Effects of Gingerols on Yeast Growth and Metabolism of the Fermentation Process

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Abstract. In this paper, the effects of gingerol on yeast growth and metabolism of the fermentation process are investigated by measuring the minimum inhibitory concentration, growth curve, alcohol, residual sugar, pH, total acid, intracellular trehalose and glycerol in different stages of yeast fermentation. The results showed that the minimum inhibitory concentration (MIC) of gingerol was 6mg/mL. In the fermentation process, with the increase of the amount of gingerol, the yeast number, the alcohol of fermentation liquor and the Glucose-lowering rate were decreased, the fermentation was incomplete, pH was lower, total acidity was increased and the content of trehalose and glycerol were increased. The inhibitory effect of gingerol amount on yeast was: 4 mg/mL>2 mg/mL>0.5 mg/mL>0 mg/mL (no gingerol was added). This showed that gingerol has an obvious inhibitory effect on the growth and fermentation of yeasts.

1. Introduction

Ginger (Zingiber officinale Roscoe, Zingiberaceae) is a medicinal plant that has been widely used in Chinese. And gingerol is a kind of plant polyphenols extracted from ginger [1-2]. Many researches found abroad, gingerol has anti-cancer, anti-oxidation, anti-inflammatory and antibacterial and other effects [3-4]. Although gingerol is a very good natural antioxidants and bacteriostatic agents in the current medicine and food preservation and other fields [5-7]. In the process, because gingerol has a strong bacteriostatic ability, and the general brewing yeast in the fermentation process will be inhibited by gingerol, fermentation stagnation, affecting the quality of ginger liquor [6]. The main production method of ginger liquor is soaked. But this process does not fully utilize the beneficial ingredients in ginger, resulting in waste of resources [9-11]. Therefore, in this experiment, the effects of different contents of gingerol on yeast growth and fermentation were investigated on the basis of sugar liquid from the production process of crystallized ginger.

2. Materials and Methods

2.1. Test Materials

Saccharomyces cerevisiae: KD (Shandong Agricultural University Food Fermentation Research Office to provide), Crystalline ginger syrup: provided by Weihai Geely Food Co., Ltd, Gingerol (active ingredient 50%), purchased from Xi’an Jinheng Chemical Co., Ltd, PDA medium: potato 200g, glucose 20 g, agar 20 g, tap water 1000ml, natural PH, 121°C autoclaved 20 min, YPD medium: yeast
extract 1%, peptone 2%, glucose 2% by 108°C autoclaved 20min after adding. Fermentation broth: sugar liquid of crystallized ginger diluted 4 times, the sugar concentration of 230 g/L, add gingerol to the final concentration of 0.5 mg/mL, 1 mg/mL, 2 mg/mL, 4 mg/mL, 8 mg/mL.

2.2. Test Methods

2.2.1. Yeast activation and preparation of suspension bacteria. Take active dry yeast at 39°C to 20 times the volume of water, 5% sucrose activation 30min, added to the YPD medium, 200 rpm in shaking culture, until it reaches $10^8$ cfu/L.

2.2.2. Determination of minimum inhibitory concentration (MIC). Adding gingerol to make the concentration of 0.5 mg/mL, 1 mg/mL, 2 mg/mL, 3 mg/mL, 4 mg/mL, 5 mg/mL and 6 mg/mL in the PDA medium, 1 mL yeast suspension was added to PDA medium and evenly coated. The medium with gingerol is used as the experimental group, the medium without gingerol is used as the positive control group, and the medium with 2 mg/mL potassium sorbate is used as the negative medium. The petri dishes were incubated in a constant temperature incubator at 28 °C for 48 h, then the yeast growth was observed.

2.2.3. Effects of different concentrations of gingerol on fermentation. The final concentration of gingerol was 0 mg/mL, 0.5 mg/mL, 2 mg/mL and 4 mg/mL, respectively in the crystalline ginger sugar solution. Then Saccharomyces cerevisiae is added to the crystalline ginger sugar solution at 500 mg/mL. Viable bacteria counts, alcohol content, total sugar, total acid, pH, glycerol and trehalose were determine during fermentation.

2.2.4. Determination of intracellular trehalose. 5 mL of fermentation broth, centrifuged at 3800 rpm for 20 min, centrifuged with cold distilled water and centrifuged twice. The supernatant was centrifuged to determine the extracellular glycerol. The cells were used to extract intracellular trehalose. After centrifugation of the bacteria with 4ml0.5 mol/L cold trichloroacetic acid (4°C refrigerator), the shock evenly placed in ice water mixture with ice for extraction, every 15 min oscillation once, oscillation 3 times , After centrifugation to collect the supernatant volume to 50 mL volumetric flask. With sulfuric acid-anthrone method to measure the trehalose content [8-9]. The trehalose concentration in the range of 0-100 mg/L, showing a good linear relationship with the OD value, the regression equation is:

$$y = 0.0107x - 0.0289 \quad (R^2 = 0.9994)$$

(1)

2.2.5. Determination of glycerol. HPLC solution: column: NH$_2$ column (250 mm×4.6 mm×5 μm); differential detector (RID); column temperature 40°C; Total flow rate: 1 mL/min; mobile phase: ultra-pure water 15% 85%; injection volume: 10 μL. The glycerol content in the range of 1-6 g/L, showing a good linear relationship with the peak area, the regression equation is:

$$y = 80159x - 10886 \quad (R^2 = 0.9999)$$

(2)

2.2.6. Determination of physical and chemical indexes during fermentation. Blood count plat method to measure Viable count; alcohol, total acid content, pH and total sugar were measured based on GB/15038-2006.

3. Results and Discussion

3.1. Determination of Minimal Inhibitory Concentration (MIC) of Gingerol on Yeast

The results of minimal inhibitory concentration (MIC) of gingerol on yeast were shown Table 1, the results indicated that the minimum inhibitory concentration of gingerol to yeast is 4 mg/mL.
Table 1. The results of different concentration gingerols antimicrobial activities

| Gingerol concentration(mg/mL) | 0.5 | 1   | 2   | 3   | 4   | 5   | 6   | Positive control | Negative control |
|-------------------------------|-----|-----|-----|-----|-----|-----|-----|-----------------|-----------------|
|                               | +++ | +++ | +++ | ++  | +   | -   | +++ |                 |                 |

Note: "+" means yeast growth, "-" means no yeast growth.

3.2. Effects of Different Concentrations of Gingerol on Yeast

It can be seen from figure 1 that the yeast was in the lag phase in the first 3 days, grows slowly, and then it entered the logarithmic phase. The number of viable bacteria in the control group was significantly higher than that in the experimental group. It was in a stable period from 18 days to 24 days, it showed that the addition of gingerol inhibited the growth and reproduction of yeast. When the concentration of gingerol was 2 mg/mL, the yeast biomass reached the maximum at 18 days. When the concentration of gingerol was 0.5 mg/mL, the yeast reached its maximum at 21 days, but the maximum biomass was slightly less than 2 mg/ml gingerol. In the first 12 days, there was little difference in biomass between the two. After both reaching the maximum number of viable bacteria, there was no stable period and directly enters the decay period, which directly affected the production of alcohol. When the concentration of gingerol was 4 mg/mL, the growth of yeast was the least in the early stage of fermentation, the fermentation was slow, and reached the maximum at 24 days. This may be due to the addition of gingerol, the nutrients in the fermentation broth were consumed in large quantities by the yeast, which was used for its own growth and defense responses. With the reduction of nutrients in the later stage, the yeast directly entered the decline phase. As the concentration of gingerol increased, the degree of inhibition of yeast growth was greater, resulting in a lower number of viable count.

![Figure 1](image1.png)

Figure 1. Effects of different concentrations of gingerol on yeast.

3.3. Effects of Different Concentrations of Gingerol on Alcohol and Total Sugar

The yeast fermentation rate in the control group was the fastest, the alcohol output was higher than the experimental group with gingerol, and when the fermentation was complete, the alcohol content was the largest, reaching 11.2% (figure 2(a)). The experimental group added gingerol had a lag period in fermentation and then entered the main fermentation period. When gingerol was 0.5 mg/mL, and 2 mg/mL, the main fermentation ended on the 24th day and entered the post-fermentation period. For the yeast cultured at 4 mg/mL, the main fermentation ended on the 27th days. The alcohol content stabilized in the later stage and then decreased, which may be due to some yeasts metabolizing alcohol into acids. With the increase of gingerol addition, the alcohol content becomes lower. It may be that the high concentration of gingerol cause the yeast to produce a corresponding stress, resulting in a lot of by-products, and a large amount of yeast death, which affects the alcohol fermentation.

Figure 2(b) showed the effect of different concentrations of gingerol on the total sugar during yeast fermentation. The yeast without gingerol had a fast sugar metabolic rate, the fermentation ended at 30...
days, and the total sugar was 9.7 g/L. The yeast added with gingerol could be started, but the fermentation was slow, and the rate of sugar metabolic was slow. After a period of 21-30 days, the fermentation was almost stopped, and the residual sugar content increased with the increase of gingerol concentration (4 mg/mL>2 mg/mL>0.5 mg/mL). This may be due to the large number of yeast’s deaths in the late fermentation period and the increase of by-products during the early fermentation process, and make it difficult to ferment. Meanwhile, the higher the amount of gingerol added, the more difficult for the yeast to ferment.

![Figure 2](image1.png)

**Figure 2.** Effects of different concentrations of gingerol on alcohol and total sugar.

### 3.4. Effects of Different Concentrations of Gingerol on Total Acid and pH.

![Figure 3](image2.png)

**Figure 3.** Effects of different concentrations of gingerol on total Acid and pH.

It can be seen from figure 3(a) that the pH value of the control group without gingerol was significantly higher than that of the experimental group added with gingerol, and it stabilized after 18 days and finally stabilized at 3.35. When gingerol is added at 4 mg/mL, the pH value was higher than 2 mg/mL and 0.5 mg/mL in the early stage, and lower than both in the later stage. However, the variation trends of the two group added the concentration at 2 mg/mL and 0.5 mg/mL tended to coincide.

It can be seen from figure 3(b) that the total acid content in the fermentation broth with the gingerol was significantly higher than the control group without the gingerol. When gingerol was added at 4 mg/mL, the total acid content continues to rise during the fermentation process, which was much higher than the control group. The acid production increased with the increase of gingerol concentration (4 mg/mL>2 mg/mL>0.5 mg/mL>0 mg/mL). This results indicated that the content of metabolic by-products, such as organic acids, had increased during the fermentation process, and resulted in an increasing total acid content. From the analysis of the total acid on the 30th day, the total acid amount increased with the increase of gingerol addition. The addition of gingerol was positively correlated with the total acid production, the correlation coefficient is 0.955, and the effect was significant (P< 0.05).
3.5. Effects of Different Concentrations of Gingerol on Trehalose and Glycerol

Trehalose is not only a storage carbon source, but also a protective substance produced by yeast in response to the harsh external environment [10]. Figure 4(a) showed the variation curve of yeast intracellular trehalose under different gingerol concentrations. With the increase of gingerol concentration, the content of trehalose increased gradually. In addition, the content of trehalose in the control group and the experimental group sharply increased on the first 5 days. The group with gingerol 4 mg/mL reached the maximum on the 5th day. The reason for this phenomenon might be that the addition of gingerol in the early stage made the yeast in a harsh environment, so the content of trehalose increased rapidly. In the same way, the yeast would also produce trehalose for self-protection because the control group contained a small amount of gingerol. However, the content of trehalose in the fermentation broth without gingerol was significantly lower than that in the experimental group with gingerol. The fermentation broth with 4 mg/mL gingerol had the highest trehalose content, which follows the order of 2 mg/mL, 0.5 mg/mL and 0 mg/mL.

Glycerol is one of the main by-products of yeast alcohol fermentation [11]. It plays an important role in maintaining the balance of NAD+/NADH and initiating alcohol fermentation. The content of glycerol increases with the increase of the concentration of gingerol (figure 4 (b)). Compared with the control group, the yield of glycerol in the experimental group with gingerol was higher than that of the control group.

The yield of glycerol in the experimental group with 4 mg/mL gingerol was higher than the experimental group with 2 mg/mL and 0.5 mg/mL. The results indicated that the high concentration of gingerol could promote the production of glycerol.

Figure 4. Effects of different concentrations gingerol on trehalose and glycerol.

4. Conclusion

The minimum inhibitory concentration (MIC) of gingerol on Saccharomyces cerevisiae KD was 6 mg/mL. Gingerol can inhibit yeast production and change the yield of its metabolites during the fermentation period, with an increasing amount of gingerol added, the number of viable yeast became lower, the alcohol content of the fermentation broth and the metabolic rate of sugar decreased. The significant achievements lay in the fact that this study will present a comprehensive understanding of the Inhibition of yeast growth Different amounts of gingerol. It will provide a good theoretical background for future research to directionally develop of ginger wine.

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