Ability to scavenge free radicals by *Malus domestica* Borkh. pulp reversed the stress profile induced by vanadium (V) in *Saccharomyces cerevisiae*

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The determination of functional value of a particular food includes the discovery and characterization of their antioxidant properties, as its phenols contents, its ability to scavenge reactive oxygen species (ROS) or its biological activity to reverse oxidative stress markers, as triggered by vanadium (V) in eukaryotic models as *S. cerevisiae*, which exhibit high homology between their enzyme antioxidant response and of the humans.

So, the aim of this work was to determine how aqueous extract of apple pulp, collected at different times of the harvest period, affect the antioxidant response of *S. cerevisiae* BY4741 treated with vanadium pentoxide (2mM).

Mesocarp fresh of *Malus domestica* Borkh. of *Bravo de Esmolfe* variety, randomly collected in orchards in the region of Celorico da Beira, in September 2012 at the beginning and end of the harvest season, were homogenized in water (2:1, w/v). In the supernatants obtained after differential centrifugation 18000 *g*, during 40 min at 4 °C, of aqueous extract were determined the phenols content [1] and the antioxidant power, using the radical DPPH$^{\bullet}$ [2]. *S.cerevisiae* BY4741, a EUROSCARF strain, were 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% of apple extract and grown during 72 h, at 28 ºC. Samples of each culture were harvested for cfu determination and cell lysates preparation by sonication in 10 mM phosphate buffer pH 7.0, to obtain post-12000 *g* supernatant, used for of glutathione GSH, glutathione disulfide (GSSG) and ROS contents determination by fluorimetry and, glutathione reductase (GR), glutathione peroxidase (GPx) and cytosolic catalase (CAT T) enzyme activities by spectrophotometry [3, 4, 5, 6, 7].

The results show that the *Bravo Esmolfe* extract at start time of harvest period was more rich in polyphenols and had higher antioxidant capacity measured by DPPH. Furthermore, it was observed a significant decrease in cell viability of *S. cerevisiae* BY4741 exposed to vanadium pentoxide (2 mM) negatively correlated with a increase of ROS content inside the cells ($p < 0.05$). However, the presence of aqueous apple extract in the culture medium significantly reversed the response to this metal. In addition, there was an increase in GSH/GSSG ratio in cells exposed to vanadium, but this marker increased 90x in cells grown in the presence of vanadium and apple extract. This stabilization of the redox status by glutathione seems strongly consolidated by the presence of the fruity extract. Cells grown in the presence of vanadium pentoxide, or in the presence of this and apple extract showed an increase in GR activity ($p < 0.05$). This response suggests that the interference of V (V) in the regeneration of GSH was not affected by the extract. However, all cells exposed to vanadium exhibited a significant decrease in GPx activity which most likely contributed to keeping down the content of GSSG and expand the GSH/GSSG ratio. This response was increased in cells exposed to the presence of apple extract. The presence of V (V) in the culture medium also caused a decrease in CAT T activity, effect which was completely reversed by the presence of apple extract. Thus, the deficit in GPx and CAT T activities induced by the presence of vanadium in culture medium contributed to the increase of ROS and loss of cell viability described above. However, the presence of apple extract rich in polyphenols and with great antioxidant reversed the response profile of *S. cerevisiae* to vanadium pentoxide.

Keywords: apple; phenols; yeast

References

[1] Singleton VL, Rossi JA (1965) *Am. J. Enol. Vitic., 16*, 144-158.
[2] Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a Free Radical Method to Evaluate Antioxidant Activity. *Academic Press.* Vol. 28, pp. 25-30.
[3] Hissin A, Hilf PJ (1976) *Ana. Bioch.*, 74, 214–226.
[4] LeBel, PC, Ischiropoulos H, Bondys, CS (1990) *Chem. Res.* 5, 227-231.
[5] Goldberg D, Spooner R (1987) Bergmeyer *Methods of enzymatic analysis*, 3rd ed., 258-265, VCH, New York.
[6] Flohé L, Gunzler W (1984) *Methods Enzymol.*, 105, 114-121.
[7] Beers R, Sizer I Jr (1952) *J. Biol. Chem.* 195, 133-140.