Chromatographic Analyses of Volatile Amines in Marine Fish

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Amines, extracted from tissues of sablefish (*Anoplopoma fimbria*), coho salmon (*Oncorhynchus kisutch*), and salmon roe (genus, *Oncorhynchus*), were analyzed by gas chromatography, and also by thin-layer chromatography–spectrophotofluorometry of the corresponding 5-dimethylamino-1-naphthalenesulfonamides. The data provide evidence for the presence of dimethylamine in long-stored frozen salmon flesh and its absence in sablefish flesh above a detection limit of 7 ng/g wet tissue. The methylamines and diethylamine were the only amines confirmed at levels above the limits of detection.

Dimethylamine and trimethylamine in fish have been important for more than three decades in problems related to spoilage and effects of bacterial action in fish flesh during storage (Shewan, 1937, 1938; Beatty and Collins, 1940; Dyer and Mounsey, 1945). During recent years, methylamine, diethylamine, triethylamine, *n*-propylamine, and *n*-butylamine, among several other primary, secondary, and tertiary amines, also have been noted in some fishery products (Sato *et al.*, 1963; Minakowski and Rzewuski, 1965; Wick *et al.*, 1967; Golovnya *et al.*, 1967a,b; Amano *et al.*, 1968). Unfortunately, quantitative information on the possible variety of amines in various fish and fishery products is minimal or nonexistent.

The quantitation of secondary amines in fish and other food products is of considerable relevance in view of nitrite-treated products and the reported involvement of the amines as potential precursors of carcinogenic nitrosamines (Ender *et al.*, 1967; Sander *et al.*, 1968; Lijinsky and Epstein, 1970). The role of nitrite in nitrosations in foods is complex and not well understood. Available evidence indicates that nitrite content drops drastically from initial concentrations in treated foods. Nonetheless, when pH and other conditions of the food media are right for nitrous acid formation from the salt, the classical amine reactions for nitrosations may occur (Geuther, 1863, 1864, 1865; Hein, 1963).

The purpose of the research, reported here, was to develop a procedure for the isolation of amines from fish tissues and to identify and quantify the amines, using readily available instrumental techniques. Mass spectrometry was not available. As in all analytical methods, the procedures given herein are not without certain limits of sensitivity and detection. Identification of amines was performed by combined thin-layer chromatography (tlc) and spectrophotofluorometry and by gas–liquid chromatography (glc). Quantitation was performed by glc. Tissue proteins were separated from organic base salts and other compounds by the method of Spinelli *et al.* (1964). Amines were recovered by steam distillation and concentrated as hydrosulfate salts by freeze-drying. Spectrophotofluorometric detection of the corresponding derivatives of 5-dimethylamino-1-naphthalenesulfonamides (DANS-amides; Seiler and Wiechmann, 1970) was performed directly on tlc plates. Results from the glc analyses on two separate columns are reported.

The development of the analytical procedures for amines in fish tissues was performed in four stages as follows: the establishment of an optimum set of conditions for the qualitative glc analyses of steam-volatile amines which are expected in fish; the confirmation of amine identifications by tlc–spectrophotofluorometry; the quantitation of amines by glc; and the examination of recoveries of the amines from other substances in fish tissues.

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EXPERIMENTAL

Materials. All chemicals were reagent grade (Eastman Organic Chemicals Division), except as specifically noted, and were used without additional purification. Mono-, di-, and trimethylamine were obtained as the hygroscopic hydrochlorides and were dried and stored over anhydrous calcium sulfate prior to use. Pyrrolidine, piperidine, and isopentylamine were obtained as practical grade chemicals (Eastman) and were used without further purification. *n*-Decane (99% purity) was obtained from Applied Science Laboratories, Inc.

Coho salmon (*Oncorhynchus kisutch*), sablefish (*Anoplopoma fimbria*), and salmon roe (genus, *Oncorhynchus*) were obtained from commercial sources. The coho were troll-caught (in 1967), iced aboard the vessel, dressed, and soon afterward glazed in commercial fashion. Two glazed coho (7–8 lb each) were then stored at approximately −28°C for 37 and 45 months, respectively. A freshly caught sablefish (3 lb) was immediately iced and stored at ice temperature for 25 days. Skeins of roe were part of a large shipment kept frozen with Dry Ice; the roe (nonnitrited) were judged to be in fair condition.

Isolation of Amines. The coho were allowed to thaw to ice temperature, then flesh samples were freed from bones, skin, and dark meat, and finally 200-g portions were weighed out. The sablefish was similarly treated. The initial weighed portion of each fish was analyzed immediately, while the remaining portions were refrozen and stored at −30°C until they could be analyzed separately. Likewise, whole skeins of salmon roe (200 g) were weighed out just prior to analysis.

The following procedure was employed for the separation of amines from flesh tissues and the isolation of the amines as nonvolatile salts. A tissue sample (200 g) was placed in a 1-qt blender jar, 400 ml of 0.7 *M* $HClO_4$ was added (Spinelli *et al.*, 1964), and the mixture blended at medium–high speed for 1 min. The blended mixture was then centrifuged in 250-ml centrifuge bottles at 2000 rpm for 20 min at 10–15°C. The combined supernatant was decanted into a 2000-ml boiling flask. (Salmon roe supernatant was filtered through glass wool.) The sides of the bottles were rinsed once with distilled water. The flask was then attached to an all-glass steam distillation apparatus and steam was introduced through an 8-mm o.d. tube reaching to the bottom of the solution. A 500-ml receiving flask containing 10 ml of 3 *M* H_2SO_4 was attached to an Allihn-Kronbitter condenser (Kontes No. K-433250). The contents of the boiling flask

Table I. Relative Retention Times and Response Factors

Compound	Stationary phase[a] Chromosorb 103 130°C	Chromosorb 103 200°C	Pennwalt 223 150°C	Response factor[b] 150°C
Ammonia	...	...	0.22	...
Methylamine	0.45	...	0.28	0.53
Dimethylamine	0.75	...	0.35	0.21
Trimethylamine	1.00 (4.06)	...	0.35	0.16
Methanol	...	...	0.41	...
Isopropylamine	...	0.46	0.44	0.18
Methylethylamine		...	0.48	0.18
tert-Butylamine		0.59	0.54	0.18
n-Propylamine		0.58	0.59	0.20
Diethylamine		...	0.65	0.23
sec-Butylamine		0.81	0.78	0.23
Isobutylamine		0.85	0.85	0.26
Diisopropylamine		1.18	0.91	0.25
n-Butylamine		1.00 (2.95)	1.00 (2.70)	0.30
Triethylamine		1.39	1.05	0.33
Isopentylamine		1.59	1.44	0.40
Pyrrolidine		1.34	1.48	0.41
Di-*n*-propylamine		1.93	1.63	0.34
n-Pentylamine		1.80	1.70	0.43
Piperidine		2.25	2.22	0.54
Pyridine		...	2.53	...
Di-*sec*-butylamine		4.00	2.54	0.60
Diisobutylamine		4.20	2.57	0.63
Tri-*n*-propylamine		21.9	3.67	0.73
Di-*n*-butylamine		6.47	4.81	0.88
n-Decane		...	5.07	1.00
Methyl octanoate		...	13.4	

[a] Retention time (minutes) for calculated relative retentions are given in parentheses. [b] Factor = peak height of *n*-decane ÷ peak height of compound.

were adjusted to pH 12–13 by the addition of 12 *M* NaOH. In the case of the salmon roe, two drops of 30% emulsion of Antifoam A (Sigma Chemical Co.) were added to the boiling flask. Approximately 500 ml of distillate was collected.

The acidic distillate (*ca.* pH 2) was extracted by methylene dichloride (2 × 60 ml), followed by diethyl ether (3 × 60 ml). The extracted distillate was then warmed to 60°C and scrubbed with purified nitrogen for *ca.* 30 min or until solvent odors could no longer be detected. Finally, the odorless solution was placed in a 2000-ml lyophilization bottle, shell-frozen in a Dry Ice/*n*-propyl alcohol mixture, and the water removed in a lyophilizer (The Virtis Co., Inc.). The amine hydrosulfates were recovered as white fluffy crystals.

In order to determine the amount of amine salts recovered, the lyophilizate was redissolved in a minimum of distilled water and quantitatively transferred to a tared 100-ml pear-shaped flask. The water was removed at 40–60°C with a rotary evaporator.

A mixture of known amounts of amines was added to a sample of salmon flesh to test the suitability of the procedure for extraction and recovery of amines. The addition was performed with a hypodermic syringe by injecting an aqueous aliquot of amine salts into thick parts of the tissues. The tissue sample was then treated according to the above procedure and percentage recoveries were determined.

Gas Chromatography. A Nuclear-Chicago Series 5000 U-shaped column model gas chromatograph with dual flame ionization detectors was used. For optimum response hydrogen flow rates were 21 ml/min for detector no. 1 (column no. 1) and 40 ml/min for detector no. 2 (column no. 2). Air flow to both detectors was 430 ml/min. Column no. 1 consisted of 8-ft × 4-mm i.d. glass column packed with 28% Pennwalt 223 + 4% KOH on 80/100 mesh Gas Chrom R (Lot SP-007 of prepared amine packing by Applied Science Laboratories, Inc.). Column no. 2 consisted of a 6-ft × 6-mm i.d. glass column packed with untreated 80/100 mesh Chromosorb 103 (lot no. 6 by Johns-Manville). Nitrogen carrier gas flow rates for columns no. 1 and no. 2 were 60 ml/min and 40 ml/min, respectively. The column oven was operated isothermally at 150°C for column no. 1 and at 130°C for column no. 2, with an on-column injection port temperature of 215°C and a (dual) detector bath temperature of 240°C.

Quantitative glc was performed by an internal standardization procedure using volumetric techniques in the following manner. Amine salts (30–60 mg) were accurately weighed into a clean, dry 3-ml volumetric flask, and 0.50 ml of 6 *N* KOH was added. An aliquot (0.165 ml) of *n*-decane in methyl octanoate was accurately added to give 3.0 mg of *n*-decane. Finally, the contents of the flask were diluted to the mark with additional methyl octanoate, the flask was vigorously shaken, and a 5-μl aliquot of the methyl octanoate phase was injected into the gas chromatograph.

Detector response factors for the quantitation of amines by the peak height method were determined for equal weights of amines with respect to *n*-decane. The factors were determined from the recorded amplitude of the *n*-decane peak divided by the amplitude of each amine peak. Quantitation factors together with relative retention times for amines are listed in Table I.

Tlc and Spectrophotofluorometry. Adsorbosil-5 plates (Prekotes "B"), 20 × 20 cm × 250-μ thickness, were obtained from Applied Sciences Laboratories, Inc. The plates were activated at 110°C for 30 min and stored in a desiccator over anhydrous calcium chloride before use. Both one- and two-dimensional ascending elution techniques were used in the separation and examination of DANS-amide derivatives of the various amines. Two solvent mixtures (A and B) were used. Mixture A consisted of chloroform:ethyl acetate (75:25, v/v). Mixture B consisted of cyclohexane:ethyl acetate (70:30, v/v). Plates were cleaned by migrating the full length with solvent mixture A (allowing the solvent to evaporate at room temperature for 5 min) just prior to spotting. For two-dimensional tlc, plates were cleaned by migrating mixture A up the total length of the plate, followed by the actual analysis beginning again with mixture A. Mixture B was used for the second dimension. Compounds were viewed under a short-wavelength uv light. Spectrophotofluorometric examinations of the separated compounds were conducted directly on the tlc plates.

DANS-amide standards were prepared from ammonia and primary and secondary amines (except diisopropylamine). DANS-amides were also prepared from the amines which were isolated from the fish samples. The preparative procedure was based on a method by Seiler and Wiechmann (1970). Briefly, the procedure involved reacting *ca.* 1 mg of amine in 0.10 ml of distilled water with 1.5 mg of 5-dimethylamino-1-naphthalenesulfonyl chloride dissolved in 0.30 ml of acetone, to which mixture *ca.* 50 mg of solid $NaHCO_3$ is added. The reaction takes place in the dark overnight, after which 0.50 ml of water and 2.5 ml of ethyl acetate are added. The DANS-amides in ethyl acetate (20-μl aliquots) are spotted directly onto the tlc plates.

Spectrophotofluorometry was performed with an Aminco-Bowman Spectrophotofluorometer (SPF) equipped with a thin-film scanner and a solid-state blank-subtract photo-

multiplier microphotometer (American Instrument Co., Inc.). Spectra were recorded on an X-Y recorder (Hewlett-Packard model 7004A). The slits of the SPF were adjusted as follows: slits A, B, C, and D, fully open; slit E, 0.5 mm; and scanner slit, 1.0 mm. The emission monochromator was calibrated against Hg lines with a "Pen-Ray" quartz lamp (Ultra-Violet Products, Inc.), while the excitation monochromator was calibrated against the emission monochromator. Corrections for photodetector response were not made.

Excitation and emission spectra were recorded for the DANS-amides on tlc plates. The plates were sprayed with 2,2′,2″-nitrilotriethanol (20%, v/v) in isopropyl alcohol and allowed to air-dry in the dark at room temperature for 16 hr before scanning them by tlc–spectrophotofluorometry.

RESULTS AND DISCUSSION

Qualitative glc. The amines listed in Table I were considered the most likely to be present, based on reports by others for fishery products (Sato *et al.*, 1963; Minakowski and Rzewuski, 1965; Golovnya *et al.*, 1967a,b). The Pennwalt 223 column provides resolution of most of the amines in a test mixture. As seen in Table I, there are three critical pairs of amines when analyzing with both columns, but fortunately the pairs are separable on one or the other column. It became apparent that the only pair that required resolution was dimethylamine (DMA) and trimethylamine (TMA), for which the Chromosorb 103 packing was satisfactory.

Retention times for the amines relative to *n*-butylamine were determined for use in identifying amines recovered from the tissue samples. Methods for transferring amines to the glc column with minimal loss, while at the same time reducing peak tailing, were also investigated. When aqueous solutions of either free amines or their salts were injected, the problem of "ghost" peaks in succeeding glc analyses was encountered with the 28% Pennwalt 223 + 4% KOH column (column no. 1). Such a problem with packings containing an alkali hydroxide has been reported by others (Simonaitis and Guvernator, 1967). Ghost peaks appeared when water was injected as a control from a fresh, new syringe. Attempts to use other packings containing tetraethylenepentamine as a replacement for KOH (Sze *et al.*, 1963) were met with failure. The problem was solved by replacing the water with an organic solvent suitable for the amines. Methyl octanoate was chosen as the solvent because it had a reasonable retention time and it eluted after the amines and the internal standard.

The methylamines in aqueous solutions were resolved satisfactorily on a freshly packed column of Chromosorb 103 (column no. 2); however, the use of *n*-decane as the internal standard precluded the use of aqueous solutions for single column analyses. Also, when methyl octanoate solutions are injected repeatedly, the retention of the ester solvent on the column necessitated changing the packing from time to time to retain consistent retention properties during analyses.

In the event that injections of alkaline solutions of amines are delayed, hydrolysis of methyl octanoate may give rise to methanol; however, methanol is separated from the amines during glc and is not necessarily a problem. For qualitative work, if delays occur, the flask containing the mixture should be chilled.

Tlc–Spectrophotofluorometry of DANS-amides. The hR_f values of each dansyl derivative of the amines separated by the two solvent mixtures are listed in Table II. Members of an homologous series of derivatives are well separated; however, this is not true for isomeric mixtures. For instance, the DANS-amides of the primary butylamines are inseparable, as well as are those of the secondary butylamines.

Table II. hR_f Values of DANS-amides with Different Solvents and Corresponding Fluorescence Spectral Values

Amine of DANS-amide	hR_f Value[a] A	hR_f Value[a] B	Fluorescence[b] Excitation, λ_{max}^{Ex} nm	Fluorescence[b] Emission, λ_{max}^{Em} nm
Ammonia	24	13	363	518
Methylamine	43	20	366	523
Dimethylamine	59	32	370	532
Diethylamine	65	39	365	527
n-Propylamine	56	33	368	522
Isopropylamine	54	33	364	522
Methylethylamine	63	36	368	528
Di-*n*-propylamine	70	49	365	526
n-Butylamine	59	37	365	523
Isobutylamine	59	39	366	522
Di-*n*-butylamine	72	55	365	522
Diisobutylamine	71	54	367	522
Isopentylamine	56	38	365	523

[a] Solvents: A, Chloroform:ethyl acetate (75:25, v/v); B, Cyclohexane:ethyl acetate (70:30, v/v). Absorbent: Silica gel 250-μ layer. [b] Tlc plate sprayed with 20% (v/v) 2,2′,2″-nitrilotriethanol in isopropyl alcohol, then dried for 16 hr at room temperature over $CaCl_2$.

Table III. hR_f Values of DANS-amides of Amines from Coho Salmon Pink Flesh and Corresponding Fluorescence Spectral Values[a]

DANS-amide spot no.	hR_f Values Solvent A	hR_f Values Solvent B	Fluorescence λ_{max}^{Ex}, nm	Fluorescence λ_{max}^{Em}, nm
1	23	11	365	520
2	39	15	362	522
3	56	29	364	531

[a] Solvents and condition as given in Table II.

Tlc analyses of the DANS-amides of the amines from fish tissues agreed reasonably well with the derivatives of ammonia, methylamine (MA), and DMA—those which were identified in the tissues by glc. Although detected by glc, not all samples from fish gave indications of diethylamine (DEA) by tlc. The corresponding fluorescence wavelength maxima for excitation and emission of the dansyl derivatives on tlc plates are given in Table II. For comparison, the fluorescence spectral values for the coho salmon DANS-amides are given in Table III, along with hR_f values. The emission spectra agreed well for the compounds identified in the salmon; however, their excitation spectra did not agree with the reference compounds.

Quantitative glc. Detection sensitivity was measured relative to DEA. Data from multiple analyses of 51 ng to 204 ng of DEA in aliquots of methyl octanoate were interpolated, and 180 ng of DEA was found to give 50% full scale deflection (fsd) on a 5-mV recorder with an electrometer setting of 1.0×10^{-11} A fsd and X4 attenuation. Relative to a 1-mV recorder and X1 attenuation, 9 ng of DEA gives 50% fsd at 1.0×10^{-11} A fsd. In practice, with the procedures discussed above, the lower limit of sensitivity is judged to be 7 ng DEA/g of wet tissue sample.

An examination of the linearity of glc detector response was made for the methylamines analyzed with the Chromosorb 103 column. By using volumetric techniques the concentrations of MA and DMA were varied in the range of 1.4 to 2480 ng/μl, and TMA varied from 7.9 to 2320 ng/μl. Log–

Table IV. Amines in Coho Salmon Pink Tissue Spiked with Amine Mixture

Amine	Level in wet tissue: Found[a] μg/g	Theoretical[b] μg/g	Recovery wt %
Methylamine	1.4	...	...
Dimethylamine	3.4	...	...
Trimethylamine	5.4	...	...
Isopropylamine	4.4	16.9	26
n-Propylamine	5.9	17.7	33
Isobutylamine	8.6	18.6	46
Diisopropylamine	8.0	17.4	46
Triethylamine	8.6	18.4	46
Di-*n*-propylamine and *n*-pentylamine	33.2	70.5	47
Di-*sec*-butylamine	8.2	19.0	43
Diisobutylamine	7.6	18.3	42
Di-*n*-butylamine	1720	3700	46

[a] Average of duplicate runs on an 8-ft × 4-mm i.d. column of 28% Pennwalt 223 + 4% KOH on 80/100 mesh Gas Chrom R at 150°C. [b] All except the methylamines were spiked in the sample.

solvent from the aqueous acidic solution. Knowing that the amines are in equilibrium with their quaternary cationic form in mildly acidic solution (pH 2), it seems reasonable to suspect that a small, but perhaps infinitestimal, amount of amines may be volatilized. (It was found necessary to maintain only mild acidic conditions in order to facilitate the amine salt recoveries during the lyophilization step.)

Losses were suspected of occurring during the final volumetric dilution step, which involved the biphasic mixture of alkaline water and methyl octanoate, just prior to glc analysis. An investigation of this possibility was made and tests with the methylamines and DEA, which were nearly quantitatively estimated, demonstrated that the losses were not caused by the method involving two phases.

Amines in Fish Tissues. Space does not warrant here a review of the many facets of the occurrence of amines in fish, since the subjects have been well aired in numerous reports by others (*cf.*, Castell *et al.*, 1971; Amano *et al.*, 1963, 1968; Dyer and Mounsey, 1945; Shewan, 1938). Suffice

Table V. Levels of Various Amines in Fish Tissues

Sample No.	Source: Tissue	Condition[a]	Amine[b]: MA	DMA	TMA	Others
			μg/g wet tissue			
158	Coho salmon flesh	Good	0.54	3.4	4.8	0.07 DEA
165[c]	Coho salmon flesh	Good	0.46	4.7	5.2	0.24 DEA
166[c]	Coho salmon flesh	Good	0.91	6.6	6.6	0.01 DEA
162[c]	Coho salmon flesh	Good	1.4	3.4	5.4	NF DEA (Spiked)
044[c]	Coho salmon flesh	Good	NF	1.1	3.6	NF
142	Sablefish flesh	Good	53	NF	147	0.56 DEA, 0.3 PA (?)
157	Sablefish flesh	Spoiled	0.8	NF	1164	NF
159	Salmon roe	Fair	0.58	4.1	7.9	NF
167	Salmon roe	Fair	1.2	3.1	5.7	NF

[a] Judged organoleptically. [b] Abbreviations: MA, methylamine; DMA, dimethylamine; TMA, trimethylamine; DEA, diethylamine; PA, *n*-propylamine. NF, none found at level >7 ng/g tissue. Amine unconfirmed by tlc indicated by (?). [c] Tissues from the same fish.

log plots of these concentrations *vs.* peak heights permitted three reasonably straight lines to pass through the corresponding points, thereby indicating linear responses for 7 ng to 12 μg of MA and DMA and 40 ng to 12 μg of TMA.

The quantitation of DMA and TMA required analyses with both columns. The peak height ratio of the two amines separated on the Chromosorb 103 column was used to correct the critical pair singlet peak for these amines analyzed with the internal standard on the Pennwalt 223 column. The response factors, given in Table I, were then applied to the corrected peak heights to calculate the amounts of amines.

Recovery of Amines. A sample of coho was spiked with a mixture of ten amines ranging from 17 to 3700 μg/g. The data from the recovery analysis are given in Table IV. The average recovery of the spiked amines was 42% of theoretical.

The losses of the spiked amines in the isolation procedure can be perhaps attributed to one or more aspects of the methods used. Initially, losses may occur if the spiked amines bind to tissue proteins and are only partially freed during the blending with the 0.7 *M* $HClO_4$. Losses may perhaps occur in the distillation step if all joints are not tight or if the rapid distillation has not proceeded long enough to enable complete removal of amines from the solution. It was believed that a distillate volume nearly equal to that of the solution to be distilled was sufficient to complete recovery. Also, losses of amines may occur during the evaporation of it to say that the amines arise from bacterial action, enzymic reactions, and nonenzymic chemical reactions (Vaisey, 1956). The amine content of fish varies widely, depending also on the species, age, and handling and storage conditions.

Contrary to what is expected, based on other researches that show the wide range of amines in fishery products (Sato *et al.*, 1963; Minakowski and Rzewuski, 1965; Golovnya *et al.*, 1967a,b), analyses of a sablefish, salmon, and salmon roe indicate that the amine patterns are relatively uncomplicated. Although the analyses here are too few to say that the results are typical for sablefish and salmon, the results show the orders of magnitude of amines that can be determined by the reported procedure. The methylamines and DEA were the only amines that were confirmed for the samples at levels above the detection limit of 7 ng/g wet tissue. The results are summarized in Table V. In the flesh of the salmon the only amines found ranged from <0.007 to 1.4 μg MA/g of tissue, 1.1 to 6.6 μg DMA/g of tissue, <0.007 to 0.24 μg DEA/g of tissue, and 3.6 to 6.6 μg TMA/g of tissue.

As seen in Table V, considering the analysis from the same salmon, MA and DEA were not found in one (sample no. 044) of four replicate runs. The analysis of the anomalous flesh aliquot was performed 6 weeks after the other three aliquots, thus suggesting that perhaps the more volatile amines can be lost during frozen storage and, thereby, escape detection. For this reason, it is necessary to know the exact conditions

in which a fish sample has been stored when attempts are made to determine the content of compounds having high volatilities, such as amines.

The sablefish, which was considered good and edible but yet below usual commercial quality, was void of DMA, and had less than 1 μg DEA/g of wet tissue. Compared to the good flesh, the level of TMA increased nearly eightfold and MA decreased nearly sevenfold for sablefish flesh (200 g of excised portion) that was stored in aluminum foil in a 6 ± 1 °C refrigerator for 28 days. The spoiled flesh was also void of DMA, which is interesting considering the evidence that has accumulated to show that the content of DMA in fish increases with storage time (Beatty and Collins, 1940; Tokunaga, 1970; Castell *et al.*, 1971).

Sato *et al.* (1963) reported that a spoiled fish was free of DMA, but that the three ethylamines and isobutylamine were detected. Also, DMA has been reported absent from the flesh of herring (Hughes, 1958), and from fillets of flounder, halibut, wolffish, and redfish (Castell *et al.*, 1971).

In addition to the simple aliphatic amines in fish, free purines and pyrimidines do occur and increase during storage (Jones, 1960; Spinelli *et al.*, 1964). To determine the latter classes of amines in fish, isolation methods other than with steam distillation are necessary. Also, in order to analyze compounds such as 6-hydroxypurine (hypoxanthine) by glc, the procedure would require preparations of stable, volatile derivatives. The isolation of all amines from fish tissues can rely on the basicity of the class of compounds, their ability to form cations, and their solubility in organic solvents, such as in the present case.

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Sucrose Accumulation in Sweet Corn Kernels: Effects of Chelators

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Application of ethylenediaminetetraacetic acid to sweet corn, as a spray on the plants or injected into the ears, increases the sucrose content of the kernels at harvest by 67%, while the amount of reducing sugar is doubled. Ethylenediaminetetraacetic acid appears to have no effect on the conversion of sucrose to polysaccharides in the endosperm during the postharvest period. Preharvest injection of pyrophosphate into the ears, however, is quite effective in retarding this process. The results indicate an effect of ethylenediaminetetraacetic acid and other chelators on sugar transport from the leaves to the ears, perhaps by altering permeability of cell membrane. We suggest that the chelator enhances the translocation of sucrose into the kernels through a barrier of cells between the vascular system and the endosperm tissue.

In a previous publication (Amir *et al.*, 1971) it was shown that the conversion of sucrose to starch in sweet corn kernels can be regulated by preharvest pyrophosphate (PP_i). The effect of PP_i on the system is associated with the inhibition of ADP–glucose synthesis, the main glucosyl donor for starch synthesis. The enzyme ADP–glucose pyrophosphorylase, the enzyme which catalyzes ADP–glucose synthesis, has an absolute requirement for Mg^{2+}. Thus, studies on the effect of ethylenediamine tetraacetic acid (EDTA) were initially undertaken to help elucidate the possible chelation of Mg^{2+} in the endosperm by PP_i as a possible mode of action. In these studies, it was found that EDTA had no effect on the conversion of sucrose to polysaccharides in detached ears.

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