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Effect of Additives on the Formation of Nitrosamines in Meat Curing Mixtures Containing Spices and Nitrite

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Studies were carried out to determine the effect of an alkaline buffer and sodium ascorbate on the formation of *N*-nitrosamines in curing mixtures containing spices and nitrite. Four samples of such premixes prepared with or without the buffer were analyzed for nitrosamines after storage for 4–15 weeks at two different temperatures. The buffering (at pH 7.5–8.2) markedly reduced the formation of nitrosamines but did not prevent it completely. The formation of appreciable levels (up to 4 ppm) of nitrosamines was observed in most of the nonbuffered samples. Higher levels of nitrosamines were formed at 38° than at room temperature. Similar studies with six samples of premixes prepared with or without ascorbate indicated its ineffectiveness in preventing nitrosamine formation. Of 30 samples of various commercial premixes analyzed 17 were positive for nitrosamines. The most commonly found nitrosamines were nitrosodimethylamine, nitrosopyrrolidine, and nitrosopiperidine, all of which are potent carcinogens.

The reported occurrence of traces of *N*-nitrosamines, many of which are potent carcinogens, in cured meat products has aroused a great deal of concern (Crosby *et al.*, 1972; Sen, 1972; Wasserman *et al.*, 1972). It is generally believed that nitrite, which is added as a preservative, reacts with the amines present in meat, during processing, storage, or cooking, to form these nitroso compounds. The formation of nitrosopyrrolidine (NPy) during frying of bacon (Sen *et al.*, 1973a; Fazio *et al.*, 1973) can be cited as an example. The recent report by Sen *et al.* (1973c) of the occurrence of fairly high levels of nitrosamines in certain types of meat curing mixtures containing spices and nitrite indicates that some of the nitrosamines in cured meat products may originate from these curing mixtures. Nitrosamines in these premixes are formed, apparently under dry condition, due to the interaction of amines in spices and nitrite both of which are major components of these formulations. Subsequently, the use of such premixes has been discontinued both in the United States and Canada.

The rate of formation of nitrosamines from amines and nitrite is dependent on many factors such as the nature of the amines, concentration of the reactants, pH, and temperature of the reaction medium (Mirvish, 1973). In general, the more alkaline the pH, the slower is the rate of the nitrosation reaction. Recent studies (Mirvish *et al.*, 1972; Fiddler *et al.*, 1973) have indicated that, in certain cases, the addition of ascorbic (or erythrobic) acid or its sodium salt can inhibit the nitrosation reaction. Therefore, it was thought that the incorporation of a mild alkali (sodium carbonate) or sodium ascorbate in these premixes may inhibit the formation of nitrosamines, and thus make them suitable for use. This paper describes the results of such a study carried out in collaboration with two manufacturers of meat curing mixtures in Canada.

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MATERIALS AND METHODS

Samples. The samples of the meat curing mixtures were prepared by the two commercial firms according to their own specifications, and shipped to us for nitrosamine analysis. The mixtures mainly consisted of spices or spice extractives, salt, and sodium nitrite. Some of them also contained wheat or corn flour, skim milk powder, tricalcium phosphate (anticaking agent), hydrolyzed plant protein, etc. A brief description of the relevant ingredients in each sample is given in Tables I–III. The buffered samples contained enough sodium carbonate so that a 10% aqueous suspension of the mixture gave a pH of 7.5–8.2. The concentration of sodium nitrite in these premixes ranged between 0.2 and 2%. In addition to sodium nitrite some of them also contained sodium nitrate (0.1–0.96%). Five samples of special premixes analyzed contained only nitrates and no added nitrites.

Nitrosamine Analysis. (*a*) *Extraction of Nitrosamines.* A 5–25-g aliquot of the sample was moistened by the addition of 5–30 ml of 3 *N* potassium hydroxide solution and then extracted in a blender for 10 min with 100–300 ml of methylene chloride. During the extraction, about 10–50 g of anhydrous sodium sulfate was added to the mixture to aid the mixing and breaking up of emulsions. The mixture was allowed to settle for a few minutes and the supernatant carefully filtered through glass wool. The filtrate was collected into a 2-l. round-bottomed distillation flask which already contained 100 ml of 3 *N* potassium hydroxide solution. The extraction was repeated once more, and the entire contents were poured on the funnel containing

Table I. Formation of Nitrosamines in Buffered and Nonbuffered Curing Mixtures Stored under Various Storing Conditions

Sample and its main ingredients	pH[a]	Storage: Days	Storage: Temp, °C	Nitrosamines, ppm: DMN	NPy	NPi
1. Buffered premix for liver sausage (white pepper, other spices, salt, sodium nitrite)	7.5	29 (100)[b]	38	N[d] (N)	N (0.006)	Trace (trace)
	7.5	36 (104)	23	N (N)	N (N)	N (N)
2. Above sample nonbuffered	6.2	43	38	N	0.008	0.6[c]
	6.2	49	23	N	N	0.2[c]
3. Buffered premix for pork sausage (paprika, white pepper, black pepper, other spices, sodium erythrobate, salt, sodium nitrite)	7.8	32	38	N	0.14[c]	0.2[c]
	7.8	36 (106)	23	N (N)	N (0.032[c])	N (N)
4. Above sample nonbuffered	5.6	47	38	0.12[c]	0.16[c]	0.4[c]
	5.6	54	23	0.08[c]	0.08[c]	1.6[c]
5. Buffered binder for frankfurter (paprika, pepperoyal, other spices, essential oils, sodium erythrobate, salt, sodium nitrite)	8.2	32	38	N	0.08[c]	N
	8.2	40 (105)	23	N (N)	N (0.012)	N (N)
6. Above sample nonbuffered	6.4	47	38	0.054	0.08[c]	4[c]
	6.4	54	23	0.02	0.04[c]	N
7. Buffered premix for chicken roll (oleoresin celery, soluble allspice, other spices, hydrolyzed plant protein, sodium erythrobate, salt, sodium nitrite)	7.8	34 (103)	38	N (N)	N (N)	N (N)
	7.8	41 (104)	23	N (N)	N (N)	N (N)
8. Above sample nonbuffered	6.6	47	38	0.2[c]	0.12[c]	0.2[c]
	6.6	54 (100)	23	N (N)	N (N)	N (N)

[a] pH of an aqueous suspension containing 10% of the premix. [b] Figures in parentheses correspond to a second analysis carried out after an extended period of storage as indicated. [c] Results confirmed by glc-mass spectrometry. [d] N = negative (detection limits, 0.004 ppm for NPy and 0.01 ppm for others).

the glass wool filter, and the filtrate was collected into the flask as before.

(*b*) *Distillation of Nitrosamines.* About 0.5–1 g of antifoam "A" (Dow Corning) was added to the mixture, and the solution was distilled under vacuum (water bath 40–50°) in an all-glass flash evaporator fitted with a vertical condenser and circulating ice-cold water as the coolant. The receiving flask was also kept immersed in an ice-water mixture. When all the methylene chloride was distilled, the receiving flask was changed and about 80–90 ml of aqueous distillate was collected. Finally, about 200 ml of fresh methylene chloride was added to the distilling flask and the distillation continued until all the methylene chloride distilled over and collected in the receiving flask. (This second distillation with methylene chloride was carried out to rinse down any nitrosamines that may have been trapped inside the condenser.) All the methylene chloride distillate (initial and the second) was saved and later used for extracting the aqueous distillate. In a few cases where the nitrosamine concentrations in the premixes were very high (>1 ppm) the extraction procedure was omitted; a 2–5-g aliquot was distilled from 50–100 ml of 3 *N* potassium hydroxide solution as described above.

(*c*) *Cleanup.* The aqueous distillate was made alkaline with the addition of 20 g of potassium carbonate and extracted twice with two 0.5 vol portions of methylene chloride that had been saved during the distillation. The methylene chloride extracts were combined and successively washed by shaking with 50 ml each of glycine–hydrochloric acid buffer (pH 2.1) and 20% potassium carbonate solution. Finally, the organic layer was dried over anhydrous sodium sulfate, filtered, concentrated, and purified by chromatography on a basic alumina column as described previously (Sen *et al.*, 1973b, 1974). The eluent from the alumina column was carefully concentrated to 1.0 ml using the micro concentration apparatus described earlier (Sen and Dalpé, 1972).

(*d*) *Semiquantitative Estimation of Nitrosamines.* The concentrations of NPy in the extracts were determined by the tlc–fluorometric method (Sen *et al.*, 1973a, 1974), and that of nitrosodimethylamine (DMN) and nitrosopiperidine (NPi) by the glc method using a nitrogen-specific Coulson electrolytic conductivity (CEC) detector operating in the pyrolytic mode (Sen *et al.*, 1972, 1973b). Where the concentrations of DMN or NPi in the sample exceeded 0.08 ppm, or 2 ppm in the final extracts an additional tlc analysis (NEDSA spray) was also carried out (Sen and Dalpé, 1972). The NEDSA spray is not sensitive below this level, and, therefore, was not used for all the samples.

In a few cases, as indicated in the tables, the identity of the nitrosamines was confirmed by glc–mass spectrometry. The high-resolution (≥10,000) mass spectrometric confirmation for each nitrosamine was carried out by specific ion current monitoring of the glc effluent from the column at the exact m/e values of the molecular ion (M^+) or NO^+ peaks. The details of the technique have been published elsewhere (Fiddler *et al.*, 1972; Telling *et al.*, 1971). The full spectral analysis was carried out by the method described earlier (Sen, 1972; Sen *et al.*, 1972, 1973a). Since about 50–100 ng of each nitrosamine was needed to obtain a spectrum distinguishable from the background, it was carried out only for those samples containing more than 0.4 ppm of nitrosamine. Below this

Table II. Effect of Sodium Ascorbate on the Formation of Nitrosamines in Premixes Containing Spices and Nitrite

Sample and its main ingredients[a]	Storage: Days	Storage: Temp, °C	Nitrosamines, ppm: DMN	NPy	NPi
1A. Black pepper, sodium nitrate, sodium nitrite	21	23	N	0.08	0.4
Above sample with 8.6% sodium ascorbate	21	23	N	0.35	1.7
1B. Composition same as in 1A but pH 7.3	26 (98)[b]	23	N (N)	0.04 (0.2)[c]	Trace (0.13)
Above sample with 8.6% sodium ascorbate	26 (100)	23	N (N)	0.08 (0.2)[c]	0.2 (0.5)[c]
2A. Oleoresin pepper, sodium nitrate, sodium nitrite	21	23	N	0.02	0.2
Above sample with 9.5% sodium ascorbate	21	23	N	0.02	0.5
2B. Composition same as in 2A but pH 7.3	28	23	N	N	N
Above sample with 9.5% sodium ascorbate	28	23	N	0.04	1.2
3A. Black pepper 88%, sodium nitrate 2%, sodium nitrite 1%, sodium ascorbate 8%)	14	38	N	0.7[c]	1[c]
3B. Above sample without sodium ascorbate	14	38	N	1[c]	3[c]
4A. Paprika 88%, sodium nitrate 2%, sodium nitrite 2%, sodium ascorbate 8%	15	38	1.2	3.6	0.8
4B. Above sample without sodium ascorbate	15	38	1.2[c]	4[c]	1

[a] Samples 1A, 1B, 2A, and 2B were provided by a commercial manufacturer; other samples were prepared in the laboratory. [b] Figures in parentheses represent data obtained from a second analysis carried out after an extended period of storage as indicated. [c] Results confirmed by glc-mass spectrometry.

Table III. Nitrosamines in Commercial Samples of Premixes Containing Spices and Nitrite

Sample and its main ingredients	Manufacturer	Approximate age,[a] days	Nitrosamines, ppm: DMN	NPy	NPi
1–3. Premixes for Mettwurst sausage[b] (3 samples) (black pepper, paprika, other spices, salt, nitrite)	A	105–204	0.85[c]	2.5–6[c]	7–25[c]
4. Premix for meat loaf (oleoresin Capsicum, oleoresin paprika, other oleoresins of spices, salt, nitrite)	B	28	N[b]	N	N
5. Premix for wiener (oleoresin paprika, Pepperoyal, black pepper, paprika, other spices, sodium erythrobate, salt, nitrite)	B	28	N	0.008	N
6. Premix for liverwurst (white pepper, Allspice, other spices, sodium erythrobate, salt, nitrite)	B	33	0.17	0.04	Trace
7. Premix for pepperoni (paprika, red pepper, other spices, sodium erythrobate, salt, nitrite)	B	33	0.1	0.04	Trace
8. Binder unit for frankfurter (paprika, other spices and spice extractives, erythrobic acid, salt, nitrite)	B	41	N	0.024	Trace
9. Binder unit for mock chicken (ground spices, spice extractives, sodium erythrobate, salt, nitrite)	C	95 at 4° (49)	N N	N (0.02)[a,c]	N Trace
10. Binder unit for bologna (paprika, spice extractives, other spices, sodium erythrobate, salt, nitrite)	C	95 at 4° (50)	N (N)	N (Trace)	N (Trace)
11. Binder unit for frankfurter (ground spices, spice extractives, other spices, sodium erythrobate, salt, nitrite)	C	95 at 4° (50)	N (0.19)	N (0.04)	N (N)
12. Binder unit for bologna (black pepper, spice extractives, other spices, sodium erythrobate, salt, nitrite)	C	95 at 4° (56)	N	N N	N N

[a] All samples were stored at room temperature except for samples 9–12 which were first stored at 4° and then kept at room temperature for 50–56 days and reanalyzed. [b] A preliminary report of these results has been published previously (Sen *et al.*, 1973c). [c] Results confirmed by glc-mass spectrometry.

level, the identity of the nitrosamines was confirmed by low-resolution specific ion current monitoring at both the M^- and NO^+ peaks.

(*e*) *Special Aqueous Methanol–n-Heptane Cleanup.* This cleanup was necessary only for extracts that produced excessive amounts of bubbles in the CEC detector cell during glc analysis. It is based on the principles previously described by Eisenbrand *et al.* (1969) and Iwaoka *et al.* (1973) but extensive modifications have been carried out to adapt the technique to work in a micro scale.

Exactly 1 ml of water was added to the final methylene chloride extract (1 ml) in the micro concentration flask, and the flask (fitted with a micro Snyder column) was heated in a water bath (55–60°) until most of the methylene chloride was distilled off. The Snyder column was disconnected and the heating continued until all traces of methylene chloride were gone. The aqueous solution was transferred with a Pasteur pipet into a 15-ml glass-stoppered centrifuge tube. About 0.5 ml of methanol was added to the micro flask, the flask briefly swirled, and all the methanol was transferred into the test tube containing the aqueous solution. *n*-Heptane (0.5 ml) was added to the mixture and the sample thoroughly mixed in a Vortex mixer for 3–5 min. The two layers were separated by slow centrifugation (the addition of a few crystals of sodium chloride helped in breaking up emulsions) and the heptane layer was removed and discarded with a Pasteur pipet. Care was taken not to discard any aqueous layer or any layer of emulsion (if any) that may have been present. A few drops of *n*-heptane were added to the aqueous solution and the mixture gently shaken for a few seconds (vigorous shaking should be avoided). The heptane layer was again discarded. Exactly 1.0 ml of methylene chloride was added to the aqueous solution and the two layers vigorously mixed for 5 min by mixing in a Vortex mixer. Aliquots of the methylene chloride layer were used for tlc and glc analysis.

Warning. Since nitrosamines are potent carcinogens, extreme care should be taken while handling these chemicals. Whenever possible, the work should be carried out in a well-ventilated fume hood, and precautions should be taken to avoid contact with the skin.

RESULTS AND DISCUSSION

Various problems can be encountered during the analysis of spice–nitrite premixes. The presence of many volatile materials in these mixtures can cause interference during glc analysis. Moreover, there is also a risk of the formation of nitrosamines, due to the interaction of the amines in spices and large amounts of nitrite present in the mixtures, during the analysis. Minor modifications of our earlier methods (Sen *et al.*, 1972; Sen, 1973) enabled us to solve these problems. The vacuum distillation of the extracts at 40–50°, instead of direct distillation by heating under normal atmospheric pressure, gives a better recovery of nitrosamines as well as better cleanup of the sample. The percentage recoveries of nitrosamines added to various samples at 0.05- and 0.4-ppm levels ranged between 60 and 80. However, no attempt was made to correct the data for recoveries. The true levels of nitrosamines in these samples were probably slightly higher than that detected.

The high pH of 3 *N* potassium hydroxide combined with a lower temperature of distillation reduces the chance of formation of nitrosamines during the distillation. Control experiments involving vacuum distillation of ground spices and nitrite under these conditions did not indicate any formation of nitrosamines (Figure 1). The high specificity of both the tlc and glc techniques, which had been discussed in detail in our earlier publications, was also helpful in carrying out these analyses. Some typical glc diagrams obtained in the study are shown in Figures 1–3. Although the nitrosamine peaks are somewhat

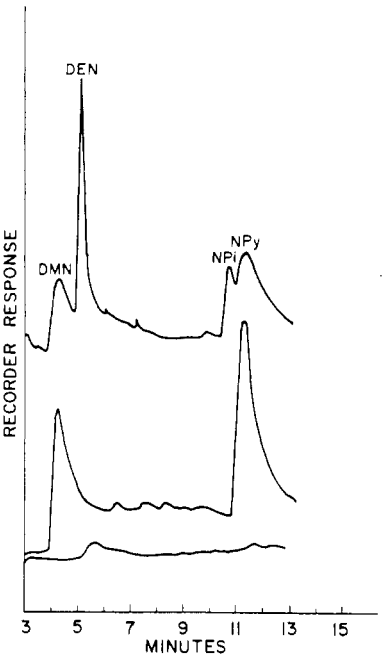

Figure 1. Glc analysis of nitrosamines in spice–nitrite premixes. Bottom diagram, paprika and nitrite mixed and immediately analyzed; middle diagram, same mixture analyzed after storage; top diagram, 12 ng of each of nitrosamine standards. Conditions: 10% Carbowax 20M on 60–80 mesh Chromosorb W (HMDS treated), 6 ft × ⅛ in. stainless steel column; column temperature 90° for first 3 min (effluent vented during this time), then programmed to 170° at 10°/min; Coulson furnace at 350°; carrier gas (He) flow, 30 ml/min; a Varian gas chromatograph, Model 2700, connected to a Coulson electrolytic conductivity detector (pyrolytic mode) was used; detector voltage, 30; and attenuator at 1.

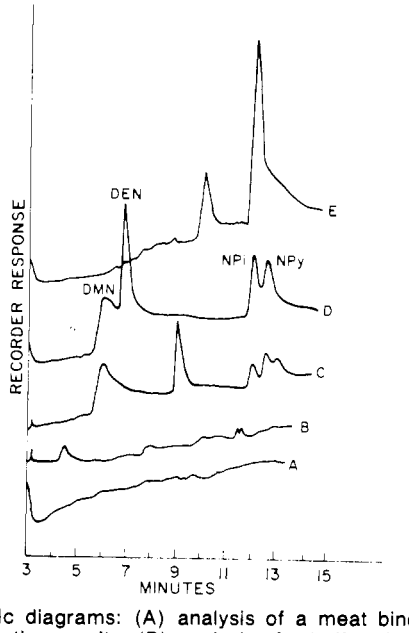

Figure 2. Glc diagrams: (A) analysis of a meat binder premix showing negative results; (B) analysis of a buffered sample (no. 7, Table I) after storing for 34 days at 23°; (C) same sample (no. 8 in Table I) nonbuffered after 47 days at 38°; (D) nitrosamine standards (12 ng each); and (E) a nonbuffered sample (no. 2 in Table I) after 43 days at 38°. Glc conditions are the same as in Figure 1.

tailing and not well resolved, we were able to carry out semiquantitative estimations without much difficulty. It can be seen from Figure 4 that peak heights are proportional to the amount injected, and by comparing peak heights we were able to estimate the nitrosamine levels in an unknown with an accuracy of ±10%. As shown for the

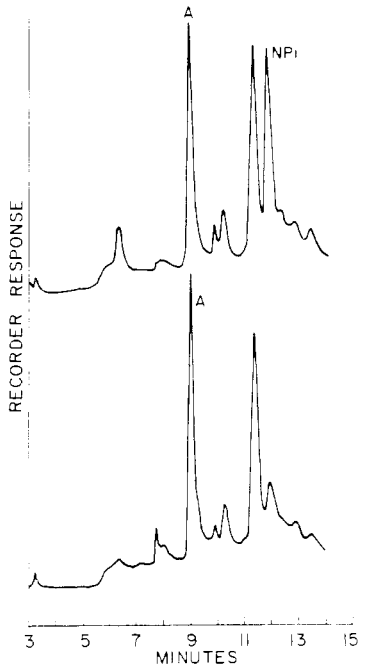

Figure 3. Glc diagrams showing the effect of ascorbate on the formation of nitrosamines in samples 3A and 3B (Table II). Top diagram: ground black pepper plus nitrite and nitrate (3B). Bottom diagram: same sample with added ascorbate (3A). Peak A is due to the added nitrosodipropylamine (internal standard) showing similar recoveries in the two samples. The addition of ascorbate markedly diminished the NPi level. NPy peaks are not visible due to inadequate sensitivity of the detector for this compound. Conditions are similar to those in Figure 1.

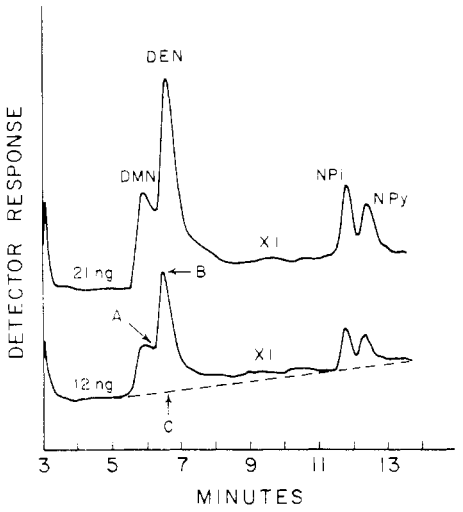

Figure 4. Glc diagrams showing the quantitative relationship between peak heights and amounts injected. For details see text.

DEN peak, the peak height can be measured either from the point B to A or B to C. In some cases, we were able to predict the amount of nitrosamines with an accuracy of ±2%.

Table I summarizes the results of the nitrosamine analysis in the buffered and nonbuffered samples of premixes. The following conclusions can be drawn from these results. (a) For each group of sample the buffered premix always contained lower levels of nitrosamines than the nonbuffered one stored under similar conditions. This is particularly noticeable in samples stored at 38°. (b) As expected, the samples stored at 38° formed higher levels of nitrosamines than those at 23°. (c) Although the buffering markedly reduced the formation of nitrosamines it did not completely prevent it. For example, two (no. 3 and 5) out of four buffered samples formed significant levels of nitrosamines within 30–40 days of storage at 38°—a temperature that is not unusual to encounter in warehouses during the summer months. Although the same samples at 23° were negative initially, subsequent analysis after 105 days of storage at 23° indicated the formation of NPy.

The data (Table II) on the effect of sodium ascorbate on the formation of nitrosamines, however, are not so clear-cut. In some samples (no. 3A–4B) ascorbate seemed to reduce the formation of nitrosamines to a small extent; however, it had an opposite effect in the case of the other samples (no. 1A–2B). The reason for this discrepancy is not clear. It should be mentioned here that the former group of samples was prepared on a small scale by mixing in a glass mortar, whereas the latter samples were obtained from the commercial firm C. Therefore, the method of mixing as well as the different brands of spices used in the two groups may account for the difference in results obtained. It is, however, clearly evident that ascorbate is not at all effective in preventing nitrosamine formation in these premixes.

The nitrosamine contents of several commercial premixes are given in Table III; the values ranged between very low (traces) to fairly high levels (up to 25 ppm). As we mentioned earlier, the concentration of nitrosamines formed depends on many factors such as the nature of the spices, temperature and duration of storage, and the presence or absence of certain additives. It is, therefore, very difficult to compare the results of one sample to those of another. In general, we observed that premixes containing only ground black pepper and/or paprika, with no other additives or fillers, formed the highest levels of nitrosamines. Also, there was some difference in results between different brands (origin) of spices used. For example, the sample of Spanish paprika (4B in Table II) formed nitrosamines very rapidly; the concentration of NPy reached 4 ppm within 15 days of storage at 38°. It may be of interest that certain samples did not form detectable levels of nitrosamines when stored at 4° even after storage for a long time. However, when the same samples were brought out of the refrigerator and stored at 23° for an additional 50–56 days three out of four samples gave positive results for nitrosamines. Besides the 12 samples in Table III, 13 more commercial samples were analyzed; of these 7 contained traces of either NPy or NPi and the remaining were negative. The five samples of premixes that contained only nitrate were also negative. These latter samples contained traces of nitrite, which may have resulted from the chemical or microbiological degradation of nitrate, but the levels of nitrite present were probably not high enough to form detectable levels of nitrosamines within the storage period of the experiment.

In conclusion, this study suggests that the practice of premixing nitrite with spices or spice extracts can be a hazard due to the formation of nitrosamines. Although buffering at pH 7.5–8.2 seems to reduce the formation of nitrosamines, the technique is not completely reliable. Under adverse storage conditions (*e.g.*, long storage or storage at warm temperature) traces of nitrosamines can be formed even in buffered samples. Therefore, it would be advisable to package nitrite separately from spices, spice extracts, and other proteinaceous materials such as milk powder, hydrolyzed protein, etc., and separately add these components to meat products at the time of processing. As nitrite-spice premixes are used in many kinds of meat products (sausages, salami, frankfurters, canned meat, meat loaf, etc.) the removal of these curing formulations would eliminate a major source of nitrosamines and make the final products much safer for human consumption.

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COMMUNICATIONS

Control of Cooked Flavor in High-Temperature Short-Time Milk Concentrates with a Sulfhydryl-Blocking Agent

Pilot plant experiments confirmed laboratory findings on the ability of organic thiolsulfonates to suppress the cooked flavor in high-temperature short-time (HTST) sterile milk concentrates. Specifically, the flavor of treated samples was improved markedly with respect to that of their untreated counterparts when 2-acetamidoethyl 2-acetamidoethanethiolsulfonate (AETS), currently not on the list of approved food additives, was added at the rate of 5 mg/100 ml of reconstituted whole milk in the operational sequence: forewarming, concentration, sterilization, homogenization, addition of AETS, and canning. Comparative keeping qualities (up to 85 days) of control and treated samples were determined at 4.4 and 21° by a panel of selected judges. Using a 10-point scale, ranging from 31 to 40, the average score of freshly made treated samples was about 3 units higher than that of freshly made untreated samples. The score difference tended to decrease slowly as the storage time increased and other off-flavors (mostly "stale") arose. No additional foreign flavors were observed in the samples treated with AETS.

Flavor problems that develop during processing and storage of high-temperature short-time (HTST) and ultra-high-temperature (UHT) sterile milk concentrates are a major obstacle to their commercialization as satisfactory beverages. The distinct cooked flavor present in recently made sterile milks fades on storage, and is replaced by another less clearly defined off-flavor which is often described as "stale." Much evidence shows that the cooked flavor is due to volatile sulfur compounds, primarily thiols, that arise on thermal breakdown of serum proteins and of the proteinaceous material associated with the fat globule membrane (Hutton and Patton, 1952; Josephson, 1954; Patton, 1958). Some speculative pathways which ascribe a key role to the Strecker degradation (Schönberg and Moubacher, 1952), and which contemplate the participation of certain natural milk constituents or of intermediate products of the Maillard reaction (Ellis, 1959; Maillard, 1912), were discussed previously (Ferretti, 1973).

A search of the literature failed to reveal any serious attempt to control cooked flavor in fluid dairy products by chemical means. Ferretti (1973) showed that the use of sulfhydryl-blocking agents, such as organic thiolsulfonates and thiosulfates, can reduce significantly the intensity of the cooked flavor that developed when milk was heated at 90° for 5 min at atmospheric pressure. Next we wanted to determine whether the same chemicals would be equally effective under pilot plant conditions.

EXPERIMENTAL SECTION

Equipment, Materials, and Methods. Plant equipment consisted of the following units: a Wiegand-Harris tubular falling film evaporator; a Mallory-type sterilizer consisting of heating, holding, and cooling sections; a high-pressure homogenizer operating at 210 kg/cm^2 and at 64°. Products were canned aseptically in a presterilized glove box fitted with a 20-l. Pyrex glass reservoir for collecting the sterile concentrate, and equipped with a 50-ml stainless steel buret and a can seamer.

Milk was obtained from the Beltsville, Md., dairy herd (U. S. Department of Agriculture). The polyphosphate used to control gelation (Leviton *et al.*, 1963) was sodium hexametaphosphate (Fisher Scientific Co., Fairlawn, N. J.). The cooked flavor inhibitor was 2-acetamidoethyl 2-acetamidoethanethiolsulfonate (AETS) which had been the most effective in the preliminary study (Ferretti, 1973); it was prepared as reported previously (Field *et al.*, 1961).

Procedure. Two sequences were used for HTST processing: (1) forewarming, concentration, addition of AETS, sterilization, homogenization, and canning; this will be referred to as sequence 1; (2) forewarming, concentration, sterilization, homogenization, addition of AETS, and canning; this will be referred to as sequence 2.

In processing according to sequence 1, milk was fore-