bamate, or ascorbate in the inhibition of *o*-diphenol oxidase of legume forages.

The ratios A_{280}/A_{260} of our fraction II preparations were lower than we had hoped to achieve but could have been increased by accepting lower yield of protein during treatment with DEAE. However, the major contaminant may not have been phenolic for it had maximum absorbance at 272–274 nm, little absorbance at 330 nm, and no absorbance between 220 and 240 nm.

Chromatography on Sephadex gave partial removal of phenolic compounds from proteins and suggested the occurrence of reversible adsorption of phenols to proteins, possibly through hydrogen bonding.

LITERATURE CITED

Anderson, J. W., *Phytochemistry* **7**, 1973 (1968).
Andrews, T. J., Lorimer, G. H., Tolbert, N. E., *Biochemistry* **12**, 11 (1973).
Chervenka, C. H., "A Manual of Methods for the Analytical Ultracentrifuge", Beckman Instruments, Palo Alto, Calif., 1969.
Cumper, C. W. N., *Trans. Faraday Soc.* **49**, 1360 (1953).
Evans, M. T. A., Mitchell, J., Mussellwhite, P. R., Irons, L., *Adv. Exp. Med. Biol.* **7**, 1 (1970).
Goldthwaite, J., Bogorad, L., *Anal. Biochem.* **41**, 57 (1971).
Gray, J. C., Kekwich, R. G. O., *Eur. J. Biochem.* **44**, 481 (1974).
Hikichi, M., Miltimore, J. E., *Lab. Pract.* **19**, 383 (1970).
Hood, L. L., *Diss. Abstr. Int. B* **34**, 1141 (1973).
Howarth, R. E., McArthur, J. M., Hikichi, M., Sarkar, S. K., *Can. J. Anim. Sci.* **53**, 439 (1973).
Jones, W. T., Lyttleton, J. W., *N.Z. J. Agric. Res.* **15**, 267 (1972a).
Jones, W. T., Lyttleton, J. W., *Phytochemistry* **11**, 1595 (1972b).
Kawashima, N., Imai, A., Tamaki, E., *Plant Cell Physiol.* **8**, 447 (1967).
Kawashima, N., Wildman, S. G., *Annu. Rev. Plant Physiol.* **21**, 325 (1970).
McArthur, J. M., Miltimore, J. E., *Proc. Int. Grassl. Congr. 10th*, 518–521 (1966).
McArthur, J. M., Miltimore, J. E., Pratt, M. J., *Can. J. Anim. Sci.* **44**, 200 (1964).
Munro, H. N., Fleck, A., *Mamm. Protein Metab.* **3**, 423 (1969).
Nishimura, M., Akazawa, T., *Biochemistry* **13**, 2277 (1974).
Reid, M. S., Bieleski, R. L., *Anal. Biochem.* **22**, 374 (1968).

Received for review January 6, 1975. Accepted March 17, 1975.

Composition of San Francisco Bay Brine Shrimp (*Artemia salina*)

Margie Gallagher* and W. Duane Brown

Brine shrimp from San Francisco Bay have been analyzed for proximate composition, including moisture, protein, crude fat fiber, and ash; 11 vitamins; 9 minerals; amino acids; cholesterol; distribution of lipid fractions; and amounts of fatty acids in the various lipid fractions.

Dried brine shrimp have long been used as food for aquaria but few reports of their composition exist. In work in progress in our laboratories, juvenile lobsters have been shown to attain excellent growth rates when fed a diet consisting solely of live brine shrimp. Also, Serfling et al. (1974) have described methods for feeding both live and frozen brine shrimp which result in good larval survival. In view of these findings, this study was undertaken to determine the composition of brine shrimp with the expectation that such knowledge would be useful in formulating diets for lobsters and other crustacea.

EXPERIMENTAL SECTION

Live brine shrimp (San Francisco Bay Brand, Metaframe Co.) were drained of water in a nylon fish net and then washed and filtered in acetone. This rinse was carried out in order to remove as much water as possible from between the swimmerets of the brine shrimp so that moisture determinations would be more accurate. The rinse was not of sufficient duration to extract any compounds from the brine shrimp. After the acetone had evaporated the shrimp were frozen and lyophilized. Moisture content was based on the original weight and that of the freeze dried material.

Amino acid composition of acid-hydrolyzed samples was done in a conventional manner using a Technicon amino acid analyzer. Cysteine was determined as cysteic acid (Moore, 1963) and tryptophan was determined by the colorimetric method of Spies and Chambers (1948).

The carotenoid index (C_I), a relative means of expressing content of carotenoids, was determined by the method of Kelly and Harmon (1972) which employs the following calculation (the absorbance (A) being that of a cyclohexane solution of extracted carotenoids):

$$C_I = (A_{474nm} \times 100)/(\text{g wet wt sample} \times \% \text{ dry wt})$$

Crude fat was determined by Soxhlet extraction with chloroform and methanol (2:1). Characterization of the lipid fraction was done by column chromatography, thin-layer chromatography, and gas–liquid chromatography of methyl esters. In general, the procedure of Medwadowski et al. (1967) was followed except that the chloroform soluble fraction of the crude fat was passed initially through a Sephadex G-25 column and the operating temperature for the gas–liquid chromatography was 190°. The methyl esters were identified and quantified according to Bartlett (1966) and Panos (1965). Cholesterol was determined by the Liebermann–Burchard method using alkaline-hydrolyzed crude fat samples (Stadtman, 1957).

Aliquots of the lyophilized brine shrimp were sent to a commercial laboratory (Ralston Purina Research 900, St. Louis, Mo.) for vitamin, mineral, and proximate analysis.

RESULTS AND DISCUSSION

As can be noted from the data in Table I, brine shrimp have a high protein content on a dry weight basis. Ash is also high as would be expected from the fact that these animals have a mineralized exoskeleton. The crude fat value is higher than that reported by Enzler et al. (1974) for Mono Lake brine shrimp. The difference could be due to variations in habitat, diet, age, or a combination of these factors. There is considerable difference in the carotenoid index between fresh and freeze-dried brine shrimp, indicating that processing changes the extractability of carotenoids. This could be of importance in formulation of diets for crustaceans requiring carotenoids.

Institute of Marine Resources, Department of Food Science and Technology, University of California, Davis, California 95616.

Table I. Composition of Lyophilized Brine Shrimp

Constituent	%, dry wt
Moisture, %[a,b]	90
Protein, % from total amino acids[b]	43
Protein, % Kjeldahl method[c]	58
Crude fat, % ether extract[c]	5.1
Crude fat, % methanol–chloroform[b]	19.3
Cholesterol[b]	0.46
Fiber[c]	3.5
Ash[c]	20.6
Carotenoid index[b]	
Fresh live brine shrimp	20.6
Freeze dried brine shrimp	0.27

[a] Moisture expressed as percent of wet weight of sample before lyophilization. [b] Analysis from this laboratory. [c] Analysis from commercial laboratory.

Table II. Vitamins and Vitamin-Like Substances in Brine Shrimp[a]

Constituent	mg/g, dry wt
Biotin	0.001
Choline chloride	6.10
Folic acid (bound)	0.007
Niacin (bound)	0.130
Pantothenic acid	0.068
Pyridoxine HCl	0.008
Inositol	1.20
Riboflavine	0.017
Thiamine	0.127
Vitamin B_{12}	0.003
Vitamin A[b]	6650

[a] Analyses from commercial lab. [b] Vitamin A is expressed as international units per gram.

Table III. Mineral Content of Brine Shrimp[a]

Constituent	%, dry wt
Copper	0.001
Magnesium	0.222
Iron	0.275
Calcium	0.100
Phosphorus	0.930
Manganese	0.013
Potassium	0.832
Sodium	5.11
Zinc	0.008

[a] Analyses from commercial lab.

Table IV. Amino Acid Composition of Brine Shrimp, Casein, and Egg Albumin (g/100 g of Protein)

Amino acid	Brine shrimp	Casein		Egg albumin
Leu	8.0	7.9[a]	9.2[b]	9.9[a]
Ile	5.3	6.4	6.1	7.0
Lys	7.6	8.9	8.2	6.5
Thr	4.6	4.9	4.9	4.0
Trp	1.0	1.6	1.7	1.2
Val	5.4	6.3	7.2	8.8
Met	2.7	2.5	2.8	5.3
Phe	4.7	4.6	5.0	7.2
His	1.8	2.9	3.1	2.9
Arg	6.5		4.1	6.0
Ala	6.9		3.2	7.6
Asp	9.2	8.4	7.1	9.3
Cys	2.2	0.4	0.34	2.8
Glu	14.2	22.5	22.4	16.5
Gly	5.3	2.3	2.0	3.6
Pro	5.2	7.5	10.6	3.8
Ser	4.8	6.3	6.3	8.2
Tyr	4.5	8.1	6.3	4.1

[a] Spector (1956). [b] Neurath and Bailey (1954).

Table V. Fatty Acid Analyses[a] of Lipid Fractions[b] from Brine Shrimp

Fatty acid	Total sample	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5
14:0	1.4	1.5	3.7	1.5	1.9	2.9
14:1	2.3	2.9	5.6	2.8	0.2	0.6
15:0	0.7	0.8	1.4	0.8	0.5	1.7
15:1	0.8	1.5	1.5	1.6	5.9	0.8
16:0	13.5	12.9	20.5	13.2	8.7	18.3
16:1	13.8	16.3	24.2	16.2	7.8	11.2
17:0	1.3	1.2	1.2	1.1	0.6	1.1
Iso 17:0		1.3	0.6	0.2	1.7	
17:1	0.9	0.5	0.5	0.5	0.4	1.1
Iso 17:1		0.3	0.5	0.4		
18:0	5.9	4.9	4.1	4.6	5.2	6.2
18:1	35.6	31.7	23.5	29.7	27.8	31.6
18:2	6.2	6.5	4.6	6.0	5.5	3.8
20:0	2.0	2.3	2.4	2.8	1.5	1.1
20:1	0.1	0.1	0.7	1.0	0.7	0.7
20:3[c] + 20:4	2.2	1.0		1.5	7.9	1.4
Iso 20:3 + 20:4				1.6	1.4	0.8
20:5	12.0	13.4	4.9	13.1	20.8	17.5

[a] Also found in all fractions and total sample, in amounts less than 1%, were: 10:0, 11:0, 12:0, 12:1, 13:0. Fatty acid 19:0 was found in small amounts in all samples except fraction 2. [b] Expressed as percent of total fraction. [c] Column used did not separate 20:3 from 20:4.

Results of vitamin and mineral analyses of brine shrimp are given in Tables II and III. The absence of any vitamin or mineral from the data in these tables merely indicates that analysis was not performed.

Amino acid analyses of brine shrimp are shown in Table IV. For purposes of comparison, values for casein and egg albumin are also given. The brine shrimp values compare favorably with those for both casein and egg albumin. Compared to the latter protein, the most limiting amino acid in brine shrimp is methionine. When the amino acid content is expressed as grams per 100 g of product, brine shrimp compare rather closely with reported values for commercial shrimp meal (March, 1962).

Silicic acid column chromatography yielded five fractions. Fraction 1 consisted of simple lipids and sterols comprising 27.7% of the total lipid. Fraction 2 contained only simple lipid with 19.3% of the total lipid. Fractions 3, 4, and 5 contained phospholipids and made up the balance of

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.