Effect of Ionizing Radiations on Stability of Fats

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Ambient temperature irradiation of unsaturated fats in oxygen initiates autoxidation, which continues at a rapid rate during subsequent storage in presence of oxygen. Even when antioxidants are present, irradiation almost completely eliminates the induction period of the fat. Storage under vacuum of fats irradiated in oxygen, however, results in some recovery of stability. Irradiation in vacuum decreases the stability of fats, so that when exposed to oxygen after irradiation they undergo autoxidative deterioration more easily. When fats, proteins, and other food components are in intimate contact, the effect of irradiation on the stability of fats is more complex. With piecrusts, ground pork, and ground beef, irradiation resulted in an immediate decrease in stability, partly regained during postirradiation storage. Irradiation of ground beef treated with antioxidants produced an immediate improvement in fat stability, which continued to increase during postirradiation storage. The effect did not occur with ground pork and appears to be associated with the free glyceride fat of beef.

IRRADIATION of fats produces free radicals (*1*) which, in the presence of oxygen, can initiate autoxidation chain reactions, and autoxidation products continue to accumulate rapidly during subsequent storage (*8*, *9*). The severity of these changes depends mainly on the conditions of irradiation and storage, composition of the fat, and type and amount of antioxidants present.

Foods can be irradiated conveniently in sealed containers in absence of oxygen, but the influence of such treatment on the ability of the fat to resist autoxidation when the container is opened and the content exposed to oxygen at some later date had not been determined. It was the purpose of this investigation to find out the effect of irradiation on the stability of fats and further changes to be expected during postirradiation storage.

Experimental

Materials Used. METHYL LINOLEATE. The methyl linoleate used has been described (*3*). Propyl gallate, butylated hydroxyanisole, α-tocopherol, citric acid, and ascorbic acid were incorporated into methyl linoleate at a concentration of 0.01% by adding 1 ml. of a 1% solution of the desired antioxidant to 100 grams of methyl linoleate and removing the solvent under vacuum in a rotating evaporator. (Ethylenedinitrilo)tetraacetic acid and methionine were completely insoluble in a number of organic solvents investigated and, when these compounds were used, an amount of the finely powdered materials sufficient to give a concentration of 0.01% was dispersed in methyl linoleate by further grinding the powder with the methyl ester in a mortar.

NATURAL FATS. Corn oil was purchased at a local market. The two lards used were prime steam lards free of added antioxidant.

GROUND MEATS. Ground beef and ground pork were purchased in local meat markets. The ground meats were reground in the laboratory and mixed thoroughly by hand. When antioxidants were used, solutions of these compounds were distributed evenly over a 1/8-inch layer of the ground meats, which were then mixed again thoroughly by repeatedly folding and spreading. Water-soluble antioxidants were used in aqueous solutions, but butylated hydroxyanisole, tocopherol, and vitamin A palmitate were dissolved in low boiling petroleum ether. Other than through normal evaporation, no effort was made to remove the small amount of solvent added to the meat in this manner.

PIECRUST. A typical homemade piecrust was prepared from 2 cups of flour, 2/3 cup of lard (antioxidant-free), 4 tablespoons of water, and 1 teaspoon of salt. A second piecrust sample consisted of the same basic formula to which an antioxidant combination of 0.01% tocopherol, 0.01% vitamin A palmitate, and 0.01% monosodium phosphate (based on the lard used) was added. The tocopherol and vitamin A palmitate were dissolved in the lard, while the monosodium phosphate was dissolved in the water used to prepare the piecrust. A third piecrust sample was a refrigerated mix purchased at a local market.

The piecrusts were prepared and rolled in the usual manner and baked directly on cookie sheets in an oven at 245° C. for 6 minutes.

RECONSTITUTED GROUND MEATS. In an attempt to explain some of the differences observed with samples of ground pork and ground beef containing added antioxidants, the free fat, bound lipids, and crude fat-free protein were isolated from samples of these tissues and remixed in various combinations before irradiation. These experiments were performed with commercially prepared freeze-dried raw beef and pork. The freeze-dried meats were extracted with low boiling (30° to 60° C.) petroleum ether in a Soxhlet apparatus to remove free glyceride fat. The residue was then extracted three times by maceration in a Waring Blendor with a mixture of chloroform and methanol (1 to 1). The residual crude protein was freed of solvent under vacuum and aliquots of each of the extracts were evaporated to dryness to determine the lipid concentration in each. The lipid and protein fractions were then cross-combined to give seven final products containing protein, bound lipids, and free fat of different origin in the proportion in which they were originally found to occur in the freeze-dried beef.

Each reconstituted meat was rehydrated with distilled water. The addition of antioxidant and any further treatment were then performed as with the fresh ground meats.

Preparation of Samples for Irradiation. METHYL LINOLEATE, CORN OIL, AND LARD. For irradiation under vacuum, 2 ml. of these materials were sealed in 15-ml. bulbs, while for irradiation under oxygen the same amount was sealed in an atmosphere of pure oxygen in glass bulbs with a total capacity of approximately 45 ml.

GROUND MEATS. Ten grams of fresh or reconstituted ground meat were introduced into 25-mm. diameter test tubes which were then alternately evacuated and filled with nitrogen five times and finally sealed under partial vacuum. The sealed samples were placed in a boiling water bath and heated to an internal temperature of 71° C. Preliminary experiments, with a thermocouple in the center of the ground meat, showed that under these conditions pork had to be kept in the water bath for 4 minutes and beef for 4.25 minutes to attain this temperature.

PIECRUSTS. Each baked piecrust was broken in small crumbs (less than 2-mm. diameter), mixed thoroughly, and introduced into test tubes which were alternately evacuated and refilled with nitrogen five times and finally sealed under vacuum.

IRRADIATION OF SAMPLES. The glass tubes were packed into metal cans and shipped to the Materials Testing Reactor at Idaho Falls, Idaho, under dry ice refrigeration for irradiation. All samples were thawed for 1 hour in a water bath at 24° C. immediately prior to irradiation at ambient temperature (approximately 21° C.). Immediately after irradiation the samples were repacked in dry ice for return shipment to our laboratory. Dose rates varied between 1.10 and 5.17 megarads per hour.

Stability Measurements. With a few samples irradiated in an atmosphere of oxygen subsequent autoxidation was followed by measuring the accumulation of peroxides in the samples stored in air at various temperatures. In all other cases stabilities were determined by the oxygen absorption method using a Warburg apparatus at 60° C.

METHYL LINOLEATE, CORN OIL, AND LARD. Individual samples irradiated in vacuum were stored as required and the stability was determined by the oxygen absorption method, in duplicate, using the contents of one tube for each determination.

When these materials were irradiated under oxygen, all the tubes of the same fat that received the same dose were opened when they were returned to our laboratory and the contents pooled to give a homogeneous starting material. Small portions were used to determine the stability before storage and the remainder was sealed under vacuum in several containers for storage as required.

GROUND MEATS. The irradiated samples were stored without any additional treatment. For stability measurements a sample was opened and immediately placed in an atmosphere of nitrogen. Approximately 5 ml. of water were mixed with the ground meat and the tube was heated on a steam bath until the melted fat floated to the surface of the water. The tube was then centrifuged briefly and placed in ice water until the fat solidified. The solid disks of fat from two tubes of ground pork or three tubes of ground beef were removed with a spatula, combined, melted, carefully dried over a small amount of anhydrous sodium sulfate, and used for stability measurements.

PIECRUSTS. The piecrusts were stored also without further treatment. Enough fat for stability measurements was obtained from these materials by a single extraction with approximately 25 ml. of low boiling (30° to 60° C.) petroleum ether. The extract was filtered and dried with a small amount of anhydrous sodium sulfate and the solvent was removed under vacuum. The residual fat was used directly for measurements of oxygen absorption.

Examination of the oxygen absorption curves showed that for many of the samples containing added antioxidant, rapid oxidation did not occur until an oxygen absorption level of 100 mmoles of O_2 per kg. of fat had been reached. For comparison purposes, therefore, the stability of these materials was arbitrarily defined as the time required to absorb this amount of oxygen. Stability measurements were usually determined in duplicate and individual values did not vary from the means by more than 3.5%.

Results and Discussion

Accumulation of Peroxides after Irradiation in Oxygen. Figure 1 shows the effect of irradiation in an atmosphere of oxygen on the accumulation of peroxides in methyl linoleate samples containing added antioxidants, when the irradiated materials are allowed to continue oxidizing in air, at room temperature. Irradiation with 4.65 megarads almost completely eliminated the induction period of the samples. Irradiated methyl linoleate containing propyl gallate absorbs oxygen at the same rate as the irradiated control free of antioxidant. The two irradiated samples containing butylated hydroxyanisole (BHA) and BHA plus citric acid show evidence of a very short autocatalytic segment in their peroxide accumulation curves. BHA, which is less effective than propyl gallate as an antioxidant for ordinary autoxidation (compare curves 6 and 7), retains a slight antioxidant activity after irradiation, which is further increased by citric acid. These results are in agreement with previous observations that propyl gallate is completely destroyed by irradiation while BHA is somewhat more resistant (*2*). Irradiation for 4.2 hours had the same effect on the rate of oxidation of methyl linoleate containing 0.1% each BHA and citric acid as storage in air for approximately 240 days.

Effect of Storage in Vacuum after Irradiation in Oxygen. Table I shows

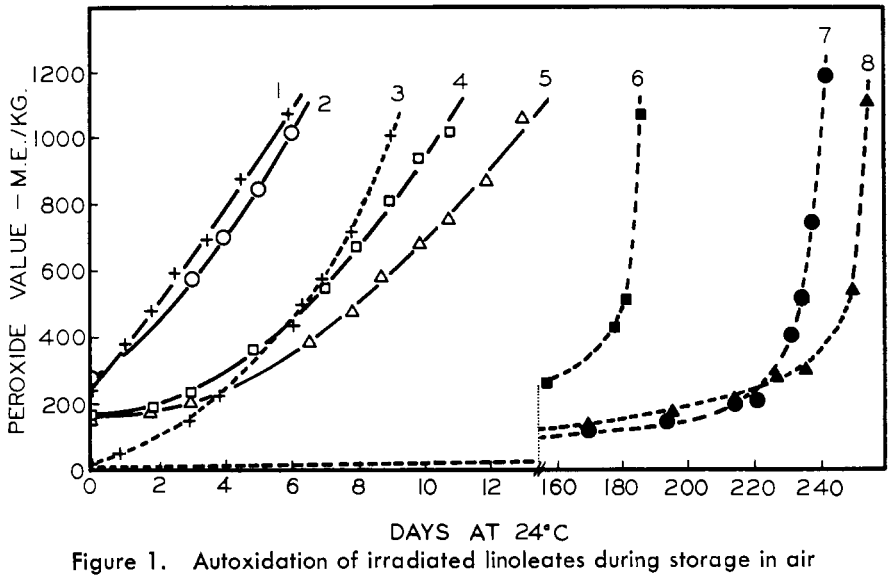

Figure 1. Autoxidation of irradiated linoleates during storage in air

———Samples irradiated with 4.65 megarads in oxygen. 1, no antioxidant; 2, 0.01% propy gallate; 4, 0.01% BHA; 5, 0.01% BHA + 0.01% citric acid
— — —Nonirradiated. 3, no antioxidant; 6, 0.01% BHA; 7, 0.01% propyl gallate; 8, 0.01% BHA + 0.01% citric acid

Table I. Effect of Storage in Vacuum on Stability of Fats Irradiated in Presence of Oxygen

Sample	Dose, Megarads	Stability[a] after Storage at 25° C. 0 week	2 weeks	8 weeks	20 weeks
Methyl linoleate	0	2.0	...	...	2.6
	5.0	1.0	1.2	1.3	1.5
Methyl linoleate + 0.01% tocopherol	0	11.8	...	...	14.9
	5.0	1.1	1.5	1.8	2.3
Methyl linoleate + antioxidant mixture[b]	0	13.7	...	...	23.1
	5.0	1.0	1.4	1.7	2.1
Corn oil	0	228	242	229	226
	4.65	45	65	83	92
Lard 1	0	59	82	72	52
	4.65	6	7	8	19

[a] Hours to absorb 100 mmoles of O_2/kg. of fat.
[b] 0.01% each α-tocopherol, vitamin A palmitate, and NaH_2PO_4.

stability decreases in all samples irradiated in oxygen and the ineffectiveness of antioxidants either added to methyl linoleate or naturally present in corn oil and lard. These changes are expected, since it has been shown that irradiation in oxygen destroys antioxidants almost completely (*2*), and initiates rapid autoxidation. In all cases, however, storage in vacuum resulted in partial recovery of stability, which continued to increase with storage time. Irradiation of unsaturated fats in oxygen initiates a large number of chain reactions which, however, must terminate when oxygen is no longer available. Storage in vacuum, therefore, should result in a stability increase because, upon reexposure to oxygen, new free radicals must be formed and new reaction chains initiated before autoxidation can again proceed rapidly.

Bradshaw and Truby (*1*), however, have found that free radicals, resulting directly from irradiation or from exposure of irradiated fats to oxygen, disappear very quickly under the conditions employed here. Consequently, stability improvement, if due to free radical decay, should appear almost immediately after access to oxygen has been denied, and should not extend over a period of 20 weeks as observed in the present study.

It is more likely that this continued stability increase is caused by gradual destruction of prooxygenic substances that are either activated molecules much more stable than free radicals or prooxidants resulting from the decomposition of peroxides. The latter is suggested also by the results in Tables II and III, where storage of materials irradiated in vacuum showed no such recovery effect; in absence of oxygen during irradiation, no peroxides are formed which can yield prooxygenic decomposition products.

Effect of Irradiation in Vacuum. Simple Fats. In Table II the loss of stability by linoleate samples treated with antioxidants may be attributed largely to destruction of the antioxidants. However, a small but definite stability decrease, proportional to dose, is shown also by the pure linoleate sample containing no antioxidant, indicating that irradiation has an adverse effect on stability independent of its destructive action on antioxidants. This effect may be due to breakdown products which have been shown to accumulate when fats are irradiated in vacuum (*3*, *4*, *6*), and which may either autoxidize more rapidly than the original methyl linoleate or possess prooxygenic activity. As would be expected, synergists improve the stability of the irradiated samples containing antioxidants by enhancing the effect of the antioxidants remaining undestroyed.

The effect of storage on the stability of these irradiated materials is not clear and it is not possible to draw definite conclusions from the limited data available. There is a tendency, however, for the stability of the samples containing no synergists to decrease appreciably during storage, while in general there is only a slight further deterioration where synergists are present.

It has been reported (*2*) that tocopherol dissolved in methyl myristate at a concentration of 0.01% is completely destroyed by a dose of 5.0 megarads. In the present study, however, methyl linoleate containing 0.01% α-tocopherol retains considerable stability after irradiation with 4.65 megarads. Although the resistance to oxidation decreases rapidly during postirradiation storage, the samples stored for 20 weeks still retain a stability 3 to 4 times greater than that of the corresponding irradiated control free of antioxidant. These observations support the findings of Rose, Lips, and Cyr (*7*) that tocopherol is more easily destroyed by irradiation in methyl myristate than in methyl linoleate.

Corn oil contains large amounts of natural antioxidants and, although ir-

Table II. Stability of Methyl Linoleates Irradiated and Stored under Vacuum

Antioxidant	Dose, Megarads	Stability[a] (Hours) after Storage under Specified Conditions: 25° C. 0 weeks	25° C. 2 weeks	25° C. 8 weeks	25° C. 20 weeks	2° C. 2 weeks	2° C. 8 weeks	2° C. 20 weeks	−20° C. 2 weeks	−20° C. 8 weeks	−20° C. 20 weeks
None	0	3.7	...	...	3.7	...	...	3.1	...	...	3.4
	1.86	3.1	2.9	2.8	2.4	2.5	2.5	2.6	2.8	2.6	3.0
	4.65	2.9	2.9	3.0	2.2	2.7	2.5	3.1	2.7	2.2	2.6
	7.44	2.7	2.8	2.3	1.8	2.7	2.3	2.6	2.6	2.7	2.9
0.01% α-Tocopherol	0	45	...	...	48	...	...	20	...	...	41
	1.86	...	...	16	...	...	...	...	...	...	...
	4.65	32	14	7	8	15	13	9	13	15	9
	7.44	...	...	7	...	...	...	...	...	...	...
0.01% Propyl gallate	0	102	...	...	90	...	...	85	...	...	106
	1.86	...	...	65	...	...	...	...	...	...	...
	4.65	52	29	47	37	37	18	38	41	36	37
	7.44	...	...	22	...	...	...	...	...	...	...
0.01% Butylated hydroxyanisole (BHA)	0	96	...	...	71	...	...	78	...	...	48
	1.86	75	80	69	50	67	52	53	96	58	45
	4.65	63	38	61	58	85	46	39	76	56	26
	7.44	49	56	32	27	57	46	36	57	54	37
0.01% BHA + 0.01% citric acid	0	131	...	...	121	...	...	128	...	...	116
	1.86	...	...	114	...	...	...	...	...	...	...
	4.65	87	87	93	95	88	87	94	93	88	80
	7.44	...	...	73	...	...	...	...	...	...	...
0.01% BHA + 0.01% ascorbic acid	0	144	...	...	118	...	...	113	...	...	108
	1.86	...	...	113	...	...	...	...	...	...	...
	4.65	...	89	92	93	92	89	80	90	83	85
	7.44	...	...	75	...	...	...	...	...	...	...
0.01% BHA + 0.01% (ethylene-dinitrilo)-tetraacetic acid	0	128	...	...	119	...	...	108	...	...	107
	1.86	...	...	90	...	...	...	...	...	...	...
	4.65	95	87	95	89	84	87	83	84	80	79
	7.44	...	...	88	...	...	...	...	...	...	...
0.01% BHA + 0.01% methionine	0	146	...	...	131	...	...	129	...	...	128
	1.86	...	...	122	...	...	...	...	...	...	...
	4.65	73	101	106	99	105	103	98	97	99	94
	7.44	...	...	80	...	...	...	...	...	...	...

[a] Hours to absorb 100 mmoles of O_2/kg. of fat.

Table III. Stability of Various Fats Irradiated under Vacuum

Sample	Dose, Megarads	Stability[a] (Hours) after Storage at 25° C. 0 week	2 weeks	8 weeks	20 weeks
Corn oil	0	252	239	236	208
	1.86	221	198	211	201
	7.44	158	156	168	144
Lard 1	0	90	79	64	74
	1.86	34	33	36	28
Lard 2	0	47	...	...	37
	2	19	24	25	22
	5	16	17	16	18
	8	12	12	15	14
Piecrust (homemade)	0	95	118	137	...[b]
	2	36	51	83	88
	5	21	34	50	51
	8	21	...[b]	35	49
Piecrust (homemade) + antioxidant mixture[c]	0	233	242	250	255
	2	132	174	202	211
	5	88	...[b]	133	...[b]
	8	55	61	71	102
Piecrust (commercial)	0	1155	1154	1097	1014
	2	686	861	768	810
	5	492	535	487	678
	8	122	435	357	494
Ground pork	0	172	...	...	...
	2	18	62	65	83
	5	15	20	26	53
	8	13	14	17	36
Ground pork + antioxidant mixture[c]	0	380	...	...	...
	2	278	...[b]	...[b]	448
	5	175	304	306	348
	8	...[b]	219	278	337
Ground beef 1	0	228	...	...	...
	2	35	62	...[b]	101
	5	30	45	67	93
	8	32	40	40	...
Ground beef 1 + antioxidant mixture[c]	0	505	...	...	...
	2	...	660	614	649
	5	630	732	731	762
	8	804	1092	1055	1133
Ground beef 2	0	271	...	...	...
	2	20	270	...	...
	8	159	30	...	...
Ground beef 2 + antioxidant mixture[c]	0	475	...	...	...
	2	494	476	...	...
	8	1130	728	...	...
Ground beef 2 + 0.01% α-tocopherol	0	620	...	...	...
	2	579	686	673	...
	8	996	1212	945	...
Ground beef 2 + 0.001% BHA	0	1020	...	...	...
	2	910	1278	927	...
	8	1460	2160	2076	...

[a] Hours to absorb 100 mmoles of oxygen/kg. of fat.
[b] Seal on these samples cracked. All had strong rancid odor and were discarded.
[c] 0.01% each α-tocopherol, vitamin A palmitate, and NaH_2PO_4.

radiation reduces its stability, it is seen in Table III that the irradiated material remains resistant to autoxidation. Lard, however, which contains only small amounts of antioxidants, suffers a proportionately much greater stability loss as a result of irradiation. Whereas a dose of 7.4 megarads reduces the stability of corn oil by only 38%, that of lard was decreased by more than 60% with a dose of only 2 megarads. Postirradiation storage of these fats has no significant effect on their stability.

FATS IN FOODS. Table III shows also that, as with lard alone, irradiation of the piecrusts resulted in a considerable decrease in fat stability. However, in the case of this more complex fat-protein system a very significant recovery of stability occurred during postirradiation storage, so that the samples irradiated with 2 megarads and stored for 20 weeks at room temperature compared favorably with the original nonirradiated materials.

Irradiation reduced the stabilities of the fat from ground pork and ground beef to 10 to 15% of the original values, but again, postirradiation storage resulted in considerable stability improvement.

The incorporation of the special antioxidant mixture did not have a great effect on the stability of the nonirradiated meats but it greatly decreased the destructive effect of irradiation. Furthermore, stability continued to increase during storage after irradiation, so that the fat from pork irradiated with 2 megarads and stored for 20 weeks was more resistant to autoxidation than the original nonirradiated pork fat.

In contrast to the other substrates studied, ground beef containing added antioxidants showed an immediate stability increase after irradiation and a continued improvement during subsequent storage. This immediate increase in stability was observed with two different samples of ground beef irradiated several months apart, but

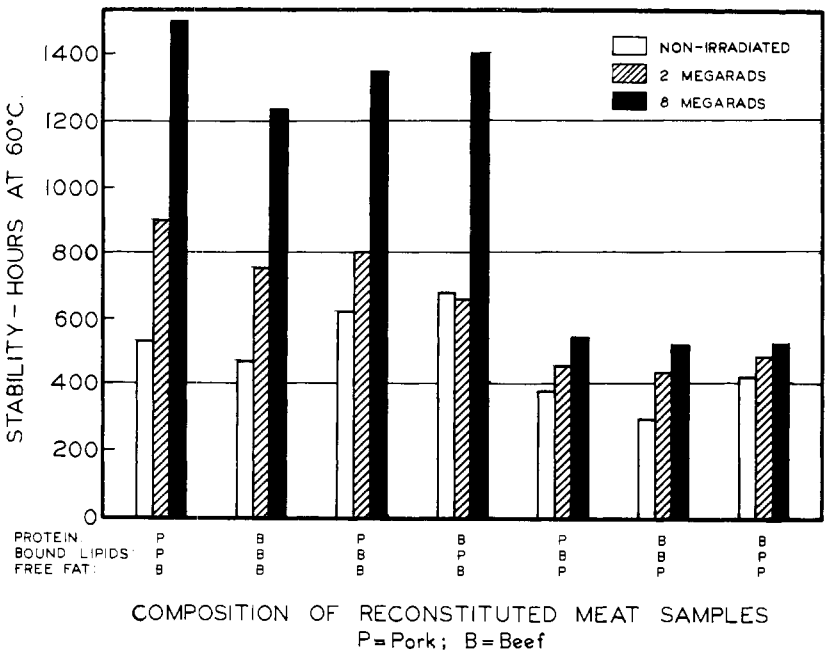

Figure 2. Effect of irradiation on stability of fat from reconstituted beef-pork combinations

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).

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FISH OIL ODORS

Odor Problems of Fish Oils. Volatile Amines of Menhaden Oil

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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