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Development of HPLC/UV method for the analysis of nitrate and nitrite in cured meat and pepper

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The presence of nitrate and nitrite in vegetable, processed meat, and fruit has shown a direct influence on the increased risk of gastrointestinal cancer and methaemoglobinemia in infants. It is correlated to the formation of nitrosamines in the body due to the reaction between secondary amines and the nitrosating agent, nitric oxide cation (NO^+) derived from nitrite in an acidic environment. Pepper is considered to be the king of spices and the dried pepper berries are used as one of the most popular spicing agent in Sri Lanka and the whole world. Comminuted cured meat sausages and meat balls, salami, etc., use nitrates and nitrites for the curing of meat, which make their way into the body through dietary intake. Therefore, determination of the level of nitrate and nitrite present in the above food categories plays a vital role in assuring the safety of human health. This study was focused on developing simple, quick method for determining the concentrations of nitrates and nitrites simultaneously using a reverse-phase high performance liquid chromatography/ultraviolet (RP-HPLC/UV) technique. This method allowed the determination of the nitrate and nitrite content simultaneously within a period of 5 minutes of running time, and the void time of the reverse-phase high performance liquid chromatography column was 1.06 min. A reverse phase Zorbax Eclipse Plus C18 column was eluted with an optimized mobile phase of 25% acetonitrile and phosphate buffer of pH = 3.5, in an isocratic elution of 20:80 ratio, under the detection of diode array detector at 222 nm wavelength and with a flow rate of 0.7 mL min^{-1} . Recoveries of nitrate and nitrite were greater than 90% and the limit of detection for nitrate and nitrite were 0.28 mg L^{-1} and 0.12 mg L^{-1} respectively. The analysis was carried out using the method developed, on 36 selected samples each of chicken sausages and chicken meatballs, and showed that the nitrate and nitrite content varied in a range of $1036.0 - 2550.0 \text{ mg kg}^{-1}$ and $15.3 - 150.0 \text{ mg kg}^{-1}$ respectively. Nitrate and nitrite content in 12 selected pepper samples varied in the range of $309.9 - 1210.3 \text{ mg kg}^{-1}$ and $63.6 - 351.6 \text{ mg kg}^{-1}$ respectively.

Keywords: Nitrates, nitrites, HPLC, reverse phase, Optimized

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