

STUDIES OF THE VITAMIN POTENCY OF COD LIVER OILS.
XII—THE COLOR REACTIONS OF COD LIVER OIL.

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In view of the fact that the U. S. Pharmacopœia has so-called color tests for cod liver oils, it has seemed of interest to check up these tests on crude fish liver oils of known origin. The results which have been obtained with a number of oils are herein reported.

Referring to Group A, it will be noted that cod liver oils Nos. 4, 5, 16, 19, 78, tested with sulphuric acid, did not give uniform color reaction. Also the results obtained with dog fish, hake, pollock and halibut were so much like some of the results obtained with cod liver oil that without a check test, it would be difficult to say definitely whether these liver oils came from cod fish or not. The situation as regards the nitric acid tests, Group B, is quite similar to the results with the sulphuric test.

In order to obtain information concerning the color reaction of the average market, medicinal oil, twelve samples of Norwegian oil were subjected to the sulphuric and nitric tests. The results obtained are reported under Groups C and D, respectively. As will be noted from these results, the nitric acid test was not exactly uniform for all the Norwegian oils. The lack of uniformity is even more pronounced in the case of the sulphuric acid test.

Inasmuch as the results with the Norwegian oils appeared to be more uniform than with the crude oils produced in our laboratory, it seemed of interest to obtain data as to the color reaction of our own medicinal (cold pressed) oils. The results are reported under Groups E and F of these tests. As will be noted, there is more uniformity in these results than in those of the Norwegian oils.

It is interesting in this connection to note that Fuller has reported,¹ "The figures and the color tests together are not limited to cod liver oil, but will apply equally well to carefully refined oils from other fish livers." Lewkowitsch² states, "The characteristic violet color reaction of liver oil is decreased considerably with the improvement in the production of the oil." This would naturally be expected since the color reaction is dependent upon some of the products of decomposition of liver tissues.

For the purpose of obtaining information concerning the effect of light, air and moisture on the color reaction of cod liver oil, sixteen samples of cod liver oil of identical source were stored for six months under the conditions noted for Groups G and H. The sulphuric and nitric color tests show that light, air and moisture cause some change in cod liver oils during a period of six months.

The general conclusion to be drawn from these results seem to be that the color tests are not sufficiently reliable to serve as a means of identifying cod liver oil, but vary according to the conditions under which the liver oils have been manufactured and stored. Even although the data are inadequate, it appears that liver oils carefully prepared from fresh livers from fish other than cod will give tests identical with those of cod liver oil prepared under comparable conditions.

¹ Henry C. Fuller, "Chemistry and Analysis of Drugs and Medicines," John Wiley & Sons, 1920, p. 921.

² Lewkowitsch and Warburton, "Chemical Technology and Analysis of Oils, Fats and Waxes," Macmillan & Co., 1922, p. 448.

CRUDE FISH LIVER OILS PREPARED UNDER LABORATORY CONDITIONS.

COD LIVER OIL COLOR TEST.

Sulphuric Acid Test.—A solution of 1 drop of the oil in 1 mil of chloroform when shaken with 1 drop of sulphuric acid acquires a violet-red tint, gradually changing to reddish brown.

Group A— Laboratory Sample No.		Results.
Codfish	4	Violet-blue, gradually changing to reddish brown
"	5	Light green-blue, gradually changing to violet-blue, and then to reddish brown
"	16	Light blue, gradually changing to violet-blue, to reddish brown, and then to greenish brown
"	19	Dirty brown at once
"	78	Light brown, gradually changing to violet-blue, to reddish brown, and then to greenish brown
Dog Fish	77	Light blue, gradually changing to red-violet, and then to reddish brown
Hake	14	Light blue, changing to violet-blue, to reddish brown
Pollock	15	Light green, changing to reddish brown
Halibut	18	Light blue to dark blue to purple, then to dark, dirty green

*Nitric Acid Test.*¹—Allow 2 or 3 drops of fuming nitric acid (specific gravity about 1.44) to flow alongside of 10 or 15 drops of the oil contained in a watch glass; a reddish or purplish color is produced at the zone of contact. On stirring the mixture with a glass rod, this color becomes bright rose-red (distinction from seal oil, which shows no change in color, and from other fish oils, which become blue).

Group B— Laboratory Sample No.		At zone of contact.	Results. After stirring.
Codfish	4	Purple	Reddish brown
"	5	Dark purple	Brownish green
"	16	Purple	to Rose-red to light reddish brown
"	19	Dark purple	to Reddish brown
"	78	Light blue	to Light purple to reddish brown
Dog Fish	77	Purple-blue	to Blue to brown to reddish brown
Hake	14	Light purple-blue to	Dark purple-blue to brown
Pollock	15	Light purple-blue to	Dark purple-blue to brown
Halibut	18	Reddish violet to	Dark bright blue to dark green

NORWEGIAN COD LIVER OILS.

Sulphuric Acid Test.

Group C— Laboratory Sample No.	Results.
8	Light violet to red-violet to reddish brown
9	Light blue to red-violet to light green-brown
10	Light blue to blue-violet to red-violet to reddish brown
11	Light brown quickly to reddish brown
12	Light brown quickly to reddish brown
26	Light blue to blue-violet to red-violet to reddish brown
37	Light blue to blue-violet to red-violet to reddish brown
43	Light blue to blue-violet to red-violet to reddish brown
44	Light blue to blue-violet to red-violet to light reddish brown
80	Light blue to blue-violet to red-violet to reddish brown
90	Light blue to blue-violet to red-violet to reddish brown
Vial	Light blue to blue-violet to red-violet to reddish brown

¹ Pharmacopœia of the United States. IX. (Revision.) P. Blakiston's Son & Co. P. 297.

Nitric Acid Test.

Group D— Laboratory Sample No.	At zone.	Results.	After stirring.
8	Light pink		Rose color to reddish brown
9	Light pink		Rose color to reddish brown
10	Light pink		Rose color to reddish brown
11	Light brown		Reddish brown
12	Light brown		Reddish brown
26	Light pink		Rose color to reddish brown
37	Light pink		Rose color to reddish brown
43	Light pink		Rose color to reddish brown
44	Light pink		Rose color to reddish brown
80	Light pink		Rose color to reddish brown
90	Light pink		Rose color to reddish brown
Vial	Light pink		Rose color to reddish brown

COLD PRESSED COD LIVER OIL—DOMESTIC.
(Prepared under plant conditions.)

Sulphuric Acid Test.

Group E— Laboratory Sample No.	Results.
17	Blue to dark blue to purple to red-violet to reddish brown
22	Blue to dark blue to purple to red-violet to reddish brown
32	Blue to dark blue to purple to green-brown
34	Blue to dark blue to purple to green-brown
73	Blue to dark blue to purple to green-brown
83	Blue to dark blue to purple to green-brown
84	Blue to dark blue to purple to green-brown
100	Blue to dark blue to purple to green-brown

Nitric Acid Test.

Group F— Laboratory Sample No.	At zone of contact.	Results.	After stirring.
17	Purple		Blue-violet to reddish brown
22	Light violet		Red-violet to reddish brown
32	Light violet		Red-violet to reddish brown
34	Light violet		Red-violet to reddish brown
73	Light violet		Red-violet to reddish brown
83	Light violet		Red-violet to reddish brown
84	Light violet		Red-violet to reddish brown
109	Light violet		Red-violet to reddish brown

SAMPLES OF COD LIVER OIL No. 17 SUBJECTED TO VARYING CONDITIONS OF LIGHT, HEAT, MOIS-
TURE AND AIR, BY STORING FOR SIX MONTHS UNDER THE FOLLOWING CONDITIONS.

Laboratory Sample No.	Storage conditions.
47	In clear glass bottle—full (room temperature)
48	In clear glass bottle— $\frac{9}{10}$ oil and $\frac{1}{10}$ water (room temperature)
49	In clear glass bottle— $\frac{1}{4}$ oil and $\frac{3}{4}$ air (room temperature)
50	In clear glass bottle— $\frac{9}{40}$ oil, $\frac{1}{40}$ water and $\frac{3}{4}$ air (room temperature)
51	In amber glass bottle—full (room temperature)
52	In amber glass bottle— $\frac{9}{10}$ oil and $\frac{1}{10}$ water (room temperature)
53	In amber glass bottle— $\frac{9}{40}$ oil, $\frac{1}{40}$ water and $\frac{3}{4}$ air (room temperature)
54	In amber glass bottle— $\frac{1}{4}$ oil and $\frac{3}{4}$ air (room temperature)
55	In clear glass bottle—full (cold cellar)
56	In clear glass bottle— $\frac{9}{10}$ oil and $\frac{1}{10}$ water (cold cellar)
57	In clear glass bottle— $\frac{1}{4}$ oil and $\frac{3}{4}$ air (cold cellar)

- 58 In clear glass bottle— $\frac{9}{40}$ oil, $\frac{1}{40}$ water and $\frac{3}{4}$ air (cold cellar)
 59 In amber glass bottle—full (cold cellar)
 60 In amber glass bottle— $\frac{9}{10}$ oil and $\frac{1}{10}$ water (cold cellar)
 61 In amber glass bottle— $\frac{1}{4}$ oil and $\frac{3}{4}$ air (cold cellar)
 62 In amber glass bottle— $\frac{9}{40}$ oil, $\frac{1}{40}$ water and $\frac{3}{4}$ air (cold cellar)

COD LIVER OIL No. 17 SUBJECTED TO VARYING CONDITIONS OF LIGHT, HEAT, MOISTURE AND AIR.

COLOR TEST.

Sulphuric Acid Test.

Group C— Laboratory Sample No.	Results.
47	Blue—dark blue—purple—red—violet—reddish brown
48	Blue—dark blue—purple—green—brown
49	Green blue—red violet—reddish brown
50	Light brown—dark reddish brown
51	Blue—purple—red violet—reddish brown
52	Blue—purple—red violet—reddish brown
53	Light brown—dark reddish brown
54	Green blue—red violet—reddish brown
55	Blue—purple—red violet—reddish brown
56	Blue—purple—red violet—reddish brown
57	Green blue, red violet—reddish brown
58	Green blue—blue black—reddish brown
59	Blue—purple—green brown
60	Blue—purple—reddish brown
61	Blue—purple—red violet—reddish brown
62	Green blue—blue black—reddish brown

COD LIVER OIL No. 17 SUBJECTED TO VARYING CONDITIONS OF LIGHT, HEAT, MOISTURE AND AIR.

COLOR TEST.

Nitric Acid Test.

Group H— Laboratory Sample No.	Results.	
	At zone of contact.	After stirring.
47	Purple	Red-violet to reddish brown
48	Purple	Red-violet to reddish brown
49	Purple	Red-violet to dark reddish brown
50	Light brown	Dark reddish brown
51	Purple	Red-violet to reddish brown
52	Purple	Red-violet to dark reddish brown
53	Light brown	Red-violet to dark reddish brown
54	Purple	Red-violet to dark reddish brown
55	Violet	Red-violet to reddish brown
56	Purple	Green to brown to dark reddish brown
57	Purple	Red-violet to dark reddish brown
58	Violet	Red-violet to dark reddish brown
59	Light violet	Rose color to reddish brown
60	Light violet	Rose color to reddish brown
61	Light purple	Purple to dark reddish brown
62	Purple	Red-violet to dark reddish brown

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