

Research Note

## Influence of temperature shock on the glycerol production in cv. Sauvignon blanc fermentation

A. KUKEC<sup>1)</sup>, M. BEROVIČ<sup>2)</sup>, M. WONDRA<sup>3)</sup>, Š. ČELAN  
and T. KOŠMERL<sup>3)</sup>

<sup>1)</sup>Bistra Scientific Research Centre, Ptuj, Slovenia

<sup>2)</sup>Faculty for Chemistry and Chemical Technology, Department of Chemical, Biochemical and Environmental Engineering, University of Ljubljana, Ljubljana, Slovenia

<sup>3)</sup>Department of Food Technology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

**Summary:** Repeating heat shocks applied during the exponential growth phase, led to high glycerol concentrations up to 10.0 g·l<sup>-1</sup> or higher. Using one heat shock 8.2 g·l<sup>-1</sup> or 2.2 g·l<sup>-1</sup> more glycerol was produced, while with a double heat shock 10.0 g·l<sup>-1</sup> or 4.0 g·l<sup>-1</sup> more glycerol was produced. Using this procedure even higher amounts of glycerol could be produced. These results could be effectively applied in wine technology.

**Key words:** glycerol production, temperature shock.

**Introduction:** In wine technology glycerol is one of those relevant compounds that, due to its non-volatile nature, actually does not contribute to the aroma of wine, but does contribute to its smoothness (EUSTACE and THORNTON 1987). An indirect contribution of glycerol to wine quality may be given by the production of glycerol by wine strains of *S. cerevisiae* improving the sensory qualities of wine (PRETORIUS and VAN DER WESTHUIZEN 1991). Several studies have shown that a slow increase of temperature resulted in an increased glycerol production (OUGH *et al.* 1972, GARDNER *et al.* 1993), and agitation was also referred to as a parameter to enhance this process (RADLER and SCHÜTZ 1982). GARDNER *et al.* (1993) reported that the strain character, temperature and agitation can influence glycerol production, while the optimal temperature for glycerol production was shown to depend on the time of agitation during cultivation.

Glycerol production during alcoholic fermentation contributes to the maintenance of the redox potential balance by osmoregulation in yeast cells (HOHMANN 1998). While glycolysis with subsequent alcoholic fermentation represents a redox-neutral metabolic sequence, the strong biomass production with corresponding production of NADH<sup>+</sup> taking place during the first third of must fermentation has to be counterbalanced within the yeast cells: increased glycerol production recycles NAD<sup>+</sup> under anaerobic conditions (VAN DYKEN and SCHEFFERS 1986).

The main objective of this work was to study the speed of temperature changes influencing the glycerol production of *Saccharomyces cerevisiae* in fermenting musts of cv. Sauvignon blanc.

**Material and Methods:** **Microorganism:** A selected dry yeast strain (*Saccharomyces cerevisiae*, Uvaferm SLO) was used in all experiments. The yeast was reactivated in water-diluted must (cv. Sauvignon blanc) at 30 °C. Fermentation processes were performed by inoculation of 0.3 g of dry yeast per litre of must.

**Fermentation substrate:** Batch fermentation experiments were carried out with grape juice of cv. Sauvignon blanc from the wine growing district of Ljutomersko-Ormoske gorice, Slovenia. The must, fermented on a laboratory scale, was not sulphurized before the onset of fermentation. The initial concentration of glucose was 95.0 g·l<sup>-1</sup> and that of fructose 102.4 g·l<sup>-1</sup>.

**Fermentor:** All laboratory scale experiments were performed in a standard type stirred 10 litre working volume tank reactor (Infors 210, by INFORSAG, CH-4103 Bottmingen and Bioengineering AG, Switzerland). The fermentor was a computer-controlled system equipped with sterilizable Ingold pH and redox electrodes, Industrial Lab MFG 509 pO<sub>2</sub> electrode, automatic foam control and a temperature control unit ( $\pm 0.5$  °C; Mettler Toledo, Switzerland). For on-line measurements SHIVA control software (BIA d.o.o., Slovenia) was implemented.

**Fermentation:** Preliminary fermentation was carried out at starting temperatures of 15, 18 and 24 °C. In heat shock experiments the initial temperature was increased from 18 to 34 °C within 2 h and then was reduced back to a final temperature of 18 °C.

To prevent oxidation of the substrate, the fermentor headspace was flushed with nitrogen. All the experiments proceeded at a low stirrer speed (N = 100 rpm). Each experiment was performed in triplicate and the average result of each series is presented.

**Analytical methods:** Glycerol in grape must and wine was determined by HPLC. Samples were filtered through a 0.45 µm membrane and analysed using a 300 x 7.8 mm diameter Aminex HPX-87H organic acid analysis cation exchange column (Bio-Rad laboratories). The eluting compounds were monitored by a fixed wavelength ultraviolet (UV-VIS) detector at 210 nm. This detector was connected in series with a refractive index (RI) detector. Glycerol and ethanol were detected by RI. Peaks were quantified using external standard calibration. The components were identified by comparing their retention times with those of the standards. Samples were quantified using external standards prepared from pure compounds. Validated methods proposed by Bio-Rad (BIO-RAD 1997) were applied.

**Biomass:** Biomass was determined gravimetrically after centrifugation (5 min) of 20 ml of fermentation broth at 4000 rpm and 24 h drying at 105 °C until constant weight was reached.

**Results and Discussion:** Several microorganisms are known to produce a large amount of glycerol as a redox balancing substance initiated by various kinds of stress

Correspondence to: Prof. Dr. M. BEROVIČ, Faculty of Chemistry and Chemical Technology, Department of Chemical, Biochemical and Environmental Engineering, University of Ljubljana, Hajdrihova 19, SI-1115 Ljubljana, Slovenia. Fax: +386-1 4760 300. E-mail: marin.berovic@uni-lj.si

(HEMMING and ADLER 1991). Glycerol is an analogue to trehalose in that it acts as an osmoprotectant redox balancing substance during osmotic stress (BLOMBERG and ADLER 1989). For yeast metabolism the glycerol metabolism was proved to be of relevant importance, especially in media with low osmotic potential (NEVOIGT and STAHL 1997) kept under anaerobic conditions (VAN DIJKEN and SCHEFFERS 1986).

In the first group of experiments heat shock was initiated 2 h after inoculation with yeast in the lag phase by a fast increase of the fermentation temperature from initially 18 to 34 °C; this temperature was maintained for 2 h and then the fermentation broth was cooled down to 18 °C.

In the second group of experiments a double temperature shock was applied. In the first part the same procedure as in the first group of experiments was applied. Two h after the first shock in the first third of the exponential growth phase a second heat shock by increasing the fermentation temperature from initially 18 to 34 °C, was induced which lasted for another 2 h. Thereafter the fermentation temperature was decreased and maintained at 18 °C until the end of the process.

These experiments indicate that production of glycerol is temperature dependent. According to the Arrhenius equation, higher fermentation temperatures hastened production of glycerol. Its biosynthesis starts during the exponential phase and is prolonged to the stationary growth phase where the glycerol produced was used to balance the metabolic process. When yeast metabolism was interrupted by a fast change of fermentation temperature, a rapid redox potential balancing was required. The microorganisms and their metabolism were pushed out of the redox balance and were forced to reach another more stable redox balance adapted to the new temperature. This resulted in marked glycerol secretion, which was significantly increased in the case of repeated temperature shocks (Figs 1 and 2). High glycerol production is also connected to fast redox potential changes in the range up to -200 mV (Fig. 2).

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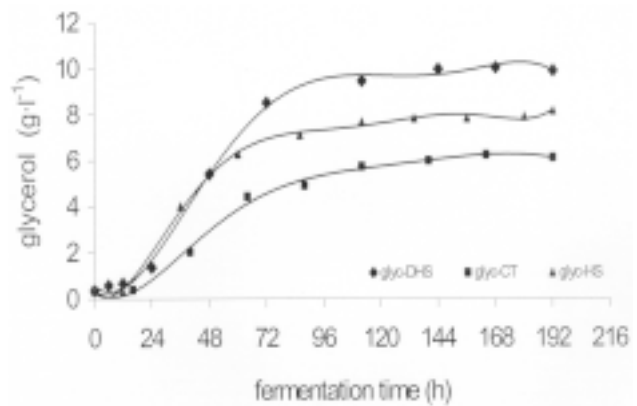


Fig. 1: Glycerol production during the fermentation with one glyo-HS (▲); double heat shock, glyo-DHS (◆); and during fermentation at constant temperature glyo-CT, 18 °C (■).

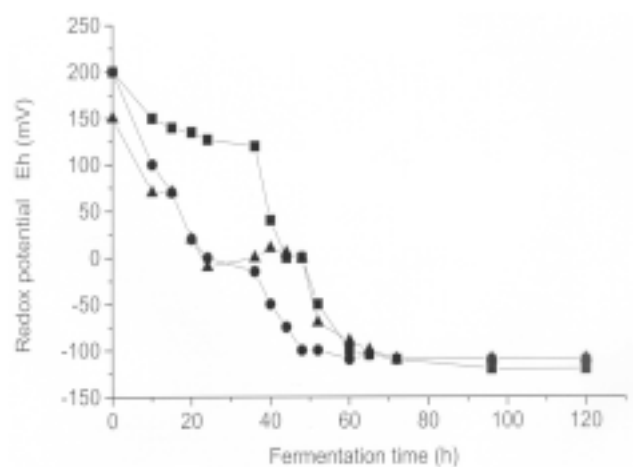


Fig. 2: On-line redox profile during the fermentation with one (HS) (●) and double heat shock (DHS) (▲) and during fermentation at constant temperature (CT; 18 °C) (■).

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