extracted by the method of this paper to give recoveries of 49 mg (23%), 56 mg (25%), 18 mg (10%), and 6 mg (50%), respectively. Thus, the official method of filtration resulted in an average loss of oil and grease of 25% of the values determined by the proposed method.

Two effluents (3 and 4) were precipitated by the method in this paper but extracted by the Soxhlet method and gave 16 and 5% low values, respectively. In addition, contamination of the oil fraction with Celite and fiber is apparent in the EPA Soxhlet method and oil and grease values are estimated to be 5-10 mg lower than reported.

Discussion

Different precipitation techniques were used in developing this method and gave valid results for specific waste effluents. For freshwater-processed shrimp, Celite, alum (200 ppm), and Magnafloc 835A (2 ppm) resulted in complete precipitation in about 15 min. The alum technique also worked on waste effluents from saltwater-processed shrimp and on snow crab, but precipitation was slower and filtration was more difficult. In general, the hexametaphosphate precipitation is the preferred technique because it resulted in a more firm, dense floc that filtered more rapidly than the alum system. In addition, the soluble proteins along with their oil content are recovered in the hexametaphosphate precipitate and included in the analysis. The soluble proteins generally are not recovered with the alum system or by the EPA method. Presumably, any reagent can be used for precipitation provided there is no carry-over into the oil fraction. Sulfuric acid was used to develop this method, but it occasionally resulted in a dark oil after drying. Consequently, the use of sulfuric acid was discontinued in favor of acetic acid. The proposed method should be tested further in comparison with the standard EPA methods for oil and grease to determine its applicability to other fishery waste effluents.

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OCCURRENCE OF VOLATILE N-NITROSAMINES IN JAPANESE SALMON ROE

Consumer interest and concern about food additives is as strong in Japan as in the United States. The possibility that secondary or tertiary amines and nitrites in fish roe products (sujiko) might combine to produce *N*-nitrosamines, known carcinogens, has received much attention and publicity. If the use of nitrites is curtailed in Japan, American salmon canners would be hurt because of loss of sales or decreased prices for roe sold to Japanese processors operating in the Pacific Northwest. The value of this business to the U.S. salmon industry is from $10 to $15 million each year.

Investigations by Howard et al. (1970) and Fazio, Howard, White, and Watts (1971) showing trace quantities of *N*-nitrosodimethylamine (NDMA) from samples of chub, sable, salmon, and shad prompted the National Marine Fisheries Service (NMFS) to be concerned about *N*-nitrosamines in smoked nitrite-treated fishery products. This concern was shared by the National Canners Association (NCA) in connection with nitrite-treated salmon roe products. Various samples of salmon roe commercially produced in canneries in the northwestern United States and Alaska were obtained by the NCA for analysis of volatile *N*-nitrosamines.

In addition to the analysis for nitrosamines which was carried out by NMFS, samples were also analyzed by NCA for residual nitrite and chloride concentrations. The results of these findings are presented in this report.

Experimental

Background

For a number of years, Japanese companies

have maintained salmon roe processing operations at canneries in the northwestern United States and Alaska. The processing of salmon roe is an art rather than a formulated production procedure, and numerous minor differences are found in the various recipes employed. The following is, of necessity, a generalized description of the production operation.

Roe from the butchered salmon is received in the egg house, cleaned of extraneous fish material, and rinsed to remove blood. From 27 to 38 kg of roe are placed in a vat containing 200 liters of saturated brine into which has been added either 0.02-0.05% nitrate or 0.05-0.07% nitrite (equivalent to 500-700 ppm.).

The mix is agitated mechanically for approximately 20 min. The actual length of time is determined by technicians who consider a range of variables, such as the size of roe, the freshness of fish from which roe was obtained, and temperature of brine solution. Larger roe, as from king or chum salmon, are held in the brine longer. Brine batches may be used for several changes of roe; normally, they are changed four or five times in an 8-h day.

After removal from the vat, the roe are drained and graded by size and color. Nitrite level of the roe at this time is about 50 ppm. The roe are then packed in 10-kg wooden boxes which are lined with sheets of plastic. After each layer is packed, it is lightly salted with a fine grind sodium chloride. The boxes are slightly overfilled, and the lids placed on without nailing. They are then stacked with weights on top to form a press. The boxes are cured in this fashion for as long as 7 to 10 days, depending on ambient temperature conditions. During the curing period, the desirable red color of sujiko develops, and nitrite residuals drop to less than 5 ppm. It is possible that the color enhancing action of the nitrite may be due to its inhibiting effect on color destroying oxidative enzymes in the roe.

Following pressing and curing, the product is inspected. If satisfactory, the lids are nailed down, and the boxes are stored at -5°F (-20.6°C) at the cannery and placed aboard transport vessels to Japan. In Japan, the same storage conditions apply until the product is sold to the retail markets.

Production Survey

Duplicate 10-kg samples of commercially produced red and pink salmon roe products were obtained from four of the five major sujiko processors. The processing plants were located on Kodiak Island in the Gulf of Alaska, southwest of Anchorage; Hawk Inlet in the Admiralty Islands, west of Juneau; Cook Inlet, large inlet which Anchorage is at the head of; and Ketchikan, southeast Alaska on the south side of Revillagigedo Island. Duplicate 10-kg samples of roe from three species of salmon—red, chum, and king—were obtained from the fifth major producer located at Puget Sound, Wash. All of these samples were obtained after their delivery to Japan. It was decided to sample the roe in Japan so that storage conditions would be more nearly identical to those received by the product going to consumers. Upon return of the samples to this country, NCA delivered them to NMFS. The samples were composited in a Hobart silent cutter, packaged in Mylar[1] bags, and sealed. A portion of the composite sample was returned to NCA for determinations of residual nitrites and NaCl content.

Experimental Pack

Using roe from the same batch of fish, one test pack and one control pack of salmon roe were prepared by NCA. The test pack was prepared in a saturated brine containing 700-ppm. nitrite, while only a saturated brine was used to prepare the control pack. The packs were cured at a temperature of 60°F (15.6°C) for 7 days and then stored for 6 mo at -5°F (-20.6°C).

Materials

The solvents—methylene chloride, pentane, and ethyl ether—were purified by distillation. Solvents, silica gel, and Celite 545 were tested prior to use to assure the absence of interfering peaks.

Analytical

The multidetection method for the analysis of volatile *N*-nitrosamines in foods developed by Fazio, Howard, and White (1971) was used in this investigation. Because of the high phospholipid content of the salmon egg samples, William T. Roubal of the Northwest Fisheries Center, NMFS, NOAA, found it necessary to make some preliminary modifications in the procedure (Fazio,

[1]Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

Howard, and White 1971). Very briefly, these modifications were as follows:

1. Initial digestion of product–30 g of KOH were employed in the digestion, and the methanolic KOH solution was refluxed for 2-5 h.
2. Distillation step–8 g of $Ba(OH)_2$ was utilized. The distillation was carried out with the aid of a magnetic stirrer.

Briefly, the procedure involved digestion of the sample in methanolic KOH, liquid-liquid extraction of an aliquot of the digest with methylene chloride, distillation of the nitrosamines from alkaline solution with further cleanup by solvent partitioning and column chromatography on silica gel and Celite 545 columns followed by GLC (gas-liquid chromatography) analysis.

A victoreen Model 4000 GLC Chromatograph equipped with a Coulson Electrolytic Conductivity Detector and an Autolab System IV Computing Integrator was employed in the analysis of salmon roe extracts. A 9 foot (2.74 m) × 4 mm inside diameter glass column coated with 10% Carbowax 1540 + 3% KOH on 80/100 mesh gas chrom Q was used. The following parameters were maintained throughout all analyses.

Temperature of injector block - 190°C
Carrier gas (helium) flow rate - 70 ml/min
GC (gas chromatograph) oven temperature - ambient for 540 s; GC oven door was closed and brought to 80°C (held at 80°C for 180 s); 80°-180°C at a program rate of 5°C/min.

Conditions of Coulson Electrolytic Conductivity Detector operated in reductive mode were:

Hydrogen flow rate - 83 ml/min
Venting helium flow - 70 ml/min
Furnace temperature - 820°C
Venting block temperature - 190°C
Conductivity bridge - 30 V
Attenuation - 1.

Moisture, nitrite, and chloride determinations were made according to the official methods of analysis of the Association of Official Analytical Chemists.

Results and Discussion

During the survey, recovery studies were conducted. A mixture of six *N*-nitrosamines was used. The *N*-nitroso compounds were NDMA, diethylamine (NDEA), dipropylamine (NDPA), dibutylamine (NDBA), piperidine (NPi), and pyrrolidine (NPy). Prior to recovery runs, however, the salmon roe samples were examined for *N*-nitrosamines. Several of the cleaner samples were fortified at the 10-ppb (parts per billion) level. In instances where a nitrosamine was found under study, appropriate adjustments were made in the recovery values. Recovery of the *N*-nitrosamines at the 10-ppb level ranged from 67 to 88%.

Representative chromatograms obtained from a fortified pink salmon roe extract together with those obtained from the corresponding unfortified samples are shown in Figure 1. This figure shows the recovery of six nitrosamines after the silica gel cleanup step. Usually, the interferences occurring at a retention time of NPy were removed by further cleanup on the acid-Celite column. During the course of this investigation, blank runs (without a salmon roe sample) were made, and the minute GLC peak (3-15 mm) with the same retention time of NDMA observed with all roe samples was not apparent in the blank. As shown in Table 1; if the peaks are calculated as NDMA, the levels range from 0 to 3 ppb. Residual nitrite and chloride concentration are also shown.

A total of 24 salmon roe samples were analyzed in duplicate. All samples contained less than 5 ppb of NDMA. The demonstrated sensitivity of the method was shown to be 10 ppb. A peak with a retention time of NDEA was found (< 1 ppb). No attempt was made to confirm the identity of NDMA or NDEA in any of the samples since all were too low for mass spectrometric confirmation. Some samples were carefully concentrated down

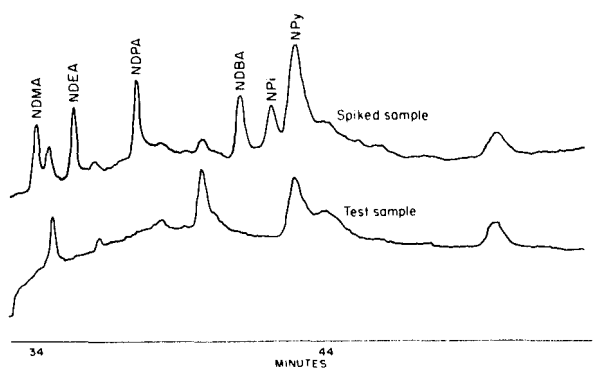

FIGURE 1.–Gas chromatograms of spiked and unspiked extracts of pink salmon.

TABLE 1.–DMNA, nitrite, and NaCl content of salmon roe samples prepared from different species of salmon at various processing plants in the Pacific Northwest.

Species	Location of processing plant	NaCl (%)	Residual nitrite (ppm.)	Apparent DMNA found (ppb)
		Production survey		
Red	Kodiak Island	8.42	0.3	1
Red	Kodiak Island	8.01	Trace	1
Red	Cook Inlet	8.75	Trace	2
Red	Cook Inlet	7.07	Trace	2
Red	Ketchikan	8.73	Trace	1.5
Red	Ketchikan	8.50	0.85	1.5
Red	Hawk Inlet	7.89	Trace	2
Red	Hawk Inlet	8.56	Trace	2
Pink	Kodiak Island	9.38	0.3	1.8
Pink	Kodiak Island	7.94	Trace	1.5
Pink	Cook Inlet	9.34	0.2	2.5
Pink	Cook Inlet	9.16	0.3	2.5
Pink	Ketchikan	9.01	0.60	2
Pink	Ketchikan	9.58	0.3	2
Pink	Hawk Inlet	9.58	0.3	2
Pink	Hawk Inlet	8.82	Trace	2
King	Puget Sound	5.19	Trace	3
King	Puget Sound	5.30	Trace	3
Chum	Puget Sound	9.53	Trace	2
Chum	Puget Sound	10.97	Trace	2
Red	Puget Sound	8.46	0.3	2
Red	Puget Sound	9.16	0.3	2
		Experimental pack		
Red	Control, NCA	9.5	Trace	0
Red	Test, NCA	8.8	Trace	3

to 100 μl. The chromatograms showed few, if any, indications of other volatile *N*-nitrosamines studied.

The multidetection method of Fazio, Howard, and White (1971) was used to prepare four runs of the same sample. The eluants from four silica gel columns were combined into a 1-liter Kuderna-Danish apparatus and concentrated to 1 ml and an aliquot injected for GLC analysis. The concentrate represented 100 g/ml of roe instead of the usual 25 g/ml. The increase in the area and height of the NDMA peak was very pronounced. The extract was submitted to an acid-Celite column cleanup. Interferring peaks were removed, but the suspected NDMA was still present (Figure 2).

In view of the above findings, it can be concluded that less than 5 ppb of apparent NDMA was found in salmon roe products of the different species of salmon having been processed at five major locations, and that no other nitrosamines were evident.

Acknowledgments

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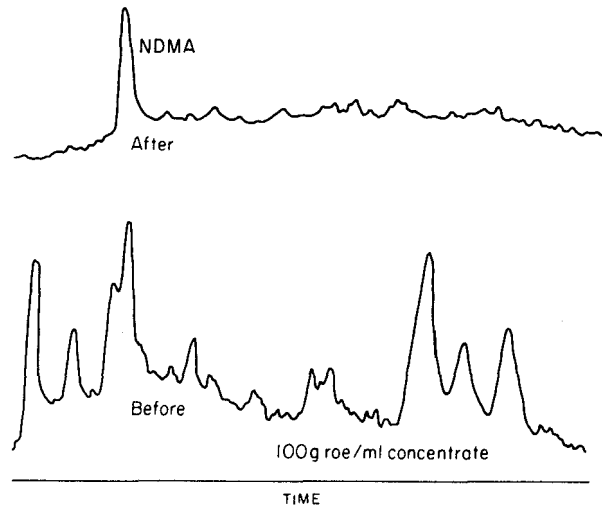

FIGURE 2.–Chromatograms of an extract of nitrite-treated salmon roe before and after being cleaned up on a column of acid Celite.

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