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The effect of baking of various kinds of raw meat from different animal species and meat with functional additives on nitrosamine contamination level

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Abstract

The studies aimed to determine the occurrence and formation of nitrosamine contamination levels with dimethylnitrosamine (DMNA) and diethylnitrosamine (DENA) in meat of various kinds, species and genders of farm animals slaughtered at meat processing plants all over Poland. The meat after cooling, cutting and jointing was classified, then comminuted and divided into several experimental variants. Moreover, the effect of the most frequent functional additives used in food industry, such as sodium chloride and sodium ascorbate, and baking process upon the level of the meat pollution was researched. Nitrosamine (DMNA and DENA) concentrations were assessed by Varian 3400 gas chromatograph coupled with Finnigan MAT ITD 800 spectrometer. The quantitative and qualitative states of respective nitrosamines were determined by comparing the chromatogram values.

The experiments conducted by the author revealed that sodium chloride or sodium ascorbate added to the meat caused a decrease in nitrosamine contamination level in comparison with meat without the additives. It was also found that under the experimental conditions and for the experimental variants, baking process leads to an increase in the levels of nitrosamine (DMNA and DENA) contamination in comparison with meat free of functional additives as compared to meat containing the functional additives.
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Keywords: Dimethylnitrosamine (DMNA); Diethylnitrosamine (DENA); Meat; Functional additives; Baking

1. Introduction

The causes and extent of current and potential eco-toxicological hazards, comprising among others water contamination with pesticides and nitroso- compounds, or chemical pollution of air, soils, agricultural products, forage or food, have been well known. *N*-nitroso compounds, represented by organic compounds, where *N*-nitroso (—N=O) group is bound with other nitrogen atoms, constitute a large group of organic nitrogen compounds which are nitrosamine precursors.

Nitrosamines are chemical substances with strong toxic, mutagenic, neuro- and nephrotoxic, teratogenic and carcinogenic effect. They form mainly in aquatic and soil environments but also in forage and food where they originate from primary, secondary and tertiary amines and amides and also result from the products of some pesticides and other precursor bio-transformations (Bogardi, Kuzelka, & Ennenga, 1991; Hill, 1988; Nitrite Safety Council, 1980; Preussman, 1983; Shamberger, 1984; Skrypec et al., 1985; Smyk, Różycki, & Dobrowolski, 1993).

Dimethylnitrosamine (DMNA) and diethylnitrosamine (DENA) reveal the strongest toxic activity. In case of secondary amines and nitrosamine formation kinetics of

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nitrosation follows the secondary equation. For the secondary and tertiary amines, the fastest reaction is observed in acid environment, i.e. at pH = 3–3.4. However, the greatest efficiency is achieved at pH = 4–5. Autotrophic (chemoheterotrophic, chemoautotrophic and photoautotrophic) and numerous heterotrophic organisms participate in biochemical and chemical processes of nitrosamine formation, which are also active in biochemical processes of nitrification and de-nitrification together with numerous bacteria. In the environment where nitrate ions are present, microorganism metabolism plays an important role, because some of them may accelerate nitrosating reaction and increase nitrosamine amount.

Experiments have demonstrated that some strains of enterococcus and *Clostridium* bacteria reveal an ability to synthesise dimethylnitrosamine (DMNA), while some *Proteus* are capable of dimethylnitrosamine production. The role of bacteria in nitrosamine formation consists in nitrate reduction to nitrites, protein degradation to secondary amines, production of an enzyme catalysing nitrosating reaction and forming a suitable reaction environment, i.e. acidification. Biocatalytic activity of some moulds in the reaction of nitrosamine synthesis is ascribed to species such as *Penicillium roqueforti*, *Penicillium camamberti* and *Rhizopus*. Also some other moulds, such as *Aspergillus oryzae*, which catalyse nitrosating of amines and participate in tri-methylamine transformation into diethylamine, are characterised by strong phytotoxic, mutagenic, teratogenic and carcinogenic effects on micro- and macro-organisms (fungi, plants, animals and humans). Studies have demonstrated an important role of NNC as initiators of cancerogenesis in humans and animals depending on ecological and geophysical conditions (King & Kurth, 1980; King, Kurth, & Shorthose, 1981; Kluczek, 1994; Smyk, Barabasz, Różycki, & Dobrowolski, 1992; Smyk, Różycki, & Barabasz, 1990; Steibert, 1982; The World Health Report, 1995; Wang, 1984; Wilkinson, 1994; Wyllie & Morehouse, 1990-1991; Zakrzewski, 1995). Nitrosamines present in food form two big classes: (1) proper *N*-nitrosamines originating from secondary amine nitrosating and (2) nitrozamides or more precisely derivatives of *N*-nitroso urethane, *N*-nitrosoguanidine and *N*-nitrosourea. The problem is that *N*-nitroso compounds may form de novo in human and animal alimentary tract. It has been demonstrated that animal organism is well prepared for *N*-compound synthesis and their amount is determined by selection of substrates and their proportional mixture.

The materials presented by WHO and prepared on the basis of research carried out by many research centres demonstrate that there are two compounds that are most often assayed in food, i.e. dimethylnitrosamine (DMNA) and diethylnitrosamine (DENA). The presence of nitrosamine results *inter alia* from various technological processes in which they form in a slightly acid environment in reaction between sodium nitrite and nitrogen oxide, and precursors present in foodstuffs, such as proteins, peptides, and amino acids (proline, orntine, glycine, alanine, choline, acetylcholine, and betanine). Basing on hitherto conducted research, it was assumed that conditions favourable for the nitroso-compounds formation occur also in meat where the precursor is creatine, i.e. one of the basic components of muscle tissue and when it occurs with phosphoric acid forming phosphocreatine. The studies have revealed that DMNA may form from quaternary ammonium salts, such as choline, acetylcholine and betaine, which are common in animal tissues. Both DMNA and DENA may form from mono-, di- and trimethylamines and from respective ethylamines. Diethylnitrosamine (DENA) forms from L-alanine in meat and meat products, the most frequently in fried bacon where much bigger quantities than in raw bacon were found. Nitrosopyrrolidine may be formed during frying of bacon through the formation of pseudo-nitroso unsaturated lipid derivatives (Skrypiec et al., 1985).

Literature and the Author's own research have identified the biggest hazards including free nitrogen concentration, temperature, pH, hydrogen ion concentration and some specific types and groups of bacteria, moulds and fungi.

Basing on the available knowledge in this area and his own research, the Author undertook the present studies to:

(1) determine nitrosamine occurrence in raw meat from various kinds, species, genders and types of farm animals slaughtered in the all year cycle;
(2) assess the importance of functional additives, such as sodium chloride and sodium ascorbate, in raw and baked meat of individual animal species for the levels of nitrosamine contamination;
(3) establish the effect of baking process on a change in nitrosamine concentrations in raw meat after slaughtering and coupling with the functional additives;
(4) carry out a comparative analysis of the obtained quantitative and qualitative results of DMNA and DENA present in raw meat, in meat with functional additives and formed as a result of baking.

2. Materials and methods

The experimental materials used for the eight-year investigations carried out in Poland were meat samples collected (under veterinary supervision) in meat factories from animals previously kept in farms and fed in the all-year-round cycle, slaughtered and subjected to after-slaughter treatment: pork from young sows, sows, hogs and boars, beef from heifers, cows, bullocks, bulls and calves, horseflesh from mares and geldings, mutton from ewes, wethers and rams, and goat meat.

The carcasses were chilled in a cooling chamber of the meat factory for twenty four hours at the air temperature range between 0 °C and 2 °C, air humidity between 85% and 90% and air velocity from 1 m/s to 2 m/s, and the inner temperature inside the muscle reached 4 °C. After this period of time after slaughter the carcasses were cut and jointed, bones were removed and the individual elements of the muscles were classified.

Samples providing the tested materials for each of the 90 test variants were collected from the hind legs – hams or haunches from 5 kinds including 15 species groups. Each species group comprised 21 animals and from each animal four samples were taken. The samples were then transported in special cooling containers to the laboratory of the Department of Microbiology, Agricultural University of Krakow, where they were kept refrigerated at 2 °C and successively used for the analysis. The same sampling method was used for each variant: I – raw meat, II – raw salted meat, III – raw meat with sodium ascorbate; IV – raw salted meat with sodium ascorbate. The external and intramuscular fat was removed from the meat samples in individual groups and respective variants. Four 200 g meat samples were collected for each experimental variant from each animal of a given kind, species and gender of the 21 slaughtered animals. Next the properly prepared samples were initially comminuted in a mincer with 2 mm mesh. Each 200 g sample for individual experimental variant was homogenised and from such prepared material 50 g was collected for laboratory analysis. One sample was always used directly for the analysis of meat from the slaughtered animal of a respective group as variant I – raw meat, whereas functional additives were injected to the other raw meat samples and these formed variant II – salted meat, III – meat with added sodium ascorbate and IV – salted meat with added sodium ascorbate. Laboratory analyses were carried out in five replications for each sample and the results were averaged. In variants II sodium chloride (NaCl) as a functional additive was applied because it is used for all meat products and plays an important role in the production technology and development of their quality and sensory features. Currently the critical amount of table salt (NaCl) according to the regulations in force in Poland ranges between 1.8% and 2.2% depending on the product and season of the year when it is produced, considering consumer health and the product quality. In order to average the permissible salt amount for the investigations, an addition of 2% of sodium chloride was used in variant II at pH ranging between 5.85 and 6.05 for all groups of raw meat. After keeping salted meat (variant II) of 48 h in cold storage (as described earlier), it was analysed in the laboratory. The samples collected for variant III – raw meat received functional additive 0.03% of sodium ascorbate which fulfils a reducing function, increases the degree of pigment reaction and lowers the residue nitrites. Next the samples were analysed in a laboratory. Functional additives such as sodium chloride (2% of the contents) and sodium ascorbate (0.03%), were added to the raw meat samples in variant IV, which were then subjected to a laboratory analysis. Subsequently, each of the samples constituting experimental material: variant I – raw meat, variant II – raw salted meat, variant III – raw meat with sodium ascorbate and variant IV – raw salted meat with sodium ascorbate, retaining their sensory and physico-chemical properties, were individually baked in an oven at 250 °C for a specified period of time to obtain the desired quality of baking. The baking periods were as follows: 60 min for veal, 75 min for pork and 85 min for beef, horseflesh, mutton and goat meat at assumed constant contents and weight of the raw material. On the basis of other experiments conducted by the Author, it was determined that the final product (variant I–IV) had the proper quality of food product good for consumption.

Experimental baking process for all experimental variants (I–IV) was carried out in an adjusted oven PPO-1e used in meat processing plants for baking meat and meat products. Baking is carried out in forced air system and the products (input) are put on cart shelves. During baking the cart rotates which ensures uniform baking of the whole product. This is a closed recirculation system forced by two centrifugal fans which suck off the air from the oven and next through a cascade of electric heaters force it back to the oven. The air flowing through the heater cascade heats up to the baking temperature suitable for individual products. Maximum air temperature inside the oven is 250 °C.

The air flows through system of slots in a shutter covering the blow-in duct and outlets in the suck up channel, which ensures uniform air flow in the whole oven capacity. The oven is equipped in the heated air humidity regulation system, which prevents overdrying of products, especially at the initial phase of baking. A pulsating spraying with warm water on heater block causing the water evaporation humidifies the air. Excessive vapour is removed from the oven during baking through the chimney with a throttle automatically shut and opened by pneumatic servo-motor. The oven is heated by electricity and baking temperature is regulated automatically. A person operating the oven can control the whole process of baking through a sight glass in the oven door. The baking chamber is lighted.

Electric heating system ensures ecologically clean and neutral environment for the baking carried out by means of PPO-1e oven and obtaining high quality final products.

PPO-1e baking oven is characterised by a high efficiency and technological effectiveness.

2.1. Technical data

- Working capacity 1 cart
- Single product input 350 kg
- Operation 1 person
- Maximum temperature 250 °C
- Installed capacity (total) 56.38 kW
  - Fan engine capacity 2 × 1.1 kW
  - Motoreductor engine capacity 0.18 kW
  - Heater wattage 54 kW
- Rotational cart speed 3 rot/min
- Water consumption 10 l/h
- Exterior dimensions
  - Width 1700 mm
  - Height 2700 mm
  - length 2200 mm
- Weight 1300 kg

The device, in which meat later used for complex analyses was baked at 250 °C during the period of time necessary for baking 1 kg of sample, is an automatic device equipped with a suitable measuring apparatus.

Properly baked sample materials from each examined animal per variant were initially comminuted in a mincer with 2 mm mesh. Each sample was then homogenised and from such prepared material 50 g was collected for laboratory analyses in five replications to detect nitrosamine (DMNA and DENA) contamination and the obtained results were averaged first for a given sample and then for all 21 samples in individual variants of the studied species and groups of slaughtered animals. Twenty-one averaged results of samples per individual group were obtained in the experiment conducted on five kinds including 15 groups of meat from males and females and each sample originated from a different animal of the slaughtered kinds, species and gender. In total 15 groups were studied, each including eight variants: I – raw meat and Ia – baked meat, II – salted meat and IIb – baked salted meat, III – meat with added sodium ascorbate and IIIc – baked meat with added sodium ascorbate, IV – salted meat with added sodium ascorbate and IVd – baked salted meat with sodium ascorbate. As a whole, it gave 120 variants and averaged results of nitrosamine (DMNA and DENA) contamination of samples.

Meat contents of nitrosamines (dimethylnitrosamine – DMNA and diethylnitrosamine – DENA) were assessed by Pancholy's method (Pancholy, 1976) adapted to nitrosamine determination in meat and meat products by Scanlan and Ryes (Scanlan, 1973; Scanlan & Ryes, 1985). DMNA and DENA concentrations were determined using a Varian 3400 gas chromatograph coupled to a mass spectrometer (Finnigan MAT ITD. 800). The samples were divided on the 0.2 μm and 25 m long Hewlett-Packard capillary column. The samples were dissolved in chloroform. After the injection of 0.5 μg of solution, a temperature gradient was applied (50–150 °C, 10 °C/min) with helium as the carrier gas. The injector temperature was set at 180 °C and the carrier gas pressure at 10 psi. The volatiles were identified by comparing their mass spectra with standards and by comparison of retention times with standards. Quantitative and qualitative analysis was conducted by comparison with *N*-nitrosamine standard solution chromatograms.

The following nitrosamine standards were applied to analyse meat:

No. N-7756 for dimethylnitrosamine (DMNA) determination and No. N-0756 for diethylnitrosamine (DENA), both produced by SIGMA, Chemical Comp., St. Louis, Mo. USA.

3. Results, discussion and conclusions

Experiments conducted to detect nitrosamine (DMNA and DENA) contamination in raw meat from different farm animals and in baked meat with functional additives produced quantitative data concerning these toxic compounds and the analysis of results allows for the following conclusions.

The experiment revealed the biggest nitrosamine concentrations in pork and beef and the smallest in veal and goat meat. Studies on nitrosamine contamination levels in meat muscles and animal products due to processing technologies are most important because of the hazards they pose for human health. Table 1 shows mean quantitative results of DMNA and DENA contamination in individual groups and variants, whereas the mean percentage variables of nitrosamine contamination in species within the group and experimental variants have been collected in Table 2 for comparison. The results of studies conducted on raw meat during this particular period of time might have been due to *inter alia* the animal being fed fodder mixtures polluted by large amounts of dioxins, which were commonly imported in big quantities to Poland from France and Germany or by chemically polluted grain forage brought from Hungary, Czech or Slovak Republic. The facts evoked a heated discussion in the mass media. Research demonstrated that serious contamination of the fodder mixture was caused by an addition of dirty, completely useless and polluted engine oil instead of animal fat, which normally is a component of such mixtures. As has been established, this highly illegal procedure lasted for many years. Statistical data show that between 1992 and 2002 the number of cancer cases increased twelvefold, which may have been caused by consumption of this seriously chemically polluted food.

Free amino acids susceptible to nitrosation, like proline, glycine, alanine, valine and biologically active ones, such as putrescine and cadaverine present in meat, also form under the influence of microorganisms and enzymes and in this way dimethylnitrosamines and diethylnitrosamines may form. The degree to which meat chemoproteins pass into nitroso derivatives depends on myoglobin and hemoglobin content in meat and on nitrite and nitrate concentrations. Nitrites present in animal body originate from various kinds of fodder and drinks, from enzymatic breakdown of L-arginine in tissues when over 10 μmol of nitrites per 1 kg of body weight is formed daily, and are due to nitrate reduction to nitrites by alimentary tract microorganisms. Nitrite uptake with food is harmful for human health because these compounds cause hemoglobin transition into methemyoglobin, which is unable for reversible oxygen binding. Reactions of nitrosation occur in the acid environment of the stomach and some bacteria develop at achlorhydria. Analysing the results obtained from the analyses of meat (at pH between 5.8 and 6.5) from slaughtered farm animals and the effect resulting from individual species, genders and environmental factors, by way of nutrition and technological processes applied in food industry, it may be seen that the quantitative and qualitative levels of nitrosamine contamination differ considerably. In order to characterise a basic chemical applied in food industry, the effect of sodium chloride on the assayed nitrosamine

Table 1
Mean quantitative nitrosamine content (μg/kg) in specifically diversified raw meat and meat with functional additives and resulting from baking ($n = 21$)

Species, kind and gender of slaughtered animals	Meat sample variant	Variant I – raw meat; Variant II – raw salted meat; Variant III – meat with sodium ascorbate; Variant IV – salted meat with sodium ascorbate						Variant I a – baked meat; Variant II b – baked salted meat; Variant III c – baked meat with sodium ascorbate; Variant IV d – baked salted meat with sodium ascorbate					
		DMNA			DENA			DMNA			DENA		
		Contents (μg/kg)		Variability coefficient	Contents (μg/kg)		Variability coefficient	Contents (μg/kg)		Variability coefficient	Contents (μg/kg)		Variability coefficient
		$\bar{x}$	±s	Z %	$\bar{x}$	±s	Z %	$\bar{x}$	±s	Z %	$\bar{x}$	±s	Z %
Young sows	I–I a	9.06	5.67	14.6	8.71	5.51	13.1	12.46	4.58	18.7	11.88	4.49	18.1
	II–II b	7.26	4.81	12.2	6.92	5.13	12.4	9.29	4.27	15.9	8.89	4.18	15.4
	III–III c	6.48	4.26	11.3	6.03	4.87	11.6	8.10	3.84	14.2	7.64	3.98	13.9
	IV–IV d	4.80	3.74	8.9	4.71	4.46	10.4	6.42	3.52	12.7	6.29	3.49	12.4
Sows	I–I a	10.44	4.98	10.9	10.11	5.59	11.8	14.62	5.68	11.8	15.26	5.79	12.7
	II–II b	8.67	4.47	9.8	8.49	4.88	10.9	11.92	4.99	10.4	12.12	5.17	11.6
	III–III c	7.68	4.05	8.6	7.55	4.29	10.2	10.21	4.37	9.6	10.04	4.62	10.8
	IV–IV d	6.72	3.72	7.2	6.66	3.86	9.8	9.30	3.84	8.9	9.19	4.14	10.1
Hogs	I–I a	9.69	5.86	13.8	9.48	5.86	12.9	12.98	5.22	19.8	13.80	5.33	19.2
	II–II b	8.13	4.94	12.9	8.04	4.93	12.1	10.73	4.88	16.5	10.85	4.98	16.1
	III–III c	7.47	4.19	11.4	7.38	4.48	11.2	9.56	4.37	13.9	9.45	4.51	13.6
	IV–IV d	6.09	3.65	10.3	6.02	4.16	10.4	8.25	3.80	12.7	8.16	4.18	12.2
Boars	I–I a	11.34	3.97	10.2	12.69	4.96	10.8	16.67	6.23	12.2	18.78	6.62	12.8
	II–II b	9.48	3.52	9.6	10.08	4.58	9.9	13.60	5.76	11.6	14.52	5.81	11.9
	III–III c	8.34	3.24	8.8	9.03	4.26	9.6	11.85	5.12	10.4	12.46	5.32	11.2
	IV–IV d	7.41	3.02	7.9	8.01	3.92	9.1	10.37	4.45	9.8	11.09	4.96	10.5
Heifers	I–I a	8.43	3.35	13.7	8.04	3.66	14.2	11.44	4.83	19.2	10.81	4.72	18.3
	II–II b	7.02	3.02	13.1	6.63	3.41	13.3	8.95	4.42	16.1	8.49	4.43	15.7
	III–III c	6.33	2.81	12.4	6.02	3.14	12.4	7.88	4.11	13.5	7.49	4.19	13.0
	IV–IV d	4.98	2.55	11.2	4.83	2.78	11.5	6.80	3.56	12.3	6.63	3.98	11.8
Cows	I–I a	8.13	4.37	16.3	8.89	4.28	15.8	10.33	5.42	19.2	11.38	5.62	19.9
	II–II b	6.90	4.06	14.1	6.75	4.03	13.6	8.66	5.17	16.3	8.99	5.28	16.8
	III–III c	6.04	3.75	12.9	6.13	3.74	12.4	7.40	4.91	13.8	7.98	5.02	14.4
	IV–IV d	4.68	3.42	11.6	5.35	3.39	11.2	6.43	4.42	12.6	6.79	4.53	13.3
Bullocks	I–I a	10.08	4.49	12.9	9.75	4.76	13.2	14.41	4.91	15.8	13.67	4.72	15.1
	II–II b	8.70	4.12	12.1	8.58	4.33	12.4	11.61	4.53	13.9	11.20	4.49	13.4
	III–III c	7.63	3.86	11.3	7.62	4.09	11.8	9.69	4.16	13.1	9.98	4.08	12.9
	IV–IV d	6.39	3.55	10.4	6.46	3.72	10.6	8.51	3.99	10.6	8.62	3.91	10.2
Bulls	I–I a	12.00	5.62	9.8	12.24	5.96	10.4	17.28	6.82	11.5	16.75	6.71	10.9
	II–II b	9.87	5.23	9.2	10.08	5.45	9.8	14.56	6.33	10.8	14.21	6.22	10.1
	III–III c	8.62	4.91	8.6	8.83	5.02	8.9	11.81	6.11	10.2	12.60	6.04	9.6
	IV–IV d	7.54	4.67	7.9	7.80	4.71	8.1	10.46	5.78	9.4	10.75	5.72	9.0
Calves	I–I a	2.94	2.54	17.6	2.85	2.82	16.9	3.85	2.73	13.8	3.56	2.68	13.6
	II–II b	2.41	2.19	14.3	2.39	2.39	13.9	2.89	2.51	12.1	2.80	2.48	12.0
	III–III c	2.11	2.03	12.9	2.10	2.16	12.2	2.59	2.29	11.3	2.55	2.25	11.1
	IV–IV d	1.68	1.92	11.4	1.66	2.03	11.1	2.10	2.11	10.4	2.08	2.07	10.2
Mares	I–I a	4.38	3.96	18.9	4.47	4.17	19.6	5.92	4.08	14.7	5.38	3.99	14.1
	II–II b	3.60	3.57	15.1	3.65	3.89	15.9	4.43	3.72	13.9	4.19	3.67	13.5
	III–III c	3.27	3.31	13.2	3.21	3.62	13.7	3.94	3.56	13.2	3.85	3.51	12.6
	IV–IV d	2.69	3.02	11.6	2.67	3.21	12.3	3.40	3.14	12.5	3.37	3.08	12.1
Geldings	I–I a	4.98	4.72	20.7	5.16	4.89	21.4	6.42	4.58	15.8	6.91	4.63	16.3
	II–II b	4.24	4.41	16.9	4.27	4.52	17.6	5.49	4.29	14.4	5.52	4.33	14.9
	III–III c	3.71	4.22	13.2	3.74	4.30	14.3	4.58	4.16	13.8	4.64	4.20	14.1
	IV–IV d	3.12	3.96	10.8	3.15	4.05	11.7	3.95	4.02	13.1	3.99	4.07	13.5
Ewes	I–I a	3.33	3.66	16.9	3.21	3.52	16.1	4.33	3.46	13.8	4.01	3.52	13.2
	II–II b	2.85	3.39	13.8	2.79	3.31	13.3	3.65	3.27	13.1	3.56	3.32	12.7
	III–III c	2.66	3.16	12.7	2.61	2.98	12.3	3.33	3.13	12.4	3.21	3.17	12.0
	IV–IV d	2.30	3.02	11.4	2.24	2.76	11.1	2.91	3.04	11.6	2.84	3.10	11.1

Table 1 (*continued*)

Species, kind and gender of slaughtered animals	Meat sample variant	Variant I – raw meat						Variant I a – baked meat					
		Variant II – raw salted meat						Variant II b – baked salted meat					
		Variant III – meat with sodium ascorbate						Variant III c – baked meat with sodium ascorbate					
		Variant IV – salted meat with sodium ascorbate						Variant IV d – baked salted meat with sodium ascorbate					
		DMNA			DENA			DMNA			DENA		
		Contents (μg/kg)		Variability coefficient	Contents (μg/kg)		Variability coefficient	Contents (μg/kg)		Variability coefficient	Contents (μg/kg)		Variability coefficient
		$\bar{x}$	±s	*Z* %	$\bar{x}$	±s	*Z* %	$\bar{x}$	±s	*Z* %	$\bar{x}$	±s	*Z* %
Wethers	I–I a	3.75	3.97	17.5	3.63	3.79	17.4	4.95	3.91	14.6	4.33	3.78	15.3
	II–II b	3.15	3.68	14.8	3.09	3.57	14.2	4.43	3.62	13.9	4.02	3.56	13.3
	III–III c	2.85	3.31	12.6	2.81	3.26	12.2	3.63	3.47	13.2	3.51	3.38	12.7
	IV–IV d	2.44	3.12	11.5	2.38	3.03	10.9	3.02	3.28	12.5	3.03	3.31	11.6
Rams	I–I a	3.99	5.35	19.8	4.32	5.06	21.7	5.91	4.72	16.3	6.65	4.89	17.1
	II–II b	3.21	5.08	16.9	3.51	4.69	17.4	4.50	4.39	14.9	4.91	4.46	15.3
	III–III c	2.79	4.79	13.8	2.94	4.36	14.3	3.76	4.17	13.7	3.97	4.25	14.1
	IV–IV d	2.62	4.42	11.9	2.70	4.19	11.2	3.30	4.02	13.1	3.38	4.14	13.4
Goats	I–I a	2.34	3.27	18.6	2.19	3.22	17.9	2.95	3.04	15.2	2.78	2.96	14.9
	II–II b	1.89	3.05	15.3	1.81	3.01	14.8	2.33	2.91	14.0	2.21	2.78	13.8
	III–III c	1.57	2.91	12.9	1.53	2.84	12.4	2.00	2.73	13.3	1.94	2.65	13.1
	IV–IV d	1.33	2.68	10.7	1.29	2.59	10.2	1.73	2.65	12.4	1.77	2.58	12.2

activity was studied. While examining the needs and possibilities of lowering sodium content in food products, one should consider the fact that NaCl plays an important role in the technology of production and development of both its quality and organoleptic features. A decreased table salt addition to meat products means possible technological problems and worse product quality. Some of the sodium chloride properties inhibit microbe development and in this way affect microbiological durability of meat products. Excessive consumption of table salt, which may cause hypertension, is a problem for communities in many countries, including Poland. The phenomenon arouses wide-ranging interest among medical doctors, nutrition specialists, consumers and food processors. A 2% sodium chloride addition to meat in experimental variants caused a decrease in nitrosamine (DENA and DMNA) contamination level in all groups and kinds as compared with raw unsalted meat. A 0.03% addition of sodium ascorbate to experimental meat variants caused a decline in DMNA and DENA nitrosamine contamination level in all groups and kinds of meat as compared with the raw meat. A 2% sodium chloride and 0.03% sodium ascorbate addition to experimental meat variants caused the highest decrease in DMNA and DENA nitrosamine contamination in all groups and kinds of meat in relation to raw meat, raw salted meat and raw meat with added sodium ascorbate. The results of the research demonstrate an apparently destructive effect of especially sodium ascorbate and lesser influence of sodium chloride on DMNA and DENA nitrosamines. On the other hand, the nitrosamine concentrations assayed in the meat samples of experimental variants subjected to baking process increased proportionally as evidenced by the results given in Tables 1 and 2. The research revealed that, considering technological properties of meat, decreasing table salt addition below 2% or increasing it is purposeless because of technological properties of meat. The above mentioned attitude was confirmed by toxicological research conducted at numerous research centres under the auspices of the World Health Organisation. Occurrence of precursors and nitrosamine formation in various animal and plant food products and their carcinogenic effect are strictly connected ecologically and toxicologically with the environment. Breathing in air containing among other things NO, NO_2, N_2O_3, nitroso halides and many other nitrosating agents as well as ingesting plant precursors with drinking water from rivers, ponds and other water reservoirs polluted by animals and sewage expresses the nitrosamine balance found in the studies. Nitrosamines may have highly diversified construction revealing the properties of solid substances, liquids, sometimes oily, and some are volatile in water vapour. Nitrosamines as compounds with specific construction present in food and fodder are not only noxious due to their toxicity as strong poisons, but they also have carcinogenic effect. Isolated nitrosamines originate from amines forming during food decay and protein breakdown in the presence of a nitrite. These amines are also present in plant environment. It is very important to avoid food rich in nitrates and nitrites or contaminated with pesticides. The compounds are frequent components of food products consumed by people and also by animals in the form of fodder. Further intensively developed research on nitrosamines should aim to determine the dependencies and their effect on the occurrence of many disease entities. According to the report of the US Academy of Sciences, further endeavours focussed on limiting the hazards connected with the mode of nutrition should involve studies on physiological, especially carcinogenic effect of nitroso-compounds.

Table 2
Mean percentage nitrosamine concentrations (μg/kg) in raw meat from various species of animals, in meat with functional additives and in meat as a result of baking ($n = 21$)

Species, kind and gender of animals	Variant I raw meat [100%]		Variant I a baked meat [%]		Variant II raw salted meat [%]		Variant II b baked salted meat [%]		Variant III meat with sodium ascorbate [%]		Variant III c baked meat with sodium ascorbate [%]		Variant IV salted meat with sodium ascorbate [%]		Variant IV d salted baked meat with sodium ascorbate [%]	
	DMNA	DENA	DMNA	DENA	DMNA	DENA	DMNA	DENA	DMNA	DENA	DMNA	DENA	DMNA	DENA	DMNA	DENA
	Contents (μg/kg)		Contents (μg/kg)		Contents (μg/kg)		Contents (μg/kg)		Contents (μg/kg)		Contents (μg/kg)		Contents (μg/kg)		Contents (μg/kg)	
	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$
Young sows	9.06	8.71	137.52	136.39	80.13	79.45	102.54	102.07	71.52	69.23	89.40	87.71	52.98	54.08	70.86	72.22
Sows	10.44	10.11	140.04	150.94	83.05	83.98	114.18	119.88	73.56	74.68	97.80	99.31	64.37	65.87	89.08	90.90
Hogs	9.69	9.48	133.95	145.57	83.90	84.81	110.73	114.45	77.09	77.85	98.66	99.68	62.85	63.50	85.14	86.08
Boars	11.34	12.69	147.00	147.99	83.60	79.43	119.93	114.42	73.54	71.16	104.50	98.19	65.34	63.12	91.45	87.39
Heifers	8.43	8.04	135.71	134.45	83.27	82.46	106.17	105.60	75.09	74.88	93.48	93.16	57.07	60.07	80.66	82.46
Cows	8.13	8.89	127.06	128.01	84.87	75.93	106.52	101.12	74.29	68.95	91.02	89.76	57.56	60.18	79.09	76.38
Bullocks	10.08	9.75	142.96	140.20	86.31	88.00	115.18	114.87	75.69	78.15	96.13	102.36	63.39	66.26	84.42	88.41
Bulls	12.00	12.24	144.00	136.85	82.25	82.35	121.33	116.09	71.83	72.14	98.42	102.94	62.83	63.72	87.17	87.83
Calves	2.94	2.85	130.95	124.91	81.97	83.86	98.30	98.25	71.77	73.68	88.09	89.47	57.14	58.25	71.43	72.98
Mares	4.38	4.47	135.16	120.36	82.19	81.65	101.14	93.74	74.66	71.81	89.95	86.13	61.41	59.73	77.26	75.39
Geldings	4.98	5.16	128.92	133.91	85.14	82.75	110.24	106.98	74.50	72.48	91.97	89.92	62.65	61.05	79.32	77.33
Ewes	3.33	3.21	130.03	124.92	85.59	86.92	109.61	110.90	79.88	81.31	100.00	100.00	69.07	69.78	87.39	88.47
Wethers	3.75	3.63	132.00	119.28	84.00	85.12	118.13	110.74	76.00	77.41	96.80	96.69	65.07	65.56	80.53	83.47
Rams	3.99	4.32	148.12	153.93	80.45	81.25	112.78	113.66	69.92	68.05	94.24	91.90	65.66	62.50	82.71	78.24
Goats	2.34	2.19	126.07	126.94	80.77	82.65	99.57	100.91	67.09	69.86	85.47	88.58	56.84	58.90	73.93	80.82

The intensity of an increase in nitrosamine concentrations greatly depends on nitrogen and phosphorus presence. Improvements of food technology should involve among others the greatest possible minimisation of amine and *N*-nitroso compound formation, e.g. by inhibitors or microorganisms, precise determination of critical nitrosamine concentrations in food, maintaining of clean environment, modernisation of technologies and improvement of food hygiene. It may be assumed that no research on the formation and occurrence of nitrosamines has been conducted so far.

Few studies on carcinogenic nitrosamine occurrence in farm animal raw meat conducted so far by the Author himself and other foreign researchers revealed nitrosamine concentrations 5–15 μg/kg DMNA and 5–20 μg/kg DENA, which is certainly not without importance for potential consumer health. The available information about nitro compounds and nitrosamines and recent results of examination of raw meat and meat half-product with functional additives commonly used in industry and processed using diversified technologies and model, reveal considerable contamination with *N*-nitrosamines of the tested samples (Rywotycki, 1997, 1998a, 1998b, 1998c, 1998d, 1998e, 1999a, 1999b, 2000, 2001, 2002a, 2002b, 2003a, 2003b). The increase in forming nitrosamines depends on the time and meat frying temperature as they form adducts with unsaturated lipid groups. The latter decompose at elevated temperature and release nitrogen oxides, which nitrosate free amines present in the environment (Lu, Conboy, & Hotchkiss, 1988; Scanlan & Ryes, 1985).

Thus, a checkup should be repeated to determine whether new factors, methods or kinds of contamination caused the presence of the studied nitroso-compounds. The results confirm a necessity to conduct both environmental and nutritional studies to explain the causes of nitrosamine formation in farm animal and wild game meat depending on fodder chemical composition, its origin and production. An increasing interest in nitrosamine occurrence in meat and meat products has been visible recently in the world literature which is primarily due to increasing cancer incidence rate. Thus, it may be assumed that both in human and animal food chain a contact with degraded (chemically polluted) natural environment of humans and animals (soil, water, food, forage, air and wrong technologies) may cause various diseases and loss of health or life.

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