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EFFECT OF CARBOHYDRATE CRYOPROTECTING AGENTS ON THE FORMATION OF N-NITROSODIMETHYLAMINE IN SURIMI-MEAT FRANKFURTERS[1]

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ABSTRACT

Previous investigations of frankfuters substituted with Alaska pollock consistently showed that, after broiling, higher levels on N-nitrosodimethylamine (NDMA) were found in the surimi containing frankfurters compared to those containing the corresponding washed mince. This was apparent in the 50%, but not in the 15% substituted frankfurters. The addition of the cryoprotecting agents, sucrose and sorbitol, to the washed mince to make surimi was the major difference in the two forms of fish. Model system experiments carried out, first in an aqueous pH 5.8 buffer and second, in a 50% fish-meat simulated frankfurter system indicated that the combination of sucrose-sorbitol increased and fructose-sorbitol decreased NDMA formation. Frankfurters prepared with 50% washed mince and containing different carbohydrates showed that sucrose-sorbitol gave higher NDMA values than fructose-sorbitol, even though all the carbohydrates tested gave NDMA results higher than the control.

INTRODUCTION

The meat and fish industry have shown increased interest in the development of new food products that use raw materials such as minced fish or surimi, either alone or in combination with meat in traditionally all-meat products. One example is the incorporation of fish protein into comminuted, cured meat products like frankfurters, which has been proposed by the National Marine Fisheries Service. Of major concern in this type of product is the simultaneous presence of amines present in the fish, primarily dimethylamine (DMA), and nitrite in the cured meat, which could result in the formation of potentially carcinogenic nitrosamines. We have previously shown that frankfurter made with either washed Alaska pollock mince or surimi at the 15% meat substitution level only contained trace levels on N-nitrosodimethylamine (NDMA), the principal volatile nitrosamine (Fiddler *et al.* 1992a). However, during these investigations we observed an interesting phenomenon. Higher levels of NDMA were found in broiled frankfurters with 50% surimi compared to the ones containing the corresponding washed mince. Surimi is washed and dewatered minced fish to which the cryoprotectant agents, sucrose and sorbitol at a total concentration of 8%, have been added to prevent myofibrillar protein denaturation during frozen storage. We therefore hypothesized that the carbohydrate-based cryoprotecting agents may have a rôle in the formation of NDMA.

The object of this paper was to determine if sucrose and sorbitol affect the formation of NDMA in model and meat-containing systems and to determine if other combinations of carbohydrates may be more suitable in controlling NDMA formation in a surimi-meat frankfurter.

MATERIALS AND METHODS

Materials

NDMA, DMA, sodium nitrite ($NaNO_2$), sucrose, sorbitol, dextrose, polydextrose, fructose, sodium ascorbate (NaAsc), sodium chloride (NaCl), sodium tripolyphosphate (STPP), sodium hydroxide (NaOH), potassium phosphate (KH_2PO_4), and methylene chloride (DCM) were purchased from local suppliers and used without further purification. A 0.5M pH 5.8 buffer was prepared from KH_2PO_4 and NaOH. Beef, pork and pork fat were obtained from a local butcher. Washed Alaska pollock mince was obtained from the National Marine Fisheries Service, Kodiak, AK. The mince was shipped via overnight express using cold packs to maintain temperature. It was requested that the mince have high levels of trimethylamine oxide (TMAO) to increase the likelihood of NDMA formation.

Apparatus

NDMA was determined with a Shimadzu gas chromatograph model GC-14A equipped with an AOC-14 auto injector and interfaced to a Thermal Energy

Analyzer (TEA) model 502A. A 3 m × 2.6 mm i.d. glass column packed with 15% Carbowax 20M-TPA on 60–80 mesh Gas Chrom P was used with the following operating conditions: column 130C; injector port, 200C; helium flow at 35 ml/min; TEA vacuum at 0.35 mm; liquid nitrogen cold trap. Minimum detectable level of NDMA was 0.2 ppb.

Aqueous Model System

This study was carried out in a model system consisting of 50 ml pH 5.8 buffer, 4.0 g carbohydrate (8%), either alone or combined at 4% each, 5 mg DMA (100 ppm), and 7.8 mg $NaNO_2$ (156 ppm) in a 100 ml round bottom flask. The mixture was stirred and heated at 68.8C (156F) for 1 h, then cooled for 5 min in an ice bath. The solution was poured into a separatory funnel, then extracted 3 times each with 50 ml DCM. The DCM was washed once with 25 ml 5N NaOH, dried with anhydrous sodium sulfate, concentrated to 1.0 ml and the amount of NDMA quantitated on the GC-TEA.

Fish-Meat Model System

This study was carried out in a model system comprised of 10.0 g washed Alaska pollock mince, 10.0 g beef or pork, 1.6 carbohydrate (8%), 2.0 mg DMA (100 ppm), and 4.0 mg $NaNO_2$ (200 ppm) in a 150 ml beaker. The above combination was thoroughly mixed, then heated for 1 h at 68.8C. This is an approximation of the time a frankfurter is near or at its internal temperature during smokehouse processing. The beaker was then placed in an oven and heated (broiled) for 5 min (280C). After broiling, the NDMA was extracted from the sample using a procedure developed previously for combination fish-meat frankfurters (Pensabene and Fiddler 1988), and then quantitated in a similar fashion.

Fish-Meat Frankfurters

Frankfurters were prepared using the following formulation: 500 g washed mince, 250 g pork, pork fat (to achieve a 30% fat level in the product), and beef to give a total product weight of 1 kg; 125 g ice; 40 g carbohydrate (8%); 27 g (2.7%) NaCl; 3 g (0.3%) STPP; 200 ppm $NaNO_2$. The carbohydrates were mixed with the washed Alaska pollock mince prior to preparation of the frankfurter emulsion. The carbohydrates were added to either fresh mince or to fresh mince, which was then frozen prior to use. The emulsion was stuffed into frankfurter cellulose casings and processed following a typical industry schedule using natural smoke (Brooker 1985). The finshed frankfurters were stored overnight in a 4C refrigerator, then skinned, cooked, and stored at −20C till analyzed (Fiddler *et al* . 1992b).

Amine Analysis

DMA, trimethylamine (TMA), and trimethylamine oxide (TMAO) were determined in the washed mince using a procedure recently developed for volatile amines in such products (Fiddler *et al.* 1991).

Statistical Analysis

Statistical analyses (ANOVA at $p < 0.05$) were carried out according to the methods of Snedecor and Cochran (1979).

Safety Note

Precautions should be taken when handling nitrosamines, since they are potential carcinogens.

RESULTS AND DISCUSSION

To determine if sucrose and sorbitol (8% total) increase NDMA formation in broiled frankfurters containing surimi, aqueous and fishmeat model systems were initially studied so as to limit the possible extraneous effects that may be associated with actual meat processing. In addition to sucrose and sorbitol, polydextrose and fructose were also studied, since polydextrose has been proposed as an alternaive cryoprotectant (MacDonald and Lanier 1991), and fructose was shown to have a marked effect in preventing freeze denaturation of fish actomyosin subjected to −30C for 7 weeks (Noguchi *et al.* 1976). Mean results from four experiments in each system are shown in Table 1. The overall repeatability of analysis was 0.54 ppb. In the aqueous model system, NDMA formation was significantly higher ($p < 0.05$) with the sucrose-sorbitol combination than with the control. There was also a difference between sucrose-sorbitol and fructose-sorbitol, with the latter combination giving lower NDMA values. There was no difference between NDMA formation in the control and sorbitol, sucrose, fructose, or polydextrose; although, NDMA formation was significantly lower with fructose compared to both sucrose and sorbitol when tested individually.

In the fish-meat model system, NDMA in the sucrose-sorbitol combination was again significantly higher than with fructose-sorbitol. There was no difference in NDMA between the control and sorbitol or fructose, but there was a difference between the control and sucrose alone or polydextrose alone with the latter two being higher than the control. These results differ from what we observed in the aqueous model system, where sucrose and polydextrose showed no significant increase in NDMA formation. It is also interesting to note that in both model systems, fruc-

TABLE 1.

EFFECT OF CARBOHYDRATES ON N-NITROSODIMETHYLAMINE FORMATION

	N-Nitrosodimethylamine, ppb		
	Model Systems		
Carbohydrate[a]	Aqueous[b]	Fish-Meat[c]	Surimi-Meat Frankfurter
None (Control)	2.1[a,b]	26.4[a,b]	12.4[a]
Sucrose	2.5[a]	32.9[c]	16.0[b]
Sorbitol	2.5[a]	27.3[a]	22.0[c]
Fructose	1.2[b]	25.1[b]	18.1[d]
Polydextrose	2.6[a]	34.2[c]	18.4[d,e]
Sucrose-Sorbitol	3.8[c]	27.1[a]	18.9[e]
Fructose-Sorbitol	1.4[a,b]	22.0[d]	16.6[b]

Within the same column, numbers with the same letter are not significantly different ($p < 0.05$) from each other; n=4.

[a]8% alone; 4% each combined.

[b]Aqueous, DMA (100 ppm) and $NaNO_2$ (156 ppm).

[c]Fish-Meat, DMA (100 ppm plus normally incurred) and $NaNO_2$ (200 ppm).

tose alone did not lower NDMA formation, nor did sorbitol alone increase it from the control, but that fructose gave significantly lower NDMA than sorbitol.

We then prepared frankfurters containing 50% washed mince and 50% washed mince with 8% carbohydrate added prior to addition of the meat. The results are also shown in Table 1. Contrary to what we observed in the model systems, the addition of all of the carbohydrates resulted in higher NDMA values than those in the control. However, the use of the fructose-sorbitol combination again gave lower NDMA levles than sucrose-sorbitol. Also, the use of sorbitol alone gave higher NDMA values compared to any other carbohydrate. There was no correlation between the amine levels in the starting mince and the resulting levels of NDMA detected in the frankfurters. The amine values in the frankfurters ranged from 9.8 to 45.8 ppm for DMA, 4.6 to 149.3 ppm for TMA and 20.6 to 848.3 ppm for TMAO.

Other reports on the effect of carbohydrates on nitrosamine formation found that glucose and sucrose catalyze the nitrosation of secondary amines under neutral or alkaline conditions (Yamamoto *et al.* 1979; Challis *et al.* 1980). However, the pH of a frankfurter made with 50% substituted mince or surimi was 5.8. Williams and Aldred (1982) found that several sugars, including sucrose and glusoce, inhibited the nitrosation of N-methylaniline. Theiler *et al.* (1981) initially found the sucrose had no effect on nitrosamine formation in a ground pork model system. They later found that a wide variety of other carbohydrates, including reducing sugars, lowered N-nitrosopyrrolidine formation in the same system (Theiler *et al.* 1984). Kurechi *et al.* (1980) found that glucose had little influence on nitrosamine formation in an aqueous model system in the pH range from 30. to 7.0; whereas, Bailey and Mandagere (1980) found that reducing sugars inhibited nitrosamine formation in an aqueous model system at pH 5.5 and 7.5. The above reports indicate the complex nature of the nitrosation reaction when carbohydrates are present. Our results show that NDMA generally increased in the presence of carbohydrates. One possible explanation for our results may involve the concentration of the carbohydrates added to either the model systems or the fish-meat frankfurters. In all our systems, we added a total of 8% carbohydrate, which is the total concentration used for the preparation of surimi. We did not attempt to vary this amount, since we were only interested in the observed effect at the recommended concentration. Enhancement may also be due to direct nitrosation of the DMA via an intermediate nitrite ester formed from the carbohydrate rather than through a reaction involving nitrogen trioxide (N_2O_3) derived from the dissociation of nitrite under slightly acidic conditions.

In conclusion, we found an enhancement in NDMA formation with a combination of sucrose-sorbitol, which is commonly used in surimi production, but inhibition with fructose-sorbitol in model systems. With the frankfurters, all of the added carbohydrates increased NDMA formation above the control, even though fructose-sorbitol was significantly lower than sucrose-sorbitol. It appears that some NDMA enhancement occurs when a combination of sucrose and sorbitol are used. This suggests that a noncarbohydrate based cryoprotectant system may be desirable for surimi used in cured meat products.

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Reference to a brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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