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**Research Project**      Development of a colorimetric method for quantification of free amino acids content in tea infusions with the pyridine-2-aldehyde and cobalt nitrate: Optimization and validation of the analytical methodology

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#### Abstract

Free amino acids are regarded as important taste components in terms of tea quality. Analysis of free amino acids has become a routine tool for quality evaluation. The aim of this work was to develop a simple and accurate method for estimation of free amino acids in tea infusions using a reaction of pyridine-2-aldehyde (P) and cobalt nitrate(C). L-glutamic acid, was used as a standard compound to find the optimal condition for the assay. The optimal conditions for color reaction were mixing of 1000  $\mu$ L of 0.5% w/v of P, 200  $\mu$ L of 0.1 M of C and 2000  $\mu$ L of tea infusion. The mixture was heated in boiling water (95°C) for 15 minutes, let stand at room temperature and then measured A580 within 60-90 minutes. The absorbance values of reaction product were linear in the range of 0.02-0.4%w/v, and the linear regression equations were  $Y = 4.337X + 0.0653$  ( $R^2 = 0.9972$ ) with LOD and LOQ of 0.01 and 0.04%w/v, respectively. Under the described condition, the average recoveries of free amino acids in 28 infusions of green, oolong tea and black tea, were 102.02-105.03% with R.S.D. of 0.55-0.73%. The comparison between the conventional ninhydrin and the developed method showed no significant difference. The developed method could be used as effectively alternative method for free amino acids quantification in tea infusions

**Keywords**                      Amino acids, colorimetric method, pyridine-2-aldehyde, tea infusions