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Short communication

Occurrence and effect of propanedial on top-fermenting yeast

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ABSTRACT

Peroxidation of polyunsaturated fatty acids caused by free radicals compromise the integrity of biological membranes. Propanedial is identified as the main product formed via the decomposition of lipid peroxidation products. Propanedial has been inferred to have mutagenic and cytotoxic roles. Top-fermenting yeast is responsible for converting fermentable sugars into alcohol. In the present paper we evaluate the relationship of lipid peroxide levels and the degree of impairment in glucose consumption of *Sacharomyces cerevisiae* cells. Results showed that cell suspensions pre-incubated with Propanedial reduced glucose consumption by about 30% resulting in a decrease in the yield of top-fermenting yeast. These findings suggest that Propanedial affects the fermentation process of *S. cerevisiae*.

1. Introduction

In the traditional ethanol process, brewer's yeast is propagated under weakly aerated conditions [1]. Oxygen is needed for the synthesis of sterols and unsaturated fatty acids, which are vital elements of cell membranes [2]. Without a supply of these lipids, the cells cannot reproduce and their viability is lessened. Free radicals and reactive oxygen species (ROS) are generated from wort oxygenation and within the cell during the limited period of yeast aerobic metabolism [3]. These components result in cell damage and potentially represent significant stress to yeast [4].

The oxidation of polyunsaturated fatty acids by ROS found in cell membranes plays a role known as lipid peroxidation, which can be measured and used as an indicator of cellular oxidative stress [5]. The products from peroxidation accumulated during aerobic metabolism induce modifications in the structure, fluidity and permeability of the membranes [8]. The oxidation of polyunsaturated fatty acids serves as a convenient index for the extent of peroxidation reactions. Among the thiobarbituric acid reactive substances (TBARS) formed, Propanedial is identified as the main product. The high content of unsaturated lipids, the aerobic conditions and the presence of metal ions are promoters of peroxidation processes [6].

Yeasts are single-celled microorganisms that reproduce by budding. They are responsible for converting fermentable sugars into alcohol and other products. *Saccharomyces*

Abbreviations used: ROS, Reactive oxygen species; TBARS, Thiobarbituric acid reactive substance assay.

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cerevisiae cells are known as top-fermenting yeast, because during the fermentation process their hydrophobic surfaces cause the flocs to adhere to CO_2 and to rise to the top of the fermentation vessel [7]. The yeast, *S. cerevisiae*, has been selected as a model for research of peroxidation reactions, due to its availability and operational simplicity. The presence of Propanedial in top-fermenting yeast may be an indicator of the degree of cell injury. The aim of this paper was to analyze the effect of Propanedial on the yield of top-fermenting yeast in order to optimize the fermentation process.

2. Materials and methods

2.1. Materials and strain of Yeast

All the solvents and other chemicals used were of analytical grade from Merck (Darmstadt, Germany). Solutions were prepared with water purified through the Milli-Q system. The strain of *S. cerevisiae* top-fermenting yeast (URM-5948) used in this study was obtained from the fungal cultures of the Federal University of Pernambuco (UFPE) - Brazil.

2.2. Preculture

Lyophilized yeast, 150 kg m^{-3}, was added into Erlenmeyer flasks with 10 mol m^{-3} phosphate buffer (pH 7.0) and incubated at 25 °C for 1 h.

2.3. Measurement of basal TBARS content in the absence and presence of glucose

Precultures (0.7 mL) of *S. cerevisae* were incubated in the presence or absence of glucose (38 mol m^{-3}) for 30 min at 37 °C and analyzed every 5 min. The basal TBARS content of *S. cerevisiae* were determined by the Buege and Aust method [9]. For this purpose, 0.5 mL of preculture was combined with 2.0 mL of reagent (3.75 kg m^{-3} thiobarbituric acid - CAS n° 504-17-6 and 150 kg m^{-3} trichloroethanoic acid - CAS n° 76-03-9). After centrifugation, the supernatant was heated for 15 min in a boiling water bath. After cooling, the absorbance was determined at 535 nm.

2.4. Treatment of top-fermenting yeast with Propanedial

Propanedial was freshly prepared through acid-hydrolysis of Propanedial diethyl acetal (1,1,3,3-Tetraethoxypropane CAS n° 122-31-6) by mixing 0.2 mL of the latter compound with 0.8 mL of NaCl (150 mol m^{-3}) and 0.03 mL of 6 kmol m^{-3} HCl, according to Jain [10]. Different concentrations (1.69 mmol m^{-3} to 25.68 mmol m^{-3}) of Propanedial were added to the yeast (0.3 mL) in phosphate buffer (2.7 mL). After incubation at 37 °C, for 0, 5 and 10 min, the remaining levels of Propanedial were quantified.

2.5. Determination of glucose consumption after treatment with Propanedial

Precultures (0.7 mL) of *S. cerevisae* were incubated for 0, 5 and 10 min, at 37 °C, for initial interaction of preculture-Propanedial, in concentrations (1.69 mmol m^{-3} to 8.42 mmol m^{-3}). In brief, 0.2 mL of glucose (38 mol m^{-3}) was added to each test and incubated for an additional 5 min. The residual glucose concentration was assayed according to Grigorian and Kiroshka [11]. This assay was performed using 0.2 mL of preculture-Propanedial combined with 1.8 mL of 150 kg m^{-3} trichloroethanoic acid. After centrifugation, 0.5 mL of supernatant was combined with 3.5 mL of reagent (2-Methyl-1-aminobenzene – CAS n° 95-53-4 and thiourea – CAS n° 62-56-6 in glacial acetic acid). This mixture was heated for 10 min in a boiling water bath. After cooling, the absorbance was determined at 680 nm and expressed as mg g^{-1} of cells.

2.6. Statistical analysis

Statistical analysis for non-paired data was carried out by Student's *t*-test using the GraphPrism (GraphPad Software Inc. San Diego. CA, USA). The accepted significant difference between the mean values of parameters was analyzed to the level of $p < 0.01$.

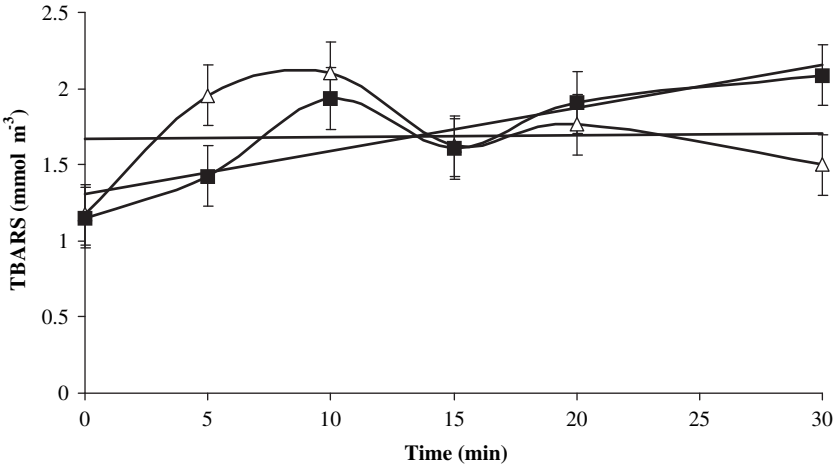

Fig. 1 – Basal TBARS content in preculture without metabolic stimulation (△) and with metabolic stimulation (■).