J Biochem Tech (2011) 3(1):230-232
ISSN: 0974-2328

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

Sevda S B*, Rodrigues L, Joshi C

Received: 7 May 2010 / Received in revised form: 8 May 2010, Accepted: 5 January 2011, Published online: 08 March 2011

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 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 1.5- to 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.

Sevda S B*, Rodrigues L, Joshi C

Department of Food Engineering and Technology, University Institute of Chemical Technology, Matunga, Mumbai-400019, India

*Tel.: 0091 9990576049, E-mail: sevdasuraj@gmail.com

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^{o}C .The starter culture of yeast containing 10^{7} cells ml^{-1}, was cooled to 18^{O}C and retained for 5 minutes. The starter culture suddenly heated and within 5 min temperature reached to 45^{O}C and maintain for 20 min at the latter temperature after which the cell suspension was quickly cooled to 18^{O}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).

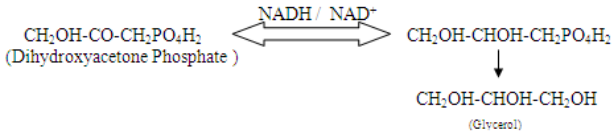

Fig1.1: Pathway of formation of glycerol

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 ^{O}C and than suddenly increase the temperature for 20 minutes.

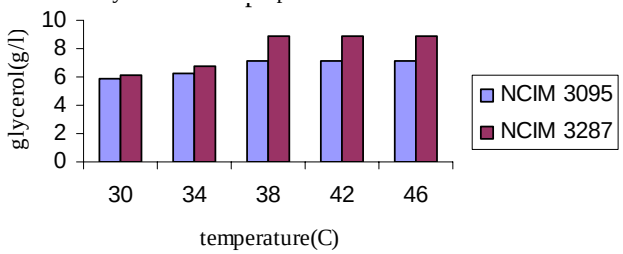

Figure 2. Effect of heat shock temperature on glycerol production in guava wine

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^{O}C to 30 ^{O}C , 34 ^{O}C , 38 ^{O}C ,42 ^{O}C and 46^{O}C then maintain later temperature for 20 min followed by cooling to 20^{O}. By seeing this fig 2 at 38 ^{O}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^{O}C and this starter culture than transferred to fermentor for fermentation.

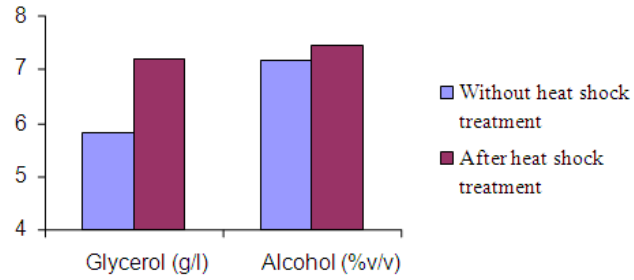

Figure 3. Glycerol production and alcohol production in guava wine before heat shock treatment and after heat shock treatment.

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).

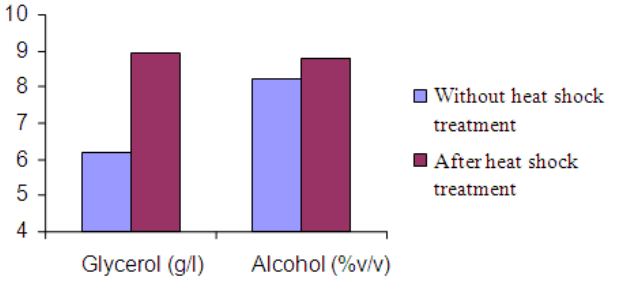

Figure 4. Glycerol production and alcohol production in guava wine before heat shock treatment and after heat shock treatment

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 .

Acknowledgements

This research was financially supported by the UGC, Government of India.

References

Albers, E., Larsson, C., Liden, G., Niklasson, C., and Gustafsson, L(1996) : Influence of the nitrogen source on *S. cerevisiae* anaerobic growth and product formation. Appl. Environ.Microbiol, 62:3187-3195

Anderson C, Badrie N (2005) Physico-chemical quality and consumer acceptance of guava wines. Journal of Food Science and Technology 42(3):223-225

Attfield PV (1987) Trehalose accumulates in *Saccharomyces cerevisiae* during exposure to agents that induce heat shock response. FEBS Lett 225:259–263

Balli, D Flari, V Sakellaraki,et al (2003) Effect of yeast cell immobilization and temperature on glycerol content in alcoholic fermentation with respect to wine making. Proc Biochem 39:499–506

Boulton, RB, Singleton VL, Bission LF, Kunkee ER (1996) Principles and practices of winemaking . Chapman and hall, New york p146-150

C.S.Ough and M.A.Amerine, (1986) Alcohols. Methods for analysis of musts and wine 2:119-123.

Gardner, N., Rodrigue, N., and Champagne, C. P.: Combined effect of sulfites, temperature and agitation time on production of glycerol in grape juice by *Saccharomyces cerevisiae*. Appl. Environ. Microbiol., 59, 2022–2028 (1993).

H.Rebelein ,Z. Lebensm. Unters. Forsch. 105 (1956) ,page 296.

Jackson, R. S.: Wine science: principles, practice, perception, p. 324–327. 2nd ed. Academic Press, Sa Diego (2000).

Jesus Torija, M., Rozes, N., Poblet, M., Manuel Guillamon,J., and Mas, A.: Effects of fermentation temperature on the population of *Saccharomyces cerevisiae*. Int. J. Food Microbiol., 80, 47–53 (2003).

Marin Berovic, Aleksandra Pivec,Tatjana Košmerl,Mojmir Wondra,and Štefan Celan : Influence of Heat Shock on Glycerol Production in Alcohol Fermentation. Journal of Bioscience and bioengineering Vol 103, no 2 , 135-139(2007).

Nevoigt E, Stahl U : Osmoregulation and glycerol metabolism in the yeast *Saccharomyces cerevisiae*. FEMS Microbial Rev 21:231–241 (1997).

Odumeru JA, D'Amore T, Russell I, Stewart GG : Changes in protein composition of Saccharomyces brewing strains in response to heat shock and ethanol stress. J Ind Microbial 9:229–234 (1992).

Ough , C.S., Fong, D., and Amenne , M.A. .Glycerol in wine ;determination and some factor affecting. Am .J. Enol. Vitic, 23, 1-5 (1972).

Oura, E: Reaction products of yeast fermentation, Process biochemistry, 12, 19-21 (1977).

Remize, F., Roustan, J. L., Sablayrolles, J. M., Barre, P.,and Dequin, S.: Glycerol overproduction by engineered *S. cerevisiae* wine yeast strains leads to substantial changes in by-product formation and to a stimulation of fermentation rate in stationary phase. Appl. Environ. Microbiol., 65, 143–149 (1999).

Remize, F., Sablayrolles, J. M., and Dequin, S.: Re-assessment of the influence of yeast strain and environmental factors on glycerol production in wine. J. Appl. Microbiol., 88, 371–378 (2000).

Roustan, J. L. and Sablayrolles, J. M.: Role of trehalose and glycogen in alcoholic fermentation in wine-making conditions. J. Wine Res., 15, 189–202 (2005).

Scanes KT, Hohmann S, Prior BA :Glycerol production by yeast *Saccharomyces cerevisiae* and its relevance to wine. A review. S Afr J Enol Vitic 19:17– 24(1998).

Wiemken, A.: The role of trehalose synthesis for the acquisition of thermotolerance in yeast. I. Genetic evidence that trehalose is a thermoprotectant. Eur. J. Biochem., 219, 179–186 (1994).

Zoecklein, W. B., Fugelsang, K. C., Gump, H. B., and Nury, S. F.: Wine analysis and production, p. 99 100. Chapman & Hall, New York (1995).