

## Apple juice reverse the oxidative effect of vanadium pentoxide in *Saccharomyces cerevisiae*

J. Agostinho<sup>1</sup>, R. Ferreira<sup>1,2</sup> and I. Alves-Pereira<sup>1,2</sup>

<sup>1</sup> Department of Chemistry, School of Sciences and Technology, University of Évora, R. Romão Ramalho, 59, 7001-554 Évora, Portugal

<sup>2</sup> Institute of Mediterranean Agrarian Environmental Sciences (ICAAM), University of Évora, Núcleo da Mitra, 7002-774 Évora, Portugal

The *Malus domestica*, Borkh, tients for over 2500 years, was domesticated and expanded in Europe by the Greeks and Romans. Due to their high adaptability to different climates and soils, the apple orchards were quickly installed throughout the world, from countries with relatively cool to subtropical climates. In Portugal, Romans have carried out their introduction and later by religious influence led to the spread of different varieties. The region of Beira Alta, Portugal, with its diversity of microclimates, of harsh winters and hot summers with high brightness, distinguished himself early as a conducive area to apple growing, being denominated IPG ( Protect geographical region) for the Golden Delicious variety. In the literature phenolic compounds of apples are described as potential inhibitors of oxidative processes, involving reactive oxygen species (ROS), implicated in chronic disorders such as cancer and cardiovascular disease. The main objective of this study was to evaluate the role of Golden Delicious apple juice in cell proliferation of yeast *S. cerevisiae* UE-ME<sub>3</sub>, and stress molecular markers, in the presence of a well-know oxidant, vanadium pentoxide.

*S. cerevisiae* inoculated in liquid YEPD medium, in the absence or presence of 2.0 mM vanadium pentoxide or in the presence of 2.0 mM vanadium pentoxide and 5% apple juice, were left to grow during 72 h, at 28 °C. Samples of each culture were harvested for determination of cfu, preparation of cell lysates, by sonication in 10 mM phosphate buffer pH 7.0, to obtain post-12000 g supernatant, used for determination of proteins, antioxidant power (DPPH) by spectrophotometry, the glutathione and ROS contents, by fluorescence and ALP and GR enzyme activities by spectrophotometry [1, 2, 3, 4, 5, 6].

The results show that 2.0 mM vanadium pentoxide (V<sub>2</sub>O<sub>5</sub>) behaved as inducer of cell death, decreasing cell viability (cfu) and ALP enzyme activity, with a significant increase of intracellular ROS and GR activity. The glutathione-mediated antioxidant system showed an increase in GSH content and a decrease in GSSG content, which reflects into a increase of the GSH/GSSG ratio. Despite also occur a rise of cytosolic free radical scavenger in cells growing in the presence of vanadium, this response was not adequate to the preservation of cell viability. The Golden Delicious apple juice increased cell viability and ALP activity as well as decreased ROS in *S.cerevisiae* grown in presence of V<sub>2</sub>O<sub>5</sub>, which seems a protector response. As the ability to free radical scavenger remained higher in the presence of apple juice and the GSH/GSSG ratio decreased, we presume that there protective effect depends on phenolic compounds (261.3 ± 23.4 g/mL ) present in apple juice which amends the response mediated by glutathione.

**Keywords** *Malus domestica*; *Saccharomyces cerevisiae*; antioxidants

- [1] Lowry OH, Rosenbrough NJ e Farr AL (1951) J. Biol.Chem 193, 265-275
- [2] Brand-Williams, W.; Cuvelier, M.E.; Berset, C. (1995). Use of a Free Radical Method to Evaluate Antioxidant Activity. *Academic Press*. Vol. 28, pp. 25-30.
- [3] Hissin, A.; Hilf, P.J. (1976). A fluorometric method for determination of oxidized and reduced glutathione in tissues. *Analytical Biochemistry*, Vol.74, pp. 214–226.
- [4] LeBel, P.C.; Ischiropoulos, H.; Bondys, C.S. (1990). Evaluation of the Probe 2, 7-Dichlorofluorescein as an Indicator of Reactive Oxygen Species Formation and Oxidative Stress. *Chem. Res.* Vol.5, pp. 227-231.
- [5] Breaudiere JP e Spillman T (1984). Bergmeyer Methods of Enzymatic Analysis, Volume II, 3rd ed., Verlag Chemie, Florida.
- [6] Goldberg, D.; Spooner, R. (1987) – Gluathione reductase, in: Bergmeyer - *Methods of enzymatic analysis*, 3rd ed., 258-265, Bergmeyer, VCH, New York.