

NITROSAMINES IN SOME EGYPTIAN CURED MEAT PRODUCTS

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INTRODUCTION

Sodium nitrite has been used for the curing of meat products from time immemorial. In addition to its microbiological stability, in particular inhibiting the growth of *Clostridium botulinum*. Nitrite has been used to provide a desirable characteristic color and flavor in the meat product (KLOMOTO et al., 1976).

Recently, however, it has been found that nitrite can react with secondary and tertiary amines to form nitrosamines,

a class of compounds found to produce tumors in a number of animal species (MAGEE and BARNES, 1967). The quantity of amines does not play an important role in the reaction but the concentration of nitrite has a major role in the formation of nitrosamines (MOHLER et al., 1972).

Reduction, or elimination, of the nitrite used in curing could reduce or prevent formation of nitrosamines.

With the decrease of the basicity of the amine and increase of the alkyl chain length the carcinogenic effect of the nitrosamines is decreased. N-nitrosodimethylamine (NDMA) and are therefore the most dangerous carcinogens in comparison to all other nitrosamines (MOHLER et al. 1972).

It is not yet known what are the toxic or carcinogenic doses of nitrosamines for man. The "permitted dose" of 5-10 µg nitrosamines/kg food is under discussion (LENGES et al., 1974).

NDMA, NDEA and N-nitrosopiperidine (NPIP) have been found in various cured meat products in concentrations mostly below 10 µg/kg in average (SEN et al. 1973a). N-nitrosopyrrolidine (NPYR) has been detected in fried and cooked bacon (GRAY and COLINS 1978). Higher concentrations (50-300 µg/kg) have been detected in some products, especially those prepared with the addition of spice-nitrite premixes (SEN and MCKINLEY, 1974 and WASSERMAN, 1978). SEN et al. (1976), working with model systems and cured meat products have demonstrated that ascorbic acid as well as ascorbyl palmitate, as reducing agents, can block the N-nitrosation reaction by reacting with nitrite.

The present investigation was conducted to determine the nitrosamine content, and other chemical properties, of three Egyptian meat products, namely, beef sausage, luncheon meat and bastirma. The effect of adding ascorbyl palmitate on the nitrosamine content of bastirma was also studied.

Experimental

Materials

9 samples representing 3 different cured meat products were purchased from the local markets of Zagazig and Cairo. Samples of fresh sausage, luncheon meat and bastirma were obtained from different shops.

Beef used for laboratory-prepared bastirma was obtained from the longissimus dorsi muscle of 2.5 years old males within 3 hours of slaughter.

Bastirma preparation: Taha (1966) technique was followed for the manufacture of Bastirma. The dry curing was carried out at room temperature for 24 hours using 10% salt (sodium chloride), containing 0% or 0.5% sodium nitrate + sodium nitrite (2:1). Ascorbyl palmitate was added to meat at the levels of 500 and 1000 ppm. Pressing lasted 18 hours and hanging

for air-drying before coating 10 hours. Applied weights during pressing were one-half the weight of the meat. Bastirma was coated with a paste made from: 5kg finely ground fenugreek + 0.50 kg ground garlic + 0.25 kg paprika + 0.25 kg cumin for each 100 kg of dried meat.

Methods

Moisture, fat (ether extract), protein ($N \times 6.25$), ash, and salt (NaCl) were determined according to the methods described by the AOAC (1970). The method of GRAU and MIRNA (1957) was used for the determination of nitrite and nitrate.

The nitrosamine content was determined according to the method described by MIRNA (1980) as follows: the sample of ground meat (25 g) was thoroughly mixed with 25 ml glycerin + 15 ml H_2O + 1 ml 40% NaOH + 0.2 ml (0.2 μg) N-nitrosodi-n-propylamine solution (as a standard) and frozen using liquid nitrogen.

The sample was then distilled at 60°C under vacuum. Three drops of 40% NaOH and 3g NaCl were then added and the distillate was used for extraction of nitrosamines with 10 ml dichloromethane (DCM) 5 times. The DCM-phase was washed with distilled H_2O , then dried through a Na_2SO_4 column and concentrated at 55°C to a volume of 1 ml.

Separation and detection of nitrosamines was carried out using gas-liquid chromatograph with a stainless steel column (3 m x 1/8 in. o.d.) of 12% carbowax 20M on 100-200 mesh of gaschrom Q and gas flow 30 ml/min helium, with a thermal energy analyzer (TEA) of Thermo Electron USA. model 502.

Results and discussion

Nine commercial samples of cured meat were purchased and analyzed for their moisture, protein, fat, ash, NaCl, nitrate, nitrite and nitrosamine content (Table 1). The gross chemical composition of the samples was generally within the legal amount permitted by the Egyptian standards for cured meats (Table 1). Exceptions were sample 7 for moisture content, sample 8 for fat content and sample 9 for moisture and fat content. The legal amounts of residual nitrite and nitrate (determined and calculated as nitrite) in sausage and luncheon (125 ppm) and in bastirma (300 ppm) were exceeded in most of the samples, with the exception of samples No. 6, 7 and 9. Samples 1-5 and 8 showed about 32-287% more total $NaNO + KNO$, (determined as $NaNO_2$) than the standards. The increase above the allowed dose was marked, particularly for samples 2, 3 and 5 (sausage samples).

NDMA, NPIP and NPYR were found in most of the cured meats examined and NDEA only in sample No. 1. Only one sample contained nitrosamines in concentration greater than 10 $\mu g/kg$ (sample of sausage No. 1). In this sample total nitrosamines were higher than the permitted dose (10 $\mu g/kg$) by about 180%; NDMA content alone was greater than allowed by about 40%. All other samples showed a nitrosamine content lower than 4 $\mu g/kg$. No association between residual nitrite content and nitrosamine concentration was found.

The Egyptian sausage and luncheon meat were characterized by the presence of NPIP and NPYR, which were found only in some cured meat products. The occurrence of NPIP may be due

Table (1) Chemical composition and nitrosamine content of some Egyptian cured meat products

ITEMS	Sausage					Egg.St.	Luncheon		Egg.St.	Bastirma		
	1	2	3	4	5		6	7		8	9	Egg.St.
Moisture%	43.1	47.8	45.3	51.1	49.8	<60	49.6	57.8	<55	45.6	48.4	< 45
Protein %	23.9	21.8	19.0	17.9	17.8	>15	22.2	20.0	>15	37.9	34.8	NL
Fat %	26.7	25.8	29.9	27.5	27.5	<30	19.5	16.5	<20	7.4	8.0	> 5
Carbohydrate%	2.8	0.8	1.0	0.5	0.3	NL	4.6	1.3	5	0.6	0.8	NL
Ash %	3.5	3.8	4.8	3.0	4.4	< 5	4.1	4.9	NL	8.5	8.4	NL
NaCl%	2.3	1.7	2.7	1.4	2.7	< 3	2.9	2.6	<3	7.1	7.0	< 8
NaNO ₂ mg/kg	78	164	215	123	203	<125	14	10	<125	119	32	<300
KNO ₃ mg/kg	132	288	269	142	263		44	62		277	86	
NDMA µg/kg	14.2	1.2	1.4	0.2	0.8	NL	1.6	1.4	NL	1.2	2.3	NL
NDEA µg/kg	0.8	--	--	--	--	NL	--	--	NL	--	--	NL
NPIP µg/kg	8.9	0.5	0.5	5.0	1.0	NL	0.4	1.5	NL	--	--	NL
NPYR µg/kg	4.5	0.8	0.5	0.5	0.5	NL	1.0	0.5	NL	0.7	--	NL

Egg. ST. = Egyptian standard

NL = No limit

TABLE (2) Effect of ascorbyl palmitate on the formation of nitrosamine in bastirma.

Item	Samples	1	2	3	4
Moisture %		46.7	45.9	47.5	48.5
Protein %		35.5	38.8	36.6	35.5
Fat %		7.8	5.7	6.0	7.0
Carbohydrate %		0.8	0.8	0.6	0.5
Ash %		8.9	8.8	9.8	8.5
NaCl%		7.8	7.5	7.8	7.0
NaNO ₂ mg/kg		4	8	8	3
KNO ₃ mg/kg		105	647	471	109
NDMA µg/kg		0.2	0.5	0.3	0.4
NDEA µg/kg		--	--	--	--
NPIP µg/kg		0.5	0.5	0.5	0.1
NPYR µg/kg		0.5	0.5	0.6	0.2

Sample 1= Bastirma produced without using nitrite or nitrate.

Sample 2= Bastirma produced using nitrite and nitrate.

Sample 3= Bastirma produced using nitrite and nitrate + ascorbyl palmitate 500 ppm.

Sample 4= Bastirma produced using nitrite and nitrate + ascorbyl palmitate 1000 ppm.

to the use of a relatively large amount of spices especially black pepper, in the preparation of Egyptian cured meat products. Piperidine, the precursor of NPIP, is a component of black pepper and other spices and can also be formed in meat by microorganisms from the amino acid lysine (SEN et al. 1973b, SEN and MCKINLEY 1974, WASSERMAN 1978 and SEN et al. 1979). NPIP was found in spice-cure mixture at levels ranging from 50 to 2000 µg/kg. Federal laws in United States, Canada and West Germany now prohibit the marketing of meat spicecure mixtures (HAVERY et al. 1976). The incorporation of sodium ascorbate does not offer protection against nitrosamine formation in permixes. Storage practice was changed and curing agents and spices are now stored separately prior to use (GOUGH and GOODHEAD, 1975).

NPYR has been detected in fried bacon, but not in raw bacon or most cured meat products. The presence of NPYR in fried bacon and the rendered fat has aroused considerable interest in its mode of formation. Consequently, various precursors have been suggested, including pyrrolidine, proline, pyrimidine and putrescine (BILIS et al. 1973, GRAY and COLLINS 1978). Fried bacon appears to be the only cured meat product that contains significant levels (an average up to 20 or 30 µg/kg) of NDMA and NPYR (SEN et al; 1979). According to GRAY et al. (1978) up to 80% of the NDMA and NDEA produced during the frying process was lost in the vapor.

Data in Table (2) indicate that ascorbyl palmitate was effective in reducing the formation of nitrosamines at the 1000 ppm level (sample 4). Studies by FIDDLER et al. (1974) have

shown that the addition of 550-5500 ppm sodium ascorbate markedly reduced the formation of NDMA. Similar studies (SEN et al. 1976) on bacon indicated that 1000 ppm of sodium ascorbate or ascorbyl palmitate considerably reduced the formation of NPYR during cooking. Frying bastirma sample indicated that ascorbyl palmitate at the two levels was effective in reducing the formation of new NDMA, NPIP and NPYR (unpublished data from this laboratory).

Moreover, the possibility exists that the addition of excess ascorbate at the initial stage of the curing process may destroy the added nitrite and reduce its inhibitory effect against *C. botulinum*. It would be more desirable to add the ascorbates or other nitrite-scavenging food additives

at the end of the curing process, because at this time the cured meat would already contain the botulinum-inhibitory factor which is believed to be formed (from nitrite) during the curing process. Recent studies have indicated that the initial level of nitrite used, and not the residual level, is the important factor (SEN et al. 1976).

From Table (2) it is evident that samples 2 and 3 had markedly higher nitrite + nitrate contents than the standard (> 300 mg/kg), while sample 4 did not, indicating that the addition of 1000 ppm ascorbyl palmitate is the only way of producing bastirma, unless added nitrite contents are reduced.

The formation of nitrosamines in control-sample No. 1 to which no nitrite and ^{nitrate} were added, means that the salt or spices

used may contain quite enough nitrite and nitrate. Nitrite can be also a result of bacterial reduction of nitrate, naturally present in the salt and spices used. The samples showed a nitrite and nitrate content of 4 and 165 mg/kg respectively. This calls for further fundamental research to determine nitrate and nitrite in salt and spices from the local market, and to determine the possibility of reducing the level of added nitrate and nitrite in the curing of meat.

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