# Influence of heat shock on yeast cell and its effect on glycerol production in guava wine production

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# Abstact

The influence of heat shock induced during the exponential growth phase of the *Saccharomyces cerevisiae* NCIM 3095 and NCIM 3287 fermentation of Guava juice were examined. A rapid temperature change from 20°C to 38°C was applied and gives maximum glycerol and ethanol production. The effect of the duration of exposure to a high temperature has been analyzed. By the influence of a heat shock treatment, glycerol production was increased by up to 28% and 44.4 % respectively in NCIM 3095 and 3287 in guava wine production. This is a noble method for high glycerol production in large scale guava wine production.

**Key words:** Heat shock, Guava wine, *Saccharomyces cerevisiae* NCIM 3095 and NCIM 3287, Glycerol production

## Introduction

Guava (Psidium guajava L.) wine is the product of anaerobic fermentation by yeast in which the sugars are converted into alcohol & carbon dioxide (Anderson C et al., 2005). In Guava wine fermentation, next to ethanol and carbon dioxide, glycerol is quantitatively the most important fermentation product. Because of its nonvolatile nature, glycerol does not contribute to the aroma of wine, but it promotes the smoothness of wine (Boulton et al., 1996). It contributes only indirectly to wine quality, but the overproduction of glycerol by wine yeast strains of Saccharomyces cerevisiae could markedly improve the sensory quality of wine (Oura,E, 1977). The increased biosynthesis of glycerol often gives wine a smoother mouth feel and an enhanced complexity (Jackson, R.S 2000). The biosynthesis of glycerol in a cell is closely associated with osmotic cell regulation (Zoecklein et al., 1995). In glycerol biosynthesis, various growth and environmental factors such as fermentation temperature, strain selection, inoculation level, sulfite concentration, sugar concentration, osmotic stress, nitrogen source and concentration, pH, aeration, fruit variety and ripeness have been reported to influence the amount of glycerol produced by yeast in

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wine (Albers et al., 1996). Several authors have also observed that glycerol is produced in response to solute stress in S. cerevisiae (Gardner et al. 1996). Several studies have shown the improvement of wine quality related to various yeast strains, using genetic or molecular techniques to manipulate glycerol formation by wine yeasts. Gene engineering of the S. cerevisiae strain resulted in a 1.5to 2.5-fold increase in glycerol production with only a slight decrease in ethanol formation in wine fermentation (Remize et al., 1999). As shown in Fig.1, during the anaerobic growth of S. cerevisiae, NADH cannot be oxidized by oxygen, but must be disposed by the formation of reduced by-products such as glycerol. The accumulation of glycerol is caused by the need to maintain a favorable redox balance (Marin Berovic et al., 2007).The interactions between the yeast strain, temperature and agitation also affects on glycerol production (Remize et al., 2000). An investigation on alcohol fermentation carried out at different temperatures could also serve to test the natural stability of indigenous strains. This could be used as a criterion for the rapid selection of one of the several microbial strains, and at the same time act as a standard for the examination of strain resistance to temperature in a controlled situation in a laboratory environment. Temperature could also be a helpful tool for evaluating the effects of the dynamics of a known population of S. cerevisiae during alcohol fermentation (Jesus Torija et al., 2003). The objective of the present study is to observe the effect of heat shock treatment on starter cultures in guava wine fermentation and corresponding the effect on glycerol and ethanol production.

## **Material and Methods**

#### Yeast and Fermentation Substrate

Saccharomyces cerevisiae wine yeast (NCIM 3095 and NCIM 3287) were used in all experiments. Yeast cultures stored in slants were reactivated in yeast extract, peptone and dextrose medium (YEPD) for 48 hr at 25°C. The starter culture of yeast containing  $10^7$  cells mI<sup>-1</sup>, was cooled to  $18^{\circ}$ C and retained for 5 minutes. The starter culture suddenly heated and within 5 min temperature reached to  $45^{\circ}$ C and maintain for 20 min at the latter temperature after which the cell suspension was quickly cooled to  $18^{\circ}$ C. For the inoculation of fruit must 20 ml suspension were used.

Fermentations were carried out on guava juice. Before the start of fermentation, the guava must was added potassium metabisulphate, diammonium phosphate and sugar. All experiments were performed in 250 ml shake flasks.

#### Analytical method

#### Determination of glycerol

Glycerol was determined by using chemical method given by Rebelein (H. Rebelein, et al., 1956).

Fig1.1: Pathway of formation of glycerol

### Determination of ethanol

Ethanol in a dilute sample can be separated from other wine components by Gas Chromatography. To improve quantification, 2-propanol (used as internal standard) solution was used to quantitatively dilute the sample. The peak area ratio for the two chromatographic peaks is compared with the area ratio obtained from injection of standard ethanol-internal standard mixture (Zoecklein et al., 1995).

# **Result and Discussion**

In guava wine fermentation, the starter culture is the main affecting factor for producing the flavors, glycerol, ethanol and ingredients. After making the starter culture, starter culture cool to 20  $^{\rm O}$ C and than suddenly increase the temperature for 20 minutes.



temperature(C)

Figure 2. Effect of heat shock temperature on glycerol production in guava wine

Fig 2 shows the temperature up to the starter culture heated suddenly in water bath and corresponding the glycerol production after heat shock treatment.

Both the strains NCIM 3095 and NCIM 3287 starter culture are suddenly heated from  $20^{\circ}$ C to  $30^{\circ}$ C ,  $34^{\circ}$ C ,  $38^{\circ}$ C ,  $42^{\circ}$ C and  $46^{\circ}$ C then maintain later temperature for 20 min followed by cooling to  $20^{\circ}$ . By seeing this fig 2 at  $38^{\circ}$ C shows the maximum glycerol production for both the strains in guava wine. Temperature higher then this not showing any increase in glycerol production. After this starter culture suddenly cool to  $18^{\circ}$ C and this starter culture than transferred to fermentor for fermentation.



Figure 3. Glycerol production and alcohol production in guava wine before heat shock treatment and after heat shock treatment.

Fig 3 showing the heat shock treatment on NCIM 3095 for guava wine production. In this the glycerol production 5.9 g/L before heat shock treatment and after heat shock treatment glycerol production is increased by 28 % and reached to 7.2 g/L in guava wine . By this alcohol production is also increased from 8.2 to 8.6 (%v/v). Heat shock has been proven to be effective in stimulating glycerol production at 40°C and 45°C. The timing of heat shock treatment does not influence glycerol production. Heat shock treatment induces S. cerevisiae to produce heat shock proteins and alters cellular characteristics.Cells subjected to heat shock acquire resistance against various stresses (Roustan et al., 2005). Many studies have shown that trehalose, which is involved in to the acquisition of various types of resistance, markedly accumulates in a cell by heat shock treatment (Wiemken et al., 1994) have suggested that trehalose may actually be a stress protecting agent, because its accumulation and the acquisition of thermo tolerance during the heat shock are partially independent of protein synthesis in S. cerevisiae. Glycerol is an analogue of trehalose in that it acts as a redox balancing substance and osmoprotectant during osmotic stress (Balli et al .,2003).

Heat-shock treatment induces *S.cerevisiae* to produce heat-shock proteins with altered cellular characteristics (Nevoigt and Stahl 1997; Roustan and Sablayrolles 2005). Cells subjected to heat shock may also acquire resistance against various stresses (Attfield 1987; Odumeru et al., 1992). In the present case, a possible explanation for the effect could be that the heat shock activated or induced greater expression of triose phosphate isomerase which then persisted during the subsequent fermentation. This, in turn, would have increased the conversion of dihydroxyacetone phosphate to glycerol (Scanes et al., 1998).



Figure 4. Glycerol production and alcohol production in guava wine before heat shock treatment and after heat shock treatment

Fig 4 shows the heat shock treatment on *Saccharomyces cerevisiae* NCIM 3287 for guava wine production Heat shock treatment on starter culture increased glycerol production by 44.4 % & ethanol production by 8.3%.

The heat shock of the yeast inoculum represents a new and easy applicable method to achieve higher glycerol and ethanol production in guava wine.

## Conclusion

Heat shock treatment gives the better result and it's an easy way to increase the glycerol content in guava wine. *Saccharomyces cerevisiae* NCIM 3287 gives better results than NCIM 3095. In NCIM 3287 glycerol production increased by 44.4 % & ethanol production by 8.3% whenever in NCIM 3095 glycerol content increased by 28 % and ethanol production by 7.2 % in guava wine.

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