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Quantification of Antioxidant Capacity with DPPH Methodology Using Methanol, Ethanol and Acetone As Diluents

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Several human pathologies are originated from free radicals that induce oxidative damage to biomolecules. The antioxidants can be powerful compounds to preventing against free radicals. One of the most common methodologies to quantify antioxidant activity in vitro uses 1,1-diphenyl-2-picrylhydrazyl (DPPH $^+$) and 6-hydroxy-2,5,7,8-tetramethylchroma $^-$ 2-carboxylic acid (trolox), both diluted in methanol. The main question that guided this paper was to evaluate the substitution of methanol for ethanol for diluting the DPPH reagent and methanol for acetone 75% and ethanol to dilute trolox. These substitutions can give analytical cost advantage and reduce analytical toxicity. Besides it has been showed that acetone 75% is better to extract polyphenol and antioxidant molecules of both pulp and skin of the fruits. Thirteen concentrations varying from 416.67 M to 81.42 ?M were used to construct the calibration curve. The results were comparated with the original methodology, using methanol as diluent. The results showed that the coefficients of the linear equations using methanol/methanol (Abs = -0.0011(trolox) + 1.2124, 2 0.9905), ethanol/ethanol (Abs = -0.0011trolox) + 1.2073, 2 0.9903) and ethanol/acetone 75% (Abs = -0.0012(trolox) + 1.1733, 2 20.9925) were similar. Samples of BSR Rúbea grape juice were used to compare the

original methodology, using methanol as the only solvent to dilute the DPPH reagent and the trolox standard, with the modified method, using ethanol to dilute the DPPH reagent and acetone 75% to dilute the trolox standard. The results obtained were 18,89 mM and 21.08 mM for the original and for modified methodologies, respectively. The statistical analysis showed that this diference was highy significant (P<0.01). The results obtained suggest that the quantification of antioxidant capacity using acetone 75% and ethanol as diluents of trolox and DPPH, respectively, besides the health and safety at work, can present analytical advantages.

Keywords: trolox, grape juice, polyphenol, free radical, oxidative damage.