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Determination of content levels of nitrogen species (Nitrite, Nitrate, and N-Nitrosamines) in processed meat consumed in Erbil City

Rebwar O. Hassan and Diyar S. Ali

Chemistry Department, College of Science, University of Salahaddin, Erbil-Iraq

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ABSTRACT

The nitrite, nitrate, and N- nitrosamines content in sixteen canned meat samples commonly marketed and consumed in Erbil city were determined. Spectrophotometric method was applied for the estimation of nitrite ions. When this ion reacts with a Griess reagent (sulphanilamide and N-(1-naphltyl) ethylenediamine hydrochloride (NED)) for color formation, and the purple colour that developed after 20 min was read spectrophotometrically at 538nm. Nitrate ion was determined by same procedure after its reduction with cadmium column to nitrite ion. While nitrosamine determination method was based on the photochemical cleavage of the N–NO bond of the nitrosamine to yield the corresponding amine and nitrite ions, followed by subsequent determination of the formed nitrite ion. None of the analyzed meat samples were found to violate the current legal limits practiced in the Iraqi food regulations.

Keyword: nitrite, nitrate, nitrosamine, meat product.

INTRODUCTION

The increasing incidence of various forms of cancer in the world at large and in Iraq in particular has been attributed to the levels of certain chemicals in our foods and drinks, in addition to the difficult conditions experienced by the country from war and embargo, which prevented the entry of many traders and this is what has led to a lot of food without paying attention to their sources, which in turn influenced the synthetic food that had existed in Iraq. Among these chemical carcinogens are N-nitrosamines and their precursors, nitrite, and nitrate. Various levels of carcinogenic volatile N-nitrosamines, nitrites, and nitrate are present in a wide variety of foods such as cured meat [1].

Alkali nitrites and nitrates, along with sodium chloride have been used in the curing of meat products to prevent bacterial spoilage and to enhance the flavor color and texture of these food products [2]. Much concern has been shown about the level of nitrite in food because the

nitrite can react in the stomach, with dietary components, to form toxic and carcinogenic nitrosamines [3]. Other danger that can occur after nitrite ingestion is that this ion in the blood steam converts oxyhemoglobin to metahemoglobin, thereby interfering with oxygen transport in the blood [4]. For these reasons, the nitrite content in meat products have been regulated and the majority of the analytical methods recommended by the legislations of different countries to determine its content.

Nitrate is a contaminant, natural contaminant and food additive that is relatively non-toxic to humans. The importance of monitoring and control of nitrate has long been recognized, but the reason for this has changed. The discovery of the beneficial effects of the L-arginine-NO-synthase and nitrate-nitrite-NO pathways in human metabolism is having a profound impact on our current view of these ions [5]. The nitric oxide plays the principal role and the nitrate ion act as product and precursors of several biochemical reactions [6].

Most N-nitrosamines have been shown to be carcinogenic in laboratory animals [7] and there is concern about health hazard to man who consumes precursors of N-nitrosamines. N-Nitrosamine term congregates a wide variety of homologous chemical substances of different molecular weights that come from the reaction of amines (especially secondary ones) and nitrosating agents, as shown in Fig. 1[8].

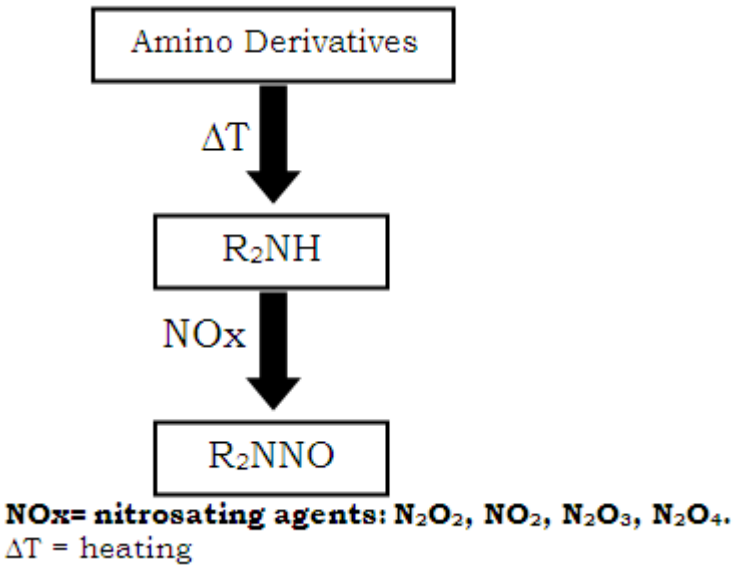


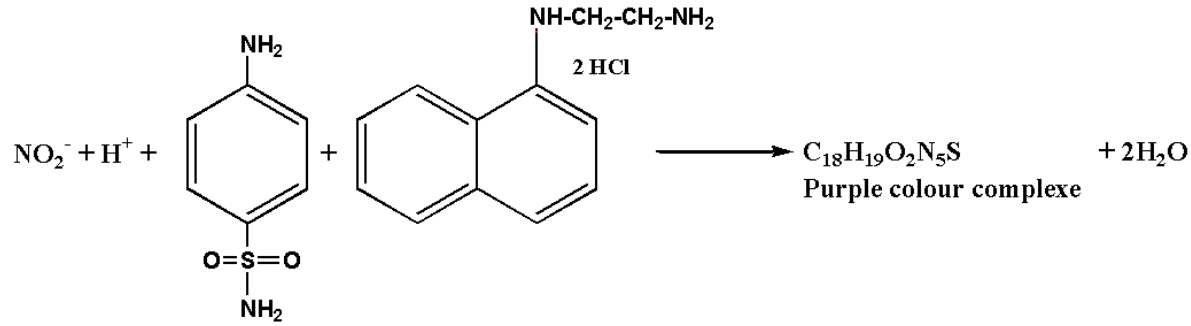
Fig. 1: N-Nitrosamines formation

The low levels at which nitrite, nitrate, and nitrosamines occur in foods and the heterogeneous nature of foods necessitate the use of sensitive methods of analysis. These methods require several steps, e.g. isolation from the food, clean-up and concentration, separation, detection, and quantification [9]. Several methods are now available for the determination of nitrite, nitrate and volatile nitrosamines in food stuffs, these have been reviewed. Among the methods more frequently used are spectrophotometer, chemiluminescence, GC and GLC with fluorescence detection by TEA [10].

The nitrite, nitrate, and N- nitrosamines content in sixteen canned meat samples commonly marketed and consumed in Erbil city were determined. Spectrophotometric method was applied for the estimation of nitrite and nitrate. Nitrosamine determination method was based on the photochemical cleavage of the N–NO bond of the nitrosamine to yield the corresponding amine and nitrite, followed by subsequent determination of the formed nitrite

ion. Nitrite ions react with a Griess reagent (sulphanilamide and N-(1-naphltyl) ethylenediamine hydrochloride (NED)) for color formation and the purple colour that developed after 20 min was read spectrophotometrically at 538 nm [11].

Rection mechanism

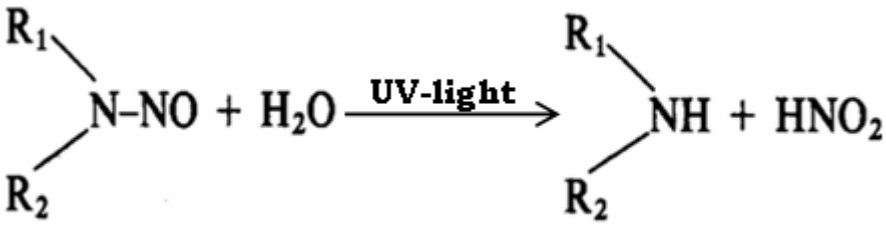


Method for analysis of NO_3^- in meat specifies the use of cadmium column to reduce NO_3^- to NO_2^-. When the sample is percolated through the column at a rate of 4.0 mL/min, 100% reduction of NO_3^- to NO_2^- can be obtained [12].

The mechanism for reduction of NO_3^- to NO_2^- by cadmium showing below:

$$NO_3^- + H_2O + Cd \longrightarrow NO_2^- + 2OH^- + Cd^{2+}$$

Analysis method of nitrosamine compounds from meat samples in which applied in this investigation was previously described in the literatures [13-15]. In this method meat extract was first passed through a 30 cm length column (1cm i.d.) filled with Deacidite MIP Cl^- - form anion exchanger, where nitrite and nitrate contained in the sample were retained to prevent their interfering with the determination method. Then nitrosamine is photochemically cleaved by UV light irradiation to yield an amine and nitrite ion as shown below:



The nitrite product from this photodegradation reaction is then detected spectrophotometrically after reaction with Griess reagent.

MATERIALS AND METHODS

Reagents

1- Disodium tetraborate, saturated solution: Dissolve 50.0 g of disodium tetraborate decahydrate in 1000 mL of tepid water and cool to room temperature.

2- Potassium hexacyanoferrate (II) solution: Dissolve 106.0 g of potassium hexacyanoferrate (II) in water and dilute to 1000 mL with water.

3- Zinc acetate solution: Dissolve 220.0 g of Zinc acetate dihydrate in a mixture of water and 30 mL glacial acetic acid then dilute to 1000 mL with water.

4- Sulphanilamide solution: Dissolve by heating on a water bath, 2.0 g of sulphanilamide in 800 mL water. Cool, filter if necessary and add 100 mL of cone. HCl while stirring. Dilute to 1000 mL with water.

5- NED solution: Dissolve 0.25 g of NED in water. Dilute to 250 mL with water store in a stoppard brown bottle in a refrigerator for not more than one week.
6- HCl solution: Dilute 445 mL of Conc. HCl (sp.gr 1.19) to 1000 mL with water.
7- Ammonical buffer solution, pH 9.6: Dissolve 37.4 g of ammonium chloride in about 900 mL of water in a 1000 mL volumetric flask. Adjust the solution to pH 9.6 with concentrated ammonia solution, and make up to the mark with water.

Standard nitrite solution
Weight 3.0 g of sodium nitrite and dissolve it in 50 mL of D.W., transfer quantitatively to 1000 mL volumetric flask, shake well and make up to the mark. Pipettes 5 mL of this solution into 1000 mL volumetric flask and make up to the mark. 1 mL of this standard solution contains 10 µg of nitrite ion (NO_2^-) [16].

Canned Food Sample
Sixteen samples of canned foods (most of them were imported) commonly marketed and consumed in Erbil City were purchased from retail outlets and were used in this study. The samples include meat, chicken luncheon and fish.

Extraction procedure
Weigh about 10.0 g of the sample, transfer quantitatively to a 500 mL beaker and adding 5 mL of saturated borax solution and 100 mL of hot water at 70°C. Heating the beaker for 15 minutes on the boiling water bath and shake repeatedly. Allow the flask and its contents to cool at room temperature and add successively 2 mL of Potassium hexacyanoferrate followed by 2 mL of zinc acetate. Mix thoroughly after each addition.

Transfer the contents to a 200 mL volumetric flask. Dilute to mark with water and mix. Allow the flask to stand for 30 min. at room temperature. Carefully decant the supernatant liquid and filter it through fluted filter paper to obtain clear extracted solution.

Determination of nitrite:
Pipette 10 mL of extracted solution into a 50 mL volumetric flask and add water to make up to 30 mL. Adding 5 mL of sulphanilamide solution followed by 3 mL of cone HC1 and leave the solution in the dark for 5 min. Adding 1 mL of NED solution and leave for 15 min. in the dark. Dilute to mark with water. Measure the absorbance of the solution in a 1 cm cell using a photoelectric colorimeter or spectrophotometer at a wave length of about 538 nm [17].

Determination of nitrate:
Pipette 10 mL of extracted solution into a 50 mL volumetric flask from clear solution, add 5 mL of buffer solution (9.6). Then passing it through cadmium column for at least 15 min. dilute to mark with water [16], after that repeating same procedure for color development.

Determination of N-nitrosamine:
Pipette 10 mL of extracted solution into a 50 mL volumetric flask from clear solution, passing it through a column filed Deacidite MIP Cl^- - form (14-52 mesh) resin for removing all nitrite and nitrate content, the final solution passing through a flow cell placed in front of UV-light for 30 min, in which the nitrosamine is photochemically cleaved to yield an amine and nitrite ion [18]. Then repeat same procedure for color development which described above for nitrite determination.

RESULTS AND DISCUSSION

The result from this study show that all the canned foods which exported from Iraq and neighbors countries to Erbil city contain a detectable amount of nitrite and nitrate, non-detectable amount of N-nitrosamine Table (1) and Table (2). The mean nitrite levels in the meat product ranged from 9.2×10^{-3} to 15.4×10^{-3} µg/mL and 1.2×10^{-4} to 8.1×10^{-3} µg/mL for nitrate. The concentration of nitrite and nitrate in the samples fall below the WHO's Acceptable Daily intake (ADI) which is set at 5.0 µg/mL body weight [1]. However, the risk to human with respect to methaemoglobinaemia and conversion of nitrate to nitrite in oral cavity and stomach leading to the possible formation of nitrosamines cannot be ignored. Chronic ingestion of small dose of nitrate can also cause dyspepsia, mental depression, and headache [1].

Table (1): Nitrite content in canned foods which exported from Neighbors Countries and IRAQ to Erbil City

Traditional name	Exported Country	Properties	Flash Code	Nitrite average as µg/mL
Al-Wadi	Jordan	Chicken Luncheon meat	13039	9.20×10^{-3}
Del Monte	Jordan	Meat Luncheon	91180	10.90×10^{-3}
Unium	Jordan	Chicken Luncheon	10222/046	9.76×10^{-3}
Bordroom	Iraq	Chicken Luncheon meat	00C00C	9.3×10^{-3}
Salem	Iran	Meat Chicken	10A17	15.4×10^{-3}

Table (2): Nitrate and N-Nitrosamine content in canned foods which exported from Neighbors Countries and IRAQ to Erbil City

Traditional name	Exported Country	Properties	Flash Code	Nitrate average as µg/mL	N-Nitrosamine average as µg/mL
Al-Wadi	Jordan	Chicken Luncheon meat	13039	1.2×10^{-4}	ND
Del Monte	Jordan	Meat Luncheon	91180	5.1×10^{-4}	ND
Unium	Jordan	Chicken Luncheon	10222/046	7.1×10^{-3}	ND
Bordroom	Iraq	Chicken Luncheon meat	00C00C	4.3×10^{-4}	ND
Salem	Iran	Meat Chicken	10A17	8.1×10^{-3}	ND

Table (3): Nitrite content in canned foods which exported to Erbil city from different countries

Traditional name	Exported Country	Properties	Flash Code	Nitrite average as µg/mL
Bordon	Brazil	Corned Beef	11991E1	1.01×10^{-2}
Zwan	Hooland	Chicken Luncheon meat	028498	1.23×10^{-2}
Groot	Holand	Chicken Luncheon meat/carne	13039	1.12×10^{-2}
Zwan	Holand	Hotdog Sausages	L2K10	1.08×10^{-2}
Dolo	France	Chicken Luncheon meat	15H35	0.97×10^{-2}
Tahani	Brazil	Beef Hotdog	902/0001	1.18×10^{-2}
Covi	France	Chicken Luncheon meat	7304902	9.30×10^{-2}
Faresco	Thailand	Sandwich Tuna Flakes	J121	1.72×10^{-2}
Tulip	EU	Chicken Luncheon meat	10222l046	1.76×10^{-2}
Al-Taghziah	Lebanon	Hotdog Chicken	39199	1.98×10^{-2}
Koutoubia	Morocco	Turkey Luncheon Viande	08252l510A	1.67×10^{-2}

Table (3) and (4) shows the result which obtained in the meat product which exported from different sources and exists in the local market of Erbil city. The concentrations of nitrite and

nitrate measured in sample of eleven types of those meat product ranged from 9.3×10^{-4} to 19.8×10^{-3} µg/mL for nitrite and from 1.2×10^{-4} to 9.1×10^{-3} µg/mL for nitrate. None detectable levels of N-nitrosamine were observed in all samples, that nitrosamines are parts of the chemical component of those products a significant finding in this study. N-nitrosamines are known toxicants as well as chemical carcinogens. Investigators have also demonstrated nitrosamine formation in food nitrite mixtures mainly in attempt to determine the extent of formation under human gastric conditions [19].

Table (4): Nitrate and N-nitrosamine content in canned foods which exported to Erbil city from different countries

Traditional name	Exported Country	Properties	Flash Code	Nitrate average as µg/mL	N-Nitrosamine average as µg/mL
Bordon	Brazil	Corned Beef	11991E1	1.4×10^{-3}	ND
Zwan	Hooland	Chicken Luncheon meat	028498	2.1×10^{-4}	ND
Groot	Holand	Chicken Luncheon meat/carne	13039	4.1×10^{-3}	ND
Zwan	Holand	Hotdog Sausages	L2K10	2.3×10^{-4}	ND
Dolo	France	Chicken Luncheon meat	15H35	4.2×10^{-3}	ND
Tahani	Brazil	Beef Hotdog	902/0001	1.8×10^{-4}	ND
Covi	France	Chicken Luncheon meat	7304902	1.6×10^{-4}	ND
Faresco	Thailand	Sandwich Tuna Flakes	J121	9.1×10^{-3}	ND
Tulip	EU	Chicken Luncheon meat	10222l046	1.2×10^{-4}	ND
Al-Taghziah	Lebanon	Hotdog Chicken	39199	1.7×10^{-4}	ND
Koutoubia	Morocco	Turkey Luncheon Viande	08252l510A	9.1×10^{-3}	ND

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