total lipid. We presume that the sterol in fraction 1 is cholesterol since Teshima and Kanazawa (1971) and Wickins (1972) found only cholesterol in the sterol fraction of brine shrimp and newly hatched nauplii. Further thin-layer chromatography of the three phospholipid fractions revealed that they were separable in a chloroform–methanol (7:1) solvent system.

Fatty acid analyses of the various lipid fractions are summarized in Table V. The major lipid components of all fractions are 16:0, 16:1, 18:1, and (tentatively) 20:5 fatty acids. These results are in general agreement with those of Enzler et al. (1974) with the exception of the large amounts of the presumed 20:5 fraction. Wickins (1972) reported high percentages of 18:3, a fatty acid which we did not find. However, Enzler et al. (1974) noted that there is a considerable variation in the lipid content of brine shrimp, due perhaps to diet. We have no information on the nature of the lipids in the diet of brine shrimp used in this study. It is noteworthy that no 22:6 fatty acid was found in this study, although it has been reported in other marine animals. This result is in agreement with Wickins (1972) and Kayama et al. (1963), who did not report this fatty acid in brine shrimp; Enzler et al. (1974) reported only 0.1%.

LITERATURE CITED

Bartlett, J. C., *J. Assoc. Off. Anal. Chem.* **49,** 21 (1966).
Enzler, L., Smith, V., Lin, J., Olcott, H. S., *J. Agric. Food Chem.* **22,** 330 (1974).
Kayama, M., Tsuchiya, Y., Mead, J., *Nippon Suisan Gakkaishi* **29,** 452 (1963).
Kelly, C. E., Harmon, A. W., *Fish. Bull.* **70,** 111 (1972).
March, B. E., in "Fish as Food", Vol. II, Borgstrom, G., Ed., Academic Press, New York, N.Y., 1962, p 384.
Medwadowski, B. D., Van der Veen, J., Olcott, H. S., *J. Food Sci.* **32,** 361 (1967).
Moore, S., *J. Biol. Chem.* **238,** 235 (1963).
Neurath, H., Bailey, K., *Proteins* **2,** 427 (1954).
Panos, C., *J. Gas Chromatogr.* **3,** 278 (1965).
Serfling, S. A., van Olst, J. C., Ford, R. F., *Aquaculture* **3,** 311 (1974).
Spector, W. S., Ed., "Handbook of Biological Data", W. B. Saunders, Philadelphia, Pa., 1956, pp 90–91.
Spies, J. R., Chambers, D. C., *Anal. Chem.* **20,** 30 (1948).
Stadtman, T. C., *Methods Enzymol.* **3,** 392–393 (1957).
Teshima, S., Kanazawa, A., *Nippon Suisan Gakkaishi* **37,** 720 (1971).
Wickins, J. F., *J. Exp. Mar. Biol. Ecol.* **10,** 151 (1972).

Received for review November 25, 1974. Accepted March 17, 1975. This work was supported in part by the NOAA Office of Sea Grant, Department of Commerce, under Grant No. 2-35208.

Isolation and Chemical Evaluation of Protein from Shrimp Cannery Effluent

Ramses B. Toma*[1] and Samuel P. Meyers

Shrimp cannery effluent, collected at a seafood plant at Westego, La., contained about 0.65% total solids. Approximately 2 kg of shrimp waste protein (SWP), drum dried at 124–127°, was obtained from 2650 l. of the effluent by treatment with HCl at the isoelectric point. Proximate analyses of SWP were: moisture, 10.00; ash, 6.33; protein, 58.98; fat, 16.97; and crude fiber, 1.62%. Gross energy was 5170 cal/g; microminerals were: calcium, 0.465%; magnesium, 471 ppm; phosphorus, 0.815%; sulfur, 0.415%; iron, 0.110%; zinc, 109.5 ppm; manganese, 18 ppm; and copper, 17.5 ppm. Total bacterial counts for shrimp cannery effluent and SWP were 1.4×10^6/ml and <200/g, respectively. SWP was analyzed for 18 amino acids. Hygroscopic properties of SWP and its defatted derivative were determined by exposure to atmospheric humidity and to 100% relative humidity in a closed container. Also, preliminary clarification tests were made with dilute solutions of four inorganic salts, namely aluminum sulfate, ferric sulfate, ferric chloride, and sodium silicate. The two iron salts were the most effective in separation.

The world-wide production and processing of shrimp represents an industry valued at several hundred million dollars. The disposal of shrimp waste materials is a serious problem of rapidly increasing magnitude. Increasing federal, state, and local regulations to reduce environmental pollution suggest examination of possible economically feasible uses for wastes from seafood processing plants. Of the 35 plants located in the Gulf area states, only three are known to produce shrimp meal from bulk waste and none of these at the present time are actively engaged in reclamation of the soluble matter (American Shrimp Canners Association, 1970), although an average capacity shrimp processing plant consumes as much as one million gallons of water per day (Robinson Canning Co., 1970). No figures are available on utilization of solids from the waste effluent, similar to those of fish solubles.

Little if any research has been done on shrimp waste effluent, because of the short canning season, variation in amount of raw material, as well as the resulted waste, the perishable nature of the waste, and the relatively isolated locations of the processing plants. Meanwhile, numerous workers are concerned with crustaceans and fish meals and separation of proteins from fish solubles. Claggett and Wong (1968, 1969) used alum sulfate, aluminum hydroxide, and lignosulfonic acid derivatives for protein recovery from salmon wastewater. Also Takahashi et al. (1969) used aluminum sulfate–ferric chloride for protein recovery from waste effluent of fish industry (Kamaboko) in Japan. Maximal removal of the nitrogenous matter was achieved at pH 7 with aluminum sulfate (400 mg/l.) and at pH 6–7 for ferric chloride (480 mg/l.). Sedimentation of protein was higher in effluent treated with ferric chloride than that treated with aluminum sulfate. However, iron salts have been used to some extent in meat rendering plants, although such salts cause corrosive properties to equipment and necessitate special precautions in selection of equipment. Dryden and Stern (1968) considered aluminum sulfate more effective than ferric chloride for increasing clarity of wastewater without pH adjustment (300 mg/l.). Recent studies by

Department of Food Science, Louisiana State University, Baton Rouge, Louisiana 70803.
[1] Present address: Department of Home Economics, the University of North Dakota, Grand Forks, N.D. 58201.

Table I. Proximate Composition of Drum-Dried Shrimp Waste Protein[a]

No. of sample	% Moisture	% Ash	% Protein	% Ether extract	% Crude fiber	Caloric value, cal/g
Mean SD[a]	10.00 ± 0.51	6.33 ± 0.28	58.98 ± 1.46	16.97 ± 0.33	1.62 ± 0.05	5170 ± 52
CV %[b]	5.1	4.4	1.9	2.5	3.1	1.00

[a] Proximate analysis was conducted for 12 samples. [b] Standard deviation. [c] Coefficient of variation; protein factor is 6.25.

Mauldin and Szabo (1974) were done on shrimp canning wastewater from shrimp plants located on the Gulf Coast of the United States, using a dissolved air flotation method along with chemical treatment.

EXPERIMENTAL SECTION

Sample Collection Procedure. Representative samples were collected from the discharge of shrimp cannery effluent by means of a portable electric pump equipped with flexible hose hooked on the discharge pipe (after screening, to remove crustaceans which are dried and used for feed and fertilizer purposes). The processed shrimp were predominately the brown species, *Penaeus aztecus* from the Gulf of Mexico. Approximately 700 gal of effluent (60–65°, pH 7.2–7.4 using portable pH meter Sargant, M434–298) was collected in 14 55-gal drums, one every 30 min. The samples were brought to the isoelectric point (pH 4.4–4.7) by addition of concentrated technical grade hydrochloric acid which contained 30% hydrogen chloride (approximately 175 ml) for total operation, at an average of 10–15 ml of HCl for every 50 gal of effluent. Samples were stirred for a few minutes and allowed to stand for 45 min to permit settlement of the suspended finely divided proteinaceous material. The collected proteinaceous materials were frozen and stored for further studies for 2 weeks.

Isolation of Shrimp Waste Protein (SWP). The frozen samples of slurry were allowed to thaw overnight at room temperature and the water was removed by processing the material at atmospheric pressure in a small counter-current double drum drier (Type 053-VIM-18, Reliance Electric & Engineering Co.). Direct steam at 33–35 psi (256–260°F) was used in the drying operation. The final dried material was light textured (200 g occupied a volume of about 1 l.), fluffy, hygroscopic, brown, with bacterial count of <200 organisms/g (Difco Laboratories Inc., 1969), and weighed approximately 2 kg. The material was stored at room temperature in five moisture-proof 2-l., polyethylene screw-capped containers for further analysis.

Proximate Analysis of SWP. Twelve aliquots of SWP were analyzed for moisture, ash, ether extract, crude fiber, and macro-Kjeldahl nitrogen, according to AOAC methods (1970), and caloric value was determined using a Parr bomb calorimeter.

The amino acid contents of SWP were determined by conventional methods using a Beckman Model 116 amino acid analyzer for 17 amino acids. Tryptophan was determined by hydrolyzing 10 mg of defatted SWP with 19 *N* sulfuric acid and treating with *p*-dimethylaminobenzaldehyde according to the method of Spies and Chambers (1948, 1949). The reaction with a 0.04% sodium nitrite aqueous solution occurred within 30 min. The intensity of the resulting color was measured on a Beckman spectrophotometer (Model DB at a wavelength of 590 mμ, compared with that of a tryptophan standard prepared under the same conditions).

Samples of SWP were analyzed for minerals, namely calcium, magnesium, manganese, zinc, and iron by means of a Jarrel Ash atomic absorption spectrophotometer; phosphorus was determined in a Technicon Auto Analyzer with vanadomolybdate solution; sulfur was determined by a turbidimetric method (Lancaster and Stanford, 1962).

Hygroscopic Properties of SWP and Its Defatted Material. Two 5-g samples each of SWP and of defatted SWP prepared by extraction with petroleum ether were weighed into tared 50-ml aluminum moisture pans which were then stored in open air at room temperature. The weights of the increased moisture content were recorded daily for 12 consecutive days or until samples attained constant weight. Similar samples of SWP and defatted SWP were kept at room temperature in a closed container equipped with a beaker of water to maintain the relative humidity at 100%.

Total Solids in Shrimp Waste Effluent. A 500-ml sample of effluent was collected from the discharge pipe every 30 min from the start to the end of the processing operation. A total of 13 samples were collected. Aliquots (100 ml each) from each sample were placed in a tared 250-ml Pyrex beaker and heated gently to dryness. The beakers were heated again in a vacuum oven (70° and 100 mmHg) to assure constant weight of residues.

Clarification of Effluent by Inorganic Salts. From a representative 2-gal sample of shrimp-cannery effluent, six 100-ml and two 90-ml aliquots were measured into eight graduated cylinders fitted with rubber stoppers.

From a freshly prepared 0.1 *M* aqueous solution of aluminum sulfate, $Al_2(SO_4)_3 \cdot 18H_2O$, 10-ml portions were added to each of the two graduated cylinders containing 90 ml of effluent. The cylinders were stoppered and the contents were mixed gently, inverting the cylinders three or four times. The pH of the mixture was measured. The cylinders were allowed to stand at room temperature and the area of clarified zone was recorded at the end of 15 and 30 min as percent of total volume.

Similarly, two 2-ml, two 4-ml, and two 8-ml portions of the 0.1 *M* aluminum sulfate solution were respectively added to each of the three remaining pairs of graduated cylinders containing 100-ml aliquots of effluent. The pH of each mixture was measured and the rates of clarification were recorded.

The concentrations of the aluminum sulfate in the four pairs of mixtures corresponded roughly to 0.01, 0.008, 0.004, and 0.002 *M*.

Determinations of the rates of clarification were repeated, using in lieu of aluminum sulfate the same molar concentrations of three other inorganic salts: ferric chloride, $FeCl_3 \cdot 6H_2O$; ferric sulfate, $Fe_2(SO_4)_3 \cdot XH_2O$ containing 72% $Fe_2(SO_4)_3$; and sodium silicate, $Na_2Si_4O_9$ (water glass).

Similar aliquots of effluent were tested with a combination of 0.1 *M* aluminum sulfate and 0.1 *M* ferric chloride in equal proportions to yield four pairs of mixtures in which the aluminum and ferric components each were present in molarities of (a) 0.001, (b) 0.002, (c) 0.004, and (d) 0.005.

RESULTS AND DISCUSSION

The proximate analysis of 12 aliquots of SWP is given in Table I. The moisture, ash, protein, and fat values of SWP all lie within the ranges that Garcia et al. (1956) reported in a study of 20 samples of fish meals; the SWP values are

Table II. Amino Acid Profile of Shrimp Waste Protein

AA	g/100 g of SWP	g/16 g of N
Essential		
Half-Cys	0.94	1.59
Ile	1.92	3.26
Leu	4.46	7.57
Lys	3.64	6.17
Met	1.68	2.84
Phe	2.69	4.56
Thr	2.53	4.28
Trp[a]	0.74	1.26
Val	2.61	4.42
Nonessential		
Ala	3.12	5.29
Arg	3.72	6.31
Asp	6.34	10.74
Glu	9.12	15.46
Gly	2.53	4.29
His	1.12	1.90
Pro	2.03	3.44
Ser	2.67	4.53
Tyr	2.14	3.64

[a] The tryptophan values obtained for four aliquots of defatted SWP were, respectively, 9368, 8353, 10,156, and 800 μg of tryptophan/g of defatted SWP. These yielded a mean value corresponding to 0.897 ± 0.098 g of tryptophan/g of defatted SWP.

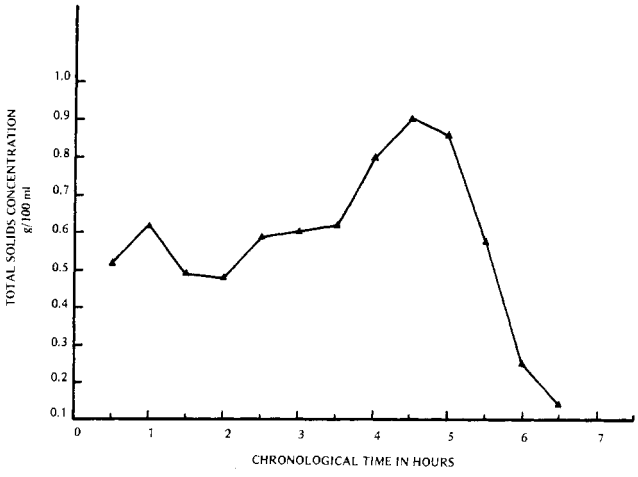

Figure 1.

also in the ranges reported by Canales (1951) for fish meals from Peru. The commercial shrimp meals analyzed by Brown (1959) and Robinson Canning Co. (1970) contained appreciably less protein and much more ash than the SWP obtained in the present study.

The smaller amounts of calcium, phosphorus, and magnesium in SWP, as compared with the quantities of these elements in fish meals, probably are due to the precipitation at the isoelectric point of SWP from shrimp cannery effluent at 60°, and removal of crustaceans from shrimp effluent. This elevated temperature would increase the solubility of minerals, whereas sources of fish meal would contain appreciably larger quantities of these minerals. Undoubtedly the discarded warm supernatant effluent, from which SWP had been removed, contained various soluble mineral constituents. It is of interest to note that the calcium/phosphorus ratio in SWP is 1/1.75, whereas in menhaden fish meal (Garcia et al., 1956) the calcium/phosphorus ratio is 1/0.567, which is equivalent to a phosphorus/calcium ratio of 1/1.76.

Table III. Absorption of Moisture from Humid Air by Oleaginous SWP and by Defatted SWP

	% increase in wt during 12 days			
	Air at 100% rel humidity		Open air (ca. 50% rel humidity)	
Days of storage	Oleaginous SWP	Defatted SWP	Oleaginous SWP	Defatted SWP
1	7.4	18.9	0.2	2.4
2	14.7	24.7	0.2	5.0
3	18.9	30.4	0.3	5.4
4	23.3	34.3	0.8	8.4
5	26.7	36.6	1.3	7.0
6	29.2	37.5[a]	2.0	5.7
7	33.3	38.3	2.2	5.4
8	35.9[a]	39.1	3.4	4.8
9	37.4	40.2	2.5	5.5
10	40.8	41.4	1.5	4.9
11	42.2	41.6	1.8	6.0
12	42.2	41.7	1.8	5.8

[a] Mold attacked the samples.

The values of micromineral contents were: calcium, 0.465%; phosphorus, 0.815%; sulfur, 0.415%; iron, 0.110%; magnesium, 471 ppm; zinc, 109.5 ppm; manganese, 18 ppm; and copper, 17.5 ppm.

The amino acid analyses for SWP are given in Table II.

Total bacterial counts for shrimp cannery effluent and SWP were 1.4×10^6/ml and less than 200 organisms/g, respectively (Difco Laboratories Inc., 1969). These results agree with the observations of Mossel et al. (1967) on fish by-products from stickwater.

Usually the concentration of total solids in shrimp cannery effluent does not exceed 1%. Total solids fluctuated between 0.48 and 0.60% for the first 2 hr following the beginning of 30 min at the start of operation (solids 0.52%). Over the next 90 min, the values rose to 0.9% and started dropping slightly to 0.86%. Then for the final 1.5 hr, the values declined rapidly (0.56, 0.25, and 0.14%, respectively). The overall mean for the day was 0.57%. The pH of the effluent throughout the operation was 7.15–7.20 (Figure 1). The total solids in the effluent are affected by various factors such as the species of shrimp being processed; whether shrimp are headed or shelled or both; type of equipment being used, that is, whether manual peeling or mechanical peeling is used; and consequently the amount of water required. Maximum values for the total solids in effluent were obtained when blanching water was discharged (Robinson Canning Co., 1970).

Daily measurements were made of the absorption of moisture from humid air at room temperature by samples of SWP and its defatted material when exposed continuously for 12 days to atmospheric relative humidity of 100 and about 50% (relative humidity for open air). At the start of measurements the initial moisture contents of the products were 10.00 and 6.60% for SWP and its defatted material, respectively. The daily increases in weight due to moisture absorption were expressed as percentages of the initial weights of samples as shown in Table III. The data indicate that SWP is readily hygroscopic, especially when stored under very humid conditions.

In isolating the SWP used in the present study, precipitation with hydrochloric acid was used because of its convenient availability and its yielding immediately a nutritionally acceptable product. More economically feasible procedures would be required in actual large-scale plant practice. Different methodologies are available for recovering

Table IV. Clarifying Action of Selected Inorganic Salts on Shrimp Waste Effluent[a]

Salt	Effects produced in effluent by addition of salt: Concn of salt in effluent, *M*	pH of effluent	Clarified zone, % total vol: After 15 min	After 30 min
Aluminum sulfate	0.002	5.6	5	12
	0.004	4.7	2	2
	0.008	4.1	2	25
	0.010	3.9	2	30
Ferric sulfate	0.002	5.8	55	63
	0.004	5.5	2	20
	0.008	3.6	2	20
	0.010	3.2	2	20
$Al_2(SO_4)_3$ + $FeCl_3$	0.002[b]	5.9	40	53
	0.004	5.7	2	12
	0.008	4.7	2	25
	0.010	4.4	2	40
Ferric chloride	0.002	6.6	75	87
	0.004	6.4	60	66
	0.008	5.8	2	2
	0.010	5.2	Nil	Nil
Sodium silicate	0.002	6.5	Nil	Nil
	0.004	7.1	Nil	Nil
	0.008	8.0	Nil	Nil
	0.010	8.2	Nil	Nil

[a] The pH of the untreated effluent was 7.2. [b] The aluminum and ferric components each were present in molarities of 0.001, 0.002, 0.004, and 0.005.

proteinaceous material from waste effluents. Such approaches include: lowering the pH of the effluent to the isoelectric point of the protein; heating sufficiently to cause coagulation and separation of the protein, followed by centrifugation; injecting air into the effluent to cause flotation of proteinaceous material; and coagulating the proteinaceous material by the addition of various chemicals to neutralize the charge of protein molecules. Some preliminary tests on shrimp cannery effluent were made using four inorganic salts, namely aluminum sulfate, ferric sulfate, ferric chloride, and sodium silicate. These salts are commonly used in the clarification of fish stickwater.

The molarities resulting when the appropriate amounts of the 0.1 *M* aqueous solutions of the inorganic salts were added to the effluent, the resulting pH of the effluent, and the relative intensity of clarification of the precipitate settled during periods of 15 and 30 min are given in Table IV.

The most effective of the agents tested were the two iron salts, ferric chloride and ferric sulfate. When either of these was added to the effluent to yield a concentration that was 0.002 *M* with respect to the ferric component, the effluent rapidly cleared. With ferric chloride, 75 and 87% clarity was achieved at 15 and 30 min, respectively, as compared with 55 and 63% clarity during the same periods using ferric sulfate. Although ferric chloride reduced the pH of the effluent from 7.2 to 6.6, whereas ferric sulfate lowered it to 5.8, the effect of chloride on clarification of the effluent was greater than that of the sulfate. High concentrations of both ferric salts were less effective than were the lower concentrations. The results are comparable with those reported by Takahashi et al. (1969) on fish effluent using ferric chloride. In spite of salt efficacy, it has corrosive properties and accelerates rancid odor in the final dried product.

The lowest concentrations used for a mixture of aluminum sulfate and ferric chloride gave a clarity of 40 and 53% at 15 and 30 min, respectively. The highest concentration of aluminum sulfate alone was relatively effective due to dissociation of salt in a small volume of effluent. The data differ from those obtained by Claggett and Wong (1968, 1969), Takahashi et al. (1969), and Dryden and Stern (1968) on various fish effluents. The immediate reason for the disagreement is not readily apparent; however, the salt can be used in addition to other treatments such as caustic soda, heating, or combination with other chemicals. Sodium silicate was not effective at any concentration tested.

The main objective of this study showed that a new potential source of protein from shrimp waste effluent could be used in various industries such as canned or processed pet foods, as an animal feed supplement in poultry or livestock feed, or as a fertilizer. However, solving the immediate problem of water pollution, with a low concentration of proteinaceous material in a tremendous amount of water, still requires higher biological and chemical oxygen demand according to Mauldin and Szabo (1974) in the United States and other countries which are involved in seafood production. The authors feel that further investigations are needed especially from the viewpoint of nutritive value of the by-product (SWP).

ACKNOWLEDGMENT

Acknowledgment is noted for the cooperation of Robinson Canning Co. in providing facilities for collection of shrimp by-products.

LITERATURE CITED

American Shrimp Canners Association, New Orleans, La., private communication, 1970.
Association of Official Analytical Chemists, "Official Methods of Analysis", 11th ed, The Association, Washington, D.C. 1970.
Brown, R. L., *Commer. Fish. Rev.* **21**(2a), 46 (1959).
Canales, H., *An. Fac. Farm. Bioquim. Univ. Nac. Mayor San Marcos*, 257 (1951).
Claggett, F. G., Wong, J., *Fish. Res. Board Can., Biol. Stn. St. Andrews, N.B., Gen. Ser. Circ.* **No. 42**, 25 (1969).
Claggett, F. G., Wong, J., *Fish. Res. Board Can., Biol. Stn. St. Andrews, N.B., Gen. Ser. Circ.* **No. 42**, 25 (1969).
Difco Laboratories Inc., "Difco Manual of Dehydrated Culture Media and Reagents", 9th ed, Detroit, Mich., 1969.
Dryden, F. D., Stern, G., *Environ. Sci. Technol.* **2**(4), 268 (1968).
Garcia, M. H., Ballester, D. C., Ariza, A., *Arch. Venez. Nutr.* 7(1), 17 (1956).
Lancaster, J. D., Stanford, J. O., *Soil Sci. Soc. Am. Proc.* **26**, 63 (1962).
Mauldin, A. F., Szabo, A. J., *Environ. Prot. Technol. Ser. EPA-660/2-74-061*, 20 (1974).
Mossel, D. A., Schothorst, V., Kampelmacher, E. H., *J. Sci. Food Agric.* **18**(8), 362 (1967).
Robinson Canning Co., Westwego, La., private communication, 1970.
Spies, J. R., Chambers, D. C., *Anal. Chem.* **20**, 30 (1948).
Spies, J. R., Chambers, D. C., *Anal. Chem.* **21**, 1249 (1949).
Takahashi, T., Kizawa, M., Miyake, M., *Mie Kenritsu Daigaku Suisangakubu Kiyo* 8(1), 53 (1969).

Received for review November 8, 1974. Accepted February 21, 1975.