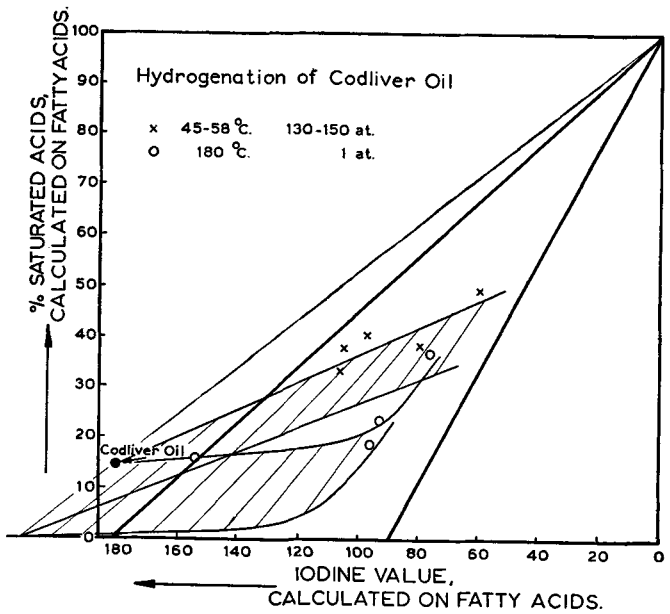

FIG. 8

known fact (5) that this catalyst shows a high selectivity in low temperature fatty oil hydrogenations (50°–60°C.).

From our considerations it can be concluded, in accordance with the opinion of Van Vlodrop (3), that, as a consequence of the extremely effective contact between the oil and this colloidal catalyst, no difficulties arise as to the transport of the reactants to and from the catalyst, notwithstanding a low reaction temperature.

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Constituent Fatty Acids of Salmon Egg Fat

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THE FAT from salmon eggs has the very high iodine value of 220 and remains fluid at temperatures as low as —35°C. (3). These properties, and the fact that large quantities of salmon eggs are discarded each year in Alaska during the salmon canning season, have stimulated an interest in research on the constituent fatty acids of salmon egg fat.

The traditional method for determining the constituent fatty acids in a natural fat is that outlined by Hilditch (7). Hilditch and others have determined the constituent fatty acids of a great number of fats by this method (1, 2, 7). Many of the constituent fatty acids in fat reported in current literature are still determined in this manner (6, 12, 13, 14). The heating of the esters of highly unsaturated fatty acids, as is required in the vacuum distillation step of the Hilditch method however alters the heat-labile unsaturated compounds. The probable limitations of the Hilditch method, when analyzing unsaturated fats such as fish liver fats, were early recognized by Farmer and Vandenheuvel (5).

Hammond and Lundberg (8) recently published a correlation of refractive index, carbon chain length, and unsaturation for methyl esters of fatty acids. The purpose of the present paper is to report the constituent fatty acids in salmon egg fat determined by the Hilditch method and to indicate, from the correlation of Hammond and Lundberg, the extent of the alteration of the fatty acids that occurred in the course of analysis.

Experimental

Sample Collection. Thirty pounds of salmon eggs were collected during the normal commercial canning of pink salmon *(Oncorhynchus gorbuscha)* at the New England Fish Company at Ketchikan, Alaska. The eggs were taken at random during the butchering of one day's catch of fish. Portions of skeins of eggs from an estimated 250 fish were sampled. The eggs were well developed but still in a tight skein and had an average maturity of 2.5 on the scale of Davidson and Shostrom (4). The eggs were frozen at —29°C. in sealed cans and stored at —18°C. until used.

Fat Preparation. Fat was separated from the eggs by the dilute brine extraction method of Sinnhuber (16). The eggs were thawed and passed through a grist mill, which broke the egg membrane. The ground egg mass was then diluted with twice its weight of 4% salt (NaCl) solution warmed to 50°C. The mixture was gently stirred, then allowed to stand for 4 hrs. with the temperature maintained at 50°C. The fat phase was siphoned off and clarified by passing through a Sharples super-centrifuge.

This gentle method of separating the fat from the egg resulted in a fat the reactive unsaturated constituents of which were unaltered as indicated by a determined extinction coefficient of essentially zero at 233 mμ. This fat however represented only ⅓ of the total lipid in the egg and consisted essentially of fatty acid triglycerides. In a preliminary report (9) a comparison was given of the constituent fatty acids in the brine-extracted oil with those in the total lipid obtained by exhaustive solvent extraction.

Analytical Procedures. The Hilditch method consists essentially of a) a preliminary separation of the fatty acids into saturate and unsaturate fractions, b) vacuum distillation of the methyl esters of these fractions into subfractions of simple composition, c) determination of iodine value and saponification equivalent on each of the subfractions from the vacuum distillation, and d) calculation of the constituent fatty acids from these data.

[1] The Fishery Products Laboratory is operated jointly by the U. S. Fish and Wildlife Service and the Alaska Fisheries Experimental Commission.

TABLE I

Analysis of Cuts from the Vacuum Distillation of Pink Salmon Egg Fatty Acid Methyl Esters

Cut number	Weight	Iodine value (Wijs)	Saponification equivalent	Refractive index (20°C.)	Calculated refractive index
	Saturate fraction				
	Grams				
S-1	9.3	1.2	247.6	1.4398	1.4374
S-2	9.14	6.7	266.0	1.4409	1.4410
S-3	8.90	8.9	268.9	1.4415	1.4413
S-4	9.14	11.3	271.5	1.4419	1.4418
S-5	8.18	11.5	273.9	1.4420	1.4421
S-6	8.82	12.7	273.7	1.4425	1.4423
S-7	9.18	17.6	274.0	1.4435	1.4428
S-8	8.93	29.4	282.3	1.4452	1.4449
S-9	9.27	51.1	287.9	1.4482	1.4477
S-10	19.14	210.4	324.0	1.4708	1.4685
	Intermediate fraction				
I-1	3.94	15.5	243.7	1.4409	1.4385
I-2	7.83	50.8	274.6	1.4466	1.4461
I-3	7.80	75.9	287.5	1.4507	1.4501
I-4	7.98	79.0	290.5	1.4515	1.4506
I-5	7.53	82.4	293.7	1.4519	1.4514
I-6	6.83	83.5	294.5	1.4519	1.4520
I-7	7.88	85.9	298.8	1.4525	1.4523
I-8	7.26	87.3	297.4	1.4524	1.4521
I-9	7.75	89.5	296.8	1.4529	1.4525
I-10	7.79	91.3	296.4	1.4531	1.4526
I-11	7.18	94.9	296.7	1.4536	1.4530
I-12	6.83	104.8	297.9	1.4551	1.4545
I-13	13.39	163.1	324.3	1.4662	1.4633
	Unsaturate fraction				
U-1	1.60	104.6	272.1	1.4531	1.4516
U-2	2.68	137.3	280.6	1.4570	1.4569
U-3	3.09	165.8	287.0	1.4609	1.4599
U-4	3.46	175.4	291.3	1.4628	1.4614
U-5	2.72	182.4	291.5	1.4639	1.4622
U-6	3.50	192.4	293.1	1.4649	1.4635
U-7	3.33	197.5	297.1	1.4659	1.4645
U-8	3.62	204.0	299.6	1.4669	1.4655
U-9	3.08	220.6	300.8	1.4681	1.4674
U-10	3.53	219.3	303.8	1.4712	1.4675
U-11	2.23	235.6	302.0	1.4722	1.4692
U-12	3.37	259.9	305.4	1.4750	1.4723
U-13	3.28	263.1	309.4	1.4765	1.4731
U-14	3.24	289.9	311.0	1.4775	1.4762
U-15	3.49	298.0	312.5	1.4786	1.4773
U-16	3.38	306.8	315.0	1.4797	1.4786
U-17	3.32	307.1	315.3	1.4814	1.4786
U-18	3.35	310.5	315.8	1.4818	1.4790
U-19	3.41	317.6	315.2	1.4828	1.4798
U-20	3.22	328.9	327.8	1.4850	1.4822
U-21	4.23	341.9	327.3	1.4867	1.4849
U-22	3.79	358.1	328.0	1.4880	1.4857
U-23	4.48	358.0	327.0	1.4894	1.4856
U-24	3.01	364.8	336.9	1.4910	1.4873
U-25	3.04	374.9	339.0	1.4917	1.4886
U-26	3.33	379.7	339.0	1.4928	1.4892
U-27	3.44	384.6	338.5	1.4939	1.4897
U-28	4.07	384.8	345.0	1.4953	1.4903
U-29	7.62	247.5	388.8	1.5131	1.4773

To minimize oxidation and isomerization of the highly unsaturated fatty acids, the salmon egg fat was converted to the fatty acid methyl esters by direct methanolysis, using the method of Winter and Nunn (17). The methyl esters were separated into saturate, intermediate, and unsaturate fractions by low temperature crystallization from acetone. The saturate fraction was crystallized from the acetone solution (100 g. of mixed esters/liter of acetone) at −50°C.; the intermediate fraction, at −67°C. The unsaturate fraction remained in solution at −67°C. Yields of 16.2%, 14.3%, and 69.5% were obtained for saturate, intermediate, and unsaturate fractions, respectively.

These fractions were then separated into a number of subfractions of increasing molecular weight by vacuum distillation. The distillation column used was similar to that described by Longenecker (10). It was 90 cm. long with an inside diameter of 17 mm. Single-turn glass helices were used to pack the column for a distance of 60 cm. The column was heat-compensated by nichrome wire heating elements, and the distillation flask was heated by an electric heating mantle. Stillhead pressures of 0.1 to 0.2 mm. were used in all distillations. The distillations required from 3 to 5 hrs. Flask temperatures varied from 165°C. at the start of the distillation to about 250°C. at the end of the distillation.

The saponification equivalent, iodine value (Wijs 1 hr.), and refractive index were determined on each of the subfractions from the vacuum distillation (Table I). From these data the constituent fatty acids in the fat were calculated by using the computation forms of Rapson *et al.* (15) (Table II).

Alteration of the Fatty Acids

Hammond and Lundberg give the following equations for the relation of refractive index of fatty acid methyl esters to carbon atom chain length and number of double bonds

$$n_D^{20} = \sqrt{\frac{25.82C + 64.48}{11.90C + 39.74}} \quad \text{for saturated esters}$$

$$n_D^{20} = \sqrt{\frac{25.82C - 7.25D + 64.48}{11.90C - 6.35D + 39.74}} \quad \text{for monoenes}$$

$$n_D^{20} = \sqrt{\frac{25.82C - 7.47D + 64.48}{11.90C - 6.57D + 39.74}} \quad \text{for polyenes}$$

where C is the number of carbon atoms in the fatty acid chain and D is the number of double bonds.

A refractive index was calculated for each of the subfractions from the vacuum distillation, using the equation of Hammond and Lundberg and the determined iodine value and saponification equivalent. The calculated refractive indexes are shown in Table I. The difference between the observed and calculated refractive index for each of the subfractions is shown in Figures 1 and 2.

Since the amount of monoenes and polyenes in the subfractions was not known, an arbitrary assumption was made that the unsaturates in all subfractions with iodine values of 100 or below were monoenes and the unsaturates in those subfractions with iodine values over 100 were polyenes. The accuracy of the assumption of the monoene and polyene content of the subfraction could not be checked. However the difference was determined between the refractive index calculated by this assumption and that calculated by the opposite and unlikely assumption that subfractions with iodine values of 100 or below were polyenes and

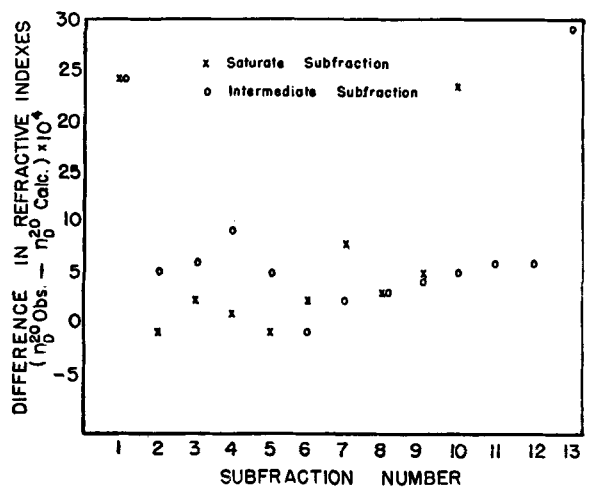

FIG. 1. Variation of the differences between calculated and observed refractive index of subfractions from the vacuum distillation of saturate and intermediate fractions.

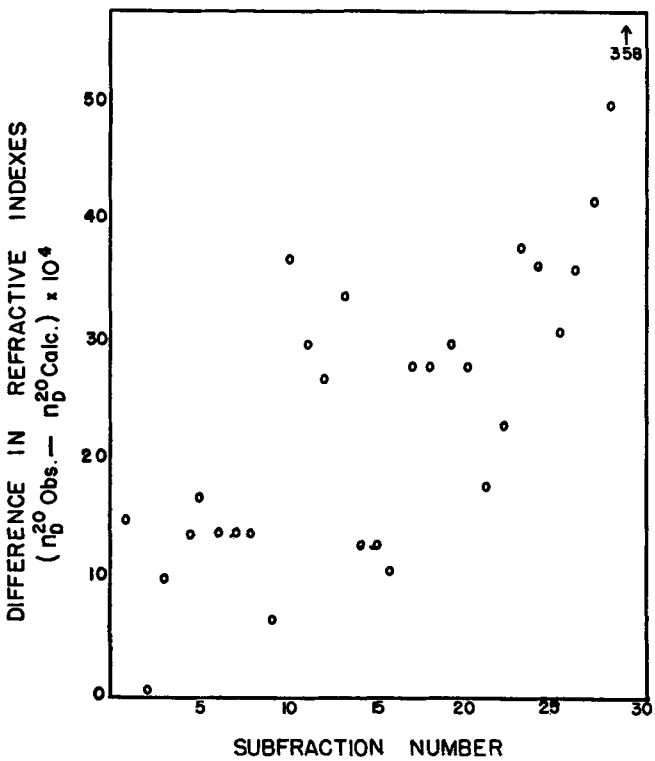

FIG. 2. Variation of the difference between calculated and observed refractive index of subfractions from the vacuum distillation of unsaturate fraction.

those over 100 were monoenes. For the saturate subfractions S-1 through S-9, the difference between the refractive index calculated on the likely assumption and that calculated on the unlikely assumption increases from 0.0000 for S-1 to 0.0002 for S-9. For the intermediate subfractions I-1 through I-11, the difference between the refractive index calculated on two assumptions increases from 0.0001 for I-1 to 0.0004 for I-11. Therefore, even if a large percentage of the unsaturates had been polyenes in those subfractions with iodine values of less than 100, only small differences in calculated refractive index would have resulted in subfractions S-1 through S-9 and I-1 through I-11. The inclusion of significant quantities of monoenes in the subfractions whose unsaturates were assumed to be polyenes would have given even lower values for the calculated refractive index indicating greater alteration.

The difference between the observed and calculated refractive index of the first subfraction from the distillation of both the saturate and intermediate fractions was caused by contamination of these subfractions by impurities held up by the column packing. The average difference between the observed and calculated refractive index for subfractions S-2 through S-9 is +0.00023 and for subfractions I-2 through I-11 is +0.00044. This small average difference may indicate a slight disagreement with the correlation of Hammond and Lundberg, but it is not greatly outside of the limits of the precision of the determinations. The effect of isomerization of the double bond caused by prolonged heating is both to increase the refractive index and to make the determined iodine value lower than theoretical. The magnitude of the difference between calculated and observed refractive index of the last subfractions of both saturate and intermediate fractions is so great that composition of these subfractions based on determined iodine value would be in error. The calculated composition would indicate more saturated compounds and compounds of lower molecular weight than actually present in the original fat.

The difference in the observed and calculated refractive index of all subfractions of the unsaturate fraction is positive. The magnitude of the difference for subfractions 1 through 9 is approximately constant and indicates significant isomerization. The large deviation of subfractions 10, 11, 12, and 13 and subfractions 17, 18, 19, and 20 perhaps indicates particular esters which are more susceptible to isomerization than those distilling in subfraction 14 through 16 or 1 through 9. Those subfractions distilling last show increasing alteration, due probably both to prolonged heating and to increasing unsaturation in the compounds distilled.

TABLE II

Distribution of Constituent Fatty Acids in Certain Salmon Egg Fats

Fatty acids	Distribution		
	O. gorbuscha	*S. salar*[a]	
	Maturity 2.5[b]	Maturity 2[c]	Maturity 4[c]
	%	%	%
C_{14} saturate	1.8	2.9	1.8
C_{14} monolefin	0.1	0.4	—
C_{16} saturate	8.3	13.4	13.0
C_{16} monolefin	5.1	10.4	9.9
C_{18} saturate	1.9	0.7	2.0
C_{18} unsaturate	31.3(3.6)[d]	25.3(3.2)[d]	36.7(2.9)[d]
C_{20} unsaturate	31.1(7.3)[d]	28.7(7.2)[d]	21.3(7.6)[d]
C_{22} unsaturate	14.7(11.8)[d]	18.1(11.4)[d]	15.3(11.1)[d]
C_{24} unsaturate	0.2	—	—
Unresolved	5.5	0.1	—

[a] Data by Lovern (11).
[b] Maturity on a scale of increasing development from 1 to 4 used by Davidson and Shostrom.
[c] Maturity on a scale of increasing development from 1 to 5 used by Lovern.
[d] The numbers in parentheses are a measure of the unsaturation and indicate the number of atoms of hydrogen required to saturate the fatty acid.

Included in Table II, for comparison, are the constituent fatty acids reported by Lovern (11) in the fat from the eggs of Atlantic salmon *(Salmo salar)*. The *O. gorbuscha* egg fat has significantly less C_{16} and more C_{20} fatty acids than the *S. salar*. The degree of unsaturation and other constituent acids are similar.

Summary and Conclusions

1. The constituent fatty acids determined by the Hilditch method in the fat from the sample of pink salmon eggs are reported. Forty-five per cent of the fatty acids were 20 and 22 carbon atoms long, with an average unsaturation of 7.3 hydrogen atoms for the C_{20} acids and 11.8 hydrogen atoms for the C_{22} acids.

2. The Hammond and Lundberg correlation of refractive index, carbon chain length, and unsaturation was used to measure the alteration that occurred in the fatty acid esters of salmon egg fat during the vacuum distillation step of the Hilditch method of analysis. The alteration by heat of the unsaturate fraction in the vacuum distillation was significant even in the first overhead subfractions. Analysis by the Hilditch method resulted in low values for unsaturation and chain length of the constituent fatty acids in the unsaturated fat.

Acknowledgment

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A Rapid Spectrophotometric Method for Determining the Linoleic and Linolenic Acid Components of Soybean Oil[1]

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THE A.O.C.S. tentative method CD-7-48 (1), when used for analyzing soybean oil, can be simplified in the type of glassware used, in the procedure of isomerization, and in the method of obtaining the specific absorption of ultraviolet light. The Spectroscopy Committee (2) recommended simplification of the analysis of soybean oil by measuring light absorption at 233 mμ and 268 mμ only. The work of Brice *et al.* (4) and of Herb and Riemenschneider (5) showed that an isomerization period of 45 min. in air with 11% KOH-glycerol reagent resulted in very satisfactory analysis of several vegetable oils. Dutton, Northern Utilization Research Branch, Peoria, Illinois, suggested that light absorption at 268 mμ and 233 mμ might be measured on the original 100-ml. solutions of isomerized material in two special test cells with quartz windows spaced to provide solution depths of about 1 mm. and 0.1 mm. to 0.15 mm., respectively. For the purpose of perfecting the method of analysis as presented here, a sample of soybean oil analyzed in 1948 by eight different laboratories to test A.O.C.S. method CD-7-48 (1, 2) was used. Our analyses of this soybean oil, which was in storage at zero degrees F. continuously since 1948, indicate no change in fatty acid composition of the oil during this relatively long period of storage.

Three different simplified spectrophotometric methods of analysis of soybean oil were tested. The simplest of these methods and the most reliable as indicated by reproducibility of analyses requires the following described equipment, reagents, and procedure:

Apparatus

1. Beckman DU Spectrophotometer with a holder for four 1-cm. square quartz cells.
2. Two special cells with quartz windows spaced 1 mm. and 0.1 to 0.15 mm.
3. 100 or more lime or Pyrex glass bottles, 125 ml., wide mouth, fitted with screw caps lined with Teflon[3] gaskets. (We use clear lime glass bottles 1¾ in. square and 4¼ in. in height.) The caps must close the bottles with a vapor-tight seal. Teflon gaskets withstand the solvent and chemical action of hot Methanol-KOH-glycerol solutions.
4. Several metal trays to hold 12 or more 125-ml. bottles tightly enough to permit vigorous shaking without dislodging the bottles. Our bottle trays were made of a long loaf bread pan and pieces of 2 in. x 4 in.-mesh welded wire fencing.
5. A 100-ml. burette, graduated to 1 ml., rapid refill and automatic zero type for dispensing a measured volume of methanol.
6. A 10-ml. and a 5-ml. hypodermic syringe.
7. Electric heating mantle and flask to heat 3 to 5 liters of KOH-glycerol reagent to 200°C.
8. A forced draft air oven thermostatically controlled to maintain 180°C. ± 3 degrees. (We use Precision Scientific Company Type A oven on full heat, 3900 watts.)
9. Three C. thermometers, 0 to 100, 0 to 200, 0 to 220.
10. Shallow, glass 1-ml. cups of large diameter to hold samples.

Reagents

1. Methanol, synthetic absolute, to pass the test of optical density in A.O.C.S. method CD-7-48 (1).
2. 11% KOH-glycerol reagent containing 17.5 g. 85% KOH (ACS reagent), per 100 ml. of glycerol prepared as described by Brice (3).
3. Several hundred grams of soybean oil of known fatty acid composition to be analyzed daily as a check sample and to provide a means of calculating the length of light paths in the special cells.

Procedure

1. Weigh .1000 to .1250 g. ± 0.2 mg. of a well mixed sample of soybean oil into a 1-ml. cup.
2. Heat a portion of the KOH-glycerol reagent to 100°C. in a large beaker to lower the viscosity of this reagent. Use a 10-ml. hypodermic syringe to transfer 8.6 ml. (11 g.) of reagent to each 125-ml. bottle. A large number of bottles may be prepared at one time since capped bottles of reagent can be stored for several weeks without change.
3. A cool or preheated air oven may be used. Place several trays of uncapped bottles containing reagent in the oven. Set the heat control for 180°C. and continue the heating until a thermometer in one bottle with the bulb immersed in the reagent reads 180°C.
4. Wear asbestos gloves to remove these racks of bottles at 180°C. from the oven. Quickly add one small cup of oil to each bottle of hot reagent in rack except the two reagent blanks. Immediately shake the bottles in this rack vigorously for three to five seconds. Proceed, likewise, to add cups of oil to each rack of bottles followed by three to five seconds of vigorous shaking. Finally shake all of the bottles in the racks for two minutes. By placing the bottle racks on the slippery surface of a laboratory bench, one technician can do a thorough job of shaking 48 samples (four trays) at one time. Inade-

[1] Publication No. 274 of the U. S. Regional Soybean Laboratory.
[2] Chemist and Agent (Physical Science Aid), respectively, Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture.
[3] E. I. du Pont de Nemours and Company, Chicago, Ill., has a listing of concerns supplying Teflon in sheet form.