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Effects of Low Nitrogen Stress on the Amount of Higher Alcohols in Wine in *Saccharomyces Cerevisiae*

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***Abstract*—Higher alcohols have a great impact on the aroma and taste of wine, meanwhile the amount of that are affected by nitrogrn nutrients. However, the accumulation mechanisms of the higher alcohols in *Saccharomyce cerevisiae* under low nitrigen condition remains unelucigated. In the present research, the determinationof higher alcohols content were analyzed in order to explore the mechanisms of the higher alcohols responses to low nitrogen stress. We found that the total amount of higher alcohols in the experimental group (310.37 mg/L) was 1.74-fold higher than that of the control group (178.12 mg/L). The results revealed that the increased accumulation of higher alcohols is due to the response to low nitrogen stress in *Saccharomyce cerevisiae*.**

Keywords—higher alcohols; saccharomyces cerevisiae; low nitrogen

I. Introduction

The nitrogen is an indispensable nutrient for *Saccharomyces cerevisiae* and plays a pivotal role in the growth and metabolism of yeast [1, 2]. Higher alcohols are by-products of alcoholic fermentation by *Saccharomyces cerevisiae* and they are generally considered as important flavour componds in wine. Higher alcohols are generally formed by two pathways: sugar metabolism and amino acid metabolism[3]. Higher alcohols are monohydric alcohols containing more than three carbon atoms, including propanol, 2-methyl-1-propanol, 3-methyl-1-butanol and 2-phenylethanol [4]. In general, higher alcohols can contribute to the improvement of wine quality when the content of higher alcohols in wine is less than 300 mg/L[3]. However, when the higher alcohol content is higher than 400 mg/L, it usually has a disadvantage effect on the wine favor and the health of the consumer[5].

Higher alcohols prodution are influenced by yeast strain, fermentation tempeture and nutrients, especially nitrogen nutrient[6, 7]. It has been reported that Nitrogen-deficient condition in must is associated with the excessive higher alcohols concentration in wine [1]. However, excessive higher alcohols often bring more complex but less desirable sensory profiles to wines [1]. Furthermore, insufficient nitrogen nutrition often occur during wine fermentation process paiticularly in white wine fermentation [8]. Therefore, revealing the synthesis mechanism of higher alcohol production under low nitrogen condition is necessary for controlling the accumulation of higher alcohols in winemaking.

II. Materials and Methods

A. Yeast Strain and Growth Conditions

S.cerevisiae strain used in the research was EC1118, which is a commercially available strain widely used in the wine industry. The synthetic fermentation media in the study were simulated standard grape juices referenced to Modified MS300 medium[9].

The following anaerobic factors were added to the medium (per liter): 15 mg ergosterol, 5 mg sodium oleate and 0.5 mL Tween 80.

B. Cultivation Conditions

The inoculation density of the bioreactor is 10^6 cells mL^{-1} and the temperature is 25℃.

The study was carried out by setting up the experimental group(180 $mgN{\cdot}L^{-1}$) and the control group(380 $mgN{\cdot}L^{-1}$) on a synthetic medium for fermentation. It has been proven that when the nitrogen concentration was about 180 mg/L,the content of higher alcohols reached its peak[1], suggesting that 180 mg/L is the proper concentration of nitrogen source to explore the mechanism of the increase of higher alcohols content. Fermentation experiments were carried out three times independently.

C. Determination of Higher Alcohols

Concentrations of 1-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol and 2-phenylethanol were determined by gas chromatography (GC) referened to Park et al[10].

III. Results

The result showed that the total amount of higher alcohols in experimental group (310.37 mg/L) was 1.74-fold higher than that of the control group (178.12 mg/L)(Fig. 1a). Among them, the contents of 2-methyl-1-propanol, 3-methyl-1-butanol and 2-phenylethanol in experimental group were increased more than 43%. In particularly, the content of 2-phenylethanol was 6.7 times higher than that of

the control group. However, the content of 1-propanol was decreased by 15% under the low nitrogen stress (Fig. 1b).

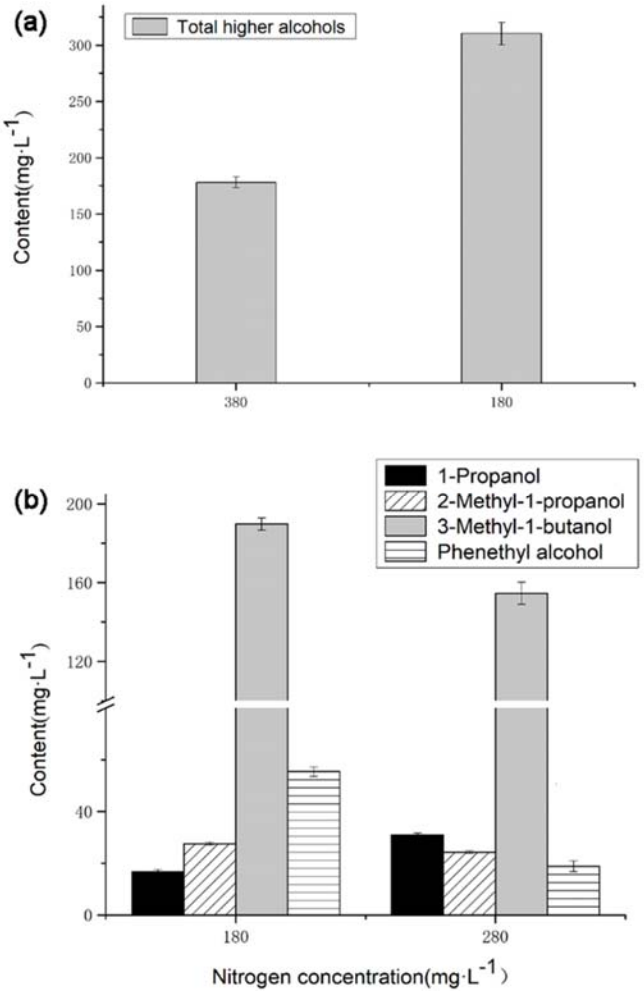

FIGURE I. HIGHER ALCOHOLS CONTENT UNDER DIFFERENT NITROGEN CONCENTRATIONS. (A) TOTAL AMOUNT OF HIGHER ALCOHOLS IN THE LOW NITROGEN TREATMENT GROUP (180 MG/L) AND CONTROL GROUP (380 MG/L). (B) THE AMOUNTS OF 1-PROPANOL, 2-METHYL-1-PROPANOL, 3-METHYL-1-BUTANOL AND PHENETHYL ALCOHOL IN THE LOW NITROGEN TREATMENT GROUP (180 MG/L) AND CONTROL GROUP (380 MG/L).

IV. DISCUSSION

Higher alcohols are monohydric alcohols which contain more than three carbon atoms, including propanol, 2-methyl-1-propanol, 3-methyl-1-butanol and 2-phenylethanol [11]. It has been shown that higher alcohols content have a strong response to low nitrogen conditions [12]. In the present study, the total amount of higher alcohols in the experimental group (310.37 mg/L) was 1.74-fold higher than that of the control group (178.12 mg/L) (Fig. 1a), indicating that low nitrogen condition significantly increased the content of higher alcohols. In addition, for different components of the higher alcohol, the contents of 2-methyl-1-propanol, 3-methyl-1-butanol and 2-phenylethanol were much higher (increased more than 43%) under low nitrogen condition. In particularly, the content of 2-phenylethanol was 6.7 times higher than that of the control group. However, the content of 1-propanol was decreased by 15% compared with the control group (Fig. 1b). These results that the increase of total higher alcohols was mainly related to the increase of 2-methyl-1-propanol, 3-methyl-1-butanol and 2-phenylethanol content under low nitrogen condition, whichis consistent with previous studies [2, 13].

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