary fermentation.In order to make yeast grow and reproduce better, 1g/100L of yeast nutrient is added to the top of fermentation tank. After primary fermentation for 2 weeks, the fermentation broth were kept at 0℃ for 7-10d to produce finished sour beer.

2.3 Optimization of Wort Acidification Process

In order to achieve the best effect of wort acidification, the technological conditions of wort acidification were optimized. The change of pH value of acidified wort can best reflect the fermentation performance of acidified strains [7]. Therefore, taking the change of pH value as the evaluation index, orthogonal test were carried out to optimize the acidification conditions of wort, with the original gravity, acidification temperature and inoculation amount as the main factors,It provide strong support for the brewing of sour beer,

2.4 Analytical Methods

Sensory evaluation is conducted by a evaluation group composed of professional teachers from China Germany Beer Technology Center. Physical and chemical index test follows GB/T4928-2008 (Recommended National Standard of the People's Republic of China). Determination of organic acids(lactic acid, acetic acid, pyruvic acid,citric acid) by high performance liquid chromatography(HPLC)[8].The volatile flavor compounds were determined by headspace solid phase microextraction and gas chromatography(HS-SPME-GC).

2.5 Statistical Analysis

The calculated results are the average values of three independent experiments, which are expressed as mean±standard deviation.Results were analyzed using one way analysis of variance (ANOVA) and Tukey' test (SPSS Corporation, version 22.0, Chicago, IL, USA).The significance of the content of flavor substance and physical and chemical indexes of sour beer and the statistical signifificance level was set at $p < 0.05$.

3. Results and Discussion

3.1 Analysis on the Optimization of Wort Acidification Process

The main factors affecting the acidification performance of *Lactobacillus bulgaricus* were the original gravity, acidification temperature and inoculation amount, and three levels were selected. orthogonal test design is carried out according to L_9 (3^3), and the pH value of acidified wort is taken as the evaluation index to determine the best acidification process of *Lactobacillus bulgaricus*. Orthogonal test factors and levels of wort acidification process are shown in Table 1; orthogonal test results and analysis are shown in Table 2; orthogonal test variance analysis is shown in Table 3.

Table 1 Factors and Level of Orthogonal Test for Acidification of Lactobacillus Bulgaricus

Levels	Factors		
	(A)Orginal gravity(°P)	(B)Acidification temperature (°C)	(C)Inoculation amount (CFU/mL)
1	12	42	1×10^6
2	14	44	1×10^7
3	16	46	1×10^8

Table 2 Orthogonal Test Results and Analysis of Acidification Process of Lactobacillus Bulgaricus

Groups	A	B	C	pH
1	1	1	1	3.51
2	1	2	3	3.56
3	1	3	2	3.64
4	2	1	2	3.47
5	2	2	1	3.53
6	2	3	3	3.59
7	3	1	3	3.48
8	3	2	2	3.54
9	3	3	1	3.62
K_1	3.570	3.487	3.553	
K_2	3.530	3.543	3.550	
K_3	3.547	3.617	3.563	
R	0.056	0.147	0.023	
Element size	B>A>C			
Optimal combination	$A_2B_1C_2$			

Table.3 Variance Analysis of Orthogonal Test for Acidification Process of Lactobacillus Bulgaricus

Factors	Sum of squared deviations	Degree of freedom	F Value	P Value	Significance
A	0.002	2	109.00	0.009	**
B	0.025	2	147.00	0.005	**
C	0.001	2	17.00	0.06	
Error	0.0002	2			

*P < 0.05, indicating significant influence of factors;**P < 0.01 indicates that the influence of factors is very significant.

From the data analysis, when the original gravity is 14°P, *Lactobacillus bulgaricus* has the best acidification performance(Fig.1a);When the acidification temperature is 42℃, the acidification performance of *Lactobacillus bulgaricus* is also the best(Fig.1b);When the inoculation amount is 1×10^7CFU/mL, the acidification performance of *Lactobacillus bulgaricus* is also the best(Fig.1c);It can be seen from Fig.1d that when the acidification time reaches 72h, the pH value almost drops to the lowest point of 3.45, and tends to be stable. In order to save time and improve production capacity, the most appropriate acidification time is 72h.Therefore, take the change of pH value of acidified wort as the evaluation index, choose the original gravity, acidification temperature and inoculation amount as the main factors to carry out orthogonal test. According to the data analysis in Table 1-3, the best conditions of acidification process of wort are: the original gravity is 14°P, acidification temperature is 42℃, inoculation amount is 1×10^7CFU/mL, acidification time is 72 h, and the pH value of final acidified wort is3.47.

The optimal acidification conditions of wort obtained by single factor analysis and orthogonal test are used for biological acidification of wort. After acidification, various indexes of wort are shown in

Table 4.

Table.4 Determination Results of Acidified Wort Indexes

Indexes	Results
Lactobacillus cells (10^8CFU/mL)	6.74±0.13
Lactic acid (g/L)	5.32±0.22
α-amino nitrogen (mg/L)	156.72±2.64
Viscosity (mPa/s)	1.61±0.03
Diacetyl (mg/L)	0.069±0.213
pH	3.48±0.32
Color (EBC)	38.71±1.24
Bitterness (IBU)	13.12±0.54

The results of Table 4 show that: the Lactobacillus cells in the acidified wort reaches 6.74×10^8 CFU/mL, the content of lactic acid is 5.32 g/L, and the pH value is 3.48; the content of α-amino nitrogen in the acidified wort is 156.72mg/L, the content of diacetyl is 0.069 mg/L, and the indexes of viscosity, chroma and bitterness also meet the requirements of the national standard.

3.2 Sensory Evaluation of Sour Beer

According to the above-mentioned optimal technological conditions of wort acidification, acid beer was brewed, and the sensory evaluation of the brewed product was carried out. The comparison results of sensory evaluation are shown in Figure 1. The results showed that the finished product of sour beer scored higher in appearance, mouthkilling power, flavor coordination and acid sense, the ester flavor and mellow sense were relatively up to standard, and the foam retention was slightly poor. All the members of the final evaluation team think that this product is a rare sour beer with mellow taste, strong killing power, strong ester flavor, balanced acidity and full body.

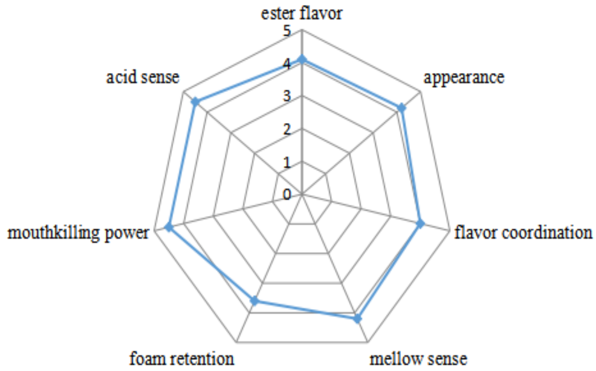

Fig.1 Sensory Evaluation Chart of Sour Beer

3.3 Analysis of Physical and Chemical Indexes of Sour Beer

Table.5 Determination Results of Physical and Chemical Indexes of Sour Beer

Indexes	Results
Original gravity (°P)	14.05±0.15
Alcohol counter (%vol)	5.92±0.08
True fermentation degree (%)	85.3±1.3
Color (EBC)	27.53±0.27
Turbitity (EBC)	0.98±0.15
Foam retention (s)	170±2
Carbon dioxide (g/L)	3.93±0.25
Bitterness (IBU)	9.21±1.73
Total acid (mL/100mL)	2.83±0.11
pH	3.52±0.06
Diacetyl (mg/L)	0.068±0.007

The results of physical and chemical index determination of finished sour beer are shown in Table

5. It can be concluded that the alcohol accuracy of sour beer is 5.92% vol, the real fermentation degree is as high as 85.3%, the total acid content is 2.83mg/l, pH value is 3.52; the turbidity value is 0.98EBC, which is relatively low, because it can promote yeast flocculation at a lower pH value, which is conducive to wine body clarification; the diacetyl content is 0.068mg/L, which is lower than the sensory threshold; the foam holding capacity is 170s. It is relatively low, and the foam retention is closely related to the CO_2 content in sour beer and the quality of malt. All other physical and chemical indexes meet the national standard GB/T 4928-2008(Recommended National Standard of the People's Republic of China).

3.4 Analysis of Organic Acids in Sour Beer

It can be obtained from Figure 3: the lactic acid content in the finished sour beer is 1830mg/L, which is higher than the sensory threshold; the acetic acid content is 148mg/L; during the yeast fermentation process, the presence of lactic acid will inhibit the production of pyruvic acid, so the pyruvic acid content is relatively Low, 83mg/L; citric acid content is 135mg/L. The content of lactic acid is higher than the sensory threshold, and the content of other organic acids is lower than the sensory threshold. The acidity of the finished sour beer is balanced. This is because the acidity is influenced by a variety of acidic substances. The right amount of acid makes the taste of sour beer more refreshing.

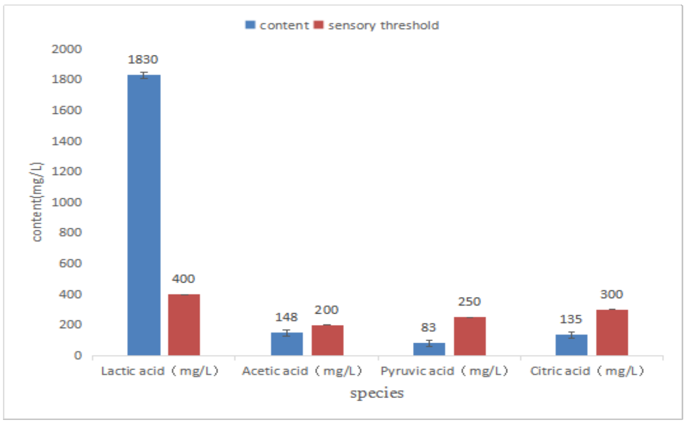

Fig.2 Determination Results of Physical and Chemical Indexes of Sour Beer

3.5 Analysis of Volatile Flavor Compounds in Sour Beer

Table.6 Types and Contents of Main Volatile Flavor Substances of Sour Beer

Flavor substance	Content (mg/L)	Sensory threshold
n-Propanol	21.35±0.31	600
Isobutanol	40.25±1.04	100
Isoamyl alcohols	47.62±1.52	50
2-Phenylethanol	28.43±2.14	40
Ethyl decanoate	0.05±0.01	1.5
Ethyl octanoate	0.21±0.02	0.9
Ethyl acetate	18.24±1.02	30
Ethyl lactate	31.02±0.52	40
Ethyl hexanoate	0.12±0.01	0.21
Isoamyl acetate	0.58±0.04	1.0
Ethyl butyrate	0.15±0.01	0.4
Isobutyl acetate	0.09±0,01	1.6
Ethyl 2-phenylacetate	0.18±0.02	3.8
Acetaldehyde	5.76±0.31	10
Dimethyl sulfide	<0.01	0.03-0.045
Diacetyl	0.06±0.01	0.1
Acetoin	1.72±0.25	50
Internal standard	5.04	
Isovaleric acids	0.57±0.02	1.5
Caproic acid	1.67±0.01	8

Caprylic acid	4.37±0.18	15
Capric acid	2.13±0.05	10

HS-SPEM-GC was used to determine the types and contents of volatile flavor compounds in sour beer, and the results are shown in Table 6. The total content of these higher alcohols is 137.65mg/L. Esters contribute to the overall flavor of the sour beer and are important aroma compounds, but excessively high levels will be regarded as off-flavors. In this study, the content of ethyl acetate in sour beer is 18.24mg/L, the content of isoamyl acetate is 0.58mg/L , ethyl lactate content is 31.02mg/L The content of esters is rich, which brings rich fruit flavor to sour beer.Tthe content of acetaldehyde in sour beer is 5.76mg/L, within the sensory threshold; the content of dimethyl sulfur is less than 0.01mg/L In the normal range, no bad flavor.

4. Conclusions

In this paper, the application of biological acidification technology in the brewing process of sour beer was studied. Using *Lactobacillus bulgaricus* to acidify the wort in the brewing process of sour beer not only reduces the pH value of wort, but also greatly improves the flavor of sour beer. The final brewed sour beer has a mellow taste, rich fruit flavor and balanced acidity. Therefore, the application of biological acidification technology provides theoretical basis and scientific basis for the industrial production of sour beer.

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