FISHERY INDUSTRIAL RESEARCH

VOLUME 5   **NO. 1**

FISHERY INDUSTRIAL RESEARCH
Volume 5 -- Number 1

PROBLEM OF "GREEN" FROZEN RAW BREADED SHRIMP

by

Mary H. Thompson and Robert N. Farragut

ABSTRACT

A green coloration has appeared sporadically on frozen raw breaded shrimp. Reported here are the results of a study made to determine the cause of the green coloration and to find a method of avoiding it. The study indicates that the coloration was caused by airborne metallic particles and that eliminiating the particles from contact with the product would therefore solve the problem.

CONTENTS

1

INTRODUCTION

At the Bureau of Commercial Fisheries Technological Laboratory in Pascagoula, Mississippi, during the summer of 1967, we became aware of a problem of green color on frozen raw breaded shrimp. We found that the green color occurred sporadically throughout the year and that when it did occur in a given lot of shrimp it affected, to some extent, nearly every shrimp in the lot.

In October 1967, a representative of a large seafood company visited the laboratory to provide us with further information. During the conference, we decided that the problem was of sufficient importance to the public and the fishing industry to warrant our studying it. In the conference, we learned that the consumer was rejecting the product because it looked moldy. We felt that rejection on such a basis might lead the consumer not just to resist buying certain particular brands, but to resist buying all frozen raw breaded shrimp.

Accordingly, this paper reports our efforts to solve this problem of the sporadic green coloration. The paper is divided into two main parts. The first reports on our attempts to determine the cause of the green coloration; the second part, our suggestions for remedying the problem.

I. DETERMINING THE CAUSE OF THE GREEN COLORATION

Our initial step was to find out the nature of the green coloration. The results of this effort indicated that it was caused by metallic contamination. Our second step therefore was to find the source of the contamination.

A. DETERMINING THE NATURE OF THE GREEN COLORATION

We tried first to determine whether the colored material was organic or inorganic in nature by suspending green breading in common organic solvents and in water. The color was not soluble in the organic solvents, nor did it change appreciably in appearance when placed in them. This stability toward organic solvents indicated that the colored material was probably inorganic in nature. Strengthening this inference was the fact that the material was soluble in water and that, upon being dissolved, it changed from a medium gray-green to a yellow green. This solubility in water and change in color indicated that the material was inorganic and further that it was loosely associated.

Despite these preliminary indications of the inorganic nature of the colored material, the tests did not rule out the possibility that the coloration might be — at least in part — microbiological in nature. So we needed to check on two hypothesis — that is, whether the coloration was microbiological in nature or inorganic.

1. Microbiological Hypothesis

In checking on the microbiological hypothesis, we took two approaches: The first was to determine the nature of any microbial growth that might result from exposure of growth media to the green material. The second was to examine the growth produced by bacteria of the genus *Pseudomonas* to see whether such growths might be similar in appearance to the green-colored material, because *Pseudomonas* species are known to produce green pigments.

a. Microbial growths resulting from exposure of growth media to the green-colored material. — Tryptone glucose beef extract agar was prepared at double strength. After the agar cooled to about 80° C., equal amounts of discolored shrimp were added, and the mixture was homogenized in a warm Waring blender jar.[1] Plates were poured and, after being cooled, were rubbed with the green portion of a discolored shrimp. The plates were

---

[1] The use of trade names is merely to facilitate descriptions; no endorsement is implied.

incubated at 35° C., 20° to 24° C., 5° C., and −20° C.

Only the plates at the two higher temperatures showed growth. At 35° C., a coarse, velvety brown growth appeared, and at 20° C. a dark, green velvety growth appeared. Both growths, after being gram stained and examined at 970 diameters, appeared to be yeasts. When we grew isolates on shrimp, the subsequent green growth was on top of the surface and differed in texture from that of the usual green spots.

b. Comparison with the appearance of Pseudomonas.

— Because *Pseudomonas* are often cited as producing colors in refrigerated foods, we secured pure cultures of *P. aeruginosa* and *P. fluorescens* from the American Type Culture Collection for our studies. We streaked 24-hour broth cultures of the true pseudomonads on agar media composed of tryptone glucose beef extract and shrimp, and cultured the media at the same four temperatures as before. The true microorganisms produced a scanty, creamy brown growth after 48 hours at 35° C. After 48 hours at from 20° to 24° C., a heavy yellow-green growth appeared. Side by side comparison with the gray-green colored shrimp lead to the conclusion that the shrimp are discolored by some source other than *P. aeruginosa* or *P. fluorescens*. The plates at 5° C. and −20° C. showed no growth after 8 days.

Conclusion from the microbial tests.

— The results of these two tests, though not conclusive, supported our solubility tests in indicating that the material causing the particular green coloration of concern here is not microbial in nature.

2. Inorganic Hypothesis

When we examined the discolored shrimp, we found three colors:

1. A medium green-gray, which turned brown on exposure to air.

2. A bright green, which did not change color on exposure to air.

3. An aqua green, which also did not change color on exposure to air.

In trying to find the inorganic basis, if any, for these colors and the changes in them, we made inorganic analyses, checked the pH of the samples, and carefully examined the physical appearance of the samples.

a. Inorganic analyses.

— In our inorganic analyses, we first ran spot tests and then analyzed the samples by the atomic absorption technique.

(1) Spot tests. — Spot tests indicated the presence of iron and copper.

By mixing several colored ions with basic solutions, such as those produced by ammonium hydroxide, and with nongreen breaded shrimp, we were able to produce the various green colors artificially. Among the colored ions we tried were those of iron, copper, and cobalt. The most prevalent gray-green was produced by the ferrous ion, not by the more common ferric ion. Ferric ion and ammonium hydroxide produced the same dull-brown color that resulted from the exposure of green shrimp to air.

Compounds in the breading (probably milk solids) apparently were capable of slowing down the normal oxidation of ferrous ion to ferric ion, particularly in the cold state, which may account for the persistence of the green color.

(2) Atomic absorption analyses. — We used a Perkin-Elmer Model 303 Absorption Spectrophotometer to gain an indication of the relative amounts of ferrous and ferric ion and of the amount of iron and copper in the samples.

(a) *Ferrous and ferric ion.* — To ascertain the presence of ferrous ion, we stripped six of the green shrimp of their breading and likewise stripped six nongreen shrimp. We also obtained samples of unused breading from the breading bins at the plant. We washed the breading with concentrated HCl to dissolve any iron present. To separate ferrous ions from ferric, we put the solutions over a Dowex 1-8X resin column and eluted it with two concentrations of HCl (7N and 1N). We then evaporated both fractions to

dryness and transferred them to volumetric flasks, which we made to volume. Finally, we measured the concentration of iron in each of the two flasks by atomic absorption.

We found that the ferrous ion was present in both green and nongreen shrimp breading but that ferrous ion was not present in as large an amount in the breading prior to its being used on the shrimp (Table 1).

Table 1.—Ferrous and ferric ions in shrimp breading

Sample	Ferrous ion	Ferric ion	Ratio: Ferrous/ferric
	$\mu g.$	$\mu g.$	
Nongreen shrimp breading:			
1	80.0	90.0	0.89
2	50.0	70.0	0.71
3	52.5	75.0	0.70
4	102.5	70.0	1.46
5	117.5	75.0	1.57
6	85.0	67.5	1.30
Green shrimp breading:			
1	52.5	155.0	0.34
2	107.5	92.5	1.12
3	95.0	90.0	1.06
4	80.0	75.0	1.07
5	55.0	90.0	0.61
6	40.0	80.0	0.50
Shrimp breading (unused from open sacks):			
1	65.0	1050.0	0.06
2	30.0	750.0	0.04

This finding indicates that enough ferrous ion exists on nongreen shrimp to produce green shrimp but that some condition for its production has not yet been met.

(b) *Iron and copper.* — Samples of green and nongreen shrimp were stripped of their breading and dry ashed. To determine the difference, if any, in total iron and copper contents, we analyzed the ashed samples by the atomic absorption technique.

Table 2 indicates that (1) most of the shrimp had been thawed and refrozen prior to our sampling time, as the ratio of shrimp to breading was low and the shrimp appeared to be very dessicated, but that the green shrimp had been mishandled with regard to temperature fluctuation to a greater extent than the nongreen shrimp, (2) the ratio of breading to shrimp was similar in the two lots, and (3) the average iron and copper contents of the

Table 2.—Iron and copper in shrimp and breading

Samples	Ratio of shrimp:breading	Moisture Shrimp	Moisture Breading	Iron μg./g. dry wt. Shrimp	Iron μg./g. dry wt. Breading	Iron μg./g. in total dry wt. Shrimp	Iron μg./g. in total dry wt. Breading	Copper μg./g. dry wt. Shrimp	Copper μg./g. dry wt. Breading	Copper μg./g. in total dry wt. Shrimp	Copper μg./g. in total dry wt. Breading
		Percent	Percent								
Nongreen:											
1	0.67	74.0	49.9	4.1	11.4	15.0	42.5	1.9	1.0	7.0	3.8
2	0.79	74.6	49.8	3.6	10.4	16.3	40.3	2.1	0.4	9.5	1.5
3	0.52	75.1	48.9	5.6	10.8	18.8	45.0	0.7	0.8	2.5	3.5
4	0.62	73.4	52.9	3.5	7.4	16.8	40.3	1.2	0.5	5.8	2.5
5	0.58	72.9	52.7	6.3	10.3	27.5	55.0	2.0	0.4	8.8	2.3
6	0.54	71.5	52.3	5.0	8.8	15.8	38.0	0.9	1.4	2.8	6.0
Green:											
1	0.67	74.4	49.6	3.9	9.1	16.0	37.5	0.7	0.4	3.0	1.5
2	0.63	79.3	49.0	2.9	8.0	15.8	43.0	1.3	0.8	7.3	4.5
3	0.80	73.9	50.3	3.7	10.5	20.0	47.8	1.5	1.1	8.0	5.0
4	0.73	75.0	48.6	5.8	10.9	32.5	55.0	0.9	0.4	5.0	2.3
5	0.73	73.9	49.0	7.0	13.1	25.0	42.5	2.2	0.6	7.8	2.0
6	0.56	74.3	49.9	3.8	7.0	20.0	43.8	1.4	0.4	7.3	2.3
Average:											
Nongreen	0.62	73.6	51.1	4.7	9.9	16.4	43.5	1.5	0.75	6.1	3.3
Green	0.69	75.1	49.4	4.5	9.8	21.6	44.9	1.3	0.6	6.3	2.9

two lots of shrimp did not differ appreciably, nor did the average iron and copper contents of the breading in the green and the nongreen lots differ appreciably, except that the iron content of the green shrimp was higher than that of nongreen shrimp on the basis of total dry weight.

b. pH. — The pH of green shrimp averaged 7.8 or higher, whereas that of nongreen shrimp averaged 7.4.

c. Appearance. — In our physical examination of the product, we noticed that some samples were desiccated and that some had minute black specks on them.

(1) <u>Desiccation.</u> — Thawing may have taken place during the distribution of the product, because most of the shrimp appeared to be desiccated upon being stripped of their breading. Furthermore, the meat of the severely green breaded shrimp was found to have a green cast.

(2) <u>Black specks.</u> — During the debreading procedure, we noticed a number of black specks located on the shrimp meat and on the tail section. Some of these particles were magnetizable, indicating the presence of iron. We therefore decided to analyze for metals in samples containing these particles.

To ensure that we were correct in our thinking that the green coloration might be caused by metallic contamination, we put our hypothesis to the test by attempting to produce the green coloration artificially.

(a) *Analysis of samples containing black specks.* — We again used the atomic absorption technique to analyze breading and batter, water samples, and water washings from the shrimp for chromium, iron, copper, nickel, and cobalt.

We solubilized breading and batter by treating them with hot concentrated sodium hydroxide (which will not dissolve most metals) in glass containers. The resulting solution and residue insoluble in the sodium hydroxide was filtered through coarse fritted glass filters. We dissolved the separated particles in hot aqua regia, boiled the solution to dryness, added water to the residue, made to volume, and analyzed.

We filtered water samples directly and treated the residue as before.

We washed shrimp samples with water until we removed all visible black particles and then filtered the washings. Again, we treated the residue as before.

Results indicated considerable metal contamination (Table 3). Although copper and iron are found to a degree in animal and plant material, the presence of this large a quantity of chromium and nickel seems to eliminate vegetable or animal contamination. These latter two metals are seldom found in living material; and if they are found, they are present in quantities 100 to 1,000 times as small. The presence of metal on or in all samples indicated an unusual source of contamination. The possibility of airborne or waterborne metal contamination from nearby metal-working plants was raised.

(b) *Production of the green color artificially.* — To see if we could produce the green color on the breaded shrimp artificially, we implanted the shrimp tissue with the metal material that we recovered from commercial green shrimp. After several experiments we found the following method to be effective in producing the green color. The shrimp (pH 7.8) were made into "butterflies" by peeling and cutting. Several applications of isolated metal residue were made to one side of each shrimp, which was then dipped in batter and breaded by hand with the appropriate breadings. These shrimp were packed 10 ounces to the carton, and each carton was sealed and left at room temperature for about ½ hour. The shrimp were frozen to 0° F. and stored for 1 week. They were allowed to come slowly to refrigerator temperature (45° F.) overnight, examined, and replaced in frozen storage. After two such temperature treatments, most of the shrimp in each package had turned green where the metal fragments had been placed. The areas not implanted with metal fragments remained a normal breaded shrimp color. Un-

Table 3.—Metal fragment contamination of samples from a shrimp-processing plant

Sample	Residue				
	Chromium	Iron	Copper	Nickel	Total
	μg.	μg.	μg.	μg.	μg.
Green shrimp breading	25.0	102.5	7.5	45.0	180.0
Green shrimp breading	25.0	147.5	7.5	70.0	250.0
Green shrimp breading	17.5	30.0	10.0	92.5	150.0
Green shrimp breading	15.0	60.0	17.5	60.0	152.5
Green shrimp breading	17.5	17.5	5.0	75.0	115.0
Green shrimp breading	7.5	87.5	7.5	40.0	142.5
Local nongreen shrimp breading	27.5	32.5	5.0	115.0	180.0
Local nongreen shrimp breading	15.0	35.0	7.5	35.0	92.5
Local nongreen shrimp breading	10.0	57.5	10.0	20.0	97.5
Batter from automatic machine, skimmed	20.0	135.0	22.5	145.0	322.5
Batter from hand machine, skimmed ..	15.0	117.5	12.5	77.5	222.5
Breading for retail	15.0	25.0	5.0	77.5	122.5
Breading for institutional	10.0	87.5	7.5	65.0	170.0
Water, thawing tank No. 1, skimmed ..	30.0	2.5	5.0	97.5	135.0
Water, thawing tank No. 2, skimmed ..	7.5	12.5	5.0	32.5	57.5
Untreated well water, skimmed	0.0	5.0	12.5	32.5	50.0
Chlorinated well water, skimmed	0.0	2.5	7.5	40.0	50.0
Raw headless shrimp after thawing ..	12.5	27.5	5.0	27.5	72.5
Shrimp after Pronto machine	0.0	17.5	5.0	50.0	72.5
Shrimp after peeling-soak tank	0.0	17.5	12.5	22.5	52.5
Peeled shrimp before breading	0.0	95.0	100.0	22.5	217.5
Peeled shrimp before breading	0.0	45.0	5.0	35.0	85.0
Shrimp after breading	0.0	62.5	10.0	32.5	105.0
Shrimp after breading	0.0	110.0	10.0	32.5	152.5
Shrimp after freezing — 2 days	12.5	92.5	7.5	65.0	177.5

Note: No cobalt residue was found.

less the shrimp went through the thawing-re-freezing cycle, the green color did not appear.

On the basis of these findings, our hypothesis as to the cause of the green coloration of the shrimp was as follows: Metal dust must be settling on the shrimp; the shrimp must be slightly ammoniacal (have a high pH); the shrimp must thaw; the shrimp juices must dissolve the metal dust releasing iron, copper, nickel, and other metallic ions; an ammonia atmosphere must be present; an anion must be available; and sufficient time must be available to form a complex chemical compound. We feel that this compound is probably a coordinated ammonia complex such as $(NH_4)Cr(SO_4)_2 \cdot 12H_2O$, $NH_4Cl \cdot NiCl_2 \cdot 6H_2O$, or $(NH_4)_3Fe(C_2O_4)_3 \cdot 3H_2O$. Such compounds are readily degradable by water, air, light, and solvent action and could produce the noted effect of changing colors (air: green to brown; water: gray-green to yellow-green). We made no attempt, however, to identify the particular compound or compounds that might be present.

B. DETERMINING THE SOURCE OF THE CONTAMINATION

From the foregoing study, we were confident that the green coloration was produced by metallic contamination. Our problem now was to pinpoint the source. To do so, we checked the breading, the water used, and the air coming into the plant.

1. Breading

We analyzed samples of institutional breading and of retail breading for metal contamination residues.

Breading that had been taken from opened bags contained a variety of metallic material; however, samples taken from unopened bags at the plant contained no metal residues.

2. Water

Two sets of water samples were analyzed. In the first set, which were general water sam-

ples, water was skimmed from the tops of the thawing tank, pump trough, and well. In the second set, only the well water was analyzed in our attempt to pinpoint further the source of the contamination.

a. General water samples. — In the analysis of the water samples, the samples were filtered through a micropore filter. The filter pad was digested with aqua regia and evaporated to dryness. The residue was treated with distilled water, and the resulting solution was made to volume in a volumetric flask. The atomic absorption technique was followed in the analysis for trace metals.

The data are shown in Table 4. The results suggest either airborne or waterborne contamination.

b. Well-water samples. — Samples were taken from each well prior to the point where the water first came into contact with air, and the samples were analyzed as before.

Table 5 shows the findings. The only iron found was in a single sample from Well No. 2, which was not operating at the time. Two samples contained manganese — one from Well No. 1 and one from Well No. 3. This contamination appears to be incidental, particularly in

Table 4.—Residues in various samples of skimmed water from shrimp-processing-plant thawing tanks and water troughs

Sample	Copper	Iron	Total residue
	μg./100 ml.	μg./100 ml.	μg./100 ml.
Thawing tank: 1st day			
1	0.3	5.0	5.3
2	1.3	5.5	6.8
3	0.5	15.0	15.5
4	0.3	6.8	7.1
5	0.3	15.0	15.3
2d day			
1	1.3	4.5	5.8
2	1.5	5.5	7.0
3	2.3	5.0	7.3
4	0.3	5.5	5.8
5	1.5	11.3	12.8
3d day			
1	1.5	5.0	6.5
2	0.0	0.0	0.0
Pump trough No. 1			
1	1.5	0.0	1.5
2	6.0	172.5	178.5
3	2.0	0.0	2.0
4	13.0	6.8	19.8
Pump trough No. 2			
1	0.0	0.0	0.0
2	1.8	6.8	8.6
3	9.0	40.0	49.0
4	6.3	110.0	116.3
Pump trough No. 3			
1	0.0	5.5	5.5
2	0.0	5.0	5.0
3	0.0	75.0	75.0
4	1.8	25.0	26.8
Well water			
1	0.0	0.0	0.0
2	0.3	5.0	5.3
3	5.3	3.8	9.1

Note: No chromium, nickel, or cobalt were found.

Table 5.—Analysis of metal residues in samples of well water

Well No.	Date of sample	Time of day	Amount of time well was in use	Iron	Nickel	Cobalt	Manganese	Copper	Total
			Percent	μg.	μg.	μg.	μg.	μg.	μg.
1	1/24/68	2:30 p.m.	60	None	None	None	None	None	None
	1/25/68	6:15 a.m.	60	None	None	None	None	None	None
	1/25/68	3:00 p.m.	60	None	None	None	None	None	None
	1/26/68	6:30 a.m.	60	None	None	None	None	None	None
	1/26/68	1:30 p.m.	80	None	None	None	None	None	None
	1/29/68	6:15 a.m.	60	None	None	None	5.0	None	5.0
	1/29/68	1:00 p.m.	75	None	None	None	None	None	None
2	1/24/68	2:30 p.m.	0	None	None	None	None	None	None
	1/25/68	6:15 a.m.	0	23.0	None	None	None	None	23.0
	1/25/68	3:00 p.m.	0	None	None	None	None	None	None
	1/26/68	6:30 a.m.	0	None	None	None	None	None	None
	1/26/68	1:30 p.m.	0	None	None	None	None	None	None
	1/29/68	6:15 a.m.	0	None	None	None	None	None	None
	1/29/68	1:00 p.m.	0	None	None	None	None	None	None
3	1/24/68	2:30 p.m.	100	None	None	None	None	None	None
	1/25/68	6:15 a.m.	100	None	None	None	None	None	None
	1/25/68	3:00 p.m.	100	None	None	None	None	None	None
	1/26/68	6:30 a.m.	100	None	None	None	None	None	None
	1/26/68	1:30 p.m.	100	None	None	None	4.2	None	4.2
	1/29/68	6:15 a.m.	100	None	None	None	None	None	None
	1/29/68	1:00 p.m.	100	None	None	None	None	None	None

view of the fact that no other trace metals were found with the manganese. Hence, the source of the metallic contamination did not appear to be waterborne.

3. Air

The air was sampled in two ways — indirectly, by taking dust samples, and directly, by taking air samples.

a. Dust samples. — Dust lifted from fan blades with care so as not to entrap rust in the ventilator system was collected both at the plant being studied and at another affected plant some 2,000 miles away. The dust samples were put into solution in the same manner as were the water samples.

Table 6, which reports the data, indicates that the dust accumulated from the fans showed a very considerable amount of iron and a considerable amount of manganese and copper.

b. Air samples. — A vacuum-pump air-sampling device was set up inside the plant and was operated intermittently for several weeks. Periods of continuous operation of the vacuum-pump sampling varied from 6½ to 72 hours. During these periods, the direction of the wind was noted.

Table 7 and Figure 1 report the results.

Continuous operation of the pump caused a variation in air flow through the filter, owing to clogging of the filter pad. Therefore, the amount of residue cannot be directly correlated with the hours of operation or with the volume of air filtered. The amount of airborne dust appears to correlate fairly closely with wind direction. In this case, wind from the north of the plant appears to be fairly heavily laden with metal contaminants. We therefore suggest that the airborne metal dust settling on open water tanks and on the shrimp and other ingredients is the initial factor causing the green shrimp.

Table 6.—Analysis of dust samples for metal residues

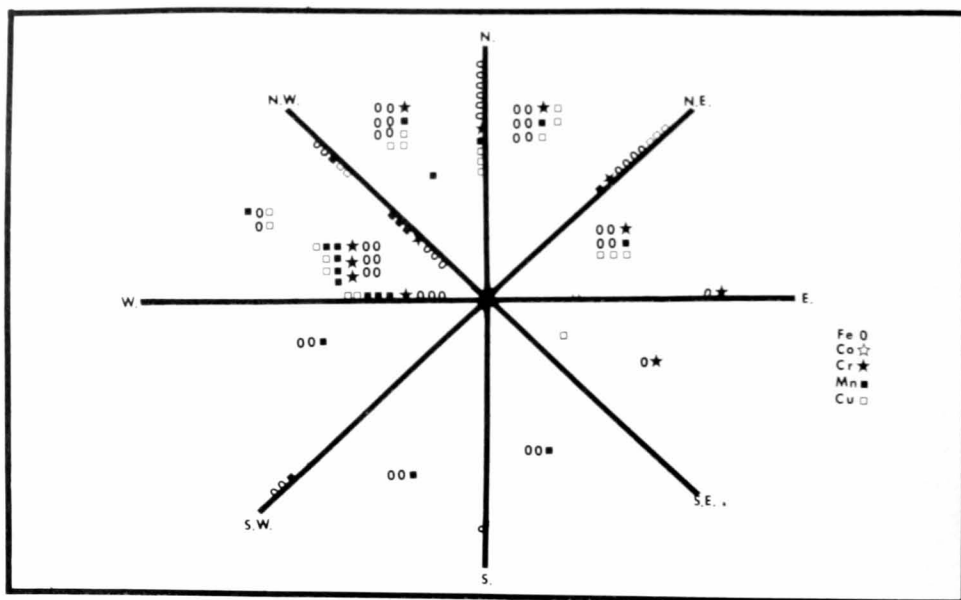| Plant	Metal residues						
	Iron	Nickel	Cobalt	Chromium	Manganese	Copper	Total
	μg./g.	μg./g.	μg./g.	μg./g.	μg./g.	μg./g.	μg./g.
A	9,160	--	--	--	123.9	404.1	9,688
B	102,919	127.2	262.0	86.0	870.0	471.5	104,736

Figure 1.—Metal contamination of air samples in relation to wind direction.

Table 7.—Analysis of air samples for metal residues

Sample	Date	Time of day[1]	Metal being worked on in plant?	Plant condition[2]	Duration of pump operation	Wind direction	Metal residues						
							Iron	Nickel	Cobalt	Chromium	Manganese	Copper	Total
					Hours		µg.	µg.	µg.	µg.	µg.	µg.	µg.
1	1/23	D	Yes	OC	7	S.E. — S.	25	--	2.5	--	3.5	--	28.5
2	1/23	N	No	C	15.5	S. — W.	25	--	--	--	3.5	--	28.5
3	1/24	D	Yes	OC	8	W. — N.W.	37	--	--	--	4	--	31
4	1/24	N	No	OC	16	N.W.	--	--	--	--	--	--	--
5	1/25	D	Yes	OC	8	N.W.	57	--	--	--	6	--	63
6	1/25	N	No	C	16	N.W.	--	--	--	--	1	--	1
7	1/26	D	Yes	C	8	N.W. — N.	--	--	--	--	4	--	4
8	1/26	WE	No	C	64	N. — N.E.	275	--	--	--	3	--	278
9	1/29	D	Yes	O	8	N.E.	--	--	--	--	--	--	--
10	1/29	N	No	C	17.5	N.E.	--	--	--	--	--	--	--
11	1/30	D	Yes	O	6.5	N.E.	--	--	--	--	--	--	--
12	1/30	N	No	C	16	N.E. — N.W.	--	--	--	--	--	--	--
13	1/31	D	Yes	OC	8	N.W — N.E.	--	--	--	--	--	--	--
14	1/31	N	No	C	16	N.E. — N.	--	--	--	--	--	--	--
15	2/1	D	Yes	OC	8	N. — E.	--	--	--	--	--	--	--
16	2/1	N	No	C	16	E. — S.E.	--	--	--	--	--	--	--
17	2/2	D	Yes	O	8	S.E.	--	--	--	Trace	--	--	Trace
18	2/2	WE	No	C	64	S.E. — E.	--	--	--	--	--	7.5	7.5
19	2/5	D	Yes	OC	8	E.	--	--	--	--	--	1.6	1.6
20	2/5	N	No	C	17.5	E. — W.	46	--	--	--	5.9	1.6	53.5
21	2/6	D	Yes	OC	6.5	W.	4	--	--	2.7	2.1	--	8.8
22	2/6	N	No	C	16	W.	--	--	--	--	0.9	1.6	2.5
23	2/7	D	Yes	C	8	W.	94	--	--	--	2.7	2.8	99.5
24	2/7	N	No	C	16	W. — N.W.	9	--	Trace	2.5	2.7	2.5	16.7
25	2/8	D	Yes	C	8	N.W. — W.	10	--	--	--	0.9	1.3	12.2
26	2/8	N	No	C	16	W.	Trace	--	--	--	--	--	Trace
27	2/9	D	Yes	C	8	N.— N.W.	16	--	Trace	1.5	1.0	1.8	20.3
28	2/9	WE	No	C	64	N.W.	16	--	Trace	1.3	1.3	Trace	18.6
29	2/12	D	No	OC	8	N.W. — E.	9	--	--	--	--	Trace	9
30	2/12	N	No	OC	16	E. — W.	9	--	--	--	--	Trace	9
31	2/13	D	Yes	O	8	W.	12	--	--	--	--	--	12
32	2/13	N	No	C	16	W. — N.W.	44	--	--	Trace	2.7	Trace	46.7
33	2/14	D	No	OC	8	N.W. — N.E.	15	--	--	--	--	--	15
34	2/14	N	No	C	16	N.E. — N.W.	7	--	--	--	--	--	7
35	2/15	D	Yes	C	7.5	N.W.	20	--	--	--	--	--	20
36	2/15	N	No	C	16.5	N.W. — W.	7	--	--	--	--	--	7
37	2/16	D	No	OC	8	W. — S.E.	5	--	--	--	--	--	5
38	2/16	WE	No	C	64	S.E. — N.W.	26	--	Trace	Trace	--	--	26

[1] D = day;   N = night;   WE = weekend
[2] O = open;   OC = medium condition;   C = closed

II. REMEDYING THE PROBLEM OF THE GREEN COLORATION

Reducing the airborne metal dust to as low a level as possible through engineering alterations and taking other appropriate steps should alleviate the problem. The following are possible measures that should help:

1. Filter the air in the plant and the warehouse.

2. Use shrimp with lower pH.

3. Use metal chelating agents (such as Na_2EDTA, $CaNa_2EDTA$, or citric acid) in the batter.

4. Control more rigidly (a) the time taken to freeze the product and (b) the temperature at which the product is stored and transported.

MS #1849

IMPROVED METHOD FOR PRODUCING PINDANG

by

Sofjan Iljas and Louis J. Ronsivalli

ABSTRACT

Use of plastic pouches reduces processing time, makes possible continuous and automated production, and enhances the wholesomeness and keeping quality of pindang, a cooked salt fish.

CONTENTS

INTRODUCTION

Boiled salt fish, or "pindang," is popular in Indonesia and in certain other countries, where it is known by various names -- for example, "sinaeng" in the Philippines and "platunung" in Thailand.

To produce pindang, the maker places layers of eviscerated fish and salt alternately in earthenware or tin containers. The ratio of fish to salt varies from 20:1 to 3:1, depending on the desired shelf life and the desired salt concentration. The method, however, does not lend itself to close control over the final concentration of salt. The fish and the salt are supported in a pot by means of a rack held above a layer of water and out of contact with the water. The water is kept boiling by holding the pot over a fire, and the pot is covered to reduce evaporation. At least once during the process, water is drained off through a port in the bottom of the pot, and then additional salt is sprinkled over the fish. The entire process takes about 8 hours. In this primitive batch method, each container yields about 1 to 15 kilograms of pindang.

The processed fish can be held for from 1 to 12 weeks at room temperature, depending on the concentration of salt, which may be as high as 25 percent. Some loss of product may occur from insects and molds during storage, because the product is not protected.

The art of preserving fish by means of salt remains primitive, principally because of the lag in technological advances and economic growth in the developing countries in which salt fish is primarily consumed; much progress has been made, however, in understanding and improving the process of making salt fish. Fougere (1952); Del Valle and Nickerson (1967a, 1967b); Nitibaskara and Dollar (1967); Aitken, Jason, Olley, and Payne (1967); and Del Valle and Gonzalez (1968) have contributed significantly toward improving the process and explaining its underlying principles.

The primitive process used today in making pindang has a low rate of production and involves sanitation problems arising from the handling of the unprotected product during processing and after processing. In addition, the cook pots accumulate microorganisms that subsequently add to the contaminants in the finished product.

We theorized that the use of small containers would serve to prevent the runoff of salt and thereby help maintain a high concentration of salt around the product and possibly hasten the penetration of salt into the product. In addition, the use of small containers would prevent contact between the product and the cooking pot, thereby eliminating this source of contamination. Further, we envisioned that placing the product in a container would make possible an automatic operation in which a conveyor and a steam chamber or pool of boiling water would be used.

Any type of container normally used in the canning process -- for example, cans or jars -- would be suitable for this purpose. In the developing countries, the use of such containers is precluded, however, because of the high cost of the cans or jars and the lack of machinery and facilities for handling them. On the other hand, plastic pouches are relatively inexpensive, require no elaborate machinery, and are easily available in developing countries. Furthermore, plastic materials are relatively inert and therefore are not likely to affect the quality of the product adversely. For the intended purpose, any of the many formulations of plastic materials that can retain their integrity in boiling water should be adequate. As examples of possibly suitable materials, saran (copolymerized polyvinylidene chloride and polyvinyl chloride) and polypropylene can withstand a temperature of up to about 150° C., and polyester (polyethylene terephthalate) can withstand a temperature of over 200° C.

The object of the research reported here was to improve the processing technique and the keeping quality of pindang by developing a method of processing the product in plastic pouches.

This paper has two main parts. Part I reports on the technical aspects of making pindang by the pouch method; Part II reports on the microbiological and sanitation aspects.

I. TECHNICAL ASPECTS

Described in this section are (A) the basic procedure used at the Bureau of Commercial Fisheries Technological Laboratory at Gloucester, Massachusetts, for making pindang by the pouch method and (B) the analyses of the finished product.

A. PROCEDURE USED IN MAKING PINDANG

In our experimental production of pindang by the pouch method, we carried out the process in three steps, using cod fillets as the source of fish material.

1. Adding Salt

The first step in our production of pindang was to add salt to the fillets in the pouches. In this step, fresh cod fillets, for which the concentrations of moisture and salt had been determined, were placed in plastic pouches of 0.05 millimeter thickness. Each pouch contained about 300 grams of fillets. (The pouches used in this experiment were made of polyethylene-coated polyester.) Salt was added directly to the product in amounts of 3, 6, 9, 12, and 15 percent of the weight of fish. For the purpose of control, however, no salt was added to several of the pouches.

2. Steaming the Fillets

The second step in our production of pindang was to steam the fillets while they were in the pouches. In this step, all of the pouches were placed in a steam chamber with their open ends up. The steam chamber was simply a large cooking pot containing a layer of water and supports to hold the pouches above the water layer. The pot was heated over a stove. After the fish had been steamed for 2 hours, their drip in the pouches was discarded by tipping the pouches manually, and more salt was added -- in amounts of 2, 4, 6, 8, or 10 percent of the weight of the fish -- to the pouches, which originally contained amounts of 3, 6, 9, 12, or 15 percent of the weight of fish, respectively, so that the total amount of salt added to each pouch was 5, 10, 15, 20, or 25 percent, respectively. The product was then steamed for an additional 2 hours. The newly formed drip was discarded as was just described.

3. Sealing the Pouches and Storing Them

The third and final step in our production of pindang was simply to seal the pouches and then to store them at room temperature.

Figure 1.—Effect of amount of salt added on the concentrations of salt and moisture in pindang produced by the pouch process.

B. ANALYSES OF THE PRODUCT

After the product had been processed, the concentrations of moisture and salt in it were determined, and the product was also examined organoleptically. Additional organoleptic examinations were made monthly thereafter.

1. Moisture and Salt Analyses

The concentrations of moisture were determined by the standard technique using an ordinary drying oven set at about 105° C. (no vacuum). The analyses for salt were made according to the rapid field method described by Greig and Seagran (1965) using No. 1177 titrators (range = from 0.4 to 20 percent salt).

Figure 1 shows how varying the amount of salt added to the fish affected the salt and moisture concentrations of pindang made by the pouch process. The data points used to obtain the curves are averages of the values found in several tests. The agreement among corresponding points from test to test was excellent. The data form relatively smooth
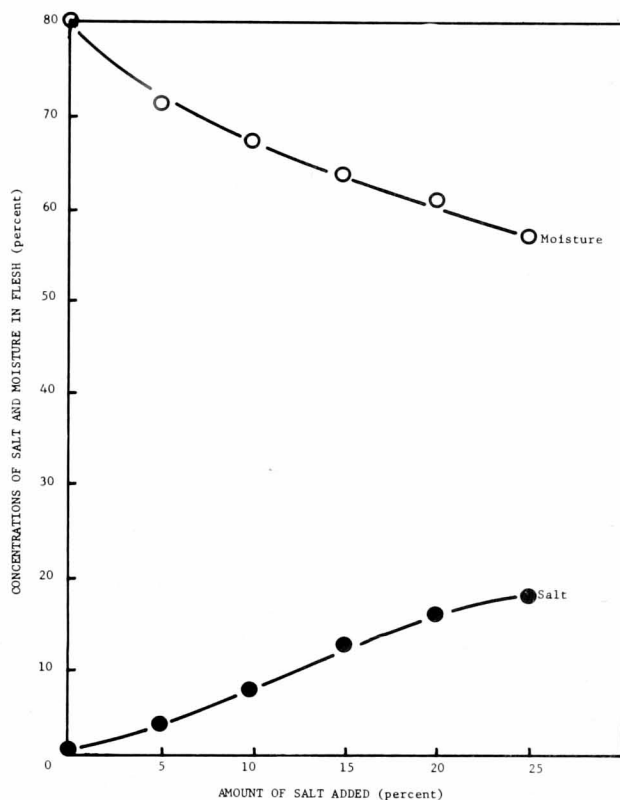
trend lines showing the decreasing concentration of moisture and increasing concentration of salt in the fish flesh as the amount of salt added was increased.

2. Organoleptic Analyses

The organoleptic analyses were made by two Indonesian and six American panelists using a 2-point scale (accept or reject). In some instances, the product was tested after it had been leached with water (to lower the salt content) and heated. Occasionally, meals were prepared according to Indonesian recipes. The Americans were not experienced in evaluating the quality of pindang, but they were experienced in evaluating New England seafood; the Indonesians, however, were experienced in evaluating pindang. The criteria of quality that the panel considered were appearance, flavor, odor, and texture.

Besides indicating whether or not the product was acceptable, the panelists were asked to comment on it. The American panelists agreed that the product, when served steamed, was acceptable; however, they recognized, of course, that the high concentration of salt was characteristic of this product. The Indonesians commented that the product was excellent. When the product was prepared according to the Indonesian recipes, the Americans also thought that the product was excellent.

The samples, after being stored for 3 months at room temperature, became slightly tough and slightly discolored, but they were still acceptable.

II. MICROBIOLOGICAL AND SANITATION ASPECTS

The literature indicates (1) that cooked, high-protein foods are involved in many of the staphylococcal outbreaks, (2) that man is a principal carrier of the infectious organisms, and (3) that some staphylococci can grow in products containing a high concentration of salt (Bryan, 1968). Therefore, an advantage of the pouch method of producing pindang is that the product does not come in contact with handlers once it has been placed in pouches.

Because the process described in the report does not sterilize the product and because the use of pouches may produce sites of low redox potential, the concentration of salt in the finished pindang should be more than 10 percent. This concentration of salt is inhibitory to spoilage and pathogenic organisms under anaerobic conditions (Ingram and Kitchell, 1967). An alternative technique is to steam the product a minimum of 6 hours, which is adequate treatment (American Can Company, 1949) for producing commercial sterility and which, therefore, permits a choice of salinity in the finished product.

The pouch process eliminates the problem involved in the sanitation of the earthenware cook pots, which was cited by Nitibaskara and Dollar (1967).

SUMMARY AND CONCLUSIONS

The method of producing pindang -- an Indonesian cooked salt fish product -- has been improved by the use of plastic pouches. The keeping quality of the product has also been improved.

Organoleptically, the product was quite acceptable for up to 3 months of storage at room temperature -- only slight changes in texture and color appeared during the third month of storage.

The process eliminates sanitation problems but requires adherence to one of two alternate guidelines -- either enough salt must be added to ensure a concentration of salt greater than

10 percent in the finished product, or else the product should be steamed for at least 6 hours in sealed containers.

The technological advantages of the method are: (1) the rate of production is nearly doubled when the method is used in batch processing because the amount of fish that can be produced per batch, regardless of the method used, remains the same; the time required, however, to process a batch by the pouch method is about half the time required by the standard method; (2) the process can probably be made continuous because the production is completely independent of cooking pots -- in fact, the process could be automated by handling the pouches similarly to products to be smoked, utilizing automatic conveyors and timing components, by substituting a steam chamber for the smoke chamber; (3) the concentrations of salt and water can be controlled closely in the finished product; and (4) losses of the product during storage that are due to such causes of spoilage as insects and molds are minimized, owing to the protection afforded by the package.

ACKNOWLEDGMENTS

LITERATURE CITED

Aitken, A., A. C. Jason, J. Olley, and P. R. Payne.
1967. Effects of drying, salting and high temperatures on the nutritive value of dried cod. Fishing News International 6(9): 42-43.

American Can Company.
1949. Canned Food Reference Manual. Third edition. Section VII, Chapter 2. American Can Company, New York, page 304.

Bryan, Frank L.
1968. What the sanitarian should know about staphylococci and salmonellae in non-dairy products. I. Staphylococci. Journal of Milk and Food Technology 31(4): 110-116.

Del Valle, F. R., and J. L. Gonzalez Inigo.
1968. A quick-salting process for fish. 2. Behavior of different species of fish with respect to a quick-salting process. Food Technology 22(9): 1135-1138.

Del Valle, F. R., and J. T. R. Nickerson.
1967a. Studies on salting and drying fish. I. Equilibrium considerations in salting. Journal of Food Science 32: 173-179.

1967b. Studies on salting and drying fish. II. Dynamic aspects of the salting of fish. Journal of Food Science 32: 218-224.

Fougere, H.
1952. The water transfer in codfish muscle immersed in sodium chloride solutions. Journal of the Fisheries Research Board of Canada 9: 388-392.

Greig, R. A., and H. L. Seagran.
1965. Technical note No. 1 -- A rapid field method for determining the salt concentration in fresh and smoked chub. Commercial Fisheries Review 27(12): 18-21.

Ingram, M., and A. G. Kitchell.
    1967. Salt as a preservative for foods.
        Journal of Food Technology 2(1):1-15.

Nitibaskara, R., and A. M. Dollar.
    1967. Quality changes during storage of
        boiled fish product prepared from Pa-
        cific herring by an improved method.
        Bulletin of the Japanese Society of
        Scientific Fisheries 33: 1028-1034.

MS #1861

IRRADIATION OF PACIFIC COAST FISH AND SHELLFISH.
7–STORAGE LIFE AT 33° F. OF IRRADIATED AND REPACKED MEAT OF DUNGENESS CRAB

by

F. M. Teeny, D. Miyauchi, and G. Pelroy

ABSTRACT

Fresh Dungeness crab meat, owing to its relatively short and variable shelf life, is ordinarily restricted to sale close to its area of production. To find a basis for widening the area of sale, we determined the shelf life for Dungeness crab meat irradiated in *wholesale* containers, stored 12 or 20 days at 33° F., and subsequently repacked into *retail* containers and again stored at 33° F. The repacked samples had adequate shelf life for marketing in retail stores. Thus, irradiation of Dungeness crab meat in wholesale containers would permit this product to be widely distributed in retail stores.

CONTENTS

INTRODUCTION

The shelf life of fresh nonirradiated Dungeness crab meat is 6 to 14 days, and this variable and relatively short shelf life has normally limited the marketing of fresh crab meat to regions near the centers of production. Miyauchi, Spinelli, Stoll, Pelroy, and Eklund (1966) have demonstrated that when Dungeness crab meat in small retail packages is irradiated at doses of 100 and 200 kilorads, the storage life of the product could be increased by two to six times over that of the corresponding nonirradiated control samples.

Processors of Dungeness crab meat usually pack the fresh product in No. 10 C-enameled cans (which contain 5 pounds of crab meat) to be kept under refrigeration for sale to retail stores and institutions. The retail-store operators, in turn, repack the meat for sale in small retail packages.

The object of the studies presented in this paper was to determine the storage characteristics of Dungeness crab meat irradiated in No. 10 cans, stored at a temperature slightly above freezing (33° F.), then repacked into smaller retail packages and stored at 33° F.

In studying the storage characteristics of crab meat, we used both sensory tests and bacteriological tests as criteria of quality. We placed major emphasis, however, on the sensory tests; the bacteriological tests were to supply insight into what occurred bacteriologically as the results of the irradiation and storage.

1. SENSORY TESTS

The samples of crab meat, which will be described later, were irradiated at levels of: (1) 0 kilorad (control), (2) 100 kilorads, and (3) 200 kilorads (Figure 1).

A. O-KILORAD IRRADIATION OF CRAB MEAT (CONTROL, NOT IRRADIATED)

1. Sources of the Crab Meat

Freshly picked crab meat, which was vacuum packed in C-enameled No. 10 cans and held in ice, was collected over a period of 5 months from two different processing plants.

2. Storage Life of the Crab Meat

a. Procedure.—At the laboratory, the No. 10 C-enameled cans were opened, and the crab meat was packed in paperboard trays with cellophane overwrap and then stored again at 33° F., simulating the normal procedure at the retail level.

Periodically the samples were rated for the quality attributes of appearance, odor, flavor, and texture by a panel of five to eight experienced judges. Representative portions of the crab meat were taken from the packages, placed in paper cups, coded, and presented to the judges together with an identified reference sample. All samples were rated on a 10-point scale (Miyauchi, Eklund, Spinelli, and Stoll, 1964) on which a mean score below 5 indicated the end of the storage life.

In addition to rating the individual attributes of each sample, the judges also gave the sample an overall score. The overall score usually reflected the lowest rating given to any of the individual attributes listed above that affected the overall quality.

b. Results.—Figure 2 shows the storage life of the nonirradiated control samples stored at 33° F. Initially the crab meat was at a high level of quality, but during the storage of the samples, the overall quality dropped. The storage life was 7 to 14 days, which is in close agreement with the findings of Miyauchi, Spinelli, Stoll, Pelroy, and Eklund (1966).
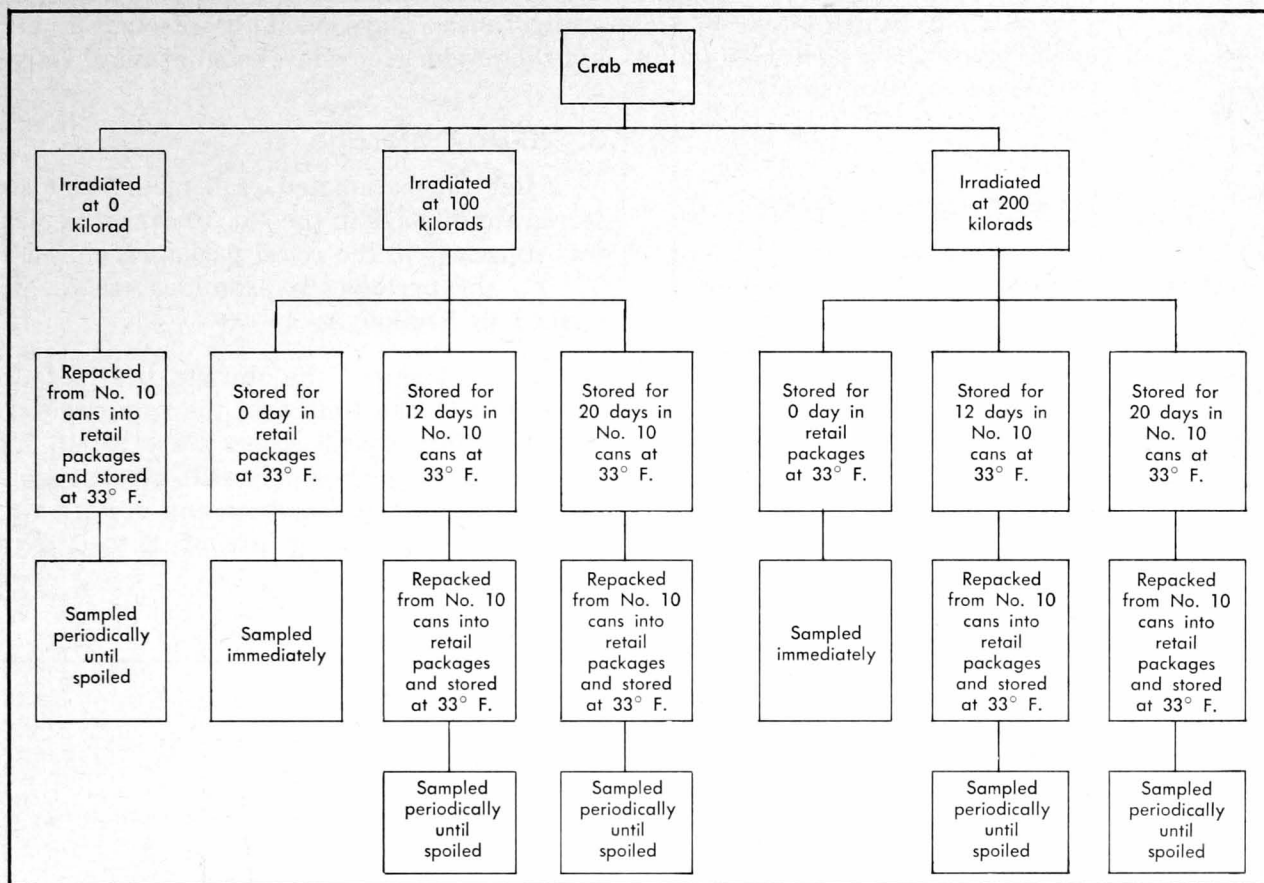
Figure 1.—Flow diagram illustrating the experimental procedure in the sensory tests.

B. 100-KILORAD IRRADIATION OF CRAB MEAT

The crab meat to be irradiated at 100 kilorads was divided into three groups. One was irradiated in the retail package and was tested immediately after being irradiated; the other two groups were irradiated in vacuum-packed No. 10 cans and stored at 33° F. for testing after storage for 12 days and after storage for 20 days.

We chose storage times of 12 and 20 days prior to repacking the crab meat into smaller retail packages to simulate the time that the crab meat might be held during: (1) storage at the processing plant, (2) transportation to the most distant parts of the United States, and (3) storage and handling at the retail market.

1. 0-Day Storage (Control)

The procedure used was the same as that described earlier under Section A except that the samples were irradiated at 100 kilorads and were tested only this one time and were not tested periodically thereafter. We irradiated the sample in the paperboard trays rather than in the No. 10 cans simply to reduce the amount of crab meat needed for the test. We assumed that the quality of the meat would be the same immediately after the samples were irradiated whether they were irradiated in the paperboard trays or were irradiated in the No. 10 cans.

Immediately after the samples were irradiated, the overall quality of the crab meat was good; the sensory scores ranged from 8 to 9 on the 10-point scale.
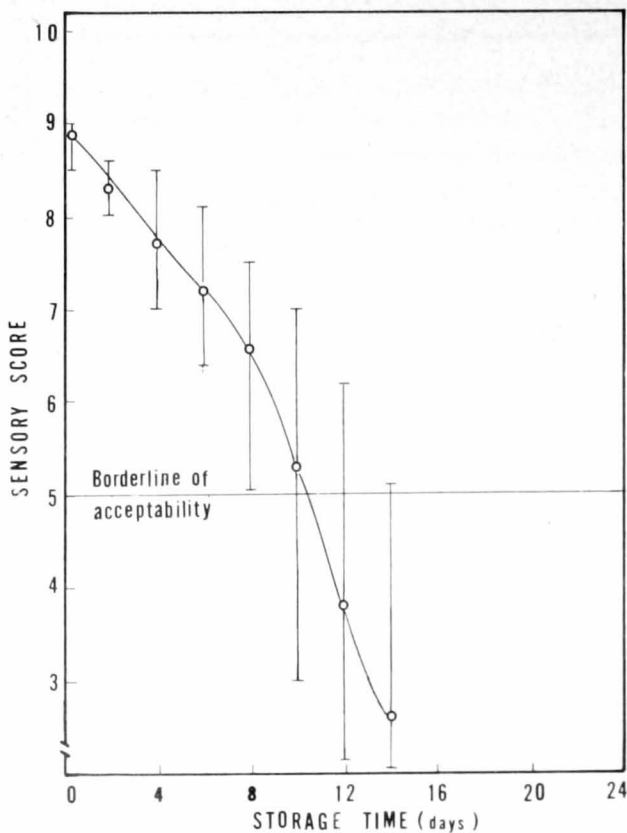
19

Figure 2.—Ranges of quality scores versus storage time of six replicate lots of Dungeness crab meat packed in paperboard trays with cellophane overwrap and held at 33° F.

2. 12-Day Storage

After the irradiated crab meat had been stored for 12 days in the No. 10 cans, the meat was repacked in the retail packages and periodically examined as was described under Section A.

At the storage time of 12 days, the overall scores ranged from 6.8 to 7.7 (Figure 3). The scores gradually dropped with storage time, and the samples had an additional shelf life of 6 to 7 days after being repacked. Thus, the Dungeness crab meat irradiated at 100 kilorads in No. 10 C-enameled cans remained in good condition for 12 days. Interestingly, with these samples as well as with the other irradiated samples to be described later, the shelf life varied less among replicates than did the shelf life of the nonirradiated samples (compare Figure 2 with Figures 3, 4, 5, and 6).

The additional shelf life of the irradiated crab meat of 6 to 7 days should be adequate to permit the product to be marketed at retail stores.

3. 20-Day Storage

After the irradiated crab meat had been stored for 20 days in the No. 10 cans, the meat was repacked in the retail packages, stored at 33° F., and periodically examined as was described in Section A.

Figure 4 shows the storage life of these samples. At the time that the samples were repacked, the overall scores were 6.7 to 7.7. The scores dropped quickly with storage time, and the samples had a short and greatly variable additional storage life of 2 to 6 days.

Figure 3.—Ranges of quality scores versus storage time of replicate lots of Dungeness crab meat initially vacuum packed in C-enameled No. 10 cans and irradiated at 100 kilorads. The cans were held for 12 days at 33° F., after which time the meat was packed in paperboard trays with cellophane overwrap and again held at 33° F.
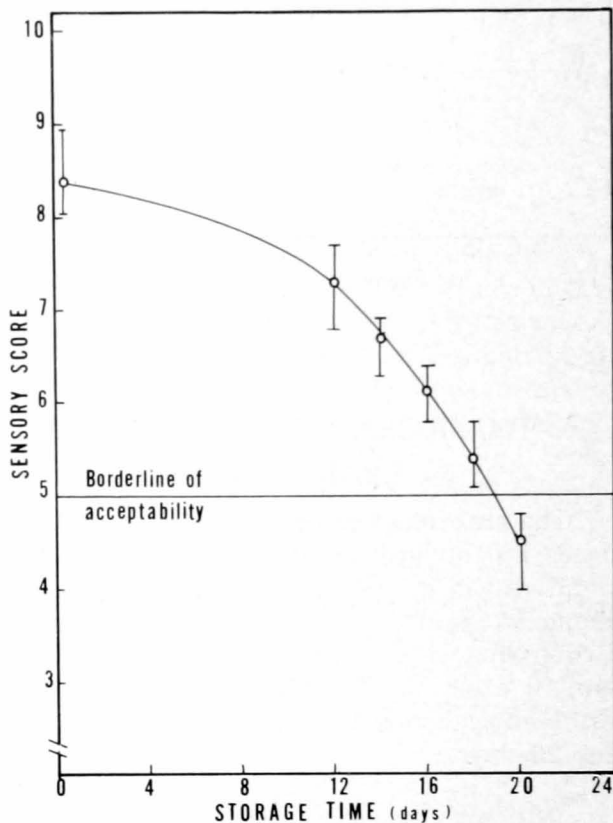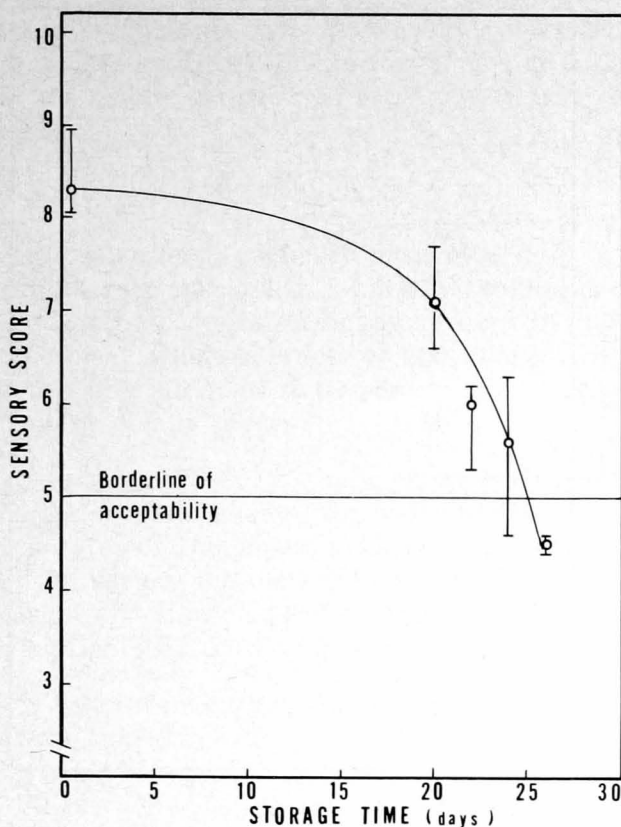
Figure 4.—Ranges of quality scores versus storage time of replicate lots of Dungeness crab meat initially vacuum packed in C-enameled No. 10 cans and irradiated at 100 kilorads. The cans were held for 20 days at 33° F. after which the meat was packed in paperboard trays with cellophane overwrap and was again held at 33° F.

Thus, irradiating Dungeness crab meat at 100 kilorads in the No. 10 cans permitted the meat to be held in good condition for up to 20 days at 33° F.; however, the additional shelf life of 2 to 6 days in the retail package was too variable. We therefore would not recommend initial storage for 20 days of samples irradiated at 100 kilorads if the samples are to be repacked for sale in retail stores.

C. 200-KILORAD IRRADIATION OF CRAB MEAT

The crab meat to be irradiated at 200 kilorads was divided into three groups. The first group was packed in paperboard trays with cellophane overwrap for testing immediately after being irradiated; the second group was vacuum packed in No. 10 cans for testing after being stored for 12 days at 33° F.; and the third group was vacuum packed in No. 10 cans for testing after being stored for 20 days at 33° F.

1. 0-Day Storage (Control)

The procedure used was the same as that described under Section B-1 except that the samples were irradiated at 200 kilorads instead of at 100 kilorads.

Immediately after the samples were irradiated, the overall scores ranged between 7.0 and 8.5. The lower overall scores generally resulted from lower odor and flavor scores due to the irradiation.

2. 12-Day Storage

The crab meat, after being stored for 12 days in the No. 10 cans, was repacked in the retail packages, stored at 33° F., and periodically examined as was described under Section B-2.

At the repacking time of 12 days, the overall scores were 7.2 to 7.9 (Figure 5). For the first 4 days after the samples were repacked, the scores showed a slight drop, after which they dropped more sharply. The additional shelf life of the samples was 7 to 9 days. Thus, irradiating, at a dose level of 200 kilorads, Dungeness crab meat vacuum packed in No. 10 cans permitted the meat to be held in good condition for 12 days at 33° F. The additional shelf life of 7 to 9 days in the retail packages should be adequate to permit the product to be marketed in retail stores.

3. 20-Day Storage

The irradiated crab meat, after being stored for 20 days in the No. 10 cans, was repacked in the retail packages, stored at 33° F., and periodically examined as was described under Section B-3.

Figure 6 shows the storage life of the samples that were repacked after 20 days of storage at 33° F. At the time the samples were repacked, the overall scores were 6.6 to
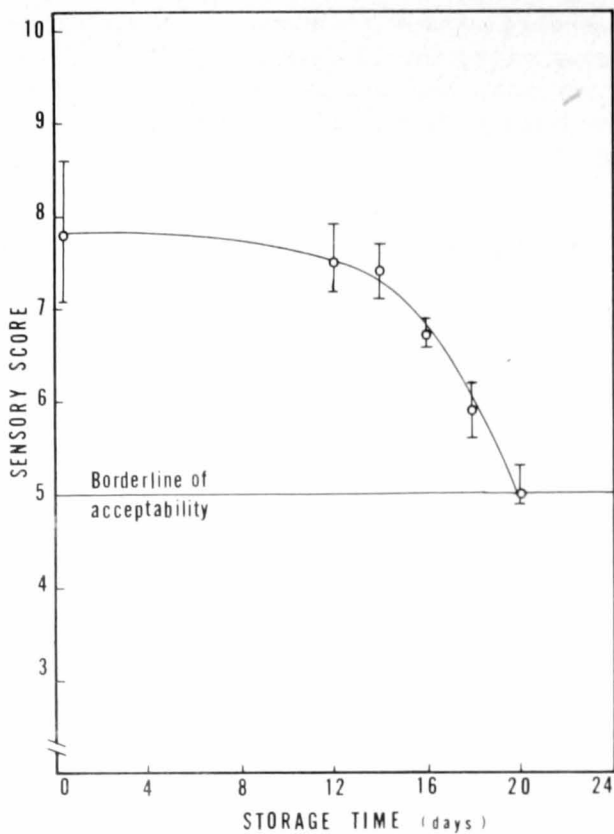
Figure 5.—Ranges of quality scores versus storage time of replicate lots of Dungeness crab meat initially vacuum packed in C-enameled No. 10 cans and irradiated at 200 kilorads. The cans were held for 12 days at 33° F. after which time the meat was packed in paperboard trays with cellophane overwrap and was held at 33° F.
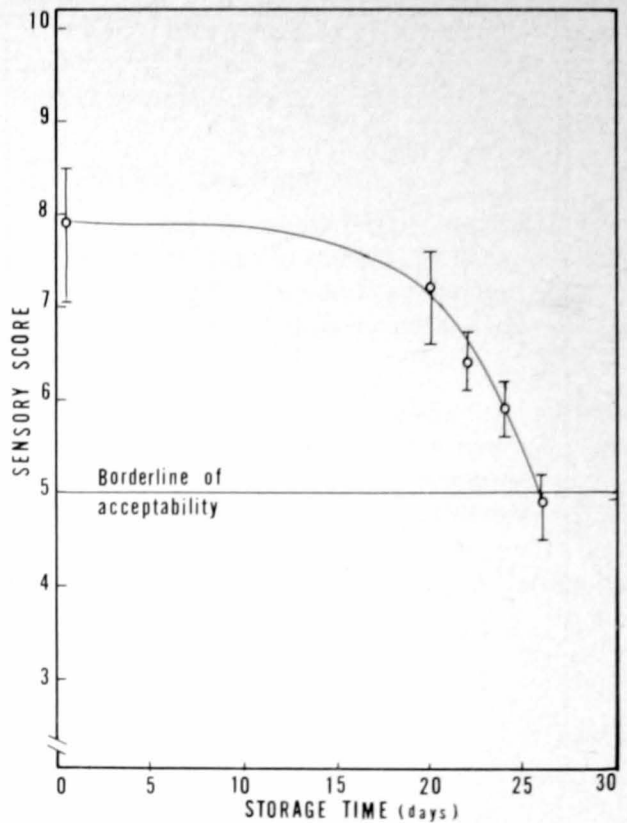
Figure 6.—Ranges of quality scores versus storage time of replicate lots of Dungeness crab meat initially vacuum packed in C-enameled No. 10 cans and irradiated at 200 kilorads. The cans were held for 20 days at 33° F. after which time the meat was packed in paperboard trays with cellophane overwrap and was held at 33° F.

7.6. A rather sharp drop in the overall scores occurred with storage time. The additional shelf life of the samples was 5 to 6 days. Thus, Dungeness crab meat that was irradiated at 200 kilorads kept in good condition for 20 days at 33° F. and still had an acceptible shelf life of 5 to 6 days, which is adequate to permit the product to be marketed at retail.

II. BACTERIOLOGICAL TESTS

A. PROCEDURE

The sampling for the bacteriological tests --total bacterial plate counts--was made by methods described by Miyauchi, Eklund, Spinelli, and Stoll (1964).

The samples for the bacteriological tests were not taken as frequently as were those for the sensory tests, because we were not trying to determine the storage life by the bacteriological tests but were merely trying to gain insight into what occurred as the result of irradiation and storage. The total bacterial counts were determined on the crab meat at the following sampling periods: (1) immediately before irradiation, (2) immediately

after irradiation, (3) at the 12-day repack time, (4) at the 20-day repack time, and (5) at the end of the storage tests.

B. RESULTS

Irradiation of the crab meat at a dose of 100 kilorads resulted in a kill of 90 to 96 percent of the bacteria originally present in the crab meat, whereas a dose of 200 kilorads resulted in a kill of 97 to 99 percent of the bacteria originally present (Table 1).

Figure 7 reports the average values of bacterial plate counts versus time for four replicates of Dungeness crab meat irradiated at the various dose levels that were described earlier and stored at 33° F. under the various conditions that also were described earlier.

With storage time, the bacterial count increased steadily. At the end of the storage tests (spoilage time), the total bacterial counts of the irradiated samples were generally higher than were those of the nonirradiated control samples. This difference may be explained in that irradiation treatment had reduced the number of, and the biochemical activities of, those organisms causing off-odor and flavor (Spinelli, Eklund, and Miyauchi, 1964). Those organisms surviving the irradiation treatment, together with others that may have been introduced during repacking, again reached sufficient numbers to cause large sensory changes
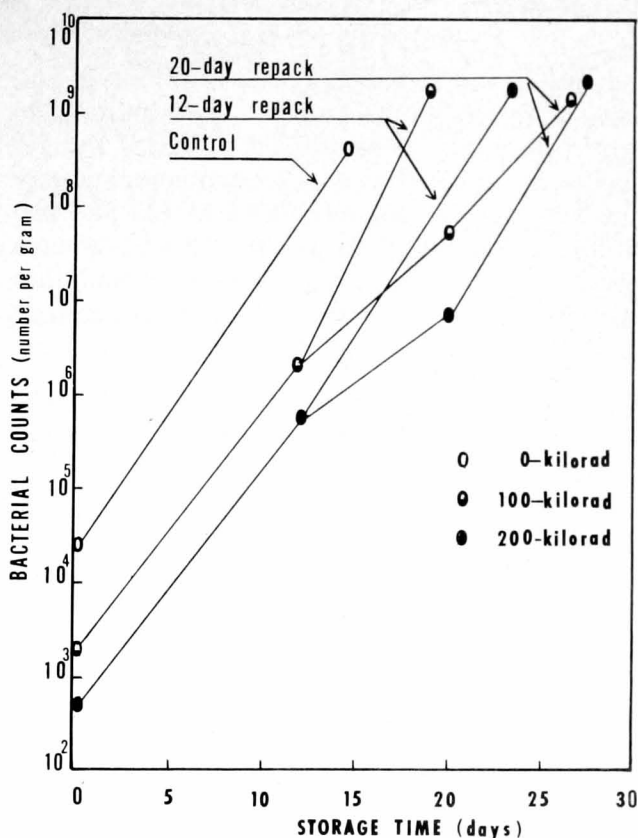

Figure 7.—Average values of bacterial plate counts of four replicate lots of Dungeness crab meat irradiated at 0, 100, and 200 kilorads and stored at 33° F.

in the crab meat when the total bacterial counts exceeded 1×10^8 per gram.

Table 1.—Total bacterial plate counts of nonirradiated and irradiated Dungeness crab meat packed in No. 10 cans for initial storage of 12 and 20 days at 33° F. and then repacked in paperboard trays with cellophane overwrap

Replicate number	Total bacterial plate counts											
	Initial			At 12-day repack time		At 20-day repack time		At end of storage period				
								Control	12-day repack		20-day repack	
	Control	100 kilorad	200 kilorad	100 kilorad	200 kilorad	100 kilorad	200 kilorad		100 kilorad	200 kilorad	100 kilorad	200 kilorad
	No./g.	No./g.	No./g.	No./g.	No./g.	No./g.	No./g.	No./g.	No./g.	No./g.	No./g.	No./g.
1	5.3×10^4	5.3×10^3	-------	5.6×10^6	-------	1.0×10^8	-------	8.8×10^8	1.5×10^9	-------	2.6×10^9	-------
2	5.7×10^4	-------	3.5×10^2	-------	1.9×10^6	-------	1.4×10^7	1.1×10^9	-------	3.3×10^9	-------	2.8×10^9
3	2.4×10^4	1.0×10^3	8.0×10^2	1.4×10^6	9.9×10^4	5.6×10^7	5.1×10^6	3.4×10^8	2.7×10^9	1.4×10^9	1.7×10^8	2.7×10^9
4	2.4×10^4	1.0×10^3	8.0×10^2	1.4×10^6	9.9×10^4	5.6×10^7	5.1×10^6	2.2×10^8	1.9×10^9	9.3×10^8	1.9×10^9	3.5×10^9
Average	3.9×10^4	2.4×10^3	6.5×10^2	2.8×10^6	7.0×10^5	7.1×10^7	8.1×10^6	6.4×10^8	2.0×10^9	1.9×10^9	1.6×10^9	3.0×10^9

SUMMARY

As an aid to widening the distribution and sale of fresh Dungeness crab meat, the storage characteristics were studied of fresh Dungeness crab meat that was packed commercially in No. 10 cans and irradiated at 100 and 200 kilorads for initial storage for 12 and 20 days at 33° F. and that was then repacked into paperboard trays with cellophane overwrap for further storage at 33° F.

After an initial storage period of 12 days in the No. 10 cans, the samples of crab meat irradiated at 100 and 200 kilorads had, after being repacked, an additional shelf life of at least 6 or 7 days, respectively. For the crab meat held for 20 days in the No. 10 cans, the samples irradiated at 100 and 200 kilorads had, after being repacked, an additional shelf life of at least 2 or 5 days, respectively. The total storage life of the nonirradiated control samples packed in retail packages ranged only between 7 and 14 days.

Total bacterial counts were determined periodically during the storage tests. At the time of spoilage, the total bacterial plate counts were found to be generally higher in the irradiated than in the nonirradiated crab meat.

The results of the several experiments show that fresh Dungeness crab meat, after being vacuum packed and irradiated at 100 kilorads or 200 kilorads in No. 10 cans, can be held for 12 or 20 days, respectively, at 33° F. and still have an adequate shelf life to permit it to be marketed in retail stores.

LITERATURE CITED

Miyauchi, D., M. Eklund, J. Spinelli, and N. Stoll.
    1964. Irradiation preservation of Pacific Coast shellfish. I. Storage life of king crab meat at 33° and 42° F. Food Technology 18: 138-142.

Miyauchi, D., J. Spinelli, N. Stoll, G. Pelroy, and M. Eklund.
    1966. Irradiation preservation of Pacific Coast fish and shellfish — IV. Storage life of Dungeness crab meat at 33°F (0.5°C) and 42°F (5.5°C). International Journal of Applied Radiation and Isotopes 17: 137-144.

Spinelli, J., M. Eklund, and D. Miyauchi.
    1964. Irradiation preservation of Pacific Coast shellfish. II. Relation of bacterial counts, trimethylamine, and total volatile base to sensory evaluation of irradiated king crab meat. Food Technology 18: 143-147.

FISH OILS—FATTY ACID COMPOSITION, ENERGY VALUES, METABOLISM, AND VITAMIN CONTENT

by

Robert R. Kifer and David Miller

ABSTRACT

This article presents a general review of fish oils.

CONTENTS

INTRODUCTION

The Bureau of Commercial Fisheries Technological Laboratory, College Park, Maryland. receives many requests for general information on fish oils.[1] The purpose of this paper is to present a general review of the subject of fish oils.

The paper is divided into two main parts. The first presents information on the sources of the fatty acids in fish oils, which will give insight into their fatty acid composition; the second part, information on the biological properties of the oils.

---

[1] For the purpose of this paper, the term oil refers to lipids in the liquid form, which are usually high in unsaturated fatty acids content. In contrast, fat refers to lipids in the solid form, which are usually high in saturated fatty acids content.

An understanding of these subjects requires a knowledge of the nomenclature of fatty acids. Accordingly, for those who may not be familiar with the nomenclature, we have explained it in the appendix.

I. SOURCES OF FATTY ACIDS IN FISH OILS

Using gas-liquid chromatography, researchers have found that fish oils contain numerous long-chain, unsaturated fatty acids, particularly of the ω3 family (Table 1). Table 2 gives the fatty acid composition of oils of various species of fish and of various animal fats. Also, for comparative purposes, Table 3 gives the composition of various vegetable oils.

The fact that fish contain considerable quantities of polyunsaturated ω3 fatty acids, however, does not necessarily indicate that fish differ markedly from land animals in their basic fat metabolic principles. The fatty acid composition of the lipids in fish, as in land animals, reflects the food they eat.

What makes the fish oils different is the ecological system accountable for the origin of the polyunsaturated fatty acids in fish. Crustaceans (zooplankton), mainly copepods, ingest phytoplankton, predominantly algae, and produce large quantities of $C_{16-18}\omega3$ polyunsaturated fatty acids, which they elongate to the $C_{20-22}\omega3$ polyunsaturated acids. Fish, which subsequently eat the algae or the crustaceans, or both, store these fatty acids in their depot oils.

Table 1.—Fatty acids commonly found in fish oils

Common name	Chemical name[1]	Simplified symbol[2]	Common name	Chemical name[1]	Simplified symbol[2]
Lauric	Dodecanoic acid	12:0	Linoleic—Con.	Nonadecanoic acid	19:0
Myristic	Tetradecanoic acid	14:0		6,9,12-Octadecatrienoic acid	18:3ω6
	9-Tetradecenoic acid	14:1ω5		11-Nonadecenoic acid	19:1ω8
	Isopentadecanoic acid	iso 15:0	Linolenic*	9,12,15-Octadecatrienoic acid	18:3ω3
	Anteisopentadecanoic acid	anteiso 15:0		6,9,12,15-Octadecatetraenoic acid	18:4ω3
	Pentadecanoic acid	15:0	Arachidic	Eicosanoic acid	20:0
	7-Pentadecenoic acid	15:1ω8		9-Eicosenoic acid	20:1ω11
	Isohexadecanoic	iso 16:0		11-Eicosenoic acid	20:1ω9
	Multiple branched acid	?		13-Eicosenoic acid	20:1ω7
Palmitic	Hexadecanoic acid	16:0		15-Eicosenoic acid	20:1ω5
	7-Hexadecenoic acid	16:1ω9		11,14-Eicosadienoic acid	20:2ω6
Palmitoleic*	9-Hexadecenoic acid	16:1ω7		8,11,14-Eicosatrienoic acid	20:3ω6
	11-Hexadecenoic acid	16:1ω5		Heneicosenoic acid (?)	21:1ω?
	Isoheptadecanoic acid	iso 17:0	Arachidonic	5,8,11,14-Eicosatetraenoic acid	20:4ω6
	Anteisoheptadecanoic acid	anteiso 17:0		11,14,17-Eicosatrienoic acid	20:3 ?
	Hexadecadienoic acid	16:2ω?		Heneicosadienoic acid	21:2(?)
	Heptadecanoic acid	17:0		8,11,14,17-Eicosatetraenoic acid	20:4ω3
	Hexadecadienoic acid	16:2ω?		5,8,11,14,17-Eicosapentaenoic acid	20:5ω3
	9-Heptadecenoic acid	17:1ω8		9-Docosenoic acid	22:1ω13
	Hexadecatrienoic acid	16:3ω?		11-Docosenoic acid	22:1ω11
Stearic	Octadecanoic acid	18:0		13-Docosenoic acid	22:1ω9
Oleic*	9-Octadecenoic acid	18:1ω9		15-Docosenoic acid	22:1ω7
cis-Vaccenic	11-Octadecenoic acid	18:1ω7		13,16-Docosadienoic acid	22:2ω6
	13-Octadecenoic acid	18:1ω5		7,10,13,16,19-Heneicosapentaenoic acid	21:5ω2(?) 24:0
	15-Octadecenoic acid	18:1ω3?		7,10,13,16-Docosatetraenoic acid	22:4ω6
Linoleic*	9,12-Octadecadienoic acid	18:2ω6		4,7,10,13,16-Docosapentaenoic acid	22:5ω6
	Unknown acid	?		7,10,13,16,19-Docosahexaenoic acid	22:5ω3
				4,7,10,13,16,19-Docosahexaenoic acid	22:6ω3
				15-Tetracosenoic acid	24:1ω9

* Parent fatty acid of a family.
[1] Geneva system whereby the unsaturated bonds were indicated by counting from the carboxyl (COOH) carbon atom.
[2] Simplified nomenclature whereby the number of carbon atoms is listed, followed by the number of unsaturated bonds and the position of the bond counting from the terminal methyl carbon atom (CH₃) or omega (ω) carbon. Thus 18:1ω3 is

$$CH_3 - CH_2 - \underset{}{\overset{H}{C}} = \underset{}{\overset{H}{C}} - (CH_2)_{13} - COOH$$
ω3
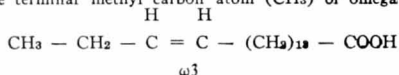
Table 2.—Approximate fatty acid composition of some fish oils and animal fats

Fatty acid	Family type	Relative amount of the given fatty acid in:							
		Menhaden	Pacific herring	Salmon	Mullet	Ocean perch	Beef tallow	Yellow grease[1]	Lard
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
14:0	--	8	8	4	6	5	4	2	--
14:1	--	--	--	--	--	--	3	1	--
15:0	--	a	a	5	6	a	--	--	--
16:0	--	29	18	16	16	13	25	25	29
16:1	ω7	8	8	6	13	8	5	4	4
16:2	ω4	a	1	1	5	1	a	a	--
17:0	--	1	a	1	1	1	--	--	--
18:0	--	4	2	4	5	4	19	14	14
18:1	ω9	13	17	19	9	22	36	41	45
18:2	ω6	1	2	1	3	2	6	7	8
18:3	ω3 ω6	1	a	1	1	a	1	a	--
18:4	ω3	2	3	2	3	2	--	2	--
19:0	--	1	a	1	1	a	--	--	--
20:1	ω9	1	9	4	a	8	--	--	--
20:2	ω6	a	--	a	1	--	--	a	--
20:3	ω6	--	--	--	a	--	--	--	--
20:4	ω6	1	a	a	3	a	--	--	--
20:5	ω3	10	9	12	10	9	--	--	--
22:1	ω9	2	12	5	a	9	--	--	--
22:5	ω6	a	--	a	1	a	--	--	--
22:5	ω3	2	1	3	3	a	--	--	--
22:6	ω3	13	8	15	8	12	--	--	--
24:1	ω9	a	1	a	1	a	--	--	--

[1] Yellow grease is an inedible grade of animal (cattle, sheep, pig, etc.) fat with a melting point of 36° C., an FAC color of 37, a free fatty acid content not exceeding 15 percent, and a M.I.U. (moisture insoluble and unsaponifiable material) content not exceeding 3 percent--FAC is the color standard established by the fat analysis committee of the American Oil Chemists' Society.
a = less than 1 percent.
Sources: Mehlenbacher, 1960; Gruger, Nelson, and Stansby, 1964; and Gruger and Robisch, 1966 (personal communication).

Table 3.—Approximate fatty acid composition of plant oils

Fatty acid	Family type	Relative amount of the given fatty acid in the oil from:								
		Coconut	Palm	Olive	Peanut	Cotton-seed	Corn	Saf-flower	Soy-bean	Linseed
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
6:00-10:0	--	16	8	--	--	--	--	--	--	--
12:0	--	48	48	--	--	--	--	--	--	--
14:0	--	16	16	1	--	a	--	--	--	--
16:0	--	9	8	14	9	22	11	7	10	13
18:0	--	2	2	5	3	2	2	3	2	3
18:1	ω9	7	16	73	52	31	28	12	23	19
18:2	ω6	2	11	6	26	45	59	79	55	16
18:3	ω3	--	a	--	a	a	1	a	8	50
20:0 22:0 24:0	--	--	6	--	--	--	--	--	--	--

a = less than 1 percent.
Sources: Deuel, 1957; and Mehlenbacher, 1960.

This ecological system varies, owing to seasonal changes in water temperature. In general, as the temperature of the water decreases, the quantity of $C_{20-22}\omega3$ polyunsaturated fatty acids increases. Thus, the difference in the degree of unsaturation in the oil of marine and fresh-water fish is probably because, in general, fresh water warms sooner and to a higher temperature. Consequently, in both marine and fresh water, the oil in phytoplankton and zooplankton becomes more saturated as water temperature increases. We can conclude that phytoplankton constitute the primary source of polyunsaturated fatty acids of the linolenic (ω3) family and that zooplankton constitutes the secondary source for

Table 4.—Fatty acid composition of the lipids from cultures of the diatom *Skeletonema costatum* at various days of growth

Fatty acid	Family type	Relative amount of the given fatty acid from cultures grown for:				
		2 days	4 days	6 days	8 days	10 days
		Percent	*Percent*	*Percent*	*Percent*	*Percent*
14.0	--	9.6	26.5	30.1	31.8	32.9
14:1	--	0.9	1.8	3.1	4.5	3.0
15:0	--	0.3	0.4	0.5	0.5	0.5
16:0	--	9.0	6.1	6.6	7.1	6.8
16:1	$\omega 7$	18.3	13.0	14.5	17.5	16.7
16:2	$\omega 7$	1.0	0.9	1.0	1.2	0.7
16:2	$\omega 4$	8.9	9.1	7.8	6.6	7.4
16:3	$\omega 4$	5.4	13.6	8.7	6.3	6.4
16:4	$\omega 1$	3.4	4.0	5.4	6.1	4.7
18:0	--	1.0	Trace	?	?	0.2
18:1	$\omega 9$	5.0	0.4	0.3	0.1	0.3
18:2	$\omega 6$	1.4	0.4	0.5	0.6	0.8
18:3	$\omega 6$	0.3	0.4	0.1	Trace	0.2
18:3	$\omega 3$	0.3	Trace	0.1	Trace	0.2
18:4	$\omega 3$	2.2	4.4	3.8	3.4	3.3
19:1	--	0.3	?	Trace	?	0.1
20:1	$\omega 9$	0.6	Trace	Trace	Trace	0.1
20:2	$\omega 6$	0.2	0.1	Trace	Trace	0.1
20:3	$\omega 6$	0.1	Trace	?	?	0.1
20:4	$\omega 6$	1.2	0.1	Trace	Trace	0.2
20:4	$\omega 3$	0.3	0.1	0.1	0.2	0.2
20:5	$\omega 3$	23.2	17.4	16.3	13.3	13.4
21:5	--	0.2	0.1	0.1	0.1	0.1
22:4	$\omega 6$	0.1	?	?	?	?
22:5	$\omega 6$	0.1	?	?	?	?
22:5	$\omega 3$	0.2	Trace	?	?	?
22:6	$\omega 3$	6.5	1.2	0.7	0.9	1.2
Unknown	--	0.4	0.1	0.1	0.1	0.1
Calculated iodine value	--	212	181	168	150	146

Source: Ackman, Jangaard, Hoyle, and Brockerhoff, 1964.

Table 5.—Fatty acid composition of algae

Fatty acid	Family type	Relative amount of the given fatty acid in:					
		A[1]	B	C	D	E	F
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
10:0	--	0	0.92	0	+[2]	+	0.42
12:0	--	0.15	+	0.47	0.63	0.13	0.26
14:0[3]	--	13.86	26.82	6.38	1.57	25.26	0.39
14:1	--	0	3.67	0	+	0.88	0.39
14:2	--	1.26	+	+	1.37	0.25	5.51
15:0	--	0.33	0	0	0	0	0
16:0	--	30.50	17.87	58.84	50.17	40.02	42.24
16:1	$\omega 7$	48.39	48.15	5.25	10.19	28.91	14.96
16:2	$\omega 4$	2.02	1.24	0.24	6.27	0.28	0.37
16:3	$\omega 4$	0.78	0	0	0.26	0	0
18:0	--	0	0	5.24	3.14	0.20	0
18:1	$\omega 9$	2.70	1.34	10.24	7.32	3.70	10.50
18:2	$\omega 6$	0	0	1.37	8.18	0.38	7.35
18:3	$\omega 3$	0	0	9.09	10.97	0	17.58
20:0[4]	--	0	0	2.86	0	0	0
22:0	--	0	0	0	0	0	0

[1] The algae are: A, *Phaeodactylum tricornutum* (Bacillariophyceae); B, *Agmenellum quadraplicatum* (Myxophyceae); C, *Amphidinium carteri* (Dinophyceae); D, *Syracosphaera caton* (Chrysophyceae); E, *Monochrysis lutheri* (Chrysophyceae); F, *Dunaliella salina* (Chlorophyceae).
[2] The + means that less than 0.01 μg. was detected.
[3] The 14:0, 14:2, 16:3ω4 and 18:3ω3 are tentative identifications.
[4] No C_{20} unsaturated fatty acids were detected.
Source: Williams, 1965. (Data were recalculated from mole percent to area percent to conform with format of Tables 1-4.)

28

fish. Tables 4, 5, and 6 give the fatty acid composition of some of these plankton groups to illustrate the most likely primary and secondary dietary sources for fishes. Various higher trophic levels in the food chains may also enter into the ecological system, because many species of fish are omnivorous; however, the basic source of oil is plankton.

Table 6.—Fatty acid composition of zooplankton samples and fish livers

Fatty acid	Family type	Relative amount of the given fatty acid in:			
		Zooplankton		Fish livers of:	
		Sample 1	Sample 2	Hake	Whiting
		Percent	*Percent*	*Percent*	*Percent*
10:0	--	0.16	+[1]	0	0
12:0	--	0.79	0.80	+	+
14:0	--	9.34	7.57	2.05	12.13
14:1[2]	--	+	+	+	+
14:2	--	0.95	0.55	0.58	0.72
15:0	--	0	0	0	0
16:0	--	34.54	30.73	30.51	23.79
16:1	$\omega 7$	16.80	10.12	11.89	12.19
16:2	$\omega 4$	2.51	1.84	1.14	0.78
16:3	$\omega 4$	1.06	0.63	0	0.53
18:0	--	3.89	3.51	4.07	3.57
18:1	$\omega 9$	17.46	15.10	24.61	15.02
18:2	$\omega 6$	0.95	1.23	1.02	0.58
18:3	$\omega 3$	0.66	0	1.02	0.82
20:0[3]	--	10.90	27.93	20.23	18.38
22:0	--	0	0	2.88	11.49

[1] The + means that less than 0.01 $\mu g.$ was detected.
[2] The 14:1, 14:2, 16:3ω4, and 18:3ω3 are tentative identifications.
[3] Includes the unsaturated C_{20} acids.
Source: Williams, 1965. (Data were recalculated from mole percent to area percent to conform with forman of Tables 1-2.)

II. BIOLOGICAL PROPERTIES

Ingested lipids, regardless of origin, function principally as a source of energy and as integral parts of cell structure. Except for a few fatty acids termed essential (linoleic, 18:2ω6, linolenic, 18:3ω3; and arachidonic, 20:4ω6), the remaining fatty acids synthesized by the body are sufficient to meet body needs for stored energy and cellular components. The extent to which ingested lipids serve to meet these needs depends upon their degree of digestibility, the quantity absorbed, and the structural characteristics of the fatty acids. In addition, certain highly unsaturated oils, including fish oil, may alter cholesterol metabolism.

This second part of the paper is concerned with the digestion and absorption of fish oils and with their biological significance.

A. DIGESTION AND ABSORPTION

Although digestion and absorption are physiologically two distinct processes, most digestibility data are derived in such a way that no distinction between the two processes is possible. Digestibility values for various oils, given in Table 7, were obtained by the difference between the quantity of lipid ingested and the quantity excreted. These data indicate that, in general, oils or fats are highly "digestible." For instance, the average "digestibility" for fish oil is 97 percent. Although no distinction is made between the physiological processes of digestion and absorption, various factors affect oil or fat in each of these two processes.

1. Digestion

Briefly, digestion of an oil or fat occurs in an animal when the oil or fat is hydrolyzed to monoglycerides and diglycerides, free fatty acids, and glycerol, with small amounts remaining as the triglyceride.

Digestibility of oils, especially those containing the polyunsaturated fatty acids, is affected by autoxidation of the fatty acids and by the type of fatty acids contained in the lipid structure. Autoxidation proceeds by a free-radical mechanism such as that illustrated

Table 7.—Coefficients of digestibility of some fats as determined by feeding them to various animals

Product	Melting point	Coefficients of digestibility of the given fat when fed to:						
		Chicken	Sheep	Guinea pig	Rabbit	Dog	Rat	Human
	° C.	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Acidulated soybean soapstock	--	75.3	--	--	--	--	--	--
Beef tallow	--	67.0	--	72.0	--	--	--	--
Butter	34.5	--	--	91.0	--	--	88.3	--
Butter	34.5	--	--	--	--	--	90.7	--
Butter	--	--	--	--	--	--	97.4	--
Butter	--	--	--	--	--	--	98.0	--
Castor oil	--	--	99.0	96.2.	92.1	--	98.0	--
Cocoa butter	28.0	--	--	--	--	--	63.3	--
Cocoa butter	28.0	--	--	--	--	--	81.6	--
Coconut oil	26.0	--	--	94.0	--	--	98.9	--
Coconut oil	26.0	--	--	--	--	--	96.5	--
Cod liver oil	--	87.0	--	93.8	--	--	98.2	--
Corn oil	33.0	92.9	--	--	--	--	--	94.7
Corn oil	43.0	--	--	--	--	--	--	95.4
Corn oil	50.0	--	--	--	--	--	--	88.5
Cottonseed oil	--	--	94.0	87.4	91.2	99.0	97.4.	--
Cottonseed oil	--	--	--	--	--	--	99.1	--
Cottonseed oil	--	--	--	--	--	--	94.8	--
Cottonseed oil	38.0	--	--	--	--	--	91.0	96.8.
Cottonseed oil (hydrogenated)	46.0	--	--	--	--	--	83.8	94.9
''	54.0	--	--	--	--	--	68.7	--
''	62.0	--	--	--	--	--	38.0	--
''	63.0	--	--	--	--	--	24.0	--
Crisco	43.0	--	94.0	73.8	91.0	--	94.8	--
Crisco	43.0	--	--	--	--	--	97.3	--
Hydrogenated fat	--	76.0	--	--	--	--	--	--
Lard	--	93.4	--	75.2.	--	97.0	97.8	--
Lard	37.0	--	--	75.2.	--	98.0	96.6	--
Lard fatty acids	--	88.4	--	--	--	--	--	--
Lard (bland)	48.0	--	--	--	--	--	94.3	--
Lard (hydrogenated)	55.0	--	--	--	--	--	63.2	--
Lard (hydrogenated)	61.0	--	--	--	--	--	21.0	--
Margarine fat	34.0	--	--	--	--	--	97.0	--
Margarine fat	34.0	--	--	--	--	--	97.0	--
Menhaden oil	--	87.8	--	--	--	--	--	--
Mutton tallow	47.0	--	--	79.8	--	--	74.6	88.0
Mutton tallow	47.0	--	--	--	--	--	84.8	--
Neat's-foot oil	--	--	--	93.5	--	--	--	--
Oleic acid	--	88.4	--	--	--	--	--	--
Oleo stock	48.0	--	--	--	--	--	74.0	--
Oleo stock	48.0	--	--	--	--	--	86.7	--
Olive oil	--	--	--	94.5	--	98.0	98.4	--
Olive oil	--	--	--	77.0	--	94.0	92.0	--
Palmitic acid	--	0.2	--	--	--	--	--	--
Peanut oil (crude)	--	--	--	--	--	--	97.6	--
Peanut oil (refined)	--	--	--	91.8	--	--	96.4	--
Peanut oil (hydrogenated)	39.0	--	--	--	--	--	92.6	--
Peanut oil (bland)	39.0	--	--	--	--	--	91.4	--
Perilla oil (hydrogenated)	67.5	--	--	--	--	--	6.0.	--
Rapeseed oil	--	--	--	--	--	--	82.0	--
Red (Ocean perch) fish oil	--	83.4	--	--	--	--	--	--
Salmon oil	--	--	--	94.0	--	--	--	--
Salmon oil	--	--	--	--	--	--	98.3	--
Shortening	--	--	--	--	--	--	98.6	--
Shortening	--	--	--	--	--	--	99.6	--
Soybean lecithin	--	86.5	--	--	--	--	--	--
Soybean oil	--	--	--	94.5	--	--	98.5	--
Soybean oil	--	--	--	--	--	--	98.3	--
Soybean oil (degummed) .	--	98.8	--	--	--	--	--	--
Soybean oil fatty acids . .	--	89.3	--	--	--	--	--	--
Stearic acid	--	3.9.	--	--	--	--	--	--
Tallow (Federal grade) . .	--	70.3	--	--	--	--	--	--
Tallow fatty acids	--	47.7	--	--	--	--	--	--
Tobacco seed oil	--	--	--	--	--	--	97.9	--

Table 7.—Continued

Product	Melting point	Coefficients of digestibility of the given fat when fed to:						
		Chicken	Sheep	Guinea pig	Rabbit	Dog	Rat	Human
	° C.	Percent	Percent	Percent	Percent	Percent	Percent	Percent
Tropical fats								
Aceituna (unrefined) ...	--	--	--	--	--	--	93.5	--
Cocoa volador	--	--	--	--	--	--	96.9	--
Corozo	--	--	--	--	--	--	97.0	--
Morro	--	--	--	--	--	--	96.4	--
Sapayulo	--	--	--	--	--	--	92.2	--
Tambor	--	--	--	--	--	--	94.5	--

Source: Primarily Deuel, 1957.

in Figure 1. It is characterized first by an induction period during which the rate of oxidation is slow and then by a period during which the rate of oxidation accelerates and during which hydroperoxides, rancid odors, and polymerized products develop. Table 8 contains a list of autoxidative decomposition products of vegetable oils. Although no detailed studies have been made of the breakdown products of fish oils, the autoxidation of these products might be expected to be more complicated, owing to their relatively high degree of unsaturation.

Table 8.—Products of decomposition of hydroperoxides of vegetable oils

Volatiles	Nonvolatile polar compounds	Polymers
Aldehydes	Aldehydyes	Dimers
Ketones	Ketones	Trimers
Hydrocarbons	Hydroxy compounds	Higher polymers
Alcohols	Acids	--
At least 200 different compounds	--	--

Source: Evans, 1961.

Thus, the net digestibility of a lipid is affected by a number of factors, such as polymerization, linkage of the fatty acids, type of fatty acids contained in the triglyceride structure, and quantity of free fatty acids fed.

Polymerization of fish oils, which lowers digestibility, usually arises from excessive heat derived externally from processing or from excessive heat derived internally from oxidation of the oil.

Another reaction, complexing, occurs between fatty acids and amino acids and is usually associated with excessive heat as a catalyst. The digestibility of the complexed fatty acids is lowered.
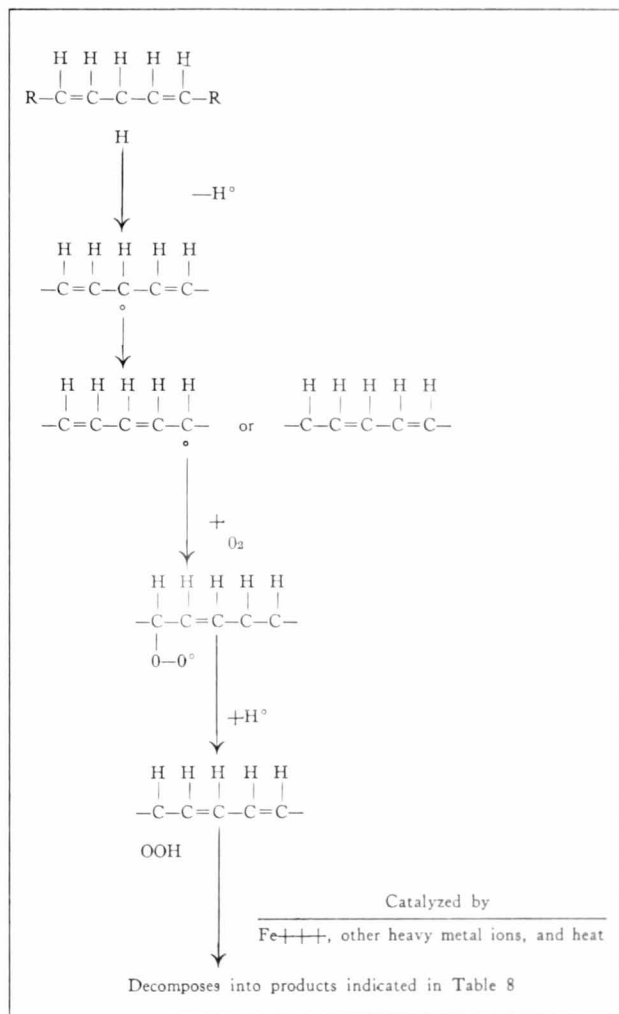
Figure 1.—Illustration of autoxidation of polyunsaturated fatty acids. Source: Olcott, 1962.

Also, maximum digestibility of oils or fats apparently occurs when the lipid fed is in the triglyceride form and when it contains at least one unsaturated fatty acid per molecule. Table 9 shows the digestibility of the saturated fatty

acids in the triglyceride form and of various individual fatty acids.

Table 9.—Digestibility of simple triglycerides and fatty acids

Product	Melting point	Digestibility of the given nutrient when fed to:		
		Guinea pig	Dog	Rat
	° C.	Percent	Percent	Percent
Triglycerides:				
Trilaurin	49	--	--	97.3
Trimyristin	56	--	--	76.6
Tripalmitin	66.5	--	95.0	27.9
Tristearin	70	--	10.0	18.9
Tristearin + Trilaurin (2:1) ..	--	--	--	39.4
Tristearin + Trilaurin (1:2) ..	--	--	--	68.6
Fatty acids:				
Lauric	44	--	--	81.5
Myristic	53	--	--	81.9
Palmitic	63	--	82.0	35.6
Stearic	69	--	--	15.8
Oleic	--	95.4	--	95.4.
Elaidic	--	55.6	--	95.6

Source: Deuel, 1957.

2. Absorption

Absorption is the process of transfer of the digested lipid material across the intestinal wall and into the blood or lymph stream. Absorption depends on certain characteristics of the undigested and digested lipid as well as on the components of the diet.

Characteristics of lipids that affect absorption are (1) the chain length of the fatty acids, (2) the degree of unsaturation, (3) the arrangement of fatty acids within the triglyceride molecule, and (4) the state of the fatty acid -- that is, whether it is free or esterified. The digestion and absorption of lipids vary widely. In general, lipids that are liquid at body temperature are absorbed efficiently. Lipids that melt well above body temperature will not be absorbed unless they are mixed with lower-melting lipids.

The absorption of fatty acids is reported to occur by three separate avenues.

In the first avenue, the short chain (10-carbon or less) fatty acids pass via the intestinal wall directly into the portal blood. These fatty acids must be in the free form and be saturated. Fish oil may contain a small quantity of these fatty acids.

In the second avenue of absorption, the monoglyceride and diglyceride of long-chain free fatty acids pass into the cells of the intestinal wall, being recombined into triglycerides and released into the lymph system. Ultimately, they enter the blood stream at the anterior vena cava and are conveyed to the tissues in a lipo-protein complex. Apparently, triglycerides per se, diglycerides and monoglycerides, and unsaturated free fatty acids enter the intestinal cells readily. However, such saturated free fatty acids as stearic (18:0) and palmitic (16:0) are poorly absorbed, although the presence of oleic (18:1ω9), polyunsaturated fatty acids, or both, enhance the absorption of these saturated acids. Data in Table 9 illustrate these relative differences expressed as "digestibility."

In the third avenue of absorption, pinocytosis -- that is, a folding of the intestinal wall around lipid particles -- occurs. Apparently, in this process, food particles that move into the base area between intestinal villi stimulate the growth of a new membrance between the villi and over the particle. Subsequently, the original base membrane disintegrates, and the particle passes into the blood or lymph system.

In addition to the characteristics of the fat already stated above, maximum absorption of any dietary fat is affected by the type and quantity of other dietary components -- protein, carbohydrates, and minerals.

The lipid material, once absorbed, may be used to supply energy for mebtabolic purposes, may be stored in depots, or may be incorporated into phospholipids of cellular structures.

The energy of fish oil, as of other oils, may be expressed as gross, metabolizable, net, or productive energy.

In poultry feeding, the most common expression is metabolizable energy, obtained by taking the difference between the energy of the food ingested and that of the excrement and urine. Fish oils yield about 3,600 calories per pound. The metabolizable energy values for a number of oils and fats appear in Table 10. Because the biological release of calories from fats is actually an expression of the

amount of a lipid digested and absorbed, any factor that alters either of these processes also lowers the amount of lipid taken into the body and concomitantly results in a lower energy value. Once absorbed, the unsaturated fatty acid yields slightly less energy per molecule than does it saturated counterpart. The unsaturated bond position of the various families apparently does not affect the caloric value of the polyunsaturated fatty acids.

Table 10.—Metabolizable energy values of various fats, oils, and fatty acids

Product	Metabolizable energy
	Calories per pound
Yellow grease	4,326
Soybean oil	4,200
Menhaden oil	4,199
Soybean oil fatty acids	4,008
Corn oil	4,000
Lard (edible)	3,980
Choice white grease	3,925
Brown grease	3,830
Oleic acid	3,770
Poultry grease	3,720
Acidulated soybean oil soapstock	3,691
Bleachable fancy tallow	3,597
Methyl esters of fatty acids	3,457
No. 2 tallow	3,451
All-beef tallow	3,451
Tallow (prime beef)	3,323
Feed grade tallow	3,230
Hydrolyzed fat	3,230
Hydrolyzed animal and vegetable fat	3,176
Soybean oil soapstock	3,150
Soybean lecithin	3,060
Beef tallow	2,860
Tallow fatty acids	2,729
Palmitic acid	- 9
Stearic acid	-17

Sources: Ault, Riemenschneider, and Saunders, 1960; Cullen, Rasmussen, and Wilder, 1962; Ewing, 1963; and Artman, 1964.

B. BIOLOGICAL SIGNIFICANCE

Fish oils are made up of two portions -- (1) a saponifiable portion containing the glycerides, phospholipids, and cerebrosides, and (2) a nonsaponifiable portion containing the fat-soluble vitamins -- A, D, and E. In our consideration of the biological significance of fish oils, we discuss first the saponifiable components, which in general constitute by far the largest part of fish oils, and then the nonsaponifiable portion containing the fat-soluble vitamins.

1. Saponifiable Components

Under the saponifiable components, we consider: (a) the essentiality of certain fatty acids in fish oil, (b) their anticholesteremic properties, and (c) their metabolic peculiarities.

a. Essentiality of certain fatty acids in fish oil. — In addition to supplying energy, certain of the unsaturated fatty acids have specific functions. Experimental evidence indicates that only linoleic acid ($18:2\omega6$) and arachidonic acid ($20:4\omega6$) will prevent the fatty acid deficiency syndromes of slow growth and dermatitis. Whether one of these fatty acids or both are the active forms is difficult to determine, for linoleic acid is a precursor of arachidonic acid. Evidence indicates that linolenic acid ($18:3\omega3$) will restore the growth retarded by essential fatty acid deficiency. The isomers $18:3\omega9$ and $18:2\omega9$ and $20:4\omega3$ of these essential fatty acid may all have potency in preventing the essential fatty acid syndrome. The complete role of the other $\omega3$ family fatty acid in essential fatty acids deficiency has not been delineated. Researchers have hypothesized that neither linoleic ($18:2\omega6$) nor linolenic ($18:3\omega3$) acid possesses essential fatty acids activity but that the elongated and more unsaturated family members are the physiologically active forms. The essential fatty acids appear to be vital components of phospholipids that are necessary for cellular metabolism and integrity.

Essentiality of fatty acids may not be restricted only to growth and dermal syndromes, because evidence indicates a need for dietary fatty acids for normal chick reproduction. With the hen, menhaden oil apparently is more effective than safflower oil in promoting greater egg production, egg size, and hatchability. Apparently, in this function, the $\omega3$ family fatty acids have greater potency than have those in the $\omega6$ family. This supposition is predicated on the fact that fish oils contain about 40 percent of $\omega3$ family fatty acids and only about 2 percent of $\omega6$ family fatty acids, whereas safflower oil contains no $\omega3$ family fatty acids and about 40 percent of $\omega6$ fatty acids.

Metabolically, the fatty acid 20:3ω9 increases markedly in tissues during essential fatty acid deficiency (Figure 2). The feeding of ω6 or ω3 fatty acid results in a significant decrease of 20:3ω9 in the tissue.
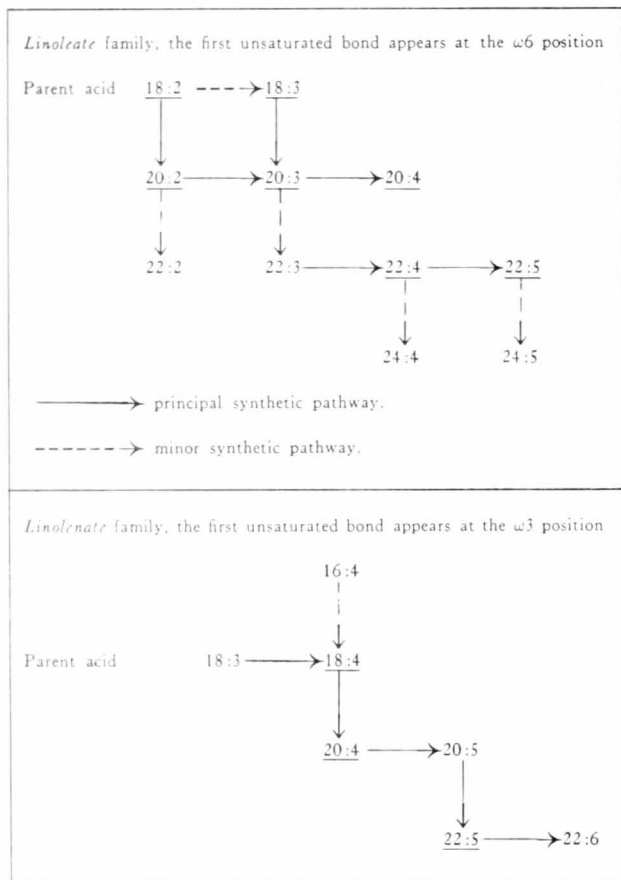

Figure 2.—Fatty acid families -- of which at least one member must be obtained from the diet.

b. Anticholesteremic properties of fish oil. —Elevated plasma cholesterol levels are the primary syndrome associated with arteriosclerosis (hardening of the arteries). Feeding dietary polyunsaturated fatty acids lowers the plasma cholesterol level and thereby lessens the incidence of arteriosclerosis. Fish oils are relatively effective in treating this condition, owing to their high content of ω3 polyunsaturated fatty acid.

c. Metabolic peculiarities of fish oil.— Feeding high levels of fish oil requires certain dietary precautions to be taken, owing to the high content of polyunsaturated fatty acids in fish oil and its resulting high reactivity. Supplementation of the diet by various nutrients -- such as vitamin E or selenium, or by synthetic antioxidants -- is therefore necessary. Lack of adequate levels of one or more of the nutrients or of the synthetic antioxidant may result in the production of certain physiological abnormalities, such as exudative diathesis, muscular dystrophy, or encephalomalacia.

In tissues of broilers or swine, another difficulty that can develop from too much fish oil in the diet is off-flavor or fishiness. This condition arises when the total content of fish oil in the diet exceeds 1.5 percent for broilers and turkeys and 1.0 percent for swine during the entire period of growth. Acceptable broiler and swine flesh are obtained, even if higher levels of fish oil are fed at the earlier stages of the animal's growth, if the oil is withdrawn from the feed 1 month in advance of marketing for broilers, 3 months in advance for turkeys, and 1 month in advance for swine.

The substitution of yellow grease and presumably any other of the more saturated fats for the fish oil during the withdrawal phase speeds the elimination of the off-flavor. Certain polyunsaturated fatty acids of the linolenic (ω3) family 18:3, 18:4, 20:4, 20:5, 22:5, and 22:6 characteristic of fish oil are correlated with the occurrence of off-flavor in the animal tissue.

2. Nonsaponifiable Fat-Soluble Vitamins A, D, and E

In general, fish-body oils either per se or as residual oil in fish meal do not contain a high or consistent quantity of the fat-soluble vitamins A, D, and E. Because the animal's requirements can be met completely by inexpensive synthetic sources stabilized at guaranteed potencies and because the amount of these vitamins that might be naturally present is variable, the presence of any of the naturally occurring vitamins is ignored in feed formulations.

In contrast to body oils, fish-liver oils, such as those from cod and halibut, ordinarily contain relatively large quantities of the fat-sol-

uble vitamins A and D, which prior to the availability of the synthetics were the main source of these vitamins for animals and humans.

LITERATURE CITED

Ackman, R. G.
1966. The superior performance of open tubular column for the analysis of mixtures of natural fatty acids. The Perkin-Elmer Corporation, G. C. Newsletter 2(4): 6.

Ackman, R. G., P. M. Jangaard, R. J. Hoyle, and H. Brockerhoff..
1964. Origin of marine fatty acids. I. Analyses of the fatty acids produced by the diatom *Skeletonema costatum*. Journal of the Fisheries Research Board of Canada 21: 747-756.

Artman, Neil R.
1964. Interaction of fats and fatty acids as energy source for the chick. Poultry Science 43: 994-1004.

Ault, W. C., R. W. Riemenschneider, and D. H. Saunders.
1960. Utilization of fats in poultry and other livestock feeds. Technology and feeding practices. U.S. Department of Agriculture, Agricultural Research Service, Utilization Research Report 2, 41 pages (see page 33).

Cullen, Marion P., Oscar G. Rasmussen, and O. H. M. Wilder.
1962. Metabolizable energy value and utilization of different types and grades of fat by the chick. Poultry Science 41: 360-367.

Deuel, Harry J., Jr.
1957. The lipids, their chemistry and biochemistry. Volume III: Biochemistry—biosynthesis, oxidation, metabolism, and nutritional value. Interscience Publishers, Inc., New York, 1065 pages (see pages 835-932).

Evans, C. D.
1961. Chemical changes accompanying flavor deterioration of vegetable oils. Proceedings, Flavor Chemistry Symposium, 1961, pages 123-146. Campbell Soup Company, Camden, New Jersey.

Ewing, W. R.
1963. Poultry nutrition. The Ray Ewing Company, Publishers, Division of Hoffman-La Roche Inc., Pasadena, California, 1475 pages (see pages 97-102).

Gruger, E. H., Jr., R. W. Nelson, and M. E. Stansby.
1964. Fatty acid composition of oils from 21 species of marine fish, fresh-water fish and shellfish. The Journal of the American Oil Chemists' Society 41: 662-667.

Mehlenbacher, V. C.
1960. The analysis of fats and oils. The Garrard Press, Champaign, Illinois, page 603.

Olcott, H. S.
1962. Oxidation of fish lipids. *In* Eirik Heen and Rudolf Kreuzer (editors), Fish in nutrition, pages 112-116. Fishing News (Books) Ltd., London.

Williams, P. M.
1965. Fatty acids derived from lipids of marine origin. Journal of the Fisheries Research Board of Canada 22: 1107-1122.

APPENDIX

NOMENCLATURE OF FATTY ACIDS

Fatty acids are named by one or more systems. Each acid may have a common name (for example, oleic acid is the common name of one of the compounds with the empirical formula $C_{18}H_{34}O_2$) and a chemical name (for example, oleic acid is called octadecenoic acid in accordance with the Geneva Convention system).

A system of nomenclature should describe the compound completely in the simplest manner. Thus, oleic acid can be indicated by the symbol 18:1, indicating 18 carbon atoms and one unsaturated bond. The position of the unsaturated bond is currently indicated by the symbol $\triangle$ followed by a number or numbers showing the location of each double bond counting the carbons from the carboxyl (COOH) end (Appendix Figure 1) or at times without the $\triangle$. Oleic acid therefore can be represented as $18:1\triangle9$ or $18:1°$.
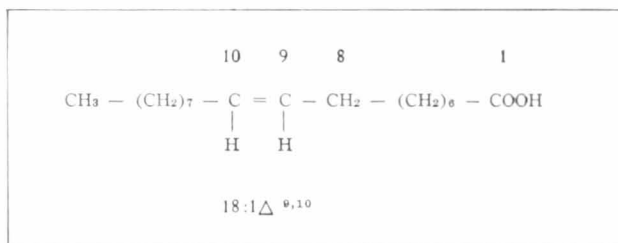
Appendix Figure 1.—Illustration of Geneva system of nomenclature.

A number of investigators have felt that a more descriptive positioning of the unsaturated bonds is needed in metabolic studies of the polyunsaturated fatty acids. So, in 1953, a biochemical system was suggested. This new system differs from the old system only in the manner that the location of the unsaturated bond is described. As in the previous system, the number of carbon atoms and unsaturated bonds are indicated as 18:1. However, in the new system, the unsaturated bonds are positioned relative to the terminal methyl group. The carbon of the terminal methyl group is designated omega (ω) and is counted as number 1. This designation of the terminal methyl group as number 1 is in contrast to the practice in the Geneva system, which designates the carboxyl (COOH) carbon as number 1.

Illustration:
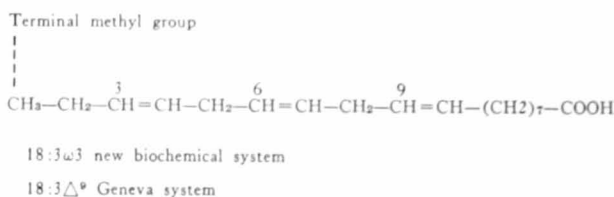
In this system of nomenclature, the polyunsaturated fatty acids fall into families based on the position of the first double bond from the terminal carbon irrespective of the chain length. Four main families of unsaturated fatty acids have been established -- designated as $\omega3$, $\omega6$, $\omega7$, and $\omega9$ -- each of which has a parent fatty acid [linolenic, $18:3\omega3$; linoleic, $18:2\omega6$; palmitoleic, $16:1\omega7$; and oleic, $18:1\omega9$ (Appendix Figures 2 and 3)].

Two of the parent acids, palmitoleic ($\omega7$) and oleic ($\omega9$), are synthesized within the animal tissue from carbohydrate or protein, or both, in the diet. The other two parent acids, linoleic ($\omega6$) and linolenic ($\omega3$), are obtainable only from the diet. In addition to these four main families, other so-called minor families have recently been found. Appendix Figure 2 shows the pathways and intermediates of two of these minor families ($\omega5$ and $\omega8$).

Acetyl CoA + CO_2 ——→ Malonyl CoA
+ (Acetyl CoA)

16:1[1] ←——— 16:0

18:1   18:0 ———→ 18:1[2] ----→ 18:2[3]

20:1   20:0   20:1   20:2 ----→ 20:3[4]

22:1   22:0   22:1

24:0   24:1

[1] Parent fatty acid of palmitoleic acid family, the first unsaturated bond appears at the $\omega 7$ position.

[2] Parent fatty acid of oleic acid family, the first unsaturated bond appears at the $\omega 9$ position.

[3] Isomers are underlined -- that is, they differ in position of unsaturated bonds in relation to the omega (ω) carbon.

[4] The quantity of this fatty acid $20:3\omega9$ increases in tissues of animals with essential fatty acid deficiency. Therefore, the increased concentration is used as an indicator of essential fatty acid deficiency

————————→principal synthetic pathway.

- - - - - - -→minor synthetic pathway.

Appendix Figure 2.—Fatty acid families -- parent acids of which may originate from carbohydrate or protein degradative products or from lipids.

The first unsaturated bond appears at the $\omega 5$ position

Parent acid   14:1

16:1

18:1

20:1

?

The first unsaturated bond appears at the $\omega 8$ position

Parent acid   15:1

17:1

19:1

?
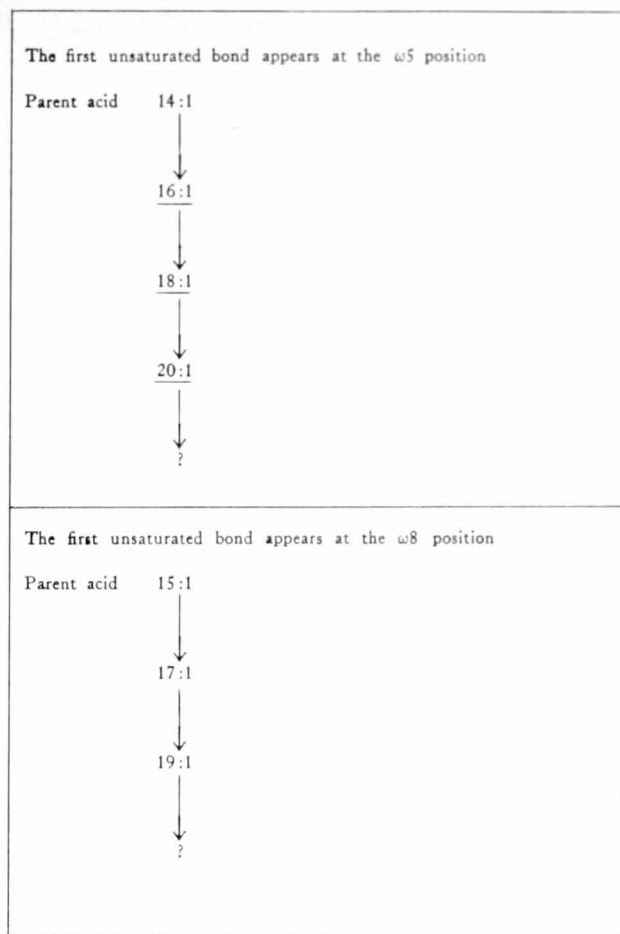
Appendix Figure 3.—Fatty acid families found in lesser quantities (origin unknown).

MS #1885

37

FISHERY INDUSTRIAL RESEARCH PUBLICATIONS

Vol. 1, no. 1 (April 1962):

Economic aspects of the Pacific halibut fishery. By James Crutchfield and Arnold Zellner.

Vol. 2, no. 1 (September 1962):

Quality changes in whiting stored in ice as indicated by organoleptic and objective tests. By Joseph M. Mendelsohn and John A. Peters.

Effect of cooking methods on the sodium content of halibut, haddock, and flounder. By Bernard I. Sohn and Maynard A. Steinberg.

Proximate composition changes in sockeye salmon (Oncorhynchus nerka) during spawning migration. By Claude E. Thurston and H. William Newman.

Mechanically deicing and weighing groundfish at the dock in New England. By John A. Peters, Joseph W. Slavin, and Arvey H. Linda.

Methods of separation of fatty acids from fish oils with emphasis on industrial applications. By Edward H. Gruger, Jr.

Storage of fish in refrigerated sea water. 1—Quality changes in ocean perch as determined by organoleptic and chemical analyses. By Edward H. Cohen and John A. Peters.

New-type multiple debreader. By Melvin E. Waters and D. J. Bond.

Vol. 2, no. 2 (December 1963):

Economic factors related to lake trout quotas on Lake Superior. By Keith D. Brouillard.

Effect of storage in refrigerated sea water on amino acids and other components of whiting (Merluccius bilinearis). By Edward H. Cohen and John A. Peters.

Drip formation in fish. 1.—A review of factors affecting drip. By David T. Miyauchi.

Storage of fish in refrigerated sea water. 2.—Quality changes in whiting as determined by organoleptic and chemical analyses. By Edward H. Cohen and John A. Peters.

Technological investigations of pond-reared fish. By Leo J. Sullivan and Harry L. Seagran.

Bibliography of publications—Division of Industrial Research, by branch, year, and author, 1955-1961 inclusive. By Virginia Whorley.

Vol. 2, no. 3 (November 1964):

Free liquid content of Gulf oysters and suggested change in standards. By Arthur F. Novak, Ernest A. Fieger, and Joseph A. Liuzzo.

Comparison of chemical and sensory tests for assessing storage life of iced calico scallops (Pecten gibbus). By Melvin E. Waters.

Cholesterol content of various species of shellfish. 1.—Method of analysis and preliminary survey of variables. By Mary H. Thompson.

Evaluation of the micro-diffusion method for the determination of tertiary volatile base in marine products. By John Spinelli.

Preparation of chilled meat from Atlantic blue crab. By David H. B. Ulmer, Jr.

Observations of the "blueing" of king crab, Paralithodes camtschatica. By Herman S. Groninger and John A. Dassow.

Comparison of the picric acid turbidity and Nessler tests with subjective evaluations of quality of shrimp. By Mary E. Ambrose, Charles F. Lee, and Frank T. Piskur.

Economic study of sea scallop production in the United States and Canada. By Richard M. Doherty, G. Paul Draheim, Donald J. White, and Charles L. Vaughn.

Vol. 2, no. 4 (May 1965):

Technological investigations of pond-reared fish. 2.—Extension of the shelf life of buffalofish products through use of antioxidents. By R. A. Greig.

Economic aspects of the U. S. albacore fishing industry. By E. A. Hale and D. B. Ferrel.

Frozen king crab (Paralithodes camtschatica) meat: Effect of processing conditions on fluids freed upon thawing. By Jeff Collins and Russel L. Brown.

Thiaminase activity in fish: An improved assay method. By R. H. Gnaedinger.

Drip formation in fish. 3.—Composition of drip from defrosted Pacific cod fillets. By David Miyauchi, John Spinelli, and John A. Dassow.

Vol. 3, no. 1 (December 1965):

Occurrence of pomfret (Brama japonica) in the Northeastern Pacific Ocean. By Charles R. Hitz and Robert R. French.

Author index of publications and addresses - 1964, Bureau of Commercial Fisheries, Branches of Economics and Technology and the Branch of Reports, Seattle. By Helen E. Plastino and Mary S. Fukuyama.

Influence of temperature on the fatty acid pattern of muscle and organ lipids of the rainbow trout (Salmo gairdneri). By Werner G. Knipprath and James F. Mead.

Costs and earnings of tropical tuna vessels based in California. By Roger E. Green and Gordon C. Broadhead.

Amino acid composition of the alewife (Alosa pseudoharengus). By Mary H. Thompson and Robert N. Farragut.

Vol. 3, no. 2 (July 1966):

Proximate composition of Lake Michigan alewife (Alosa pseudoharengus). By Donald R. Travis.

Accumulation of organic acids during cold storage of shucked soft clams, Mya arenaria (L.), in relation to quality. By Baruch Rosen.

Value of menhaden fish meal as a protein supplement to cottonseed meal-corn diets fed to rats. By Robert R. Kifer, Edgar P. Young, and Kam C. Leong.

Value of menhaden fish meal as a protein supplement to meat-and-bone meal-corn diets fed to rats. By Robert R. Kifer, Edgar P. Young, and Kam C. Leong.

Value of menhaden fish meal as a protein supplement in soybean meal-corn diets fed to rats. By Robert R. Kifer, Edgar P. Young, and Kam C. Leong.

Proximate composition of Gulf of Mexico industrial fish. By Mary H. Thompson.

Vol. 3, no. 3 (December 1966):

Proximate composition of the Pacific Coast Dungeness crab (Cancer magister). By Robert N. Farragut and Mary H. Thompson.

Microbial analyses of frozen raw breaded shrimp. By Bobby J. Carroll, Travis D. Love, Benjamin Q. Ward, and Melvin E. Waters.

Recent technological studies of Dungeness crab processing. Part 4 — Preliminary report on salt uptake and heat penetration in whole-cooked crab. By Harold Barnett and Richard W. Nelson.

Commercial fisheries of the Columbia River and adjacent ocean waters. By A. T. Pruter.

Vol. 3, no. 4 (May 1967):

Extending the shelf life of frozen cisco (Coregonus artedii) products through the use of water-soluble antioxidants. By R. A. Greig, J. A. Emerson, and G. W. Fliehman.

Technological studies of Dungeness crab processing. Part 3—Laboratory experiments in the control of drain time. By Harold J. Barnett, Richard W. Nelson, and John A. Dassow.

Temperature, weight, and drip changes during precooking of tuna. By Alexander M. Dollar, Andrew Goldner, and Harold S. Olcott.

Efficacy of fish oils in healing wounds and burns. By Maurice E. Stansby, Paul E. Zollman, and R. K. Winkelmann.

Chemical composition of commercially important fish of the United States. By Maurice E. Stansby and Alice S. Hall.

Author index of publications and addresses - 1965, Bureau of Commercial Fisheries, Division of Economics and Branches of Reports (Seattle) and Technology. By Helen E. Plastino and Mary S. Fukuyama.

MANUSCRIPTS APPROVED

FOR PUBLICATION IN *FISHERY INDUSTRIAL RESEARCH*

DIVISION OF FOOD SCIENCE

Title and author(s)	Laboratory[1]
Proximate composition of commercial fishes from the Mediterranean Sea and the Red Sea. By A. Herzberg and Rachel Pasteur.	Washington, D.C.
Green algae, *Chlorella*, as a contributor to the food supply of man. By Norman W. Durrant and Carol Jolly.	Washington, D.C.
Evaluation of muscle hypoxanthine and total volatile bases as potential quality indices for industrial bottomfishes from the Gulf of Mexico. By Enrique J. Guardia and Gerhard J. Haas.	Washington, D.C.

[1] For further information about these papers, please contact the Division Chief at the appropriate address:

Chief, Division of Food Science
Bureau of Commercial Fisheries
U.S. Fish and Wildlife Service
Washington, D. C. 20240

DIVISION OF EXPLORATORY FISHING

Title and author	Base[1]
Explorations for calico scallop, *Pecten gibbus*, in the area off Cape Kennedy, Florida, 1960-66. By Shelby B. Drummond.	Pascagoula

[1] For further information about this paper, please contact the Base Director at the following address:

Base Director
Bureau of Commercial Fisheries
Exploratory Fishing and Gear Research Base
P.O. Drawer 1207
Pascagoula, Mississippi 39567

CONTENTS

Return this sheet to above address, if you do NOT wish to receive this material ☐, or if change of address is needed ☐ (indicate change and give ZIP Code).