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Study of Sulphides Production Affecting the Fermentation Process of the Lager Beer

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Abstract: In the lager brewing production process, volatile sulphides such as sulphur dioxide, hydrogen sulphide, thiols and thioesters are produced by yeast metabolism during the fermentation of lager beer. Fluctuations in malt raw materials, yeast strain characteristics and fermentation process parameter adjustments can all have an impact on sulphides, and the concentration of sulphides in finished beer can have a negative impact on the sensory tasting of lager beer. This study investigated the fermentation of sulphides in lager beer under the conditions of dissolved wort oxygen, yeast recovery, fermentation temperature and pressure, and the results showed that the longer the yeast growth time, the later the fermentation of sulphur dioxide and the lower the sulphur dioxide content in the final beer by 10mg/L. The longer the yeast recovery days, the higher the fermentation of sulphur dioxide and hydrogen sulphide. The higher the content, the higher the fermentation temperature of the lager and the faster the fermentation rate, which favours a low sulphur dioxide content at the end of the main fermentation.

Keywords: Lager Beer; Fermentation Process; Sulphur Dioxide; Hydrogen Sulphide.

1. Introduction

Sulphides are mainly produced by yeast metabolism during the main fermentation phase of beer. Sulphur dioxide and hydrogen sulphide produced during beer fermentation are the result of the biosynthesis of the sulphur-containing amino acids methionine and cysteine via the sulphate reduction pathway. Inorganic sulphate is absorbed via sulphate permease, and SO_2 reductase reduces SO_2 to HS_2[1]. The yeast uses water or malt raw material to synthesise Sulphur containing amino acids to produce the intermediate product sulphur dioxide, which has an antioxidant effect. Sulphur dioxide plays a vital role in the flavour stability of the finished lager beer, while H_2S has an adverse effect on the flavour of the beer.

During the fermentation of lager beer, SO_4^{2-} from the fermentation broth is transported into the yeast cell, where it is converted to SO_3^{2-} by a series of catalytic reactions with ATP sulfidase, APS kinase and PAPS reductase, and then to S^{2-} by the action of sulfite reductase, and then on to the downstream sulfur-containing amino acids. When there is a significant accumulation of SO_3^{2-} in the yeast cell due to certain factors, the yeast's own protective mechanism kicks in and pumps it into the extracellular fermentation broth, causing a rise in the sulphur dioxide content of the beer [2]. Large amounts of sulphur dioxide can cause an undesirable sulphur odour in the fermentation broth, which not only affects the organoleptic taste of lager beer, but also acts as an antioxidant and antibacterial agent when lagering. However, a small percentage of the population suffers from sulphite sensitivity, with side effects including a range of skin conditions, lung, gastrointestinal and cardiovascular symptoms. It is therefore important to control sulphur dioxide levels to avoid these adverse effects on human health. The European Parliament and Council Regulation on the provision of information to consumers on food products lists the sulphur dioxide substances that cause allergies or intolerances [3]. If a total sulphur dioxide level of 10 mg /L or more is detected, the total sulphur dioxide level must be stated on the label of alcoholic beverages [4].

The factors affecting the levels of sulphur dioxide and hydrogen sulphide include wort oxygen content, yeast activity and yeast proliferation. To improve the sensory quality and flavor stability of lager beer during shelf life, and to accumulate production experience and theoretical basis for regulating the production of sulfur dioxide and hydrogen sulfide during the fermentation process of lager beer, this study is of importance.

2. Materials and Methods

2.1. Materials and Reagents

2.1.1. Materials

French malt, imported American hops, lager yeast A.

2.2. Reagents

Paraffin; iodine solution; formaldehyde; sodium hydroxide; sulphuric acid, Sinopharm Chemical Reagent Co. All reagents are analyticall grade.

2.3. Analysis Methods

2.3.1. Plato Test

Transfer the fermentation broth to a funnel with filter paper, place the filtered fermentation broth in a stainless steel cylinder, hold the upper end of the saccharimeter, slowly place the saccharimeter with a sugar range of 0-20°P into the fermentation broth, wait for the saccharimeter to stabilise, read the scale value of the cut-off line, check the correction value corresponding to the mark at the bottom of the saccharimeter, when the fermentation broth is below 20°C, the value displayed by the saccharimeter minus the correction value, the corrected reading is the fermentation broth sugar level.

2.3.2. The Detection of Wort Oxygen Content Level

Connect the sampler and the oxygen meter inlet hose, put the outlet hose into the waste liquid bucket, rotate the instrument flow rate adjustment is open, open the sampling valve, wait for the measurement pool is full of beer liquid, press the start button to measure, adjust the outlet flow rate should flow down vertically and the flow rate remains stable, wait for the data to stabilize and record the data.

2.3.3. The Determination of Sulphur Dioxide Content

Add 1 drop of defoamer to the blank sample, 5 ml of un-degassed sample to a small measuring cylinder, then add 45 ml of distilled water, then add 0.3 ml of 0.025 mol/L iodine solution and 10 ml of p-phenylene red and shake lightly, add 10 ml of formaldehyde and shake lightly, set the volume to 100 ml and set aside, water bath 25°C to reach 30 min, zeroing at 570 nm visible light.

Add 1ml of mercury stabiliser to the test sample, then add 25ml of sulphuric acid and shake gently to mix, add 1 drop of defoamer and 5ml of beer sample into a small measuring cylinder, prevent the liquid from splashing during light shaking, add 7.5ml of 0.1mol/L NaOH, then add 5ml of sulphuric acid and 30ml of distilled water, then add 10ml of p-phenylene red, shake well and add 10ml of formaldehyde, fix the volume to 100 ml, water bath 25 °C to 30 min, with 570 nm visible light test, check the standard curve graph to get the results.

2.3.4. The Test of Yeast Slurry Concentration

Using an electronic balance, weigh an empty centrifuge tube G_1, take a yeast sample from this tube and measure the weight of the centrifuge tube containing the yeast slurry G_2 and place this tube on the centrifuge tray. Take a water-filled tube of the same weight as G_2 and place it on the symmetrical tray with the top cover. Turn on the centrifuge power switch; set the speed to 3000 rpm; set the time knob to 15 min and press the "Start" switch to run centrifuging for 15 min. When the centrifuge has completely stopped working, open the top cover, remove the centrifuge tube with the yeast and pour out the liquor from the tube. Use an electronic balance to weigh the yeast G_3.

The yeast slurry concentration $G = [(G_3-G_1)/(G_2-G_1)] \times 100\%$.

2.3.5. The Detection of Sulphur Dioxide

HS-GC-PSPD method [5] was used.

2.3.6. Yeast Counting Method

A blood cell counter was used to count yeast cells.

2.4. Experimental Design

The lager beer process parameters were adjusted to 10 mg/L of dissolved oxygen in the wort prior to yeast addition by wort oxygenation. Yeast was pitched at a rate of 12×10^6 cells/mL. The main fermentation temperature was 10°C and the top of the fermenter was not under pressure. All trials were carried out in scale lager fermenters with a batch size of 310m³.

2.4.1. Effect of Wort Oxygen Content on the Contents of Sulphur Dioxide and Hydrogen Sulphide in Lager Beer Fermentation

The lager beer fermentation process parameters were fermentation temperature of 10°C, yeast inoculum of 10×10^6 cells/mL and yeast recovery on day 9. Under these same conditions, Wort oxygen content in the wort was 8 mg/L, 9 mg/L, 10 mg/L and 11 mg/L respectively to analyze the effect on sulphur dioxide and hydrogen sulphide in the fermentation broth under different conditions of dissolved oxygen in the wort.

2.4.2. Effect of Harvest Yeast on Sulphur Dioxide and Hydrogen Sulphide Content in Lager Beer Fermentation

The effect of different harvest yeast on fermentation broth sulphur dioxide was analysed under different fermenter harvest yeast time conditions.

2.4.3. Effect of Fermentation Rate on the Content of Sulphur Dioxide and Hydrogen Sulphide Produced by Lager Beer Fermentation

The fermentation rates were analyzed at 1.4°P/day, 1.5° P/day, 1.6°P/day, 1.7°P/day, 1.8°P/ day in lager beer and the effect of different fermentation temperatures on the concentrations of sulphur dioxide and hydrogen sulphide was researched in the fermentation broth.

3. Results and Analysis

3.1. Effect of Wort Oxygen Content on the Contents of Sulphur Dioxide and Hydrogen Sulphide in Lager Beer Fermentation

Under different wort oxygen content conditions, as wort oxygen content increased, the peak sulphur dioxide content gradually decreased, the peak sulphur dioxide content was 18.5mg/L, 17.1mg/L, 16.3mg/L, 15.0mg/L, respectively, with a negative correlation, as shown in Figure 1, lager beer produced sulphur dioxide and hydrogen sulphide slowly at the beginning of fermentation, and as fermentation was basically completed on the 5th day of fermentation, the peak sulphur dioxide content was in the range of 14-18mg/L, and hydrogen sulphide was in the range of 0.10-0.15mg/L. The peak sulphur dioxide was in the range of 14-18mg/L and hydrogen sulphide was in the range of 0.10-0.15mg/L. Wort oxygen content was low and lager beer produced higher levels of sulphur dioxide and hydrogen sulphide during slow fermentation at the start of the main fermentation, with higher wort oxygen content in the wort reducing the amount of sulphur dioxide and hydrogen sulphide produced. During the fermentation of lager beer, after the peak sulphur dioxide yeast, the sulphur dioxide content decays, see Figure 2, while hydrogen sulphide decreases rapidly, the consumption of fermentable sugars is a key factor in the rapid reduction of hydrogen sulphide, the fermentable sugar assimilation rate drops below 0.05 w/w%/h, we observe a rapid H_2S reduction, during its growth phase, the yeast absorbs sulphate, which is then reduced to sulphite, then sulphide, and for the synthesis of sulphur-containing amino acids. As metabolism slows, sulphite and sulphide are released from the yeast cells. When yeast outgrowth occurs, the H_2S in the fermenting wort falls very rapidly, probably through yeast uptake, making the sulphide demand greater than the intracellular supply of sulphide through the reduction of sulphate and sulphite [6].

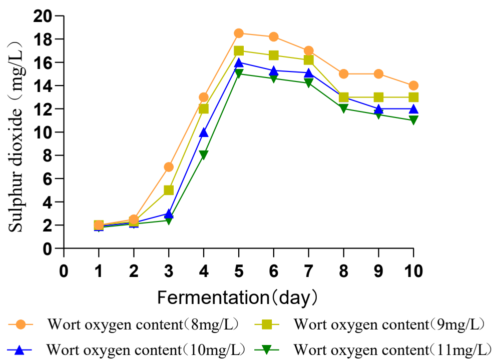

Figure 1. Sulphur dioxide production curve per day on fermentation

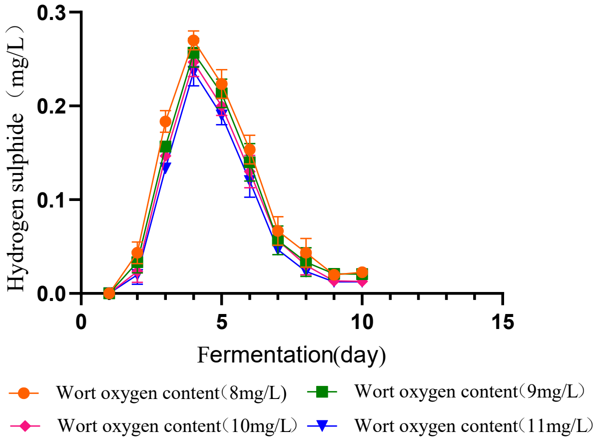

Figure 2. Hydrogen sulphide production curve per day on fermentation

Lager beer in the fermentation process wort oxygen content determines yeast proliferation, as shown in Figure 3 , under the conditions of wort oxygen content 8 ~ 10mg/L, lager beer logarithmic period to maintain aerobic yeast growth, yeast absorption of sulfur-containing amino acids, the later the production of sulfur dioxide, fermentation peak yeast number 40 ~ 45 x 10^6 Cells/mL, fermentation broth sulfur dioxide peak number of 16.3mg/L, with a positive correlation with the peak The later the fermentation process enters the yeast anaerobic fermentation stage, the more sulphate is absorbed by the yeast and converted to SO_2 and the shorter the anaerobic fermentation formation time of sulphur dioxide, therefore, the lower the sulphur dioxide and hydrogen sulphide content in the fermentation broth end product.

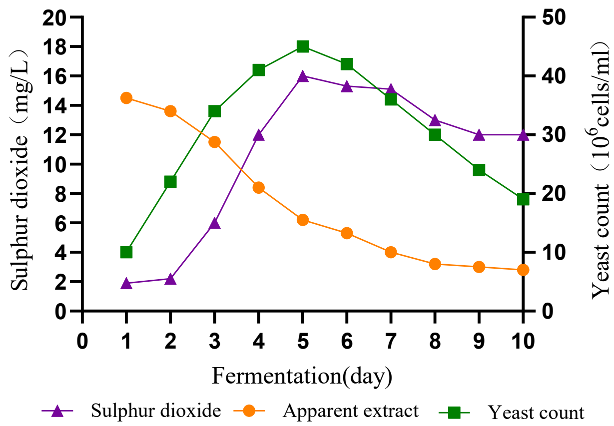

Figure 3. Changes in sulphur dioxide content during Fermentation

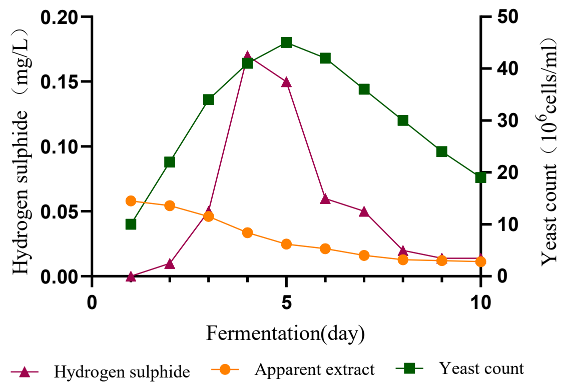

Figure 4. Changes in hydrogen sulphide content during Fermentation

In the scale lager brewing process in Figure 4, the fermentation process can result in high sulphur dioxide in the fermentation broth, insufficient wort oxygenation can trigger a too short aerobic phase of yeast, insufficient yeast proliferation and low fermentation rate, and a long anaerobic fermentation phase of yeast, which produces high sulphur dioxide content, and also exclude low levels of amino nitrogen wort, zinc ions, and excessive sulphate content in the water, all of which have a high impact.

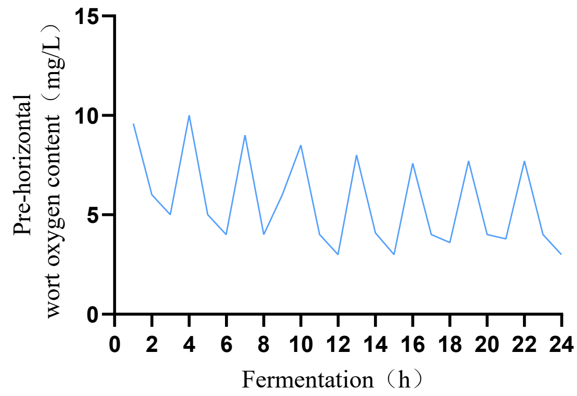

Figure 5. Variation in wort oxygen content of pre-horizontal fermentation

The wort oxygen content required to ferment yeast in lager beers is mainly achieved by controlling the full tank time within 24h, as shown in Figure 5, during the 10 batches of wort into the tank, all the oxygen is not consumed by the yeast. Control the fermentation solution dissolved oxygen at 8-6mg/L during tank feeding. If the wort oxygen content is too low it will lead to low provision of yeast oxygen and the lager beer fermentation produces increased levels of sulphur dioxide and hydrogen sulphide. Increasing wort oxygenation reduces sulphur dioxide production and promotes yeast growth, and if the wort is rich in esters, then sulphur dioxide and hydrogen sulphide production is reduced.

3.2. Effect of Harvest Yeast on Sulphur Dioxide and Hydrogen Sulphide Content in Lager Beer Fermentation

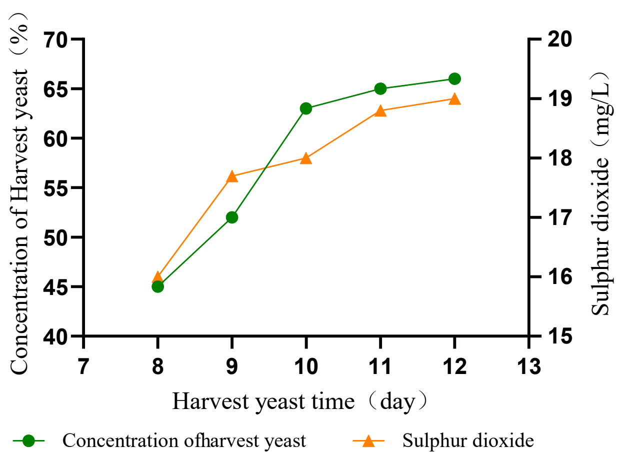

Figure 6. Effect of harvest yeast on sulphur dioxide content

As shown in Figure 6 and 7, the later the yeast is recovered, the yeast slurry concentration rises gradually from 45% to 66% after recovery from day 8 to 12, lager beer fermentation produces a positive correlation from 16 mg/L to 19.1mg/L gradually, lager beer fermentation drops sugar more than 1.8°P/day, after the peak of yeast, yeast settles rapidly to the bottom of the cone, yeast will undergo The degree of yeast autolysis is related to yeast mortality to a certain extent, and yeast autolysis is an inevitable process in beer fermentation. Yeast autolysis is caused by a reduction in cell viability when process conditions are poor or nutrients are depleted, triggering the breakdown of autolysis enzymes that enable the cells to digest their own structures [7]. These will lager beer fermentation sulphur dioxide and hydrogen sulphide levels rise.

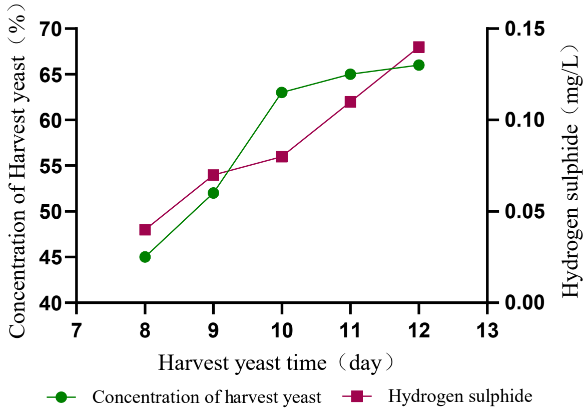

Figure 7. Effect of harvest yeast on sulphur dioxide content

High yeast metabolism and faster fermentation rates allow more sulphites to be metabolised by the yeast to synthesise the sulphur-containing amino acids required for its own metabolism, producing less SO_2. Factors that are not conducive to yeast activity levels, such as insufficient yeast inoculum, can lead to increased SO_2 and hydrogen sulphide.

3.3. Effect of Fermentation Rate on Sulphur Dioxide and Hydrogen Sulphide Content Produced by Lager Beer Fermentation

In the main fermentation process of lager beer, temperature control is crucial. A low main fermentation temperature results in a slow decrease in wort concentration and a longer fermentation time; conversely, a high main fermentation temperature results in a fast decrease in wort concentration and a shorter fermentation cycle. When the temperature is raised, the metabolism of the yeast changes and many volatile substances are produced, which can seriously affect the flavour of the beer.[8] This will seriously affect the flavour of the beer. As in Figure 8, the higher the fermentation temperature of lager beer, the higher the fermentation temperature is controlled at 10 ± 0.5 °C, the fermentation rate reaches 1.4 to 1.8 °P/day, the fermentation rate is fast, the main fermentation period drops sugar rapidly, which is conducive to the production of sulphur dioxide in the fermentation boom and shortens the main fermentation period of sulphur dioxide, and the sulphur dioxide content is low at the end of the main fermentation. During the fermentation of lager beer, the sugar drop is slow, which can lead to high carbon dioxide levels during the storage period. Yeast activity factors are influenced by the fermentation environment, such as excessive wort concentration, high yeast mortality and insufficient wort oxygenation, which can cause high sulphur dioxide and hydrogen sulphide.

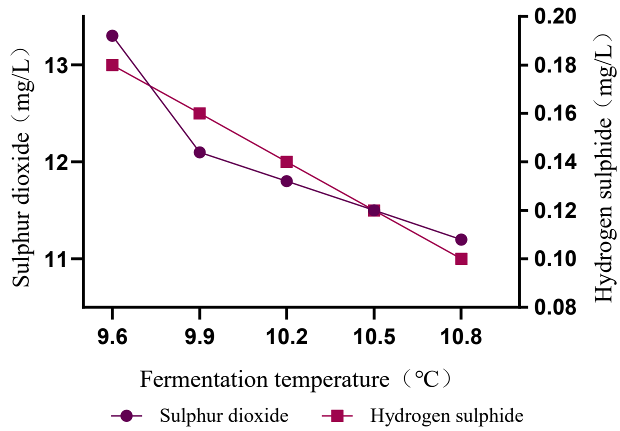

Figure 8. Effect of fermentation temperature on sulphur dioxide and hydrogen sulphide

4. Conclusion

A preliminary knowledge of the relationship between sulphur dioxide production in lager beers during fermentation and fermentation process factors enables an explanation of the mechanism of sulphur dioxide production by the sulphate metabolic pathway in yeast cells. According to the characteristics of the yeast strain under different fermentation process and working conditions, the regulation mainly optimises the process parameters such as dissolved oxygen in the wort, yeast recovery use, fermentation temperature and pressure during the fermentation process, and the regulation results are discussed in relation to the production practice data. The conclusions show that: the later the production of sulphur dioxide, the lower the SO_2 content in the final product of the fermentation broth, the longer the yeast growth time, the later the fermentation production of sulphur dioxide, the lower the sulphur dioxide content in the final finished beer by 10mg/L. The longer the yeast recovery days, the higher the fermentation production of sulphur dioxide and hydrogen sulphide the longer the yeast recovery days, the higher the content of sulphur dioxide and hydrogen sulphide produced by the fermentation process. The sulphide content produced by lager beer fermentation process gradually decreases with increasing temperature. The higher lager beer fermentation temperature, the faster the fermentation rate and the lower the sulphur dioxide content at the end of the main fermentation. Lager brewing in fermentation optimises process conditions, particularly wort oxygen content and yeast recovery, to accumulate production practice data for the regulation of sulphur dioxide production during lager beer fermentation.

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