Table III. MH Contents of Flue-Cured Tobacco and Assorted Cigarettes

Sample	MH content, ppm
Untreated, hand-suckered flue-cured tobaccos	24, 35
MH-treated flue-cured tobaccos	136, 176
Experimental cigarettes, University of Kentucky, type 1-R-1	103
Experimental cigarettes, University of Kentucky, type 1-A-4	120
Havana cigars, 1959	5
Commercial cigarettes:	
85 mm, filter, Japan	88
85 mm, filter, U. S.	98
85 mm, filter, U. S.	113
85 mm, filter, U. S.	79
85 mm, filter, U. S.	68
85 mm, filter, U. S.	133
85 mm, nonfilter, U. S.	61
100 mm, filter, U. S.	119

ative of maleic hydrazide. Apparently, MH from treatments in previous years remains in the soil and is absorbed by the tobacco plant. Large quantities of MH may enter the soil. Generally, MH is applied to tobacco in the field at a rate of about 6 pints of 30% MH per acre. Some overzealous farmers apply much more. For flue-cured tobacco the average yield is 2000 lb/acre. If all of the MH were to remain on the tobacco and not be degraded, this would amount to about 900 ppm of MH. Obviously, a significant portion of the MH will find its way into the soil and even with some degradation there still remains a sufficient amount in the soil to be absorbed by subsequent crops. There is no literature to substantiate this point, but it is certainly in need of investigation.

This method was applied to the determination of MH in various tobaccos and in commercial and experimental cigarettes. The results are summarized in Table III. MH contents of commercial cigarettes and experimental cigarettes, designed to simulate commercial cigarettes, fall in the range of 60–120 ppm. The value for flue-cured tobacco was quite representative. Other batches of hand-suckered tobacco gave values similar to the domestic types. The lowest value found was for a brand of 1959 Havana cigars, a surprisingly low 5 ppm.

LITERATURE CITED

Anglin, C., Mahon, J. H., *J. Ass. Offic. Agr. Chem.* **41**, 177 (1958).
Hoffman, I., *J. Ass. Offic. Agr. Chem.* **44**, 723 (1961).
Lane, J. R., Gullstrom, D. K., Newell, J. E., *J. Agr. Food Chem.* **6**, 671 (1958).
Wood, P. R., *Anal. Chem.* **25**, 1879 (1953).

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COMMUNICATIONS

The Lipids of Mono Lake, California, Brine Shrimp (*Artemia salina*)

Lipids were extracted from Mono Lake freeze-dried brine shrimp (*Artemia salina*) by chloroform–methanol (2:1) and examined by standard procedures for fatty acid composition and other parameters.

Mono Lake is alkaline (pH 9–10) and contains about twice as much dissolved solids as the ocean. The 7.4% total salts are accounted for mostly as ions of sodium, chloride, and bicarbonate (Mason, 1967). A species of brine shrimp that flourishes seasonally in this lake is harvested in ton lots mostly for aquaria feed (Whitney, 1967). Brine shrimp are of interest not only because they seem to be uniquely useful for feeding all types of aquaria fish but, in addition, they show promise in the feeding of lobsters (*Homarus americanus*) (Shleser and Gallegher, 1974) and other species (Wickins, 1972) that might be adapted to aquaculture. They also furnish an example of how the many underutilized salt lakes of the world might be used for the production of high quality protein for food and feed.

Previous workers (Bowen, 1964; Mason, 1967) have indicated that the brine shrimp of Mono Lake are physiologically and morphologically distinct from those that occur in Great Salt Lake or San Francisco Bay. We report some observations on the composition of the lipids of Mono Lake brine shrimp.

EXPERIMENTAL SECTION

Freeze-dried brine shrimp were received from the California Koi Co. at ambient temperature and stored at −18° pending analysis. Protein, fat, fiber, moisture, and ash were assayed by Ralston Purina Laboratories, St. Louis, Mo.

In this laboratory, moisture was determined by a standard AOAC method (1970a) with the following modification: the drying period was interrupted after 30 min in order to break up the caked powder with a glass rod, the powder adhering to the rod was brushed back into the container used for drying and weighing, and the drying was resumed.

Lipids were extracted from weighed portions of the dried brine shrimp in large Soxhlet extractors with chloroform–methanol 2:1 (Medwadowski *et al.*, 1971). A slow stream of nitrogen was passed through the solvent during the extraction periods to minimize oxidation. The lipid extracts were purified by Sephadex G-25 chromatography, and yields of extractable lipid were determined by drying and weighing aliquots (Smith *et al.*, 1973).

Activated Mallinckrodt Silica AR CC-7, 100–200 mesh columns were used to separate fractions containing (a) mainly neutral lipids and free fatty acids, (b) sphingolipids, and (c) phospholipids (Medwadowski *et al.*, 1967).

Preliminary results for unsaponifiables determined with a standard AOAC method (1970b) were high and irreproducible. Thin-layer chromatography indicated the presence of significant amounts of esters that had escaped sa-

Table I. Composition of Mono Lake Freeze-Dried Brine Shrimp (*Artemia salina*)

	Composition, %
Protein, N × 6.25, d.b.[a]	58.5
Lipid, ether extract, d.b.[a]	5.1
Lipid, chloroform–methanol, d.b.[b]	10.6
Fiber[a]	3.5
Moisture	9.5,[c] 13.1[a]
Ash[a]	20.6

[a] Commercial laboratory. [b] This laboratory, after 110 hr of extraction, 9.4%; after 164 hr, 10.5% and 10.8%. [c] This laboratory.

ponification. For subsequent trials, aliquots of the extracts were heated in sealed vials in a boiling water bath with 0.5 *N* potassium hydroxide (Kayama *et al.*, 1963). An equal volume of water was added and the unsaponifiable fraction was extracted with three to four equal volumes of diethyl ether. The ether fractions were combined and washed with water, 0.5 *N* potassium hydroxide, water, 0.5 *N* potassium hydroxide, and twice more with water. The ether was evaporated on a warm water bath and the unsaponifiable residues were dissolved in chloroform and transferred to volumetric flasks. Thin-layer chromatography (Medwadowski *et al.*, 1967) of the extract and unsaponifiable fractions indicated that most of the fatty acid and cholesterol esters were hydrolyzed by this procedure.

Fatty acid compositions were determined as described previously (Medwadowski *et al.*, 1971; Smith *et al.*, 1974). Results are reported as weight percentages.

RESULTS AND DISCUSSION

The composition of Mono Lake freeze-dried brine shrimp is shown in Table I. Lipids accounted for 10.6% of the dried product.

Column separation of the extracted purified lipids indicated that 40–50% of the total appeared in the fraction containing neutral lipids, 15–18% appeared in the sphingolipid fraction, and 34–43% appeared in the phospholipid fraction, in three separate runs. No further analytical work was done with them.

Five separate determinations of the amount of unsaponifiables in the lipid fraction determined by the procedure described ranged from 3.9 to 5.9%, with a mean of 5.1%. The main component, by thin-layer chromatography, was cholesterol. Teshima and Kanazawa (1971) reported 32% unsaponifiable in the lipid of the brine shrimp analyzed by them. Henderson *et al.* (1972) reported that cholesterol was the main sterol in the lipids of Mono Lake brine shrimp; according to Payne and Kuwahara (1972) cholestanol is also present. However, Teshima and Kanazawa (1971) found that the sterols of brine shrimp, even of those fed diets with other sterols added, contained only cholesterol. Wickins (1972) also found only cholesterol in the sterols of San Francisco brine shrimp nauplii.

The fatty acid compositions previously reported for brine shrimp differ from our observations (Table II), accountable in terms of the differences in feed and environment in which they were grown and possibly to the differences in the physiology of the Mono Lake shrimp. Hinchcliffe and Riley (1972) compared the fatty acids of four different phytoplankton species with those of brine shrimp that had fed on them. There were some points of similarity but many points of difference, suggesting that the metabolic need of the animal is at least of equal significance. The analyses obtained in our study differ strikingly in some instances from previous reports. Kayama *et al.* (1963) found 45% 16:1 compared to our 16%, but their animals were being fed a plankton, the lipids of which contained 48% of this fatty acid. Jezyk and Penicnak (1966) recorded 45% 18:3 in the neutral lipids of their shrimp fed algae, the lipids of which contained 43% 18:3. Our value of 7.4% 18:3 is closer to the average of those examined by Hinchcliffe and Riley (1972). No information is available on the lipids of the natural food ingested by the Mono Lake shrimp.

Table II. Fatty Acid Composition of Mono Lake Freeze-Dried Brine Shrimp (*Artemia salina*)

Fatty acid	%[a]
14:0	2.4, 2.9
16:0	17.0, 19.2
16:1	14.5, 19.3
18:0	6.7, 5.9
18:1	38.8, 28.6
18:2	3.9, 3.5
18:3	7.4, 7.4
20:1	1.7, 1.2
20:3 + 20:4	2.2, 2.0
20:5	5.9, 5.4

[a] Additional identified fatty acids that were present in amounts less than 1% were: 15:0, 0.4%; iso-16:0, 0.6%; 17:0, 0.7%; 17:1, 0.7%; iso-20:0, 0.5%; and 22:6, 0.1%.

Wickins (1972) observed that freshly hatched nauplii from Utah brine shrimp eggs provided poorer nutrition for the larvae of a prawn (*Palaemon serratus*) than did those from the San Francisco area. The differences were not directly attributable to pesticide residues, minerals, fatty acid composition, or other factors studied. More detailed studies of the effects of feed and environment on the composition of the brine shrimp from different sources are needed for optimal utilization of this resource.

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LITERATURE CITED

Association of Official Agricultural Chemists (AOAC), "Official Methods of Analysis," 11th ed., 14.004, 1970a, p 211.
Association of Official Agricultural Chemists (AOAC), "Official Methods of Analysis," 11th ed., 28.063, 1970b, p 457.
Bowen, S. T., *Biol. Bull.* **126**, 333 (1964).
Henderson, W., Reed, W. E., Steel, G., "Advances in Organic Geochemistry, 1971," Pergamon Press, Oxford, 1972, p 335.
Hinchcliffe, P. P., Riley, J. P., *J. Biol. Ass., U. K.* **52**, 203 (1972).
Jezyk, P. F., Penicnak, A. J., *Lipids* **1**, 427 (1966).
Kayama, M., Tsuchiya, Y., Mead, J. F., *Bull. Jap. Soc. Sci. Fish.* **29**, 452 (1963).
Mason, D. T., "Limnology of Mono Lake, California," University of California Press, Berkeley, Calif., 1967.
Medwadowski, B., Haley, A., Van der Veen, J., Olcott, H. S., *J. Amer. Oil Chem. Soc.* **48**, 782 (1971).
Medwadowski, B. F., Van der Veen, J., Olcott, H. S., *J. Food Sci.* **32**, 361 (1967).
Payne, T., Kuwahara, S. S., *Experientia* **28**, 1022 (1972).
Shleser, R., Gallegher, M., "Proceedings of the World Mariculture Society," Jan 1974.
Smith, V., Lin. J. S., Olcott, H. S., *Fish. Bull.*, in press (1974).
Teshima, S., Kanazawa, A., *Bull. Jap. Soc. Sci. Fish.* **37**, 720 (1971).
Whitney, R. R., *Contrib. Mar. Sci.* **12**, 262 (1967).
Wickins, J. F., *J. Exp. Mar. Biol. Ecol.* **10**, 151 (1972).

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