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NITROSAMINES AND THEIR PRECURSORS IN SOME KAZAKH FOODSTUFFS

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INTRODUCTION

Food as a complicated pharmacological complex may be the carrier of procarcinogens or the source of potential precursors for their formation. In order to protect the 'chemical purity' of an organism, it is necessary to direct extensive efforts towards the removal of such substances from food. This applies particularly to *N*-nitroso compounds, many of which are potent procarcinogens for various animal species (Druckrey et al., 1967; Magee et al., 1976) and presumably for man (Bogovsky, 1981), since their presence has been detected in some foods (Scanlan, 1975).

The high incidence of oesophageal cancer among both men and women of the Kazakh people (more than 70% of all registered cases of oesophageal cancer in Kazakhstan) has been reported to be associated with dietary peculiarities (Kolicheva et al., 1970). Although the modern diet of the native kazakh population is characterized by an increasing tendency to include some half-finished and canned foods, a traditional assortment of mealy, meaty and milky dishes is still widely consumed, particularly by the rural population. We thus decided to examine typical kazakh foods for contamination with nitrosamines, nitrates and nitrites. The present paper describes the first results obtained.

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[1] This paper is included in the proceedings although the authors were unable to be present at the meeting.

MATERIALS AND METHODS

Gas Chromotograph (GC) - Thermal Energy Analyzer (TEA)

For GC-TEA analysis, a 'Tracor-560' temperature-programmable gas chromatograph was used, with a 1.83 m × 2 mm i.d. coiled glass column, packed with 10% Carbowax 20. M and 2% KOH on 80/100 mesh Chromosorb W-AW. The column temperature was 150°C and the argon carrier gas flow rate was 70 ml/min. A TEA-502 LC was used as a specific detector for *N*-nitroso compounds, with a furnace temperature of 500°C and a cold-trap temperature of -150°C (isopentane + liquid nitrogen).

High-pressure liquid chromotograph (HPLC)

A high-pressure pump (Altex, Model 100), an HPLC sample injection valve (Rheodyne, Model 7120) and a Lichrosorb Si-60, 5μ, column (4.6 mm × 250 mm) were interfaced directly to the TEA-502 LC for HPLC-TEA analysis. A mixture of 1% isobutanol, 4% acetone and 95% isooctane was used at a flow rate of 2 ml/min.

A Hewlett-Packard integrator, HP 3380-A, was used to record chromatograms and for the assessment of TEA-response.

Glassware

The glassware used in these investigations has been described by Havery et al. (1978).

Reagents

All reagents used for HPLC were of high purity or 'HPLC grade'. Methylene chloride was purified and tested prior to use, to ensure the absence of interfering peaks. Standards of 20 *N*-nitroso compounds were purchased from the USA.

Procedure

Several modifications of the mineral oil distillation technique reported by Fine et al. (1975) have been used routinely in many laboratories equipped with TEA. Wolf & Aidjanov (1979) have slightly modified the original procedure to make this method more applicable to a wide range of different commodities and to take into account the opinion of Krull et al. (1978) concerning the possibility of false-positive and false-negative findings. The modified procedure is as follows:

Accurately weighed 25 g samples of the well-minced solid or semi-solid food were transfered to a 500 ml round-bottomed flask, to which was added 50 mg of ascorbic acid, 4 ml of 0.1 mol/l sodium hydroxide, 50 ml of mineral (paraffin or castor) oil and, as an internal standard, *N*-nitrosodipropylamine (NDPA). The flask was attached to a vacuum pump

and the contents were heated slowly, avoiding 'bumping', until the temperature reached 110°C (after approximately 50 min). The distillate was collected in a glass trap at -150°C. The melted distillate was placed in a separating funnel and, after mixing with 0.1 mol/l hydrochloric acid (4 ml), was extracted with three equal volumes of dichlormethane (3 × 10 ml). The organic phase was dried (anhydrous sodium sulfate) and con-centrated in a Kuderna-Danish evaporator to 2 ml, then concentrated (after cooling) to 0.5 ml under a gentle stream of nitrogen. This procedure yielded reproducible results, the recoveries being close to 95-100% for the most volatile nitrosamines.

The levels of nitrate were determined by the method of Mori et al. (1972) and the Modified AOAC method was used for the determination of nitrite concentrations (Fiddler, 1977).

Collection of food samples

Food samples were collected from the Ghurjev and the Alma-Ata regions of Kazakhstan, which show considerable contrast in incidence of oesophageal cancer. In most cases, the samples were frozen in dry-ice, shipped by air and stored frozen until analysis. Some samples from the outskirts of Alma-Ata were analysed immediately upon arrival in the laboratory. Meat samples were collected three times a year: at the end of autumn, when meat and meaty products ('kazi' and 'chuzhuck') were usually laid in store for the winter; in spring, when the supply of these products was exhausted, and in summer when fresh meat was dried in the sun.

Local features of pickling and storing meat products

Fresh slices of beef or horse-flesh were salted (the sodium chloride content ranged from 3 to 8%) and left overnight. The next day, the slices were hung in the sun to dry, then were laid away for long-term storage at below 10°C. Lower levels of sodium chloride (no more than 3%) are used for salting fresh meat in summer.

Commercial 'Kazi' is a smoked horse sausage, canned with added sodium nitrite. A domestic kind of 'Kazi' is made by packing the salt-dried meat into horse gut.

As a rule, commercial and home-made varieties of 'chuzhuck' (fermented sausage) consist of minced horse-meat, sodium chloride, sodium nitrite (used only in the industrial product), ground black pepper and garlic. However, some housewives add other ingredients, such as uncooked rice, to the above components.

RESULTS AND DISCUSSION

The levels of volatile nitrosamines in the most typical kazakh foodstuffs are presented in Tables 1 and 2.

Table 1. Occurrence of NDMA in foodstuffs

Commodity	No. of samples	No. of positive samples 0.1-0.9	1-5	> 5 (µg/kg)	
Fresh meat	11	0	0	0	
Frozen meat	7	0	0	0	
Salted, sun-dried meat					
I[a]	10	2	0	0	
II[b]	8	1	1	0	
Salted air-dried meat					
I[a]	14	2	0	1	(8)[c]
II[b]	9	0	0	2	(9,54)
Smoke-dried meat	6	0	2	0	
Home-made 'Kazi'	9	2	0	0	
Commercial 'Kazi'	5	0	1	1	(6)
Home-made 'Chuzhuck'	7	2	0	0	
Commercial 'Chuzhuck'	5	0	1	0	
'Kurt' & 'Irimshick'	20	3	0	0	
'Koumiss' & 'shubut'	12	0	0	0	
Local bread	16	0	0	0	
Rock & table salts	10	0	0	0	
Rice	10	0	0	0	
Raw vegetables	38	0	0	0	
Daily rations	56	3	2	0	

[a] Stored 2 weeks at below 10°C

[b] Stored 8 weeks at below 10°C

[c] The NDMA content is shown in parentheses (µg/kg) for those samples containing > 5 µg/kg.

Table 2. Occurrence of volatile heterocyclic nitrosamines in foods.

Commodity	No. of samples	No. of positive samples 0.1-0.0	1-5	> 5 (μg/kg)	
Fresh meat	11	0	0	0	
Frozen meat	7	0	0	0	
Salted sun-dried meat					
I[a]	10	0	0	0	
II[b]	8	0	0	0	
Salted air-dried meat					
I[a]	14	1	0	0	
II[b]	9	1	0	0	
Smoke-dried meat	6	0	0	1	(5.6)[c]
Home-made 'Kazi'	9	1	0	0	
Commercial 'Kazi'	5	0	0	1	(12)
Home-made 'Chuzhuck'	7	1	0	1	(7)
Commercial 'Chuzhuck'	5	0	1	0	
'Kurt' & 'irimshick'	20	0	0	0	
'Koumiss' & 'shubut'	12	0	0	0	
Local bread	16	0	0	0	
Rock & table salts	10	0	0	0	
Rice	10	0	0	0	
Raw vegetables	38	0	0	0	
Daily rations	56	2	1	0	

[a] Stored 2 weeks at below 10°C

[b] Stored 8 weeks at below 10°C

[c] The nitrosamine content is shown in parentheses (μg/kg) for those samples containing > 5 μg/kg.

No nitrosamines were detected in 11 samples of fresh meat and 7 samples of frozen meat.

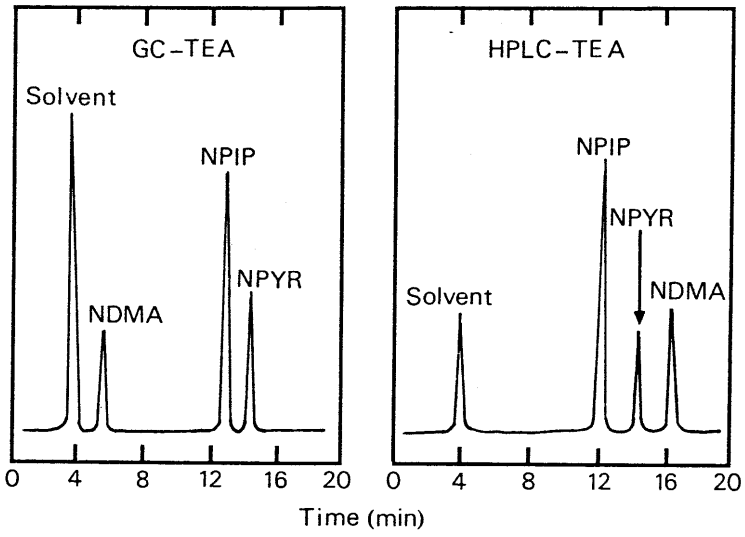
Two of 10 samples of salted, sun-dried meat contained *N*-nitrosodimethylamine (NDMA) (0.1 and 0.4 µg/kg). Greater amounts of NDMA (0.6 and 4.2 µg/kg) were found in 2 of 8 samples of the same kind of meat which had been stored for a longer time (8 weeks) at about 10°C.

In contrast with salted, sun-dried meat, the content of NDMA was higher in those samples of salted meat which were air-dried without exposure to the sun. Three of 5 positive samples of salted air-dried meat contained NDMA in the 8-54 µg/kg range and, in addition, the heterocyclic nitrosamines *N*-nitrosopyrrolidine (NPYR) and *N*-nitrosopiperidine (NPIP) (each at 0.8 µg/kg) were found in these samples. The chromatograms shown in Fig. 1 illustrate the simultaneous presence of NDMA, NPYR and NPIP in a sample of 'chuzhuck'.

FIG. 1. GLC-TEA AND HPLC-TEA CHROMATOGRAMS FOR A 10-µl DCM EXTRACT OF HOME-MADE 'CHUZHUCK'.

Left: GC-TEA chromatogram. Glass column (1.83 m × 2 mm i.d.) packed with 10% Carbowax 20 M and 2% KOH on chromosorb W-AW, 80-100 mesh. Carrier gas was argon at flow rate of 70 ml/min. Column temperature was 150°C.

Right: HPLC-TEA chromatogram. 5µ Lichrosorb Si-60 column; solvent, 1% isobutanol, 4% acetone and 95% isooctane.



In 6 samples of smoke-dried meat, two contained NDMA up to 4 µg/kg and one sample contained 5.6 µg/kg NPYR. NDMA and NPYR were detected in samples of home-made 'kazi' in amounts not exceeding 0.9 µg/kg, but the levels of NDMA in the commercial product were in the range of 4-6 µg/kg and the NPYR level was 12 µg/kg. NDMA (up to 1 µg/kg) and the NPIP (0.8 and 7 µg/kg) were detected in 2 of 7 samples of home-made 'Chuzhuck'. The same amount (4 µg/kg) of both NDMA and NPIP was found in one sample of commercial 'chuzhuck'.

No nitrosamines were found in samples of 'koumiss' (fermented mare's milk) and 'shubut' (fermented camel's milk). Three of 20 samples of sour-mild products ('kurt' and 'irimshick'), classifified according to cheese type, contained NDMA in concentrations of 0.1-0.7 µg/kg. Neither dialkyl nor heterocyclic nitrosamines were detected in 16 samples of local bread and in 38 samples of different local raw vegetables.

The present findings show that nitrosamines in the daily diet arise predominatly from the consumption of foods containing smoke-dried or salted, air-dried meat. In addition, certain differences of nitrosamine content depended not only on the composition of the food, but also on the manner of preparation. Thus, if the daily rations contained fried meat, the content of NPYR was somewhat higher, although this effect was far less pronounced than that reported by Pensabene et al. (1974).

Calculations based on the average *per-capita* consumption for some meat products and on the direct analysis of daily rations both indicate that the daily intake of volatile nitrosamines for male persons in early spring was in the range of 0.1-1 µg for NDMA and 0.1-0.35 µg for NPYR and NPIP. In late autumn, the corresponding intakes were in the range of 0.1-0.3 µg and 0-0.1 µg, respectively.

We have not yet sufficient data concerning regional variations of the nitrosamine content of foods (Tables 1, 2 and 3 show the combined results for the two regions).

Preliminary results from this laboratory[1] have shown that meat proteins irreversibly dissociate to polypeptides and low molecular weight nitrogenous compounds, in particular amino acids and amines, and that the content of some vitamins is also reduced (especially ascorbic acid, which is an effective inhibitor of the nitrosation reaction) by canning and by prolonged storage of meat at approximately room temperature instead of refrigeration. It is likely that under these circumstances the necessary conditions were created not only for exogenous but also for endogenous formation of NPYR and NPIP.

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[1] Aidjanov et al., unpublished results.

Although the concentrations of nitrite found in salt-preserved meat products (Table 3) are low (the permissible level of nitrite in meat products is 40 μg/kg in the USSR), preventative measures should be taken without delay. These measures should concern the technology of meat-product preparation and the conditions of food storage.

Table 3. Nitrite and nitrate contents of foods[a].

Product	No. of samples	NO_2^- (mg/kg)	NO_3 (mg/kg)
Fresh meat	7	0.1 ± 0.02	10.2 ± 2.4
Salt-drying meat	10	0.2 ± 0.05	20.8 ± 3.2
Smoke-drying meat	6	1.2 ± 0.12	18.4 ± 2.5
'Kazi' of shops	6	4.1 ± 0.36	60.5 ± 9.1
Home-made 'Chuzhuck'	5	0.4 ± 0.06	41.8 ± 7.9
'Kurt' & 'irimshick'	10	0.2 ± 0.07	8.5 ± 1.5
'Koumiss'	5	0	1.4 ± 0.8
Wheat bread	12	0.1 ± 0.03	19.0 ± 2.0
Potato	8		80.0 ± 10.5
Onion	14	0.3 ± 0.04	995.5 ± 75.0
Rock salt	5	0.2 ± 0.45	92.4 ± 51.6
Table salt	5	0.1 ± 0.07	54.5 ± 11.8

[a] Averages ± standard deviations.

SUMMARY

Numerous samples of various foods, such as salt-dried and smoke-dried meats, fermented sausages, sour-milk products, fermented mare's and camel's milk, raw vegetables, rice, rock and table salts, local bread and daily food rations were analysed by GC-TEA and HPLC-TEA for the presence of volatile nitrosamines.

Apart from the observation of NDMA (up to 0.7 μg/kg) in three samples of Kazakh cheeses, the volatile nitrosamines were detected only in some meat and meat products. Salted air-dried meat containined NDMA in amounts up to 54 μg/kg. NPYR (12 μg/kg) was found in one sample of nitrite-cured sausage. The highest content of NPIP (7 μg/kg) was

detected in fermented, home-made sausage. It is clear that the only significant contribution of these dialkyl and heterocyclic nitrosamines to the diet is from stored, salt-dried meat and nitrite-cured meat products.

Analyses of nitrate and nitrite in various local products are reported.

The validity of our assumption that the high incidence of oesophageal cancer can be lowered by realistic improvements in the processing and storage of meat and meat products requires further evaluation.

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