

CHANGES IN TREHALOSE CONTENT OF BAKER'S YEAST AS AFFECTED BY OCTANOIC ACID

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SUMMARY: Octanoic acid inhibited ethanolic fermentation by *Saccharomyces cerevisiae* (bakers yeast) and the trehalose accumulation, however did not affect the endogenous degradation of trehalose. This inhibition may be explained by the binding of octanoic acid to hexokinase or other proteins of plasma membrane because they are not necessary for endogenous fermentation. The degradation of trehalose may be due to an activation of trehalase.

Key Words: trehalose, yeast, *Saccharomyces*, octanoic acid.

ALTERAÇÕES NO TEOR DE TREALOSE DE LEVEDURA DE PANIFICAÇÃO PROVOCADAS POR ÁCIDO OCTANÓICO

RESUMO: A adição de ácido octanóico inibiu a fermentação alcoólica realizada por *Saccharomyces cerevisiae* (levedura de panificação) e o acúmulo de trealose, contudo não afetou a degradação endógena de trealose. Esta inibição poderia ser explicada pela ligação do ácido octanóico a hexoquinase ou outra proteína da membrana plasmática porque não são necessárias para a fermentação endógena. A degradação da trealose poderia ser devida a uma ativação da trealase.

Descritores: trealose, leveduras, ácido octanóico, *Saccharomyces*.

INTRODUCTION

Octanoic and decanoic acids are minor components produced during alcoholic fermentation (LAFON-LAFOURCADE, 1983) but, even in low concentration, it has been found that they have a significant effect on alcoholic fermentation (LAFON-LAFOURCADE et al., 1984). The toxicity of both acids increased in the presence of ethanol which could explain the higher effect of ethanol produced during fermentation than the added ethanol (VIEGAS et al., 1985; SÁ-CORREIA, 1986).

In an investigation of the effect of octanoic and decanoic acids on the inhibition of fermentation by yeasts, it was observed that both acids decreased the biomass at 30°C and that decanoic acid was more toxic than octanoic acid (VIEGAS et al., 1989).

NORDSTROM (1964) reported that the inhibitory action of lower fatty acids on yeasts could be partly due to the interference with essential metabolic activities requiring acyl-CoA-compounds while FERDINANDUS & CLARK (1969) verified an inhibition of bacterial enzymes

phosphofructokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase by octanoic acid.

Studying the effect of acetic acid on ethanolic fermentation, GUTIERREZ et al. (1991) verified that there was less accumulation of trehalose with lower biomass production.

This paper describes the effect of octanoic acid on trehalose accumulation and suggests that its mode of action could be explained by activation of trehalase.

MATERIALS AND METHODS

Microorganism. The yeast used in this study was a strain of baker's yeast (*Saccharomyces cerevisiae*), trade mark Fleischmann.

Fermentation conditions. Fermentation experiments were carried out in 500 ml conical flasks capped with aluminium foil, containing 250 ml of potassium citrate buffer pH 4.0 or 5% glucose in the same buffer. Additions of ethanolic solutions of octanoic acid were made to produce final concentrations of 48, 96, 120, 180 and 240 mg/L. They were inoculated with 20 mg/ml of fresh

bakers yeast cells and incubated at 40°C. Samples were withdrawn at zero time and as appropriate thereafter for determinations of trehalose and reducing sugars. All analyses were made in triplicate and all the reagents were of analytical grade.

Trehalose. Yeast cells were collected by centrifugation at 1,000 x g for 10 min and washed with cold distilled water. Trehalose was extracted from yeast cells by 0.5 M trichloroacetic acid and determined by the anthrone method (BRIN, 1966).

Reducing sugars. Sugars present in the medium were estimated by the modified method of Somogyi-Nelson (AMORIM et al., 1982).

RESULTS AND DISCUSSION

Octanoic acid inhibited exogenous fermentation of glucose by *Saccharomyces cerevisiae* (bakers yeast) in nonproliferant conditions (Fig.1) as has already pointed out (LAFON-LAFOURCADE et al., 1984; VIEGAS et al., 1985; VIEGAS et al., 1989; VIEGAS & SACORREIA, 1991).

Figure 2 shows that octanoic acid inhibited trehalose accumulation in the 5% glucose medium, and did not affect the endogenous degradation of trehalose in citrate buffer (Fig.3).

The lack of inhibition of endogenous fermentation by octanoic acid at concentrations which suppressed exogenous fermentation indicates that the primary point must be on hexokinase and/or sugar transport across the plasma membrane in a mechanism similar to iodoacetate (BRADY et al., 1961) and uranyl ion (ROTHSTEIN et al., 1951).

The means by which octanoic acid prevents glucose fermentation is still not clear. Some bacterial enzymes of glycolysis were inhibited by octanoic acid (FERDINANDUS & CLARK, 1969). Besides inhibiting phosphate uptake by yeast cells, there is some evidence that at least two glycolytic enzymes are inhibited by short-fatty acids (SAMSON et al., 1955). The effect of lower fatty acids may be explained by a decrease in

internal pH values which are inhibitory to hexokinase and phosphofructokinase activities (KREBS et al., 1983) however VIEGAS & SACORREIA (1991) did not observe differences in the internal pH in yeast cells incubated with octanoic acid.

The synthesis of trehalose in yeast was reduced in the presence of 2,4-dinitrophenol (BERKE & ROTHSTEIN, 1957; AMIN et al., 1984; GUTIERREZ, 1990). Dinitrophenol makes membrane permeable to protons inhibiting the yeast protonpumping ATPase (SERRANO, 1980). The reduction of trehalose levels by octanoic acid shown in Figures 2 and 3 may be explained by changes in the activity of plasma membrane ATPase because VIEGAS & SACORREIA(1991) observed a higher activity of the enzyme in yeast cells grown in the presence of octanoic acid and BORST et al.(1962) reported that long-chain fatty acids were also effective in stimulating ATPase activity of rat-liver mitochondria.

The activation of trehalase by octanoic acid could explain the immediate increase in trehalose endogenous degradation observed in Fig.3 in the same way as 2,4-dinitrophenol (THEVELEIN, 1984) and also explain the level of reducing sugars found in the medium (Fig.4).

But VALLE et al. (1986) have reported that the activation of trehalase is mediated by an intracellular acidification caused by glucose and uncouplers. Irreversible toxicity resulting in the fall of trehalose contents may explain cell death of baker's yeast when exposed to octanoic acid.

The low concentration of short-chain fatty acids necessary to inhibit exogenous but not endogenous fermentation in yeast cells could be explained by the binding of octanoic acid to hexokinase and/or proteins of membrane because they are not necessary for endogenous fermentation.

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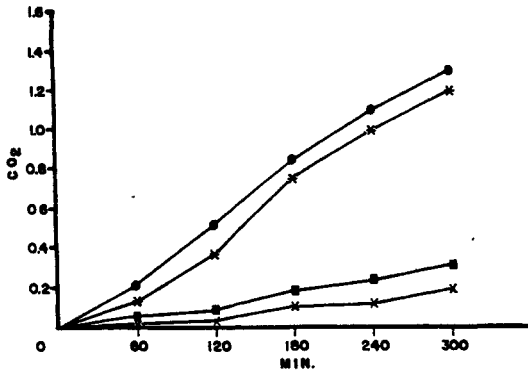


FIGURE 1. EFFECT OF OCTANOIC ACID ON ALCOHOLIC FERMENTATION (20 mg/mL FRESH BAKER'S, 5% GLUCOSE IN K CITRATE pH 4.0). TOTAL OCTANOIC ACID CONCENTRATIONS: CONTROL (●), 48 (✱), 96 (■), 120 (X) mg/L.

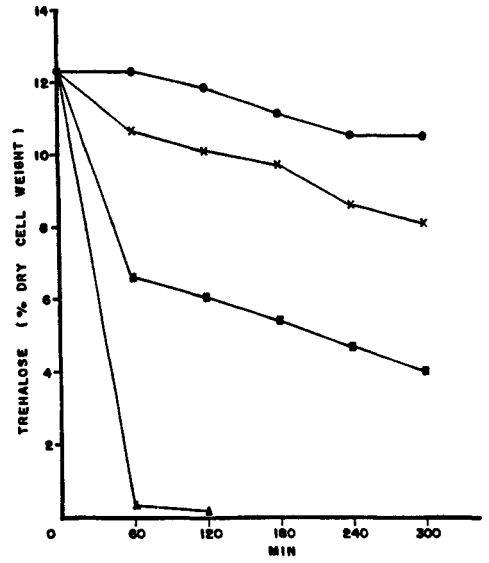


FIGURE 3. EFFECT OF OCTANOIC ACID ON TREHALOSE LEVELS DURING ENDOGENOUS FERMENTATION (20 mg/mL FRESH BAKER'S YEAST, K CITRATE pH 4.0). TOTAL OCTANOIC ACID CONCENTRATIONS: CONTROL (●), 120 (X), 180 (■), 240 (Δ) mg/L.

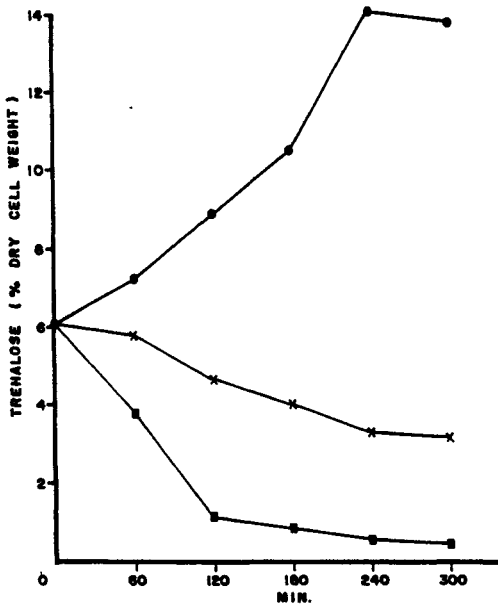


FIGURE 2. EFFECT OF OCTANOIC ACID ON TREHALOSE LEVELS DURING EXOGENOUS FERMENTATION (20 mg/mL FRESH BAKER'S YEAST - 5% GLUCOSE - K CITRATE pH 4.0). TOTAL OCTANOIC CONCENTRATIONS: CONTROL (●), 120 (X), 180 (■) mg/L.

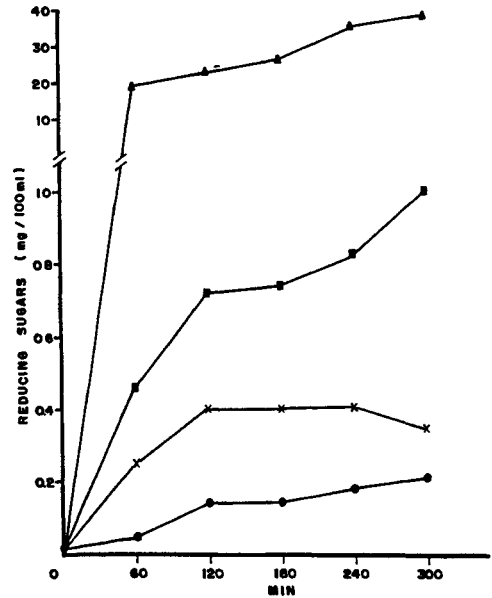


FIGURE 4. EFFECT OF OCTANOIC ACID ON MEDIUM REDUCING SUGARS (20 mg/mL FRESH BAKER'S YEAST, K CITRATE pH 4.0). TOTAL OCTANOIC ACID CONCENTRATIONS: CONTROL (●), 120 (X), 180 (■), 240 (Δ) mg/L.

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