# The Standardization of Cod Liver Oil

## Further Suggestions on the Use of the Uranium Nitrate Color Test

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IN last September's edition of this journal the writer published an article on the behavior of fish oils with uranium nitrate and pyrogallic acid. At that time a method[1] was proposed to differentiate between cod liver oil (U. S. P.) and contaminated cod liver oil. Since then a slight modification of the test has been suggested by a prominent chemist who found it more convenient to heat the test tube containing the oil and reagent by letting it stand in a 400 cc beaker with about 80 cc of water and heating so that the water just boils; instead of placing the tube in a steam bath as published.

### Modification

Modifying the test so that the following data could be assembled 10 milligrams of uranium nitrate and 3 cc of the oil to be tested were placed in a 6 inch glass tube with an inside diameter of 20 mm. The tube was then placed upright in a 400 cc beaker containing 80 cc of water which was heated, so that it just boiled, keeping this temperature for 20 minutes, after which the oil was allowed to cool and the color measured. Failure to pay attention to these details will without doubt result in condemning good oils. The method, if carefully conducted, I believe will have a value as a rapid sorting test to separate good oils from those contaminated with oils other than those from the livers of fish related to the cod.

### Green Color

Further investigations indicate that certain grades of sardine oil such as Northern California give a deep green color like that of chlorophyll in 2 minutes with the uranium nitrate, at boiling water temperature gradually changing to red on further heating. The characteristics of the California sardine oil are Sp. Gr. @ 15.5° C 0.9319, Iodine Value 186.7, Saponification value 190.2, Unsaponifiable matter 0.54%, Free Fatty Acid 1.50%, Index of refraction @ 40° C 1.4747, Cold Test 36° F.

Hake liver and dogfish liver oils will also give a decided green color which on further heating changes to a dark amber or reddish brown color.

### Color Reading Experiments

Experiments and analysis were conducted to prove that the fish oil actually changed to give a definite color reading with the uranium nitrate. Samples were collected and analyzed according to the United States Pharmacopoeia method. The color of the oil was read, before and after heating with uranium nitrate, in a Wesson tintometer[2] using standard Lovibond

[1] Ten milligrams of uranium nitrate plus 3 cc. of fish oil placed in a test tube and heated in a steam bath for 20 minutes.

[2] As adopted by the American Oil Chemists Society.

glasses. Samples of pure cod liver oil were tested for unsaponifiable matter and the uranium nitrate test with the following results.

| Cod Liver Oil | Before Heating | After Heating | Unsapon. |
|---|---|---|---|
| U. S. P. oil..... | 1 yellow 0.1 red | 35 yellow 3.8 red | 1.20% |
| U. S. P. oil..... | 1 yellow 0.1 red | 35 yellow 3.7 red | 1.19% |
| U. S. P. oil..... | 1 yellow 0.1 red | 35 yellow 3.8 red | 1.20% |
| Poultry grade oil... | 1.5 yellow 0.2 red | 35 yellow 5.3 red | 1.46% |
| A refined[3] oil..... | 5 yellow 0.3 red | 35 yellow 5.0 red | 4.15% |

[3] The oil was too dark to be read, so it was first refined with sodium hydrate.

The above poultry grade of cod liver oil was unsatisfactory for a medicinal oil and gave the following characteristics, Sp. Gr. @ 15.5° C 0.9309, Iodine Value, 161.8, Saponification Value 185.5, Unsaponifiable matter 1.46%, Index of Refraction 1.4726 @ 40° C, Free Fatty Acid 0.30% and Cold Test 16° F.

## Experimental

A cod liver oil (U. S. P.) containing 5% of California sardine oil was analyzed and the analysis compared thus:

| | U. S. P. Cod Liver Oil | 5% Mixture | California Sardine Oil |
|---|---|---|---|
| Specific Gravity 15.5° C. ..... | 0.9235 | 0.9264 | 0.9319 |
| Iodine value ..... | 162.6 | 163.8 | 186.7 |
| Saponifiable value ..... | 180.5 | 188.1 | 190.2 |
| Unsaponifiable ..... | 1.20% | 1.16% | 0.54% |
| Free fatty acid..... | 0.60% | 0.60% | 0.50% |
| Refraction index @ 40 C. .... | 1.4728 | 1.4729 | 1.4747 |
| Cold test liquid..... | 0° F. | 8° F. | 36° F. |
| | Color Reading | | |
| Before heating ..... | 1 Y. 0.1 R. | 2 Y. 0.5 R. | 35 Y. 1.2 R. |
| After heating ..... | 35 Y. 3.8 R. | 35 Y. 4.9 R. | 35 Y. 5.0 R. |

Other mixtures were made using the same cod liver oil with sardine oil having an unsaponifiable matter of 1.08%. The samples were tested for color readings together with Whale, Menhaden, Pilchard, Dogfish, Seal, and Herring oils and the data tabulated.

| | Color Before Heating | Color After Heating |
|---|---|---|
| Cod Liver (U. S. P.) oil..... | 1 yellow 0.1 red | 35 yellow 3.8 red |
| 1% Sardine in cod..... | 1 yellow 0.1 red | 35 yellow 4.0 red |
| 5% Sardine in ocd..... | 1 yellow 0.2 red | 35 yellow 5.2 red |
| 10% Sardine in cod..... | 1.5 yellow 0.3 red | 35 yellow 6.0 red |
| Seal oil (unsaponifiable 0.41%)..... | 1 yellow 0.2 red | 35 yellow 4.0 red |
| Sardine oil (unsaponifiable 1.08%)... | 35 yellow 3.5 red | 35 yellow 6.0 red |
| Whale oil (unsaponifiable 1.50%).... | 35 yellow 3.3 red | 35 yellow 9.7 red |
| Menhaden oil (unsaponifiable 1,23%). | 35 yellow 4.1 red | 35 yellow 12.0 red |
| Pilchard oil (unsaponifiable 0.96%)... | 35 yellow 3.7 red | 35 yellow 10.0 red |
| Herring oil (unsaponifiable 1.00%)... | 35 yellow 4.5 red | 35 yellow 15.0 red |
| Dogfish oil (unsaponifiable 10.18%)... | 35 yellow 4.7 red | 35 yellow 27.6 red |

## COMMERCIAL COD LIVER OIL

Samples of commercial cod liver oil were analyzed and the colors compared in the following manner:

| Sp. Gr. @ 15.5° C. | Iodine Val. | Sapon. Val. | F. F. A. | Un-sapon. | Index Ref. @ 40° C. | Color | |
|---|---|---|---|---|---|---|---|
| | | | | | | Before Heating | After Heating |
| 0.9202 | 134.9 | 177.2 | 3.00% | 5.52% | 1.4695 | 5 y–1.0 r | 35 y–11.6 r |
| 0.9240 | 151.8 | 178.8 | 3.50% | 4.58% | 1.4725 | 5 y–1.3 r | 35 y–11.2 r |
| 0.9250 | 154.7 | 183.7 | 4.00% | 3.15% | 1.4719 | 5 y–0.5 r | 35 y– 7.7 r |
| 0.9225 | 144.8 | 187.3 | 4.10% | 3.12% | 1.4697 | 5 y–2.1 r | 35 y–12.0 r |
| 0.9238 | 152.9 | 181.5 | 4.55% | 2.70% | 1.4610 | 5 y–1.0 r | 35 y– 8.1 r |
| 0.9214 | 145.2 | 183.5 | 3.05% | 4.16% | 1.4700 | 5 y–1.0 r | 35 y– 7.7 r |
| 0.9229 | 152.0 | 188.0 | 4.25% | 2.75% | 1.4690 | 5 y–1.0 r | 35 y– 8.2 r |
| 0.9198 | 144.2 | 178.8 | 4.19% | 8.73% | 1.4695 | 5 y–2.7 r | 35 y–28.5 r |
| 0.9250 | 153.0 | 180.9 | 3.99% | 3.72% | 1.4724 | 5 y–2.1 r | 35 y–14.1 r |
| 0.9210 | 135.6 | 182.0 | 16.40% | 1.47% | 1.4672 | 35 y–1.9 r | 35 y– 9.8 r |

## COLD PRESSED HERRING OIL

Comparing characteristics of the Herring, Sardine, Whale, Dogfish and Cod Liver Oils we have the following:

| | Herring Oil | Sardine Oil | Dogfish Oil | Whale* Oil | Poultry Cod Liver Oil |
|---|---|---|---|---|---|
| Specific gravity @ 15. 5° C..... | 0.9219 | 0.9319 | 0.9175 | 0.9257 | 0 9319 |
| Iodine value ..... | 135.7 | 186.7 | 133.2 | 136.0 | 161.8 |
| Sapon. value ..... | 189.7 | 190.2 | 166.3 | 183.3 | 185.5 |
| Unsaponifiable ..... | 1.63% | 0.54% | 10.18% | ..... | 1.46% |
| Free fatty acids..... | 6.60% | 1.50% | 2.20% | 0.18% | 0.30% |
| Cold test ..... | 26° F. | 36° F. | 14° F. | ..... | 16° F. |
| Color before heating..... | 35 y 5.3 r | 35 y 1.2 r | 35 y 4.7 r | ..... | 1.5 y 0.2 r |
| Color after heating..... | 35 y 13.0 r | 35 y 5.0 r | 35 y 27.6 r | ..... | 35 y 5.3 r |
| Index refraction @ 40° C..... | 1.4689 | 1.4747 | 1.4698 | ..... | 1.4726 |

* Antarctic Right Whale (America) Lewkowitsch, sixth edition (vol. 11), page 470.

Comparing the values of the commercial cod liver oils we find but one that passes the U. S. Pharmacopoeia for unsaponifiable matter, and that gives characteristics similar to that of Whale Oil, and yet these are all Cod Liver Oils. Therefore, I would suggest, from the experiments that have been made above: ("A") that for medicinal cod liver oil a maximum of 1 yellow 0.1 red before heating with uranium nitrate and 35 yellow 3.8 red after heating be allowed on a basis of approximately 10 mm x 20 mm column of oil, (which equals 3 cc of oil); ("B") that for poultry oil 1.5 yellow 0.2 red in the cold and 35 yellow 5.0 red after heating be the established standard in the uranium nitrate test conducted as described above.