OIL AND GREASE: A PROPOSED ANALYTICAL METHOD FOR FISHERY WASTE EFFLUENTS

The published procedures (American Public Health Association 1971:407-413; Environmental Protection Agency 1974) for determining oil and grease in industrial wastes are generally unsuitable for fish-processing waste effluents, especially for such high-load effluents as occur during the processing of salmon for canning. These wastes cannot be filtered satisfactorily by the method described. In addition, a Soxhlet extraction of the fish proteinlike material after drying for 30 min gives low values because of the inefficient extraction of protein-bound lipids.

These inadequacies of the published methods for the analysis of oil in fish-processing waste streams indicate a need for an alternate method that is simple and accurate. Accordingly, a method was worked out using portions of the published oil and grease methods and using techniques developed by Kelley and Harmon (1972) for the analysis of carotenoids. The method involves a precipitation of protein and particulate matter to allow easy filtration and subsequent extraction of oil from the residue under anhydrous conditions, using 2-propanol (IPA) and petroleum ether (PE). The method is proposed as an alternate method for determining oil and grease in fishery waste effluents.

Materials and Methods

Reagents and Equipment

Celite[1] 503, Johns-Mansville (filter aid): For best results, Celite should be washed with water and solvents because a slight oil residue may carry over into the oil fraction. Blend about 100 parts of Celite by weight with 500 parts water, filter, reblend with 500 parts (vol) IPA, filter, reblend with 500 parts (vol) PE, filter and apply suction until reasonably dry. Air dry and store in a jar. Filter paper dispersion: Blend 20 7-cm filter paper disks (Whatman 1 or 40) with distilled water in a blender for 5 - 10 min. Bring volume to 2,000 ml. Sodium hexametaphosphate in water: 250 mg/ml, use 1 ml per analysis, i.e., 250 ppm. Other materials required are: filter flasks (250 ml and 2,000 ml), graduated cylinder (1,000 ml), filter pump (water aspirator), filter funnel (fritted disc, 350 ml coarse, 150 ml medium), blender and jars (Virtis Model 23 and 200-ml blender jars), rotating evaporator with 250-ml flask, film to seal cylinder (parafilm "M," American Can Company, Marathon Products), 50% acetic acid, anhydrous magnesium sulfate (powdered), reagent grade IPA, and reagent grade PE (bp 40°-60°C).

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

Preparation of Filter Funnel

Assemble filter flask and a 350-ml "c" sintered glass filter funnel. Add about 3 g filter aid and 100 ml filter paper dispersion directly to the funnel. Fill funnel with water, stir and allow to partly drain without vacuum. Apply vacuum, rinse briefly, and press down along edge of mat to ensure a good seal.

Preparation of Sample and Filtering Step

Pour well-mixed sample of effluent to the 1,000-ml mark in the graduated cylinder. Add 3 to 6 g filter aid to aid precipitation. In its absence, flotation and precipitation both occurred. Add 1 ml hexametaphosphate solution, seal cylinder with film, and mix by inverting cylinder about 12 times. Add 2 ml acetic acid. The amount of acid will vary with the type of effluent and is not critical provided enough is added; the pH must be lower than 4.2, but precipitation works equally well at several levels between pH 2.1 and 4.2. Invert three or four times. Excessive mixing inhibits rate of precipitation. Wait about 2 min and add more acid if top inch or so is not clear. Solids in salmon waste effluents are slow to settle and are best handled by allowing the mixture to settle overnight in the refrigerator. Salmon waste, after 2-h settling, can be filtered but with difficulty. If filtration is started too soon, the sample often must be discarded because it will not filter. Shrimp and crab waste usually can be filtered in 15 to 30 min. Filter clear supernatant fluid under vacuum through the prepared filter funnel (very rapid), and transfer more slowly the precipitate (50-75 ml vol) and rinsings to the funnel. Use about 200 ml water to remove excess acid and to rinse graduate and filter. Continue vacuum 5 to 10 min to remove as much water as possible because the next step, the extraction, must be anhydrous.

Extraction of Oil

Carefully transfer solid material, including

Celite and filter paper, to the 200-ml blender jar plus about 15 g anhydrous $MgSO_4$ and 75 ml IPA. The desiccating step with $MgSO_4$ is not effective if volumes of IPA are excessive. In addition, all volumes should be maintained as specified to allow rinsing without exceeding the capacity of the 250-ml evaporating flask. The IPA should be measured in the liter graduate and shaken or rotated to wash cylinder. Blend at high speed for 5 min, then pour contents of blender jar into 150-ml dry filter funnel (M-porosity), apply vacuum until dripping ceases, rinse briefly with PE (wash bottle), then repeat extraction with 75 ml PE. The second extraction with PE removes about 2.5% of the total oil.

Quantitatively transfer filtrate to a pre-dried and weighed 250-ml 24/40 standard taper round-bottom flask, and flash evaporate using a rotating vacuum evaporator and warm water bath. This method takes from 5 to 10 min, but other techniques of evaporating would be suitable. When solvents are removed, add about 10 ml PE to determine if water or solid materials are present. If clean, evaporate to dryness, wipe outside of flask, and place in drying oven for exactly 30 min to remove traces of solvent or water. Cool in air for 1 h and weigh. Subtract tare weight and record weight of oil directly as milligrams per liter. The common practice of storing the dry flasks in a desiccator was not necessary because there was little change in weight with subsequent exposure to air. The oil apparently reached nearly constant weight (oxidation) during the 0.5-h drying step. Exposure of the dry oil and the flasks to air for 15 and 40 min resulted in 2.2 and 2.6 mg gain in weight for 1,684 mg oil and only 3.2 mg gain with overnight exposure. Consequently, because the 250-ml round-bottom flasks were difficult to weigh in a rapid manner, weights were obtained after oven drying for 0.5 h and air cooling for 1 h.

If the above PE solution is not free of water or solid particles, add 10-15 g anhydrous sodium sulfate and sufficient PE to mix well. Let sit a few minutes, and filter through sodium sulfate on a 60-ml medium- or fine-porosity fritted-glass funnel, rinse with PE, and transfer back to evaporating flask. The pre-weighed 250-ml flask should be washed out with water and solvents before reuse. This step is time-consuming and is never necessary if the previous extraction and desiccating steps are done properly.

Accuracy and Precision

The results of replicate analyses on eight effluent samples indicate that the proposed method gives acceptable precision (Table 1).

The mean standard deviation of these data on three different species is 5.3, and the mean is 552 mg/liter. The published mean standard deviation for the three methods given in the Environmental Protection Agency (EPA) manual is 1.1, with a mean of 15.0 mg/liter. To compare standard deviations with different means, the coefficient of variation (*CV*) is used, and for the data in this paper the *CV* is 1 as compared with 7 for the data given in the EPA manual. This means that a sample of waste effluent having 100 mg oil and grease/liter will have a comparative standard deviation of 1 or 7 mg/liter, depending on the method used.

The accuracy of the proposed method was evaluated by comparing the EPA Soxhlet method with the method given in this paper, using seven grab samples of king crab, snow crab, and shrimp waste effluents. The data in Table 2 show that the official EPA Freon 113 Soxhlet method gave oil and grease values that were consistently low, varying from 6 to 48% and averaging about 30%.

The filtrates from the EPA method of filtration from samples 3, 4, 5, 6, 7 were precipitated and

TABLE 1.—Oil and grease values expressed as milligrams per liter for eight effluent samples.

Sample	Replicate oil and grease values		
	I	II	III
1. Snow crab effluent	158	154	153
2. Snow crab effluent	251	250	248
3. Shrimp effluent	397	399	
4. Shrimp effluent	432	404	
5. Salmon effluent	844	847	
6. Salmon effluent	231	221	
7. Salmon effluent	923	925	
8. Salmon effluent	1,200	1,190	

TABLE 2.—Comparison of oil and grease values expressed as milligrams per liter determined by the EPA Soxhlet method and the proposed method.

Sample analyzed	EPA method		Proposed method	
	A	B	C	D
1. King crab effluent	41	39	68	70
2. King crab effluent	37	28	59	54
3. King crab effluent	(1)	(1)	225	225
4. King crab effluent	(1)	164	221	225
5. Shrimp effluent	179	182	215	209
6. Snow crab effluent	161	164	174	174
7. Snow crab effluent	5	8	12	13

[1]Samples 3A, 3B, and 4A could not be filtered except by changing filters.

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