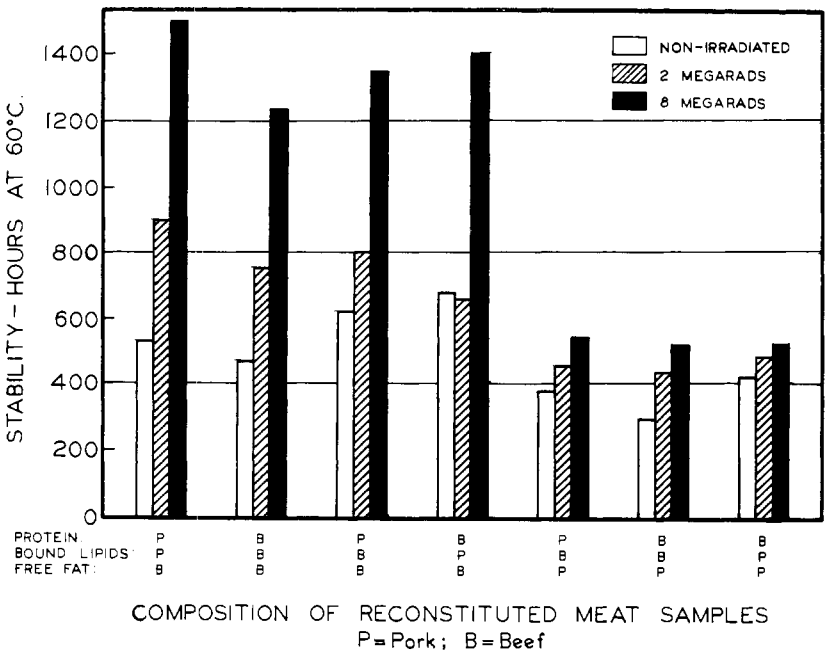

Figure 2. Effect of irradiation on stability of fat from reconstituted beef-pork combinations

not with ground pork, and only when antioxidants had been added to the beef. It was first thought that this effect might be due to the formation of new antioxygenic substances or synergists resulting from the effect of irradiation on the proteins of the meat, since many amino acids or peptides show synergistic activity with phenolic antioxidants (*5*). However, the failure of pork to show the same effect is difficult to explain.

In an attempt to determine the cause of this effect, the free glyceride fat and bound lipids from samples of freeze-dried beef and pork were extracted separately and the meats reconstituted using various cross-combinations of lipid and nonlipid components. As shown in Figure 2, all the irradiated samples showed an immediate increase in stability, but only the four samples containing free fat from beef showed large increases, the largest being obtained with a sample consisting of pork protein and phospholipids with free fat derived from beef. The three samples containing free fat from pork showed only very small stability improvements. Storage of these irradiated samples for 2 weeks at room temperature showed no significant or consistent effect; some of the stored samples showed an increase in stability and others a decrease but, in all cases, these changes were small. It appears, then, that this immediate increase in stability as a result of irradiation is associated mostly with the free fat of beef in combination with added antioxidants. The reason for this effect, however, remains unknown at this time.

Literature Cited

(1) Bradshaw, W. W., Truby, F. K., Office of Technical Services, U. S. Dept. of Commerce, PB Rept. **154306** (1962).
(2) Chipault, J. R., Mizuno, G. R., J. AGR. FOOD CHEM. **14,** 221 (1966).
(3) Chipault, J. R., Mizuno, G. R., *J. Am. Oil Chemists' Soc.* **41,** 468–73 (1964).
(4) Chipault, J. R., Mizuno, G. R., unpublished results.
(5) Clausen, D. F., Lundberg, W. O., Burr, G. O., *J. Am. Oil Chemists' Soc.* **24,** 403–4 (1947).
(6) Merritt, C., Jr., Walsh, J. T., Bazinet, M. L., Kramer, R. E., Bresnick, S. R., *Ibid.*, **42,** 57–58 (1965).
(7) Rose, D., Lips, H. J., Cyr, R., *J. Food Sci.* **26,** 153–155 (1961).
(8) Sedláček, B. A., *Nährung* **2** (6), 947–56 (1958).
(9) Tonnelat, J. P., Flanzy, J., *Ann. Zootechnol.* **10** (2), 97–104 (1961).

Received for review October 4, 1965. Accepted January 26, 1966. Presented in part before the Division of Agricultural and Food Chemistry, Symposium on Radiation Preservation of Foods, 150th Meeting, ACS, Atlantic City, N. J., September 1965. Research supported by the U. S. Army Natick Laboratories, Natick, Mass., and The Hormel Foundation, Austin, Minn.

FISH OIL ODORS

Odor Problems of Fish Oils. Volatile Amines of Menhaden Oil

G. R. MIZUNO, EVELYN McMEANS, and J. R. CHIPAULT

The Hormel Institute, University of Minnesota, Austin, Minn.

The basic constituents of a highly volatile fraction collected during molecular distillation of menhaden oil have been examined by paper chromatography and thin-layer chromatography. Tentatively identified were ethylenediamine and 1,4-butanediamine as major components with smaller amounts of propyl- and hexylamines. Secondary and tertiary amines or quaternary ammonium bases were not detected.

THE nature of the compounds responsible for fishy odors remains largely unknown. Some investigators believe that carbonyl compounds resulting from the oxidative deterioration of highly unsaturated fatty acids are responsible for fishy odors (*9*). Others, however, have suggested that these odors are caused by noncarbonyl constituents (*4*) and that certain nitrogen-containing compounds may be involved in their formation (*7, 14*).

During a study of the nature of fish oil odors samples of volatile compounds collected during molecular distillation of menhaden oil were made available to us. The origin of the samples and the nature of the volatile acidic constituents of the highly volatile fraction have been described (*6*). This report describes the basic constituents of this fraction.

Experimental

Starting Material. Nitrogen determinations and qualitative tests for

amines were performed on the forerun distillate and the aqueous and non-aqueous fractions of the cold trap condensate collected during molecular distillation of menhaden oil (*10*).

Amines were isolated only from the aqueous portion of the cold trap distillate. For preliminary experiments the condensate recovered after isolation of the acids as their sodium salts (*6*) was used, but for final fractionation and identification, larger amounts of material were obtained directly from the total aqueous volatile fraction.

Preliminary Measurements. Total nitrogen was obtained by Kjeldahl analysis, using the digestion mixture and procedure of Noble (*12*) in combination with the colorimetric determination of ammonia described by Russell (*13*).

Spot tests as described by Feigl (*8*) were used to determine the presence or absence of the various classes of amines in these fractions. Tests for tertiary amines were performed with a citric acid–acetic anhydride reagent which gives a red to purple color when heated in the presence of these compounds. The test for secondary amines was the blue-violet color formed when they are treated with a mixture of sodium nitroprusside and acetaldehyde, while the iodine-azide test for dithiocarbamates was used to detect primary and secondary amines as a group after they had been treated with carbon disulfide.

Isolation of Amines. Either the entire condensate recovered from the isolation of the sodium salts of the acids (*6*), or 1 to 2 ml. of the aqueous portion of the original cold trap condensate was acidified to a pH of 2.0 with 2*N* hydrochloric acid. The hydrochlorides of the bases were then isolated after distillation of the water and other volatile compounds, using the procedure and apparatus employed for preparation of the acid salts (*6*). The residual amine hydrochlorides were dissolved in 100 to 200 μl. of water and used for qualitative tests and for paper and thin-layer chromatography.

Separation of Amines. PAPER CHROMATOGRAPHY. For separating the aliphatic primary monoamines, strips of Whatman No. 1 filter paper, 11.5 × 45 cm., were treated by dipping once in 0.15*N* aqueous solution of sodium acetate and drying in air at room temperature (*15*). The solvent system was the upper layer obtained from an equilibrated mixture of butanol–water–acetic acid (50:49:1, v./v.). The chromatograms were developed for 18 hours at room temperature and the amine spots were revealed by dipping the paper in a ninhydrin solution made from 200 mg. of ninhydrin and 15 mg. of ascorbic acid dissolved in 100 ml. of absolute ethanol. With this system primary monoamines with one to six carbon atoms were well separated, while more polar diamines or amino alcohols remained near the solvent front, with R_f values less than that of methylamine.

THIN - LAYER CHROMATOGRAPHY. Chromatography of the volatile bases of menhaden oil on paper with the solvents recommended by Bremner and Kenten (*2*) for separation of diamines was not successful because of excessive streaking and tailing. Much better results were obtained by using thin-layer chromatography. The procedure was similar to that described by Brenner, Niederwieser, and Pataki (*3*). The Silica Gel G plates were dried at 105° to 110° C. for at least 4 hours and stored under atmospheric conditions. Aqueous solutions of the amine hydrochlorides were spotted on the plates, air-dried, and chromatographed with a solvent consisting of 75 grams of phenol, 25 ml. of water, and 4 ml. of glacial acetic acid. The spots were revealed by spraying the plates with a 0.1% ninhydrin solution in chloroform.

Results and Discussion

Table I shows the results of the qualitative tests performed on these menhaden oil volatile fractions. The forerun was the first distillate of the molecular distillation and represents low molecular weight lipid components such as free fatty acids, partial glycerides, unsaponifiables, and breakdown products of unsaturated fatty acids that could be collected on the molecular still condensing surface. No amines could be detected in this material or in the organic upper layer of the more volatile compounds accumulated in the molecular still cold trap. However, both these fractions contained approximately 30 μg. of nitrogen per gram as determined by Kjeldahl analysis. This amount of nitrogen is very close to the limit of detection of the qualitative amine tests employed and it is possible that, even if all the nitrogen present were in the form of amines, it would have been undetected in these samples.

The aqueous layer from the cold trap contained 15 times this amount of nitrogen (482 μg. per gram) and tests for tertiary and secondary amines were negative, while the dithiocarbamate test, which detects both primary and secondary amines, was positive, indicating that the amines in this fraction were all primary in nature.

In preliminary exploratory experiments the hydrochlorides of the bases isolated from the aqueous cold trap layer were chromatographed on untreated Whatman No. 1 filter paper using several solvent systems and indicator solutions. During these experiments the absence of secondary and tertiary amines and of choline or other quaternary ammonium bases was indicated by the failure of the chromatograms to develop spots when they were treated with sodium nitroprusside–sodium carbonate (*1*) and phosphomolybdic acid (*5*), respectively.

These amine hydrochlorides were chromatographed on acetate paper using the butanol–water–acetic acid solvent, and when the chromatogram was dipped in the ninhydrin solution, pink to purple spots appeared within a few minutes (Figure 1). This is characteristic of primary amines and again confirms the presence of these compounds in the menhaden oil volatiles. Secondary amines do not react with ninhydrin until the paper has been heated in an oven at 65° to 70° for several minutes and, in all cases, the unknown material gave no additional spots upon heating after ninhydrin treatment.

As shown also on Figure 1, the primary monoamines with one to four carbon atoms (*D*-1 to *D*-4) give compact well-separated spots, but the more polar diamine (*A*) or ethanolamine (*B*) migrates only a very short distance from the base line and their R_f values are less than that of methylamine. The unknown amines from menhaden oil (*C*) gave a long yellowish streak from the base line to the solvent front on which were superimposed four light pink to purple spots. The major portion of these unknowns was concentrated in an area consisting of a dark purple spot (*C*-2) with considerable tailing which moved slightly above the base line with an R_f value less than that of methylamine. There was some slight evidence of separation within the area (*C*-1, *C*-2), but no conclusion could be drawn regarding the nature of these compounds except that they were not primary monoamines. In addition, two very faint light pink spots (*C*-3, *C*-4) could be detected when enough material was used on the chromatogram. One corresponded to propylamine, while

Table I. Presence of Amines in Volatiles from Menhaden Oil

Sample	Total Nitrogen Content,[a] μg./G.	Response to Tests for: Primary and secondary amines	Secondary amines only	Tertiary amines only
Standards				
n-Propylamine	...	+	−	−
Di-*n*-propylamine	...	+	+	−
Tri-*n*-propylamine	...	−	−	+
Blank	...	−	−	−
Menhaden oil volatiles				
Forerun distillate	30	−	−	−
Cold trap upper (organic) layer	30	−	−	−
Cold trap lower (aqueous) layer	482	+	−	−

[a] Procedures described in text.

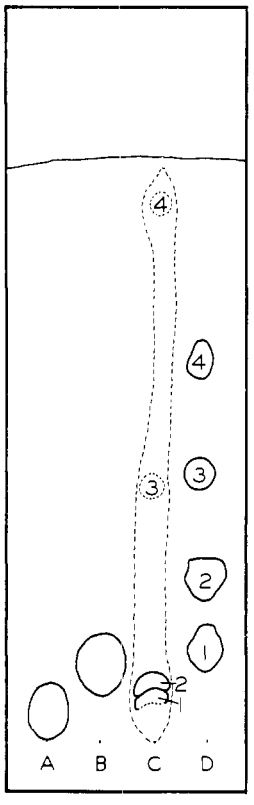

Figure 1. Separation of primary amine hydrochlorides on paper

A, 1,4 - butanediamine (putrescine). *B*, ethanolamine. *C*, volatile amines from menhaden oil. *D*, methylamine (1), ethylamine (2), propylamine (3), butylamine (4)

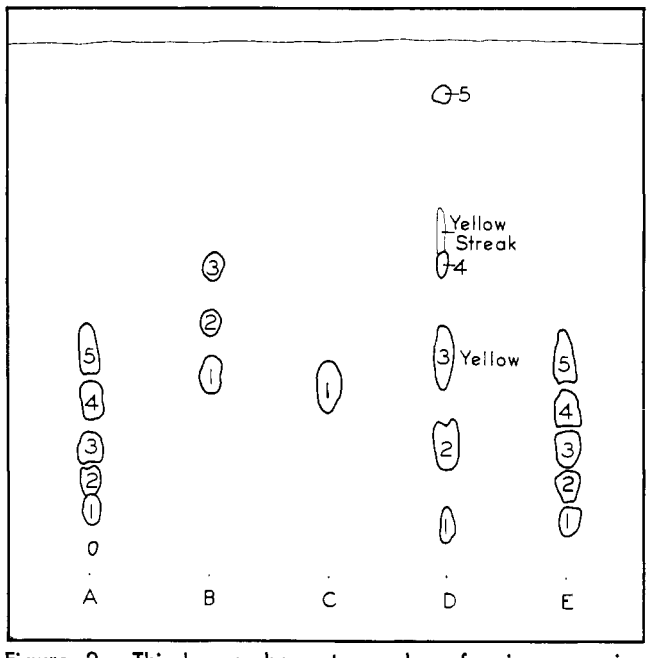

Figure 2. Thin-layer chromatography of primary amine hydrochlorides

A and *E*, ethylenediamine (1), 1,3-propanediamine (2), 1,4-butanediamine (3), 1,5-pentanediamine (4), 1,6-hexanediamine (5). *B*, methylamine (1), ethylamine (2), propyl amine (3). *C*, ethanolamine. *D*, volatile amines from menhaden oil

the other was close to the solvent front. When log R_m values, calculated according to the formula proposed by Bremner and Kenten (*2*) ($R_m = 1/R_f - 1$), were plotted against the carbon number of the primary monoamines, this farther spot corresponded closely to hexylamine.

A typical tracing of a thin-layer chromatogram, developed with the phenol–water–acetic acid solvent designed to separate diamines, is shown in Figure 2. Under the conditions employed here, the unknown material (*D*) gave five distinct spots. The first two spots had R_f values lower than those of the monoamines and corresponded closely to ethylenediamine and 1,4-butanediamine (putrescine), respectively. Spot *D*-3 was yellow with no tinge of pink and, therefore, is not considered to be due to an amine. Spot *D*-4, which showed a slight pink tinge preceded by a yellow streak, corresponds to propylamine, and *D*-5, which was also very weak, is probably due to the hexylamine detected on the paper chromatogram.

The amines present in this volatile fraction from menhaden oil consist mainly of ethylenediamine and butanediamine with minor amounts of C_3 and C_6 monoamines. More polar polyamines such as spermine and spermidine have lower R_f values than the diamines (*11*) and, therefore, can be considered absent from this material.

If the total nitrogen content of this aqueous fraction, as determined by Kjeldahl analysis, is expressed in terms of butanediamine, it is equivalent to a concentration of 0.15% of this compound in the volatile aqueous phase and of approximately 0.7 p.p.m. in the original menhaden oil. Undoubtedly, only a small fraction of the amines present in the original oil was recovered and detected but, even if we assume that 90% of these compounds were lost, their original concentration in the oil must have been extremely low. The odor of these compounds is not pleasant, but, in spite of the names given to some of the members of this series (putrescine, cadaverine), it is not of a putrid character, but rather similar to that of ammonia, although considerably weaker. Although these compounds may play a role in the over-all odor of fish oils, the nature of their odor and their low concentrations suggest that their contribution must be rather small.

Trimethylamine, which has often been associated with fishy odors (*14*), was not detected in this sample of volatile material.

Acknowledgment

The authors are grateful to Maurice Stansby and Eric Gauglitz, Seattle Technological Laboratory, Bureau of Commercial Fisheries, for supplying the starting materials.

Literature Cited

(1) Bregoff, M., Roberts, E., Delwiche, C. C., *J. Biol. Chem.* **205,** 565 (1953).
(2) Bremner, J. M., Kenten, R. H., *Biochem. J.* **49,** 651 (1951).
(3) Brenner, M., Niederwieser, A., Pataki, G., "Aminosäuren und Derivate," in E. Stahl, "Dünschicht-Chromatographie," Springer-Verlag, Berlin, 1962.
(4) Chang, S. S., Masuda, Y., Mookerjee, B. D., *Chem. Ind. (London)* **1962,** 1023.
(5) Chargaff, E., Levine, C., Green, C., *J. Biol. Chem.* **175,** 67 (1948).
(6) Chipault, J. R., McMeans, Evelyn, J. AGR. FOOD CHEM. **13,** 15 (1965).
(7) Davies, W. L., Gill, E., *J. Soc. Chem. Ind.* **55,** 141-T (1936).
(8) Feigl, F., "Spot Tests in Organic Analysis," 5th ed., Elsevier, New York, 1956.
(9) Forss, D. A., Dunstone, E. A., Stark, W., *J. Dairy Res.* **27,** 211, 373 (1960).
(10) Gauglitz, E. J., Gruger, E. H., *J. Am. Oil Chemists' Soc.* **42,** 561 (1963).
(11) Herbst, E. J., Keister, D. K., Weaver, R. H., *Arch. Biochem. Biophys.* **75,** 178 (1958).
(12) Noble, E. D., *Anal. Chem.* **27,** 1413 (1955).
(13) Russell, J. A., *J. Biol. Chem.* **156,** 457 (1944).
(14) Stansby, M. E., *Food Technol.* **16,** No. 4, 28 (1962).
(15) Steiner, M., Stein von Kamienski, E., *Naturwissenschaften* **40,** 483 (1953).

Received for review December 8, 1965. Accepted March 14, 1966. Study supported by the U. S. Department of the Interior, Fish and Wildlife Service, and by The Hormel Foundation.