Comparison of Several Methods for Recording Color of Fish Liver Oils

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SOME definite, reproducible method should be available for recording color of fish liver oils since color is often an important characteristic in determining quality. Although visual comparison methods are usually relied upon, a more reproducible, objective method such as spectrophotometric measurements would be desirable.

The following experiments which compared visual color description, Lovibond tintometer comparison, and spectrophotometric readings using one series of grayfish liver oils (*Squalus suckleyi*) are not extensive enough to permit recommending a procedure, but the results should indicate the reproducibility of methods. The livers used for subsequent oil preparation were ground, thoroughly mixed, and 100-gram portions weighed into each of a series of pint fruit jars. The samples were then placed in an incubator at 90° F., and every few days thereafter a jar was removed and the contents transferred to a 400-ml. beaker containing 100 ml. of water. This mixture was boiled for 20 minutes, placed in a 250-ml. bottle, and centrifuged for 15 minutes. The resulting oil was clarified by filtration through anhydrous sodium sulfate and cotton.

In the series of oils thus obtained, the depth of color was roughly proportional to the time of incubation of the original livers. Consequently, the lightest oil was prepared from the fresh livers while the darkest oil was from livers which had been incubated the longest period of time, or in this case 96 days.

Four observers were asked to arrange the oils in sequence of color (Table 1). They were all in agreement except for sample numbers 127 and 141. Additional observers decided that oil number 141 was lighter than oil 127. When these oils were again submitted to observers 1 and 2, as unknowns, they also agreed on this order. It thus appears that the difference in the color of these oils was great enough so that independent observers could arrange the oils in approximately the same order.

A qualitative identification of the color can be obtained from the descriptions given by the four observers (Table 2). Very likely if the observers had been trained in color description, their terms would have been in better agreement as well as being more suggestive of the actual color of the oils.

When asked to specify whether the difference in the color of the oils was slight, definite, or very definite, all the observers were in good agreement (Table 3).

TABLE 3

Degree of Difference in Color Recorded by Observers

Samples	Observer			
	1	2	3	4
42- 82	definite	definite	slight	slight
82-102	slight	slight	slight	slight
102-105	slight	slight	slight	slight
105-110	slight	slight	slight	slight
110-112	very definite	definite	very definite	definite
112-111	slight	slight	slight	slight
111-126	slight	slight	slight	slight
126-141	very definite	definite	very definite	very definite
141-127	slight	slight	slight	slight
127-156	very definite	definite	very definite	very definite
156-158	very definite	definite	very definite	definite

TABLE 1

Arrangement of Samples by Visual Comparison of Color of a Series of Grayfish Liver Oils

Observer	Order of Oil Samples											
	42	82	102	105	110	111	112	126	127	141	156	158
1	1	2	3	4	5	7	6	8	9	10	11	12
2	1	2	3	4	5	7	6	8	9	10	11	12
3	1	2	3	4	5	7	6	8	10	9	11	12
4	1	2	3	4	5	7	6	8	10	9	11	12
5	....	....	....	....	....	....	....	....	10	9	....	....
6	....	....	....	....	....	....	....	....	10	9	....	....
7	....	....	....	....	....	....	....	....	10	9	....	....

TABLE 2

Description of Color by Observers

Sample	Observer			
	1	2	3	4
42	greenish yellow	greenish yellow	greenish lemon yellow	lemon yellow
82	greenish yellow	greenish yellow—deeper	greenish lemon yellow	lemon yellow
102	greenish yellow—trace red	greenish yellow—deeper	greenish lemon yellow	lemon yellow
105	greenish yellow—trace red	greenish yellow—deeper	greenish lemon yellow	lemon yellow
110	greenish yellow—some red	greenish yellow—deeper	greenish lemon yellow	lemon yellow
112	reddish yellow	light amber	orange	orange
111	reddish yellow	light amber	orange	orange
126	reddish yellow	light amber	orange	orange
141	very reddish yellow	medium amber	light brownish yellow	orange amber
127	very reddish yellow	medium amber	brownish yellow	orange amber
156	light brownish red	dark amber	brownish yellow	amber
158	dark brownish red	reddish amber	yellowish brown	dark amber

The data in Table 2 show that the readings made on a Rosenheim-Schuster colorimeter [1] by any one observer follow the visual arrangement quite closely. Differences in readings by two observers indicate that there is a considerable subjective factor in the estimation of color by means of the tintometer. However, the observers taking part in these experiments were untrained in the use of the instrument. With more experienced observers, the readings might have been in closer agreement.

Isopropanol was used as the reference liquid in making the measurements with a Beckman spectrophotometer (Table 5). This choice was arbitrary on the basis that isopropanol is the reference liquid and oil solvent often used in the estimation of vitamin A by this instrument. In this experiment, the measurements were made with undiluted oil. A one-centimeter absorption cell was employed. When extremely dark oils are used, it may be necessary to dilute them with a solvent. In that case, isopropanol would be unsatisfactory since oil dissolves in it slowly. A solvent such as chloroform would probably be more suitable as a reference liquid under these conditions.

The results (Table 5) indicate that transmission measurements follow closely the order made by visual comparison and that a fairly reliable estimation of the change in the depth of color can be made. For any one oil, three readings of per cent transmission will probably be adequate, such as at wave lengths of 500, 600, and 700 millimicrons. Further measurements with other oils would have to be made before a procedure could be recommended. It would appear, however, that the spectrophotometric method promises reproducible results which can be correlated with visual observations.

[1] This instrument is based on the Lovibond color system. A one-centimeter absorption cell was employed.

TABLE 4

Rosenheim-Schuster Colorimeter Readings

Sample	Observer					
	1		2		3	
	Red	Yellow	Red	Yellow	Red	Yellow
42	0.0	4.4	0.0	5.1	....	....
82	0.4	5.4	0.0	6.0	....	....
102	0.5	7.2	0.5	8.0	....	....
105	0.6	8.4	1.0	8.4	....	....
110	0.6	8.4	1.0	7.2	....	....
112	1.3	16.0	2.3	10.3	....	....
111	1.6	11.0	1.9	10.0	....	....
126	1.4	14.0	2.0	12.2	....	....
141	2.4	20.0	3.1	20.0	2.5	22
127	2.4	24.0	3.3	20.2	3.1	23
156	5.1	28.0	5.1	27.0	5.1	22
158	7.5	29.9	9.0	26.0	....	....

TABLE 5

Per Cent Transmission Using Isopropanol as the Reference Liquid

Sample	Wavelength in Millimicrons							
	450	500	550	600	650	700	750	800
42	40.0	82.2	97.3	99.1	99.1	99.4	99.3	100.0
82	30.7	74.5	93.7	97.1	98.2	98.9	99.3	99.0
102	20.0	66.1	90.0	96.5	98.2	99.2	99.8	100.0
105	19.2	64.8	88.0	95.1	98.0	99.2	99.7	100.0
110	21.3	61.9	85.9	93.8	97.0	98.8	99.5	100.0
112	5.9	39.7	72.5	88.0	94.0	97.1	98.6	100.0
111	6.7	41.0	72.9	87.2	93.7	96.7	98.1	99.5
126	5.1	41.0	73.0	88.6	94.6	97.5	98.8	100.0
141	2.3	28.7	64.0	83.9	92.9	96.9	98.6	100.0
127	1.2	23.6	57.5	78.5	89.7	95.0	97.4	99.3
156	0.2	11.1	44.2	71.2	85.7	93.3	96.8	99.2
158	0.0	2.3	23.2	52.7	75.2	87.6	93.6	97.5

Report of the Referee Board

AGAIN for the year 1945-46 the Referee Board has little more than routine activity to report. Twenty-eight Referee Certificates were issued, of which twenty-six were renewals. The usual ten check samples of cottonseed were distributed. For the second time a single series of check oil samples, three each of crude cottonseed and of crude soybean oil, were distributed. All three of the soybean oil samples were degummed, i.e., water-washed; fortunately for purposes of checking the accuracy of the different laboratories the new refining procedure for degummed oil gave normal variations for refining loss.

The Referee Board has found it very satisfactory to make regular use of the check meal samples sponsored by the Smalley Foundation Committee and heartily approves the recent change of policy whereby that Committee will also take charge of distributing check seed and oil samples. Sponsorship of the latter samples by the Referee Board has been due to lack of any other committee responsible for them. We acknowledge with great appreciation the help received from R. T. Doughtie and from the Atlanta Laboratory of Law and Company on the cottonseed samples, from F. G. Dollear on the cottonseed oil samples, and from R. T. Milner on the soybean oil samples.

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