

## Stimulatory Effect of *Citrus* Extract on Nitrite Reduction to Nitric Oxide at Acidic pH

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Currently, the importance of nitric oxide (NO) for both plant and animal metabolism is well known. This compound can be obtained from diet through nitrite reduction at acidic pH. This is a slow process, but it can be facilitated by reducing compounds, such as flavonoids and organic acids. The aim of this study is to develop a method that allows simultaneous identification by HPLC of flavonoids and organic acids in *Citrus sinensis* extracts, and check the nitrite-reducing potential of its components. Three methods were evaluated and modified regarding its chromatographic parameters. NO formation was followed with specific electrode. Aqueous extracts obtained from *Citrus* juice were analyzed by a C<sub>18</sub> reversed-phase column, using a linear gradient of sulfuric acid and acetonitrile, and the compounds were detected at 215 and 278 nm. Running time was 60 min under a flow-rate of 0.5 mL/min, at 28 °C. Using this approach, malic, ascorbic and citric acids were eluted in the first 20 min and all phenolic compounds in the following 40 min. Most of the eluted compounds were identified using individual standards. The spontaneous reduction of nitrite (50 µM) at pH 2.0 produced 230 µmol NO, and this amount was 4.5 fold increased by an aliquot of *Citrus* extract (10 µL). With the addition of quercetin (50 µM), one of the *Citrus* flavonoids identified in the extract, NO production from nitrite was 12 to 50 fold increased, depending on nitrite concentration. These results suggest a potential effect of *Citrus* extracts in stimulating NO production from nitrite at stomachal pH.

Keywords: nitric oxide, nitrite, *Citrus* extract

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