

Solid-Phase Extraction Method for Volatile *N*-Nitrosamines in Hams Processed with Elastic Rubber Netting

A method was developed for the determination of volatile *N*-nitrosamines in hams processed in elastic rubber nettings. The method was based on a modification of a solid-phase extraction (SPE) procedure used in the past to determine selected nitrosamines in different types of cured meat products. The nitrosamines detected in ham most likely originate from the amine precursors in rubber and from the nitrite commonly used in the meat curing process. The method was compared with 2 established procedures for *N*-nitrosodibutylamine (NDBA) analysis in cured meat products: the mineral oil distillation procedure (MOD) and the low temperature vacuum distillation procedure (LTVD). All 3 methods used the same gas chromatographic/chemiluminescent detection conditions and system. No significant difference was found between the MOD and LTVD methods. These methods were found to yield significantly higher NDBA levels than the SPE procedure. When 2,6-dimethylmorpholine was added to the sample before analysis in the MOD and LTVD procedures, artifactual nitrosamines were formed. No artifactual formation was noted in the SPE method. We propose that the new SPE method replace the current methods being used for analysis of netted, cured meat products.

Fajen et al. (1) first reported volatile *N*-nitrosamines in the air of rubber and tire manufacturing plants in 1979. Since then, several reports have been published about detection of nitrosamines in the precursors used in the production of natural and synthetic rubber (2, 3) and in the finished products themselves. The source of these nitrosamines was attributed to rubber vulcanization accelerators, which contain a dialkylamine or acyclic amino group. The nitrosamines found range from simple dialkyls such as *N*-nitrosodimethylamine (NDMA), *N*-nitrosodiethylamine (NDEA), and *N*-nitrosodi-

butylamine (NDBA) to alicyclics such as *N*-nitrosopiperidine (NPIP), *N*-nitrosopyrrolidine (NPYR), and *N*-nitrosomorpholine (NMOR). For instance, Lakritz and Kimoto (4) reported nitrosamines in rubber-stoppered blood collection tubes, and Fiddler et al. (5) found them in disposable rubber gloves. Ireland et al. (6) found nitrosamines in a wide variety of finished rubber products including gloves and condoms. The most widely publicized reports concerned their detection in infant pacifiers and baby bottle nipples (7-9). Regulatory action initially limited the total nitrosamine content in the rubber nipples to 60 ppb (10). This was eventually lowered to 10 ppb, and the rubber industry complied with this limit (11). A similar reduction in nitrosamine content of nipples and pacifiers was observed by Sen et al. in Canadian investigations (12, 13).

The finding of nitrosamines in rubber products raised concern about the possible hazards of rubber-containing products in contact with food and the possible migration of preformed nitrosamines into the food. Sen et al. (14) reported finding NDEA and NDBA in cured meats held in elastic rubber netting during smokehouse processing. They found trace quantities of these nitrosamines in the unused netting and high levels (up to 504 ppb NDBA) in the used netting. The corresponding meat samples also contained NDBA (up to 29 ppb). Recently, the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture, while examining a new process for preparing hams, found significant levels of NDBA in the product. This was attributed to rubber in the elastic netting.

A comprehensive FSIS monitoring program of hams and other products processed in these elastic rubber nettings is expected to determine the extent of the occurrence of nitrosamines, before regulatory action. However, the available methodology creates problems with conducting an extensive survey. First, only limited numbers of samples can be analyzed by methods currently in use by FSIS. Second, the reliability of this methodology needs to be demonstrated for volatile nitrosamines, particularly NDBA. Third, the simultaneous presence of both nitrite in the cured meat product and amine from the rubber, which may have migrated into the meat product, may artifactually produce NDBA as a result of analysis (14). Therefore, an alternative method based on a nondistillation technique had to be developed to minimize the potential for artifact formation. This method was then compared with those currently in use.

METHOD

Caution: *N*-Nitrosamines are potential carcinogens. Exercise care in handling these compounds.

Reagents

(a) *Celite 545*.—Not acid-washed (Fisher Scientific Co.). Test reagent blank before starting sample analysis, particularly if new lot of Celite is used. If interfering chromatographic peaks are noted, prewash twice with dichloromethane (DCM), filter, then dry 4 h in 120°C vacuum oven before use.

(b) *Sodium sulfate*.—Anhydrous, granular (Mallinckrodt No. 8024).

(c) *Silica gel*.—70–230 mesh (EM 7734). Prewash twice with DCM, filter, and dry 4 h in 60°C vacuum oven before use.

(d) *Propyl gallate*.—Aldrich Chemical Co.

(e) *Morpholine*.—Aldrich Chemical Co., doubly distilled before use to remove traces of NMOR.

(f) *2,6-Dimethylmorpholine*.—Aldrich Chemical Co., checked for nitrosamine contamination before use.

(g) *Dichloromethane (DCM), pentane, ethyl ether*.—LC grade (Burdick and Jackson).

(h) *N-Nitrosodipropylamine (NDPA) internal standard solution*.—0.10 µg/mL in DCM.

(i) *Gas chromatography working standard solution*.—NDMA, *N*-nitrosomethylethylamine (NMEA), NDEA, NDPA, *N*-nitrosoazetidine (NAZET), NDPA, NPIP, NPYR, NMOR, and *N*-nitrosohexamethyleneimine (NHMI), each 0.10 µg/mL in DCM. These nitrosamines were either purchased or synthesized from their corresponding amines and sodium nitrite according to general procedure published previously (15). *N*-Nitroso-2,6-dimethylmorpholine was also synthesized as above.

(j) *Ham samples*.—Random samples were obtained from local suppliers or FSIS and analyzed without further heating. Two samples were obtained from each ham: outer 1/4 in. and second 1/4 in. of product. Grind samples through 1/16 in. plate before analysis and store in -20°C freezer until analyzed.

Apparatus

(a) *Mortar and pestle*.—Glass, 473 mL (16 oz., A.H. Thomas).

(b) *Chromatographic columns*.—(1) Glass, 350 × 32 mm id with 60 × 6 mm id drip tip, no stopcock, prepared by glassblower. (2) Glass, 300 × 19 mm with 250 mL reservoir (Lurex Scientific).

(c) *Tamping rod*.—Glass, 450 mm long with 12 mm diameter disk on end, prepared by glassblower.

(d) *Evaporative concentrator*.—Kuderna-Danish (K-D), 250 mL; concentrator tube, 4 and 10 mL, Snyder (3-section) and micro-Snyder distilling columns (Kontes Glass Co.).

(e) *Gas chromatograph-Thermal Energy Analyzer (GC-TEA)*.—Shimadzu gas chromatograph Model GC-14A equipped with AOC-14 auto-injector or equivalent, interfaced to Thermal Energy Analyzer Model 502A (Thermedics, Inc.). Operating conditions: 2.7 m × 2.6 mm glass column packed with 15% Carbowax 20M-TPA on 60–80 mesh Gas Chrom P;

He carrier gas 35 mL/min; injector 180°C; TEA furnace 475°C; TEA vacuum 0.4 mm; liquid nitrogen cold trap; column programmed from 120 to 200°C at 4°C/min.

Determination

(a) *Solid-phase extraction (SPE)*.—Weigh 10.0 ± 0.1 g sample into mortar, and add 250 mg propyl gallate directly to sample. Spike sample with 1.0 mL internal standard solution (equivalent to 10 ppb), using transfer pipet. Add 25 g anhydrous sodium sulfate and mix with pestle ca 15 s; then add 20 g Celite and again mix with pestle 15–20 s until Celite is thoroughly mixed with sodium sulfate and sample. Grind entire mixture with moderate pressure for additional 1 min. Using powder funnel, quantitatively transfer mixture into glass column (350 × 32 mm) containing glass wool plug at bottom. Tamp with tamping rod to achieve height of ca 75 mm. Add 20 g anhydrous sodium sulfate to top of column. Rinse mortar, pestle, and tamping rod with 20 mL DCM and add rinse to top of column. Immediately add additional 130 mL DCM to column (column will darken when solvent elutes through it). Collect eluate in 250 mL K-D flask equipped with 10 mL concentrator tube. When column stops dripping, remove K-D flask (discard contents of glass column), add boiling chip, attach Snyder column, and concentrate eluate on steam bath until DCM stops distilling. There will be ca 3–7 mL of concentrate remaining in concentrator tube. Add 4.0 g silica gel to glass column (300 × 19 mm with 250 mL reservoir) containing glass wool plug and 25 mL pentane, and top it with 5.0 g anhydrous sodium sulfate. Using disposable glass pipet, quantitatively transfer concentrate to silica gel column; then rinse concentrator tube with two 4 mL portions of pentane and add to column. Collect eluate in 250 mL Erlenmeyer flask (flow rate ca 2–3 drops). When liquid level in column reaches top of sodium sulfate, add 150 mL wash mixture (25% DCM in pentane). When liquid level in column again reaches top of sodium sulfate, change collection vessel to 250 mL K-D flask equipped with 4 mL concentrator tube (discard contents of Erlenmeyer flask). Add 150 mL elution solvent (30% ether in DCM). When column stops dripping, remove K-D flask, add boiling chip, attach Snyder column, and concentrate on steam bath to 4 mL. Remove Snyder column and K-D flask, add new boiling chip, attach micro Snyder column, and concentrate to 1.0 mL in 70°C water bath. Do not concentrate sample with stream of nitrogen. (Note: Room temperature should be less than 24°C during SPE procedure.)

(b) *Low temperature vacuum distillation (LTVD)*.—Samples were analyzed by technique developed by Sen et al. (16) and described in detail in *USDA, FSIS Chemistry Laboratory Guidebook* (17). Briefly, 25 g sample, without any nitrosation inhibitors, was distilled under vacuum (20 torr) from base in 2 L pear-shaped flask immersed in 45–46°C water bath. Aqueous distillate was acidified and extracted with DCM. DCM was washed with acid and base, dried with anhydrous sodium sulfate, and concentrated.

(c) *Mineral oil distillation (MOD)*.—Samples were analyzed by method originally developed by Fine et al. (18) as specified in *USDA, FSIS Chemistry Laboratory Guidebook*

Table 1. Recovery of volatile *N*-nitrosamines in ham at the 10 ppb fortification level

<i>N</i> -Nitroso compound	Recovery, %			
	Range	Mean (<i>n</i> = 12)	SD	CV
Dimethylamine	67.8–96.0	81.7	8.7	10.7
Methylethylamine	67.0–91.0	77.9	6.4	8.3
Diethylamine	68.9–90.5	77.8	6.9	8.9
Dipropylamine	71.4–108.2	89.6	10.5	11.7
Azetidine	77.5–105.4	91.7	8.6	9.4
Dibutylamine	72.0–102.4	87.7	11.2	12.8
Piperidine	85.8–105.6	96.0	6.7	7.0
Pyrrolidine	83.3–109.1	97.5	9.1	9.3
Morpholine	81.2–102.4	94.8	7.4	7.8
Hexamethylenimine	85.6–109.5	99.5	7.6	7.7

(19). Briefly, 25 g sample, without any nitrosation inhibitors, was distilled under vacuum (<2 torr) from base and mineral oil to temperature of 120°C. Aqueous distillate was extracted with DCM, dried with anhydrous sodium sulfate, and concentrated.

(d) *Nitrosamine determination*.—Quantitate volatile nitrosamines as described previously (20), using 5.0 µL injection. Minimum detectable level (signal:noise >2) of NDMA, NMEA, and NDEA, 0.2 ppb; NAZET, NPIP, NPYR, NMOR, and NHMI, 0.5 ppb; and NDBA, 1.0 ppb.

(e) *Sodium nitrite analysis*.—Residual sodium nitrite was determined in 10.0 g sample by Griess-Saltzman procedure as modified by Fiddler (21).

(f) *Statistical analysis*.—Data were analyzed by General Linear Model and Means procedures (ANOVA and Student's paired *t*-test) of Statistical Analysis System PC software distributed by SAS Institute, Inc. (22). These results were then interpreted according to methods of Snedecor and Cochran (23) and Youden and Steiner (24).

Results and Discussion

There is an ongoing need to improve and expand the capabilities of the methodology used in the analysis of cured meat products for volatile nitrosamines, with assurance that nitrosamines will not artifactually form during analysis. We have previously shown that our solid-phase extraction (SPE) procedure is versatile. It enabled us, with solid support and solvent modifications, to determine NPYR in pumped and dry-cured bacon (20, 25) and nitrosoamino acids in a variety of cured meat products (26). The analysis of frankfurters containing fish protein in the form of Alaska pollock mince and surimi posed a special problem with regard to artifactual NDMA formation because of the presence of both nitrite in the meat and dimethylamine in the fish. This problem was resolved by using 2 chromatographic columns. In the first, the amine and nitrosamine were separated from the nitrite-containing sample, and in the second, the nitrosamine was isolated from the retained amine (27). Although this SPE method has been used for the isolation and quantitation of selected nitrosamines in specific sample types, its potential applicability has not been fully investigated. For

example, the SPE method with acid-Celite in the bottom column of a 2-column system could only be used to isolate NDMA, NAZET, NPYR, and NMOR, because of the acidified Celite's retention characteristics. To isolate any other nitrosamines, a third column containing silica gel or alumina was required (20, 27). The use of a second column containing silica gel was based on a modification of the method originally developed by White et al. (28). Because NDBA was the nitrosamine of primary interest in the elastic-netted cured meat products, a modification in the solid support was required. First, changing the acid-to-Celite ratio was tried, but NDBA was not retained. Next, substituting silica gel for acid-Celite in the lower column was attempted. The amount of silica gel in the lower column and the solvent system used to elute the NDBA contained in the lipids were both varied, but there was still too much lipid material in the extract for quantitation to be practical. Therefore, this approach was abandoned. The use of an SPE column containing the meat sample, anhydrous sodium sulfate, and Celite with direct DCM extraction followed by a separate silica gel column was found to give the best results.

A ruggedness test of the SPE procedure was performed on ham containing naturally incurred 15.6 ppb NDBA. Deviations in the normal grinding, packing, and solvent elution steps in the first column and packing and elution steps in the second column indicated that the results were not significantly different except for the effect of room temperature. When the room temperature exceeded 24°C, the use of the pentane-containing solvent system with the silica gel column caused separating and channeling. This resulted in lower recoveries of both NDBA and the internal standard, NDPA. In addition, during the development of this method, 50 ppm morpholine, a rapidly nitrosated amine, was added to the sample before analysis to assess artifact formation; no NMOR was detected.

The recoveries of 10 volatile *N*-nitrosamines added to nitrosamine-free ham at the 10 ppb level are shown in Table 1. Recovery of NDBA, the nitrosamine commonly found in netted hams, was 88%. The mean recovery of all other nitrosamines was >78%. Statistical analysis of the data by Student's paired *t*-test showed no significant difference in recovery between NDPA and NDBA ($P < 0.05$, $n = 12$). For this reason, and be-

Table 2. Determination of *N*-nitrosodibutylamine in netted ham by 3 methods

Sample	NaNO ₂ , ppm	SPE ^a		LTVD ^a		MOD ^a	
		NDPA, %	NDBA, ppb ^b	NDPA, %	NDBA, ppb ^b	NDPA, %	NDBA, ppb ^b
A	ND ^c	83.7	15.8	87.8	19.6	106.0	18.7
B	1.0	87.9	22.2	89.8	28.8	101.3	33.0
C	1.5	104.6	41.8	84.6	53.6	83.1	55.3
D	1.6	86.3	14.4	95.1	26.6	112.9	24.5
E	1.9	95.9	19.9	88.5	23.6	83.4	23.4
F	2.1	80.5	49.9	97.5	50.9	97.3	37.3
G	2.5	85.3	26.3	85.6	30.2	95.6	32.1
H	3.5	94.5	17.8	88.1	15.8	97.2	17.9
I	5.1	90.0	10.8	81.4	10.7	98.8	10.6
J	7.6	93.9	22.5	87.5	22.9	102.6	26.0
K	10.1	84.3	50.1	83.4	43.5	98.7	54.6
L	12.1	95.7	22.4	85.0	28.6	89.0	30.7
M	12.7	83.1	18.2	92.0	15.3	104.6	18.2
N	13.4	89.6	11.1	91.7	9.8	97.4	10.4
O	16.0	83.9	14.0	101.3	16.6	92.5	15.0

^a Results are averages of duplicate determinations.

^b Data corrected for recovery of the NDPA internal standard.

^c ND, none detected, <1 ppm.

cause NDPA has not been reported in any food or rubber products and is used as the internal standard in the MOD and LTVD methods, it was chosen as the internal standard for our SPE procedure.

After the reliability of the SPE procedure was determined, NDBA was determined in commercial ham samples in duplicate by each of 3 methods: SPE, MOD, and LTVD. Results, averaged over 2 determinations, are shown in Table 2. Residual sodium nitrite was also determined in all 15 hams. No statistical correlation ($P < 0.05$) was found between residual nitrite and NDBA values in any of the methods. Individual NDBA values ranged from 10.3 to 51.2 ppb for SPE, 9.6 to 54.8 ppb for LTVD, and 10.3 to 58.3 ppb for MOD. Mean recoveries for the internal standard were 89.3, 89.3, and 97.3% for the SPE, LTVD, and MOD methods, respectively. Data were analyzed by ANOVA, and the means of the methods were further examined by Duncan's multiple range test at the $P < 0.05$ level. The repeatabilities were as follows: 1.3 ppb, CV 6.2% (0.7 ppb, CV 2.8%, corr.) for the SPE procedure; 2.65 ppb, CV 11.2% (1.5 ppb, CV 5.8%, corr.) for the LTVD procedure; and 1.6 ppb, CV 6.0% (2.26 ppb, CV 8.3%, corr.) for the MOD procedure.

As shown in Table 3, with the uncorrected data, the methods were significantly different from each other. With the data corrected for the recovery of the internal standard, no significant difference between the MOD and LTVD was detected; however, the SPE differed significantly from both. The MOD and LTVD values in both the uncorrected and corrected data were higher than the SPE data. This suggests artifactual formation of NDBA during the MOD and LTVD sample analysis. The procedures currently being used by FSIS to determine NDBA in ham samples (MOD and LTVD) do not use any nitrosation inhibitors during analysis. The SPE procedure uses propyl gallate to inhibit artifact formation. The MOD and LTVD methods

rely on alkalization to prevent artifact formation during distillation, but Challis and Kyrtopoulos have shown that nitrosation can occur even under alkaline conditions (29).

To determine whether nitrosamines could form artifactually in any of these procedures, 50 ppm 2,6-dimethylmorpholine, a rapidly nitrosated secondary amine, was added to several ham samples before analysis. *N*-Nitroso-2,6-dimethylmorpholine was detected in 11 of 11 samples analyzed by the MOD method (7.0–492.0 ppb; mean, 73.1 ppb) and in 4 of 6 samples analyzed by the LTVD method (8.5–36.8 ppb; mean, 23.8 ppb). None was detected in 10 of 10 samples analyzed by the SPE procedure. Artifactual nitrosamine formation during MOD analysis was previously demonstrated when additional nitrite or amine was added to the cured meat samples before analysis (20, 24, 30). There is an indication that nitrosating species can be generated in cured meat products even if the measured residual nitrite is low or not detected. For example, Hotchkiss et al. (31) demonstrated that lipid–nitrite reaction products have nitrosative ability, and others have successfully formed nitrosamines by transnitrosation of nitrosothiols (29, 32). Therefore, artifact formation during MOD and LTVD was not completely unexpected.

Table 3. Comparison of the 3 methods for analysis of NDBA in hams

	<i>n</i>	SPE	LTVD	MOD
Mean	30	21.23 ^a	23.54 ^b	26.12
Mean (Corr.)	30	23.80 ^a	26.43 ^b	27.19 ^b

^a Not significantly different ($P < 0.05$) from each other.

^b Not significantly different ($P < 0.05$) from each other.

In conclusion, the newly developed SPE procedure is distillation-free, offers an opportunity to perform more analyses than the current methods, and gives good recoveries for a wide variety of volatile nitrosamines. It is not susceptible to artifactual nitrosamine formation, as might occur when the sample contains either high levels of residual nitrite or a nitrosamine precursor. Therefore, we propose that this SPE procedure represents a reliable alternative to the MOD and LTVD methods for determining nitrosamines in cured meat products processed in elastic rubber nettings.

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References

- (1) Fajen, J.M., Carson, G.A., Rounbehler, D.P., Fan, T.Y., Vita, R., Goff, U.E., Wolf, M.H., Edwards, G.S., Fine, D.H., Reinhold, V., & Biemann, K. (1979) *Science* **205**, 1262–1264
- (2) Yeager, F.W., VanGulick, N.N., & Lasoski, B.A. (1980) *Am. Ind. Hyg. Assoc. J.* **41**, 148–150
- (3) Spiegelhalter, B. (1983) *Scand. J. Work. Environ. Health* **9**, 15–25
- (4) Lakritz, L., & Kimoto, W.I. (1980) *Food Cosmet. Toxicol.* **18**, 31–34
- (5) Fiddler, W., Pensabene, J.W., & Kimoto, W.I. (1985) *Am. Ind. Hyg. Assoc. J.* **46**, 436–465
- (6) Ireland, C.B., Hytrek, F.P., & Lasoski, B.A. (1980) *Am. Ind. Hyg. Assoc. J.* **41**, 895–900
- (7) Havery, D.C., & Fazio, T. (1983) *J. Assoc. Off. Anal. Chem.* **66**, 1500–1503
- (8) Babish, J.G., Hotchkiss, J.H., Wachs, T., Vecchio, A.J., Gutenmann, W.H., & Lisk, D.J. (1983) *J. Toxicol. Environ. Health* **11**, 167–177
- (9) Osterdahl, B.-G. (1983) *Food Chem. Toxicol.* **21**, 755–757
- (10) *Fed. Regist.* (1984) **49**, 50789–50790
- (11) Havery, D.C., & Fazio, T. (1985) *Food Technol.* **39**, 80–83
- (12) Sen, N.P., Kushwaha, S.C., Seaman, S.W., & Clarkson, S.G. (1985) *J. Agric. Food Chem.* **33**, 428–433
- (13) Sen, N.P., Seaman, S.W., Baddoo, P.A., & Weber, D. (1988) *J. Food Sci.* **53**, 731–734
- (14) Sen, N.P., Baddoo, P.A., & Seaman, S.W. (1987) *J. Agric. Food Chem.* **35**, 346–350
- (15) Pensabene, J.W., Fiddler, W., Dooley, C.J., Doerr, R.C., & Wasserman, A.E. (1972) *J. Agric. Food Chem.* **20**, 274–277
- (16) Sen, N.P., Seaman, S.W., & Miles, W.F. (1979) *J. Agric. Food Chem.* **27**, 1354–1357
- (17) *USDA, FSIS Chemistry Laboratory Guidebook* (1986) Revised Ed., U.S. Department of Agriculture, No. 5.021, 5–113 to 5–119
- (18) Fine, D.H., Rounbehler, D.P., & Oettinger, P.C. (1975) *Anal. Chim. Acta.* **78**, 383–389
- (19) *USDA, FSIS Chemistry Laboratory Guidebook* (1986) Revised Ed., U.S. Department of Agriculture, No. 5.020, 5–105 to 5–112
- (20) Pensabene, J.W., Miller, A.J., Greenfield, E.L., & Fiddler, W. (1982) *J. Assoc. Off. Anal. Chem.* **65**, 151–156
- (21) Fiddler, R.N. (1977) *J. Assoc. Off. Anal. Chem.* **60**, 594–599
- (22) SAS (1985) *SAS User's Guide: Statistics*, SAS Institute, Inc., Cary, NC
- (23) Snedecor, G.W., & Cochran, W.G. (1979) *Statistical Analysis*, 6th Ed., Iowa State University Press, Ames, IA
- (24) Youden, W.J., & Steiner, E.H. (1975) *Statistical Manual of the Association of Official Analytical Chemists*, AOAC, Arlington, VA
- (25) Gates, R.A., Pensabene, J.W., & Fiddler, W. (1984) *J. Assoc. Off. Anal. Chem.* **67**, 236–239
- (26) Pensabene, J.W., & Fiddler, W. (1990) *J. Assoc. Off. Anal. Chem.* **73**, 226–230
- (27) Pensabene, J.W., & Fiddler, W. (1988) *J. Assoc. Off. Anal. Chem.* **71**, 839–843
- (28) White, R.H., Havery, D.C., Roseboro, E.L., & Fazio, T. (1974) *J. Assoc. Off. Anal. Chem.* **57**, 1380–1382
- (29) Challis, B.C., & Kyrtopoulos, S.A. (1976) *J. Chem. Soc. Commun.* **21**, 877–878
- (30) Pensabene, J.W., & Fiddler, W. (1982) *J. Assoc. Off. Anal. Chem.* **65**, 1346–1349
- (31) Hotchkiss, J.H., Vecchio, A.J., & Ross, H.D. (1985) *J. Agric. Food Chem.* **33**, 5–8
- (32) Dennis, M.J., Davies, R., & McWeeney, D.J. (1976) *J. Food Sci.* **30**, 639–645