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Nitrosamines in cured pork products packaged in elastic rubber nettings: An update

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Previous research has shown that traces to fairly high levels of certain *N*-nitrosamines can form in cured pork products packaged in elastic rubber nettings. The *N*-nitrosamines are formed due to the interaction of nitrite additive in the meat and amine additives in the rubber that are used as accelerators in the curing of rubber. This paper briefly reviews earlier findings and presents some recent results. Of twenty samples of such pork products analyzed, one was negative, six contained 12–32 μg/kg levels of *N*-nitrosodibenzylamine (NDBZA) but no *N*-nitrosodi-*n*-butylamine (NDBA), and the remaining samples contained appreciable levels of both the compounds (NDBA up to 48 μg/kg and NDBZA up to 520 μg/kg). These results suggest that the problem of *N*-nitrosamine formation in these products is yet to be resolved.

INTRODUCTION

During the past 10 years, considerable attention has been paid to the occurrence and formation of *N*-nitrosamines in rubber-based food contact materials such as baby bottle rubber nipples and elastic rubber nettings used for packaging cured pork products (Havery & Fazio, 1982; Spiegelhalder & Preussmann, 1982; Sen *et al.*, 1984; Gray & Stachiw, 1987; Sen *et al.*, 1987; Sen, 1988). These *N*-nitrosamines are believed to be formed due to the interaction of various nitrosating agents (e.g. nitrite, nitrogen oxides) and amine additives in the rubber that are used as accelerators in the curing of rubber. Since most *N*-nitrosamines have been shown to be carcinogenic in laboratory animals (Preussmann & Stewart, 1984), there is concern about health hazards to man due to the migration of these compounds from the rubber to the foods. There is also a possibility that additional amounts of *N*-nitrosamines could be formed during cooking or processing of foods that have come in contact with rubber products. This paper mainly discusses the *N*-nitrosamine contamination problem of cured pork products packaged in elastic rubber nettings.

Elastic rubber nettings are widely used to package various cured pork products (e.g. smoked ham, cottage rolls, pork picnic shoulders). These nettings help to hold the meat pieces together and retain the specific shapes of the individual products during the entire course of processing and marketing. Consumers often warm or cook (for raw products such as sweet pickled pork) these items with nettings on them. Some items are processed with the nettings, but the nettings are removed before the products are sold to consumers. The fact that such a practice could contaminate the finished meats with traces of *N*-nitrosamines and, therefore, would be undesirable, was first pointed out by Sen *et al.* (1987). The major *N*-nitrosamine detected in such products was *N*-nitrosodi-*n*-butylamine (NDBA) with lower levels of *N*-nitrosodiethylamine (NDEA) present in some samples. There also appeared to be a definite concentration gradient in the levels of NDBA and NDEA across the cross-section of the packaged meat. The used nettings contained the highest levels (up to 504 μg/kg) followed by decreasing levels in the meats taken from the ~5 mm outermost (up to 64 μg/kg) and ~5 mm second outermost (up to 45 μg/kg) layers. Meats taken from the center were either negative (< 0·3 μg/kg) or contained only traces (< 1 μg/kg) of NDEA and/or NDBA. Furthermore, when unused nettings were deliberately nitrosated (without meat) with dilute nitrite solution under acidic conditions, the same two *N*-nitrosamines were formed in fairly large quantities. The above findings clearly supported the theory that these *N*-nitrosamines were first formed due to the interaction of nitrite and the amine additives in the nettings, and then migrated into the meat. In each meat sample, the total amounts of *N*-nitrosamines detected (including that in the used netting) were much higher than that present in the original unused netting.

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Therefore, the major concern appeared to be the formation of these compounds from the nettings during curing and processing, and not the preformed *N*-nitrosamines present in the original nettings.

Following the above discovery, the industry introduced a newer type of netting that produced lower levels of NDBA and completely eliminated NDBA formation (Sen *et al.*, 1988; Sen, 1991). The new nettings, however, promoted the formation of a previously undetected *N*-nitrosamine, NDBZA, which was reported to be non-carcinogenic to rats (Druckrey *et al.*, 1967) but also later reported to be genotoxic in some in-vitro bio-assay systems (Schmezer *et al.*, 1987, 1990; Boyes *et al.*, 1990). It was concluded that even though NDBZA formation in such products appeared to be preferable to that of NDBA and NDEA, because of the conflicting toxicological data, excessive formation of NDBZA might be a matter of concern as well. Further research was recommended towards developing improved nettings that do not produce or produce only negligible levels of *N*-nitrosamines in cured meat products.

More recently, the USDA (Food Chemical News, 1990) has also detected fairly high levels of NDBA in US-produced netted hams. This prompted us to revisit the Canadian situation. In the present study, 15 samples of unused elastic rubber nettings, procured during 1989–90, were analyzed for their *N*-nitrosamine contents as well as for their *N*-nitrosamine-forming potential. A total of 20 samples of netted cured pork products representing 12 producers and procured during May 1991–February 1992 were also analyzed for both volatile *N*-nitrosamines and NDBZA. The results of these studies are presented in this report.

MATERIALS AND METHODS

Methodologies used for the determination of *N*-nitrosamines in meats

The details of the methods used for the determination of volatile *N*-nitrosamines and NDBZA have been described previously (Sen *et al.*, 1987, 1988). Briefly, the volatile *N*-nitrosamines (mainly NDBA) were isolated from the cured meat samples by low-temperature vacuum distillation from acidic sulfamic acid, the aqueous distillate extracted with dichloromethane (DCM), and the DCM extract was concentrated to a small volume using a Kuderna-Danish concentrator. A 2–6 μl aliquot of the concentrated extract was analyzed by gas chromatography–thermal energy analyzer (GC–TEA) or GC–high resolution mass spectrometry (GC–MS). To compare results, a few samples were also re-analyzed by distillation from 3 N KOH solution (Sen *et al.*, 1987).

Two minor modifications were made in the previously published method for the determination of NDBZA in cured meats (Sen *et al.*, 1988). First, the basic alumina used for clean-up of the meat extract was deactivated with the addition of 2% water instead of the 1·5% used previously. The other change made was that after loading the alumina column with meat extract, it was washed successively with 50 ml *n*-pentane and a 20 ml mixture of *n*-pentane and DCM (1 : 1). Previously, only 50 ml *n*-pentane was used for washing. Both the washings were discarded. NDBZA was then eluted with DCM as before. These changes resulted in a much cleaner extract than obtained previously.

Determination of *N*-nitrosamines in nettings

Cut pieces of the nettings were extracted and analyzed for both volatile *N*-nitrosamines and NDBZA as described previously (Sen *et al.*, 1987, 1988).

Rapid test for determining *N*-nitrosamine-forming potential of elastic rubber nettings

Since the main concern appeared to be the formation of *N*-nitrosamine from the nettings during processing of the packaged meats, a simple and rapid test was developed to determine the *N*-nitrosamine-forming potential of such nettings. This was done by treating cut pieces of the unused netting with dilute nitrite solution at pH 3–4, and then measuring the amounts of *N*-nitrosamines formed as described previously (Sen *et al.*, 1987, 1988). Excess nitrite was destroyed by treatment with sulfamic acid before analyzing for the respective *N*-nitrosamines.

Precaution to avoid artifactual formation of *N*-nitrosamines

As reported previously (Sen *et al.*, 1987. 1988), both the methods used for the determination of volatile *N*-nitrosamines and NDBZA have been shown to be free of artifactual formation. However, to be absolutely sure, all the meat samples analyzed in this study for their volatile *N*-nitrosamine contents were distilled from acidic sulfamic acid solution which is a strong inhibitor of artifactual formation (Hotchkiss *et al.*, 1980). This was verified by analyzing cured meat with 5 mg/kg added methylbutylamine or morpholine. No evidence for artifactual formation of the corresponding *N*-nitrosamines was observed. Therefore, it is highly unlikely that the volatile *N*-nitrosamines detected in the meat samples were formed as an artifact. Furthermore, as mentioned above, five meat samples were re-analyzed using the alkaline distillation method. The two sets of results agreed well with each other, suggesting that the alkaline distillation method normally used in this laboratory for the determination of volatile *N*-nitrosamines in cured meat is also unlikely to be prone to artifactual formation. Similarly, the method used for the determination of NDBZA included *N*-nitrosation inhibitors that prevent or minimize artifactual formation. The addition of dibenzylamine to a meat, which was negative for NDBZA, did not form any detectable level of NDBZA.

Table 1. Levels of *N*-nitrosamines detected in various unnitrosated and nitrosated elastic rubber nettings[a]

Sample	NDBA		NDBZA	
	No. positive / total	Mean level (range) (μg/kg)	No. positive / total	Mean level (range) (μg/kg)
Unnitrosated nettings	3/15	1·2 (N^b–8·7)[c]	15/15	284 (50–641)[c]
Nitrosated nettings	15/15	253 (101–763)[c]	15/15	4960 (810–12 800)[c]

[a] All results are expressed on the basis of the weight of the nettings.
[b] *N* = negative.
[c] Results in selected samples were confirmed by gas chromatography – mass spectrometry as described previously (Sen *et al.*, 1987, 1988).

RESULTS AND DISCUSSION

The data on the levels of *N*-nitrosamines in the unused nettings (unnitrosated) and those detected in the nitrosated nettings are presented in Table 1. Only three samples contained traces of preformed NDBA and all contained appreciable levels of preformed NDBZA. But upon deliberate nitrosation, all formed much higher levels of both NDBA and NDBZA; none formed or contained NDEA. It was concluded that these nettings, if used for packaging cured pork products, had the potential to form both NDBA and NDBZA in the finished meats. In fact, our 1987–88 monitoring data (Sen, 1991) were in good agreement with these findings, namely that none of the packaged meats contained NDEA but contained traces of NDBA and slightly higher levels of NDBZA.

It should be emphasized that the above nitrosation test was not designed to simulate conditions existing during processing of cured meats. It should be considered simply as a rapid screening test that will be useful for predicting the nitrosamine-forming potential of newly formulated nettings. The actual levels of *N*-nitrosamines that might be formed in the packaged meat would be much lower—most likely in the 1–100 μg/kg range.

Table 2. Levels of various *N*-nitrosamines detected in recent samples of netted cured pork products[a]

Item	Producer	Month of procurement	Netting on or off	μg/kg *N*-nitrosamine detected[b]		
				NDEA	NDBA	NDBZA
Smoked pork cottage rolls						
(1)	A	May, 1991	On	10·6[c]	8[c]	N[d]
(2)	B	May, 1991	On	N	24[c]	N
(3)	B	January, 1992	On	N	48[c]	520
(4)	B	February, 1992	On	N	7	96
(5)	C	February, 1992	On	N	31·6[c]	N
Smoked ham						
(6)	D	May, 1991	On	N	38·6[c]	N
(7)	D	January, 1992	On	N	2·9	62
(8)	E	February, 1992	Off	N	N	12
(9)	B	February, 1992	Off	N	N	25[c]
(10)	F	February, 1992	Off	N	N	13[c]
(11)	E	February, 1992	Off	N	19·8[c]	28
(12)	G	February, 1992	Off	N	N	28·6[c]
(13)	H	February, 1992	Off	N	1[c]	34
Smoked pork shoulders (picnic, boneless)						
(14)	I	May, 1991	On	N	32·4[c]	N
(15)	I	January, 1991	On	N	23·3[c]	226[c]
(16)	I	February, 1992	On	N	16·5	316[c,e]
Sweet pickled pork cottage rolls (raw)						
(17)	J	May, 1991	On	N	N	N
(18)	B	February, 1992	On	N	N	32·5[c]
(19)	K	February, 1992	On	N	N	13[c]
(20)	L	February, 1992	On	N	0·6[c]	18

[a] All samples, except the sweet pickled cottage rolls (raw), were analyzed without any cooking or warming. The raw samples were cooked with the netting on as per instructions on the package or as described previously (Sen *et al.*, 1987).
[b] All results are based on the analysis of the whole homogenized meat (after removal of the netting).
[c] Confirmed by gas chromatography–high resolution mass spectrometry (Sen *et al.*, 1987, 1988).
[d] N = negative (detection limit = 0·1 μg/kg for NDEA, 0·3 μg/kg for NDBA, and 2 μg/kg for NDBZA).
[e] Also verified by high performance liquid chromatography–thermal energy analysis.

The data on the *N*-nitrosamine levels in the recent samples of netted cured pork products are presented in Table 2. As can be seen, some of the samples contained fairly high levels of *N*-nitrosamines. This was unexpected because, using the same or similar methods of analysis, our 1987–88 survey data (Sen, 1991) indicated a significant decrease in the levels of *N*-nitrosamines in such products. However, there were some encouraging results as well in the present study. This is reflected in the results on some hams (Nos 7–10 and 12–13) and the sweet pickled cottage rolls (Nos 17–20). These samples contained either none or negligible levels of *N*-nitrosamines. This suggests that it is technically feasible to produce nettings that do not form excessive levels of *N*-nitrosamines in the finished packaged meats.

Some nettings, currently in use, are still producing high (> 10 μg/kg) levels of NDBA or both NDBA and NDBZA. The findings with respect to NDBA residues suggest that nettings are of older types, whereas those with respect to NDBA/NDBZA indicate that the rubber used in the manufacture of the nettings contained high levels of both NDBA- and NDBZA-producing rubber curing accelerators. The latter findings are consistent with our data on the nitrosamine-forming potential of various nettings (Table 1). It was understood that during reformulation of newer nettings, the rubber industry had reduced the level of NDBA-producing accelerator and substituted another that produces NDBZA. But our latest data on packaged meats (Table 2; sample Nos 1–3, 5–6, 11, 14–16) indicate that this did not happen in all cases. Therefore, a better control of the input of the rubber curing accelerators is being pursued further.

The highest levels of *N*-nitrosamines were found in smoked pork cottage rolls (Nos. 1–5) and smoked pork shoulders (Nos 14–16). It is not clear whether this is due to the type of netting used or to special curing and smoking conditions (e.g. temperature and duration of smoking and curing, nitrite concentration) employed in the preparation of such products. Further research on this aspect will be desirable. It would also be of interest to determine if spraying the netted meats (before curing and smoking) with an appropriate solution of *N*-nitrosation inhibitor (e.g. sodium ascorbate, α-tocopherol) will reduce such *N*-nitrosamine formation. If encouraging results are obtained, industry would need approval from Health and Welfare Canada for such usage.

CONCLUSION

The problem of *N*-nitrosamine formation in cured pork products packaged in elastic rubber nettings still remains, although some progress has been made in reducing the levels of NDBA and NDEA in certain products. Research should continue toward developing much more improved nettings that do not form or form only negligible levels of *N*-nitrosamines. It is also recommended that monitoring of cured pork products, packaged in such nettings, for their *N*-nitrosamine contents be continued in order to assess trends, and have a better control of the situation.

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