RESEARCHES ON FORMATION OF NITROSAMINES IN MEAT PRODUCTS

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ABSTRACT

Dosage of nitrosamines was performed by high performance liquid chromatography with UV detection. The formation of nitrosamines in meat products, on the basis of residual nitrite, was emphasised by monitoring nitrate, nitrite and nitrosamine concentrations over a 28-day period for both meat products purchased directly from the producer on the first day they could be marketed and in samples of traditionally prepared at home sausages. There was a decrease in the nitrate and nitrite concentration in parallel with the occurrence and increase of nitrosamines concentration.

Research has shown that the process of nitrosamine formation in meat prepoducts is slow and progressive; the range of the first measurable nitrosamines was between 14 and 21 days. We note that for some industrial products the NA level determined after 28 days was above the maximum allowed for this type of product (1µg/kg).

In industrial meat products, at the end of the study, NDMA (nitrosodimethylamine) concentration varied between 0.80-23.40 µg/kg and the NDEA concentration varied between 11.60 and 61.90 µg/kg. For samples of pork sausage prepared at home, at the end of the study, nitrosamines concentrations ranged from 0.28-0.56 µg NDMA/kg and (0.13-0.29) µg NDEA/kg.

INTRODUCTION

In all the recommendations, the EU Scientific Advisory Committee on Nutrition included the need to reduce exposure to pre-formed nitrosamines in food by reducing the amount of added nitrite to the minimum necessary to ensure microbiological conservation and safety throughout the life of the product (EFSA, 2003).

Research in the field has identified the following sources of nitrosamines: food (meat products, fish), drinks (beer, whiskies), substances used in industry and agriculture (pesticides), cigarette smoke, exhaust gases, cosmetics, pharmaceutical and rubber products.

Nitrosamines can cause cancer in different organs, in a wide variety of animal species (Andrade *et al*. When metabolically activated, nitrosamines may be the cause of human cancer (Arranz *et al*., 2007). The formation of nitrosamines in meat products depends on: nitrite concentration used as an additive, residual nitrite concentration, ascorbic acid, ascorbate or α-tocopherol concentration, nitrosamines precursors, pre-processing procedures and conditions, moisture, percentage of adipose tissue, presence of catalysts/nitrosation reaction inhibitors, smoking process and culinary preparation method (Byun *et.al.*, 2004).

Lijinski (1999) states that in food, nitrocompounds concentration is often very low and thus human exposure to these compounds is low but should not be ignored due to the high carcinogenic potential of these compounds.

The mechanism and extent of nitrosoamine formation are dependent on both the amino acid structure and the

origin of the nitrosating agent, as well as the reaction conditions (Challis, 1985). Therefore, the pH of the medium, amine base and temperature are important factors that influence the rate of formation of nitrosoamines (Mirvish, 1975; Ward *et al*, 1987).

The tolerance level of human exposure to nitrosamines varies between 5-10 μg/kg body weight. In many countries a program for monitoring the concentration of volatile nitrosamines in food is applied; for example, a maximum permitted concentration of 10 μg N-nitrosopyrrolidine (NPYR)/kg for placing foods on the market (Ventanas *et al.,* 2006).

The formation of nitrosamines in food is the result of the use of nitrite, smoking, hot-drying, marinating, fungal contamination or food contact with some packaging. (Thicker, 2000).

The main cause in the formation of volatile nitrosamines is residual nitrite; Lijinsky (1999) determined N-nitrosodimethylamine, N-nitrosopyrrolidine and N-nitrosopiperidine in sausage samples, but the species and content of nitrosamines may vary from country to country depending on dietary habits.

MATERIAL AND METHOD

The research carried out by DSVSA Dolj aimed to dynamically determine the nitrate, nitrite, nitrosodimethylamine and nitrosodietylamine concentrations for industrially prepared products, and traditional household products, marketed within the city of Craiova for a period of 28 days. Dynamic analysis of nitrate, nitrite content and nitrosodimethylamine (NDMA) and nitrosodiethylamine (NDEA) formation were performed on meat samples (chicken ham, Banatean dry salami, French dry salami, traditional sausages and pork pastrami), purchased from the manufacturer on the first day they could be marketed.

The second set of determinations was made on batches of five types of meat products (sausages, wieners, loin, salami, ham) purchased from the commercial network.

The third set of samples consisted of 5 batches of traditional pork sausages. The determinations were initially performed at 7 day intervals for 28 days.

Nitrites were determined by the spectrophotometric method with Peter-Griess reagent; nitrates were determined by the same method, after their reduction to nitrites, with cadmium powder. Determination of nitrosamines was carried out in several stages: separation of nitrosamines from samples, calibration curves, determination of nitrosamines.

Chromatograms were processed using the LC Solution Software.

The concentrations of nitrosodimethylamine and nitrosodiethylamine were calculated automatically by the software based on the analytical data using the peak area and the external standard method. The calibration curve was obtained by fitting data using linear regression.

RESULTS AND DISCUSSIONS

During the study the concentration of nitrate and nitrite in meat samples (chicken ham, Banatean dry salami, French dry salami, traditional sausages and pork pastrami) purchased directly from the producer on the first day they could be marketed, decreases, as shown in Figure 1.

The concentration of nitrosamines increases throughout the study for all the preparations analyzed (Table 1).

Table 1

The variation of NDMA and NDEA concentration in industrially prepared meat products

Sample	Initial concentration		Concentration after 28 days	
	NDMA mg/kg	NDEA mg/kg	NDMA mg/kg	NDEA mg/kg
1	nd	0.43	29,00	11,60
2	nd	nd	10,50	22,50
3	nd	2,10	5,30	27,50
4	nd	2,70	0,80	61,90
5	nd	nd	23,40	40,00

Legend: 1- chicken ham; **2**-Banatean dry salami; **3-**French dry salami; **4**- traditional sausages; **5**-pork pastramy; **nd**- undetectable

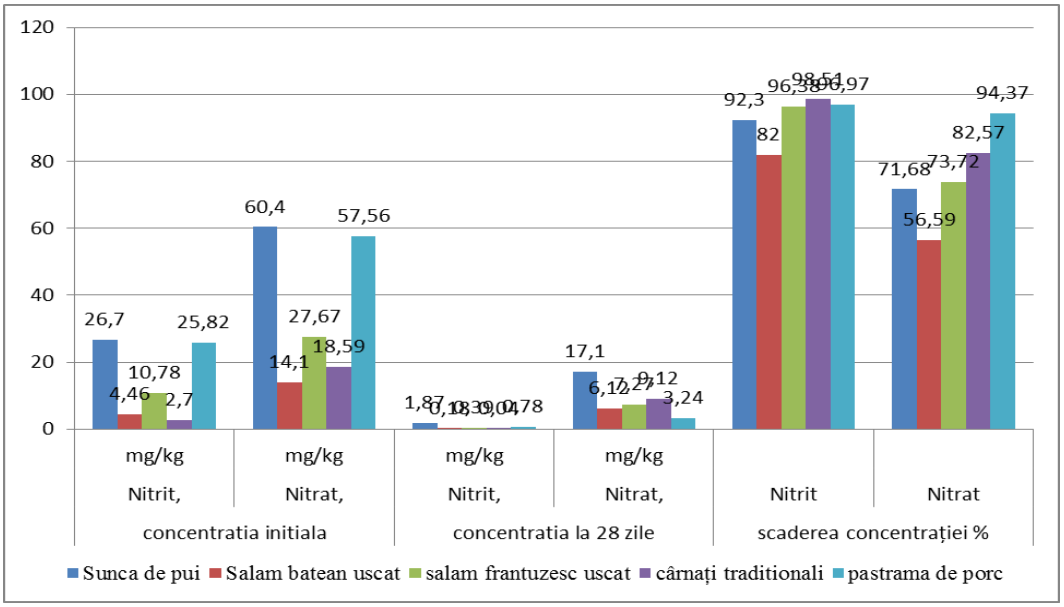

Figure 1 Variation of nitrate concentration and NDEA in industrially prepared meat products

Evolution of concentrations of nitrates, nitrites and nitrosamines in samples of pork sausages prepared in the laboratory are shown in Tables 2, 3, 4 and 5, respectively

Table 2

Evolution of the concentration of nitrite ions (mg/kg) in pork sausage samples prepared in the laboratory

Date /Batch	Initial	7 days	14 days	21 days	28 days
L1	0,35	0,34	0,25	0,21	0,17
L2	6,73	6,62	5,04	4,47	3,81
L3	0,42	0,38	0,29	0,21	0,15
L4	10,16	9,11	8,01	7,53	6,47
L5	9,74	8,10	7,60	6,81	6,69

Legend: Batch 1: Pork, table salt (4%).Batch 2: Pork, table salt (4%), sodium nitrite (100 mg/kg), garlic extract (25 ml).Batch 3: Pork, table salt (4%), potassium nitrate (200mg / kg), garlic extract (25ml). Batch 4: Pork, ktable salt (4%), sodium nitrite (100 mg / kg), potassium nitrate (200mg / kg), garlic extract (25ml). Batch 5: Pork, table salt (4%), Sodium nitrite (100 mg / kg), Potassium nitrate (200mg / kg), garlic extract (25ml) .

Table 3

Evolution of the concentration of nitrate ions (mg/kg) in samples of pork sausages prepared at home

Date /Batch	Initial	7 days	14 days	21 days	28 days
L1	6.28.	5.16	4.02	2.52	2.20.
L2	7.78	4.16	2.56	2.44	1.98
L3	53.09	36.23	33.27	25.23	22.94
L4	194.90	126.38	119.18	82.69	50.92
L5	193.17	174.79	110.19	70.30	49.92

Legend: Batch 1: Pork, table salt (4%). Batch 2: Pork, table salt (4%), sodium nitrite (100 mg / kg), garlic extract (25 ml).Batch 3: Pork, table salt (4%), potassium nitrate (200mg / kg), garlic extract (25ml). Batch 4: Pork, table salt (4%), sodium nitrite (100 mg / kg), potassium nitrate (200mg / kg), garlic extract (25ml). Batch 5: Pork, table salt (4%), Sodium nitrite (100 mg / kg), Potassium nitrate (200mg / kg), garlic extract (25ml) .

Table 4.

Evolution of NDMA concentration (µg/kg) in samples of pork sausages prepared at home

Date / Batch	Initial	7 days	14 days	21 days	28 days
L1	nd	0,11	0,18	0,27	0,28
L2	nd	0,15	0,29	0,47	0,47
L3	0,09	0,17	0,27	0,30	0,31
L4	0,16	0,30	0.39	0,47	0,56
L5	0.10	29	0,37	0.42	0,48

Table 5.

Evolution of NDEA concentration (µg/kg) in samples of pork sausages prepared at home

Date / Batch	Initial	7 days	14 days	21 days	28 days
L1	nd	nd	0,09	0,11	0,13
L2	nd	0,09	0,14	0,20	0,24
L3	nd	nd	0,08	0,14	0,16
L4	nd	0,11	0,18	0,21	0,25
L5	nd	0,10	0,12	0,15	0,29

Legend: Batch 1: Pork, table salt (4%). Batch 2: Pork, kitchen salt (4%), sodium nitrite (100 mg / kg), garlic extract (25 ml).Lot 3: Pork, table salt (4%), potassium nitrate (200mg / kg), garlic extract (25ml). Batch 4: Pork, table salt (4%), sodium nitrite (100 mg / kg), potassium nitrate (200mg / kg), garlic extract (25ml). Batch 5: Pork, table salt (4%), sodium nitrite (100 mg / kg), potassium nitrate (200mg / kg), garlic extract (25ml) .

In the chicken ham test, the nitrate concentration decreased by 71.68% and the nitrite level by 92.3%; in the Banat dry salami sample, the nitrate and nitrite concentrations decreased by 56.59% and 82%, respectively; in dry French salami nitrate and nitrite concentrations decreased by 73.72% and 96.38% respectively; in the traditional sausage sample, the decrease in the concentrations was 82.57% for nitrate and 98.51% for nitrite, and in the pork pastrami sample 94,37% for nitrate, respectively 96,97% for nitrite.

CONCLUSIONS

Dosage of nitrosamines was performed by high performance liquid chromatography with UV detection. The formation of nitrosamines in meat

products, on the basis of residual nitrite, was emphasised by monitoring nitrate, nitrite and nitrosamine concentrations over a 28-day period for both meat products purchased directly from the producer on the first day they could be marketed as well as samples of sausage prepared experimentally in the laboratory. There was a decrease in the nitrate and nitrite concentration in parallel with the occurrence and increase of nitrosamines concentration.

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Within the batches of fresh homemade sausages, decreases in nitrate and nitrite concentrations were observed in lower percentages compared to the decrease in analyte concentrations in industrial preparations, which ranged from 31.31% to 64.28% for nitrite, respectively 56.79% and 74.55% for nitrate.

Research has shown that the process of nitrosamine formation in meat preoducts is slow and progressive; the range of the first measurable nitrosamines was between 14 and 21 days. We note that for some industrial products the NA level determined after 28 days was above the maximum allowed for this type of product (1µg/kg). In industrial meat products, at the end of the study, NDMA (nitrosodimethylamine) concentration varied between 0.80-23.40 µg kg and the NDEA concentration varied between 11.60 and 61.90 µg/kg. For samples of pork sausage made at home, at the end of the study, nitrosamines concentrations ranged from 0.28-0.56 µg NDMA/kg and (0.13-0.29) µg NDEA/kg.

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