RESULTS ON QUANTITATIVE ANALYSIS OF NITRATES AND NITRITES IN SOME FOOD PRODUCTS OF ANIMAL ORIGIN

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Keywords: nitrate; nitrite; myoglobin; amino acids

ABSTRACT

The toxicity of nitrates is also manifested by its ability to convert to nitrosamines; nitrosamines can form in both food products (exogenous origin) during storage and in the digestive system (endogenous origin), especially in the stomach. The nitrites diasotize the sulphanilic acid in an acidic medium and the formed diazonium salt is coupled with alpha-naphthylamine to give a pink-colored azoic compound with a maximum absorption at λ = 520nm. In the meat products analyzed the nitrate content recorded large variations, ranging from 27.54 mg/kg to the Polish sausage sample and 79.31 mg/kg in the pork pastrami sample.
Nitrate concentrations were within a broad range without exceeding the maximum permissible concentration (0.74 mg/kg in the half-smoked sausage sample, respectively 32,24 mg/kg in the chicken sausage.

INTRODUCTION

Sodium and potassium nitrates and nitrites (E 249 - E 252) are used for both preservative action and especially for colouring meat and meat preparations, along with sodium chloride, ascorbic acid and potassium sorbate. The nitrate ion as such has no effect on the colour of the meat, being used as a source of nitrite. Transformation of nitrate into nitrite occurs bacterially by nitrate reductase at pH higher than 5.8.

The mechanism of action of nitrites is explained by the reaction of nitrogen monoxide with myoglobin, with the formation of nitroso-myoglobin (the specific colouring of salted meat, thermally untreated); by boiling, the nitroso-myoglobin passes into the nitroso-hemocromogen. Haemoglobin reacts in the same way as nitrites added as an additive.

In addition to the action of fixing the colouring the meat, nitrates introduced into food play an important role in the formation of the flavour and taste of the meat; it is widely accepted that the flavour of foods in which nitrates are incorporated is more pleasant. This phenomenon would be explained by the participation of the microbial flora, which reduces the nitrates to nitrites, to the formation of the flavour and taste of the meat.

Nitrites, added as such in the food or from nitrate reduction, exhibit moderate antimicrobial activity (especially in combination with sodium chloride in acidic medium), antioxidant action (due to the reduction character); nitrites are involved in the formation of an effective inhibitor of growth of sporulating anaerobic bacteria, *Clostridium botulinum*, which produces very active neurotoxins. The high risk of using nitrites is their ability to react with amines, amino acids, and form nitrosamines, highly carcinogenic compounds; in fact, even nitrites as such may be carcinogens. The value of pH and temperature influences the consumption of nitrite in meat products in the sense that at low pH and high temperatures the disappearance of this reddening agent is rapid (Banu *et al.,* 1997). The optimum pH is 5.8-6.0 when $NaNO_2$ is unstable. Under the pH value of 5.6, the nitrite rapidly disappears from the environment, and below the pH value of 5.2 the NO formation is practically inhibited. Over pH 6.0 the nitrite becomes stable, oxidizing and toxic.(Madrid *et al*., 2000).

MATERIAL AND METHOD

The research carried out was aimed at evaluating the nitrate and nitrite content of some food products of animal origin (meat products and fermented cheeses) marketed in specialized stores in Craiova. Within DSVSA Dolj, 30 samples of fermented cheeses and meat products were analyzed to determine the nitrate and nitrite content.

Determination of nitrites is performed by spectrophotometric method with Peter-Griess reagent. The nitrites diasotize the sulphanilic acid in an acidic medium and the formed diazonium salt is coupled with alpha-naphthylamine to give a pink-coloured azo compound with a maximum absorption at λ = 520nm. The quantitative determination of nitrites is carried out in several stages: extraction of the analyte from the sample to be analyzed, purification of the extracts and determination itself (drawing of the calibration curve, determination of nitrites).

Determination of nitrates is carried out in the form of nitrites (after their reduction with cadmium powder), using the spectrophotometric method with Peter-Griess reagent. The nitrites diasotize the sulphanilic acid in an acidic medium and the formed diazonium salt is coupled with alpha-naphthylamine to give a pink-coloured azo compound with a maximum absorption at λ = 520nm.

In order to calculate the evaluation of the yield of the reduction reaction of nitrate to nitrite, the calibration curve for the nitrite-nitrate system reduced to nitrite was designed as it follows:

- in a series of tubes, volumes of nitrite standard solution containing concentrations between 0 and 10 micrograms of nitrite, volumes of nitrate standard solution, respectively, with concentrations between 0 and 10 micrograms of nitrate, are introduced and brought to 19 ml with distilled water; 1 g of cadmium is added, shaking for 30 minutes to carry out the reduction of nitrate to nitrite.
- after the removal of cadmium, add 0,5 ml of Griess I reagent and Griess II respectively and read extinctions at λ = 520 nm against the control sample.

The yield of the reduction reaction is calculated, according to the data in Table 1, for each concentration level in the calibration scale, according to the relation: $\eta = C_2 \cdot 100 / C_1$

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Table 1.

The yield of the nitrate reduction reaction at nitrite

C_1 (µg nitrite)	C_2 (µg nitrite obtained after reduction)	η %	η medium
4	3494	87.36	94.25%
8	7254	90.68	
12	11.634	96.95	
16	15.827	98.92	
20	19.632	98.16	

RESULTS AND DISCUSSIONS

The results obtained in the determination of nitrates in the meat products samples and fermented cheeses are shown in the figures below. The results are the average of three distinct determinations

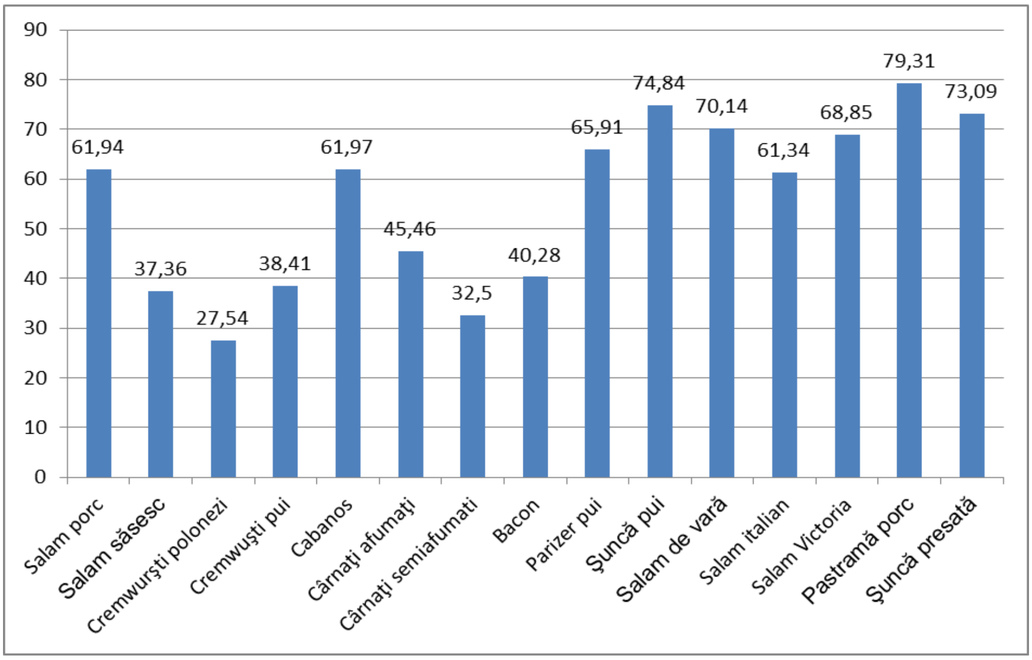

Figure 1 Nitrate and nitrite content of preparation of meat mg NO3-/kg

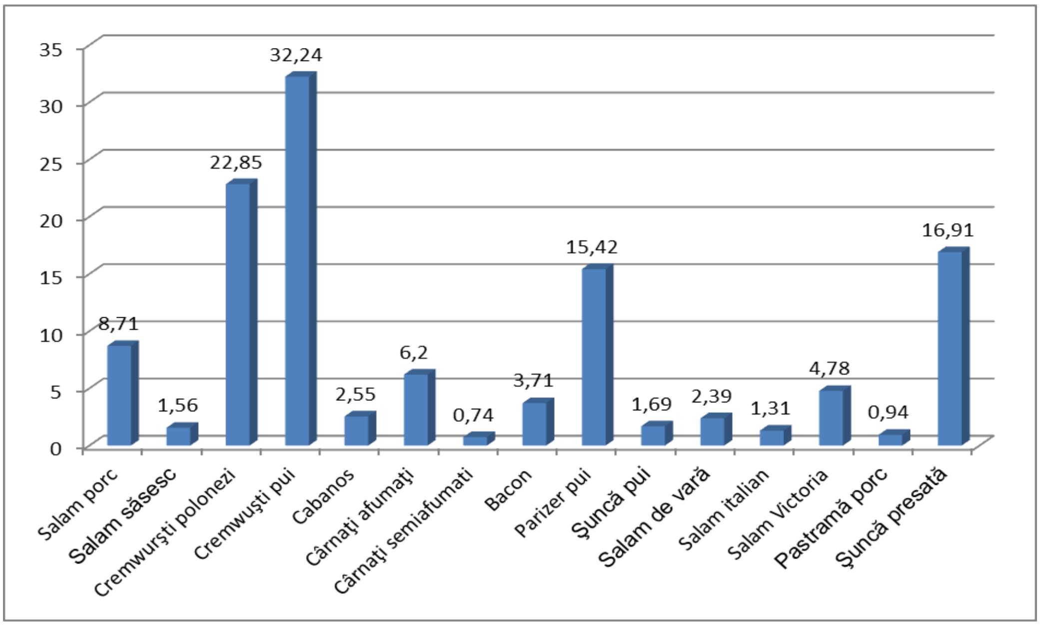

Figure 2 Nitrate and nitrite content of meat preparation mg NO2-/kg

In the meat products analyzed the nitrate content recorded large variations, ranging from 27.54 mg/kg to the Polish sausage sample and 79.31 mg/kg in the pork pastrami sample. Nitrate concentrations were within a broad range without exceeding the maximum permissible concentration (0.74 mg/kg in the half-smoked sausage sample, respectively 32.24 mg/kg in the chicken sausages).

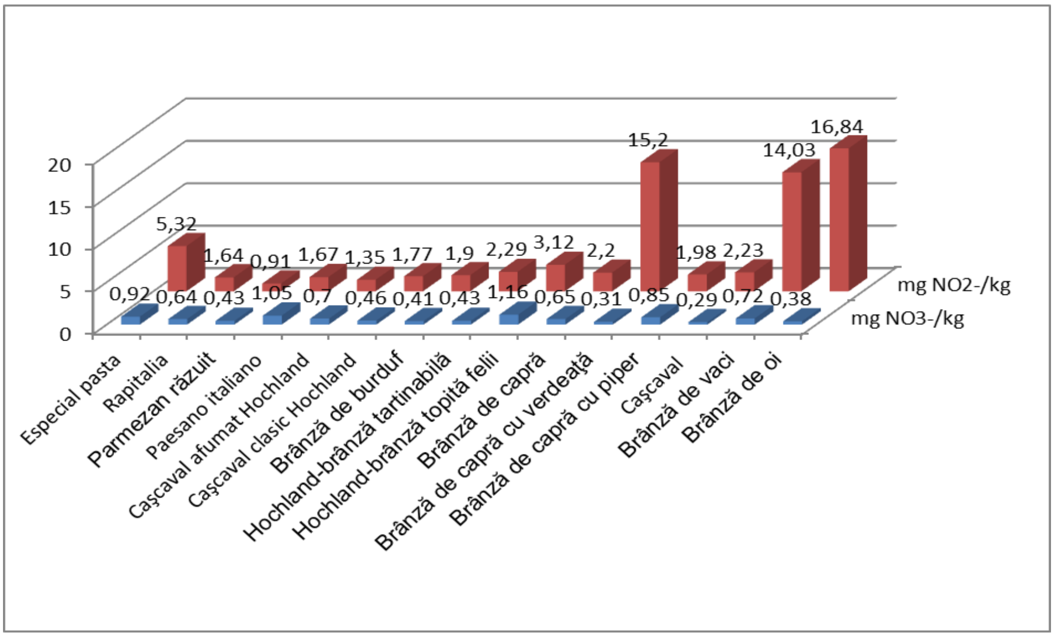

Figure 3 Compozition in nitrates and nitrites of cheeses fermented

In the fermented cheese samples nitrate and nitrite concentrations were well below the maximum permitted levels. The variation ranges for analyte concentrations were: 0.91 mg nitrate/kg (grated parmesan sample) -16, 84 mg nitrate/kg (sheep milk cheese sample) and 0.29 mg nitrite/kg pressed cheese sample) - 1.16 mg nitrite/kg (for the Hochland processed cheese sample).

CONCLUSIONS

For the determination of nitrite in food products (meat products and fermented cheeses) the spectrophotometric method with Peter-Griess reagent was applied. Determination of nitrates was done by the same method, after their reduction to nitrites, with cadmium powder. The yield of the reduction reaction was over 90%.

30 food samples, of which 15 samples of meat productss, 15 samples of fermented cheeses were analyzed, yielding results consistent with those in the literature. In the meat products analyzed the nitrate content recorded large variations, ranging from 27.54 mg/kg to the Polish sausage sample and 79.31 mg/kg in the pork pastrami sample. Nitrate concentrations were within a broad range without exceeding the maximum permissible concentration (0.74 mg/kg in the half-smoked sausage sample, respectively 32.24 mg/kg in the chicken sausage).

In the fermented cheese samples nitrate and nitrite concentrations were well below the maximum permitted levels.

BIBLIOGRAPHY

1. **Andrade R, Reyes FGR, Rath S. A.**, 2005 - *Method for the determination of volatile N nitrosamines in food by HS-SPME-GC-TEA. Food Chemistry 91: 173–179.*
2. **Banu C, Alexe P, Vizireanu C**. 1997 - *Procesarea industrială a cărnii.București: Ed. Tehnnică, 208-213, 455.*
3. **Honikel K.** 2008 - *The use and control of nitrate and nitrite for the processing of meat products, Meat Sci ; 78: 68–76.*

4. **Madrid VA, Madrid CJ.** 2000; - *Los aditivos en los alimentos (Según la Unión Europea y la Legislación Española), 1[a] Edición, AMV Ediciones, Mundi Prensa*,
5. **Menard *et al*.** 2008 - *Menard C, Heraud F, Volatier J.L, Assesment of dietary exposure of nitrate and nitrite in France. Food Additives and Contaminants, 25: 971-988.*
6. **Regulamentul (UE)** nr. 1129/2011 al Comisiei din 11.11.2011 de modificare a anexei II la Regulamentul (CE) nr. 1333/2008 al Parlamentului European şi al Consiliulu