Relation Between the Iodine Number and Refractive Index of Crude Soybean Oil

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IN view of the recent work of Zeleny and Coleman (4) and Hopper and Nesbitt (1) on the relationship between the refractive index and iodine number of linseed oil, it was thought that a similar relationship might hold in the case of soybean oil. The validity of this relationship is based on the assumption that the oils consist almost wholly of the glycerides of long-chain aliphatic acids essentially free of cyclic groups and hydroxy acids, and that the refractivities of the double bonds in the compounds present are additive. In this study, determinations of both refractive index and iodine number were made on a large number of soybean oils, and the relationship between these characteristics was derived by statistical analysis. The factors which may affect this relationship or be of importance when the refractive index is used as a measure of the iodine number have been studied.

The samples of oil were obtained from the soybeans either by cold pressing in a hydraulic press or by extraction with Skellysolve F. Most of the work herein reported was done on oils which were obtained in the course of the regular analytical determination of the oil content of soybeans. These oils were extracted in a Butt apparatus and subsequently freed of solvent by heating on a steam bath for one-half to one hour. All iodine number determinations were made by the official A. O. C. S. Wijs method. Agreement within 0.8 unit was considered as satisfactory for duplicate determinations. The refractive indices were measured with sodium light on a Zeiss dipping-type refractometer equipped with jacketed double prisms maintained at 25 ± 0.05°C. During the course of this study, the refractometer was checked repeatedly against two glass prisms the indices of which had been determined by the National Bureau of Standards. The indices of these two test prisms were such that the scale readings observed with them were at the extremes of the scale of the instrument. Under the above conditions, duplicate readings of oils and test prisms agreed to within 0.00003 units of refractive index.

The equation relating the iodine number to the index of refraction was calculated by the method of 270 samples of extracted oil from least squares (3) from the data on the 1937 crop of soybeans. Assuming the iodine number to be the dependent variable (Relation I):

$$\text{I. No.} = 8661.723n\frac{25}{D} - 12{,}626.174.$$

The standard error of estimate is 0.78 units of iodine number. If the index of refraction is assumed to be the dependent variable (Relation II):

$$\text{I. No.} = 8845.037n\frac{25}{D} - 12{,}896.050.$$

The most probable equation lies somewhere between the two equations given, but the least squares solution does not give a line which allows for errors in both variables.

Sixty-five of the same samples

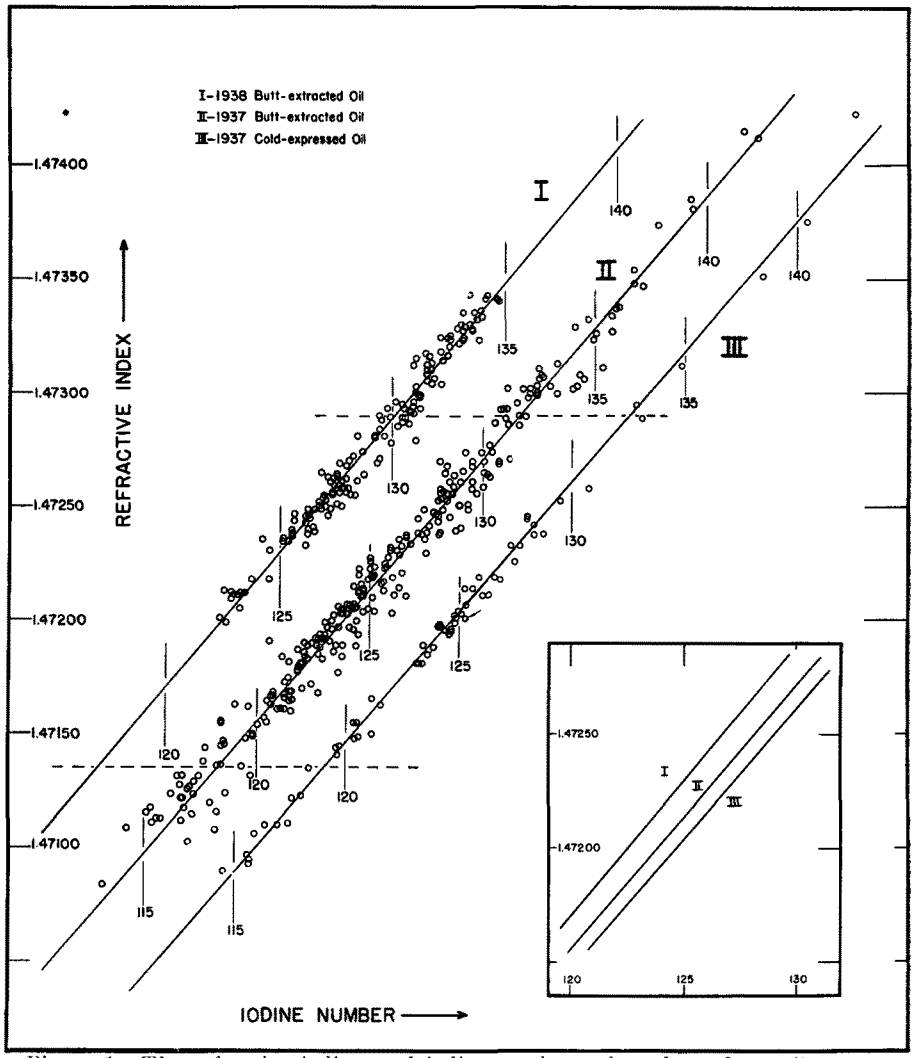

Figure 1. The refractive indices and iodine numbers of crude soybean oils. Horizontal broken lines indicate the portion of the figure shown in insert.

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covering a wide range in iodine number were cold-pressed and were found to give for Relation I:

$$\text{I. No.} = 8751.942n_D^{25} - 12{,}758.140,$$

with the standard error of estimate as 0.45, and for Relation II:

$$\text{I. No.} = 8806.667n_D^{25} - 12{,}838.694.$$

One hundred fifty-six samples of extracted oil from 1938 soybeans gave for Relation I:

$$\text{I. No.} = 8482.631n_D^{25} - 12{,}363.875,$$

with the standard error of estimate as 0.53, and for Relation II:

$$\text{I. No.} = 8724.710n_D^{25} - 12{,}720.404.$$

The three sets of data are plotted in Figure 1, and the line representing Relation I is drawn through the corresponding set of points to illustrate the distribution of values about the line; the relative positions of the lines are shown in the inset.

Values of iodine number in different refractive index ranges are given in Table I for both Relations I and II in comparison with values computed by extrapolation of the equations for the data from linseed oil given by Zeleny and Coleman, and Hopper and Nesbitt mentioned above. In Table I there are also shown corresponding values obtained from equations representing all the 1937 and 1938 extraction data presented in this paper, combined with the data from 1,485 samples of linseed oil used by Hopper and Nesbitt in their work which were made available through the kindness of T. H. Hopper. The 1,911 samples of soybean and linseed oils thus included were found to give, for Relation I:

$$\text{I. No.} = 8626.877n_D^{25} - 12{,}575.226,$$

and for Relation II:

$$\text{I. No.} = 8657.167n_D^{25} - 12{,}619.958.$$

As can be seen, the differences between the equations derived in this paper for the three groups of soybean oils are outside the probable experimental error. For a given index of refraction, the iodine number for the oil from 1938 soybeans is lower that that from the 1937 soybeans by about 1.0 to 1.7 units. The explanation is not apparent. The difference between cold-pressed and extracted oil may be explained on the basis of differences in composition, since these two methods of obtaining the oil no doubt give varying amounts of constituents other than glycerides.

TABLE I.
IODINE NUMBERS CALCULATED FROM EQUATIONS FOR VALUES OF REFRACTIVE INDEX: COLUMNS I AND II REPRESENT EXTREME VALUES FOR SOYBEAN OIL, AND COLUMN III REPRESENTS VALUES IN THE HIGH RANGE FOR LINSEED OIL.

Equation	I n_D^{25} = 1.47000	II n_D^{25} = 1.47400	III n_D^{25} = 1.48100
Soybean oil			
Cold-pressed, 1937			
Relation I	107.21	142.22	—
Relation II	107.11	142.33	—
Butt-extracted, 1937			
Relation I	106.56	141.20	—
Relation II	106.15	141.53	—
Butt-extracted, 1938			
Relation I	105.59	139.52	—
Relation II	104.92	139.82	—
Linseed oil			
Hopper and Nesbitt			
Relation I	—	141.58	200.67
Relation II	—	140.83	201.27
Zeleny and Coleman	—	140.42	200.51
Combined			
Butt-extracted 1937 and 1938 soybean oil data, and Hopper and Nesbitt data			
Relation I	106.28	140.79	201.18
Relation II	106.08	140.71	201.31

The effect of the length of heating of the oil on a steam bath was studied on samples treated in four ways: cold-pressed, cold-pressed to which solvent (Skellysolve F) was added, hot solvent-extracted, and cold solvent-extracted. As can be seen from Figure 2A, heating up to two hours produced no significant changes in either iodine number or refractive index of the cold-pressed oil as compared with a freshly cold-pressed sample which is represented in Figure 2A by the dotted lines designated "No Heat." When solvent was added to this oil, the iodine number while decreasing with time did not differ by more than 0.8 unit from the original oil, while the refractive index approached the original value only very slowly. The differences in iodine number for all samples were scarcely more than the experimental error, whereas the very marked difference in refractive index between the extracted oils and cold-pressed oils should be noted.

Since the oil obtained by cold solvent extraction (percolation) gave the same results as that obtained in the Butt extractor, no ef-

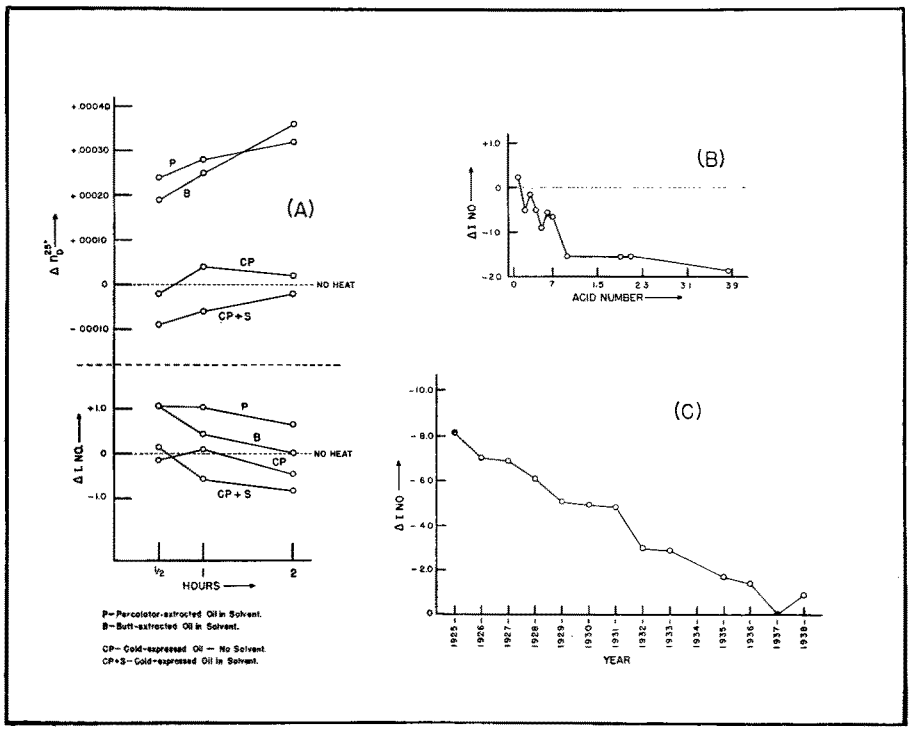

Figure 2. Factors influencing the relationship between iodine number and refractive index. A. Solvent and Oil on the steam bath, B. Acid number, C. Age of the soybeans.

fect of the four hours' heating during extraction was apparent. Since the cold-pressed oil to which solvent was added did not differ in properties from the cold-pressed oil without solvent, it might be concluded that no effect on the oil was produced by the removal of solvent on the steam bath. The difference between solvent-extracted and cold-pressed oil appears to be caused by compounds of a non-glyceride nature which are present in one oil to a greater extent than in the other. A study of the change in refractive index of several extracted soybean oils as added solvent was removed by heating on the steam bath, confirmed the general slope of the refractive index curves in Figure 2A for oils in which solvent was present. The index of refraction rose rapidly for about 30 to 40 minutes and then showed a much slower rate of increase, indicating that almost all of the solvent had been evaporated in this length of time.

The effect of moisture content of the soybeans on the relationship between iodine number and refractive index was examined by pressing oil from two varieties of beans at several moisture contents ranging from 1.5 to 11.5 percent. The differences between the iodine numbers observed and those calculated from the equation ranged from 0 to 0.8 unit and no correlation was observable between these deviations and the moisture content.

In order to determine whether the pressure at which the oil is expressed from the beans altered the composition of the oil, equal-weight samples of the same variety of beans were pressed at pressures of 4, 8, 12, and 20 tons per square inch for 5 minutes each. The amount of oil obtained decreased slightly at a pressure of 12 tons per square inch and decreased still further at 20 tons' per square inch pressure. The maximum spread in iodine number was 0.83 and in index of refraction, 0.00010; no trend in the determinations was apparent.

The container and conditions under which the sample of oil was stored were investigated as factors which might influence the relationship between iodine number and refractive index. Samples of cold-pressed oil were stored in both glass and steel containers (1) in a refrigerator, (2) at room temperatures exposed to light, and (3) at room temperatures in the dark. These samples were examined the day on which the oil was expressed, again on the following day, and then one month later. None of the samples showed a significant change in either iodine number or index of refraction.

Pickering and Cowlishaw (2) have previously pointed out an effect of free fatty acids on the relationship between iodine number and index of refraction. In the very large number of routine soybean samples which have been examined at the U. S. Regional Soybean Industrial Products Laboratory there have been very few cases in which the acid number of the oil has exceeded 0.7. An examination of a series of eleven oils ranging in acid number from 0.1 to 3.8 showed a regular decrease in iodine number below that calculated from the refractive index measured. For the extreme case of the acid number of 3.8, the decrease amounted to 1.9 units; and for an acid number of 0.8, it amounted to 1.0 unit of iodine number. In almost every case where the soybeans were sound, unshriveled, and had not been stored for a long time, the average acid number of the oil was about 0.3. Therefore, errors in iodine number from this cause may be expected to lie within the limits of experimental accuracy. This is shown in Figure 2B.

Another and more serious cause for the deviation of the observed iodine numbers from those calculated by means of the equation has been found to be the age of the soybean sample. As shown in Figure 2C, with soybeans grown 14 years ago, the average deviation from the curve for cold-pressed oil from soybeans grown in 1937 is 8 units of iodine number. The figures shown in the graph represent the averages of 4 samples for the years 1925 to 1933, inclusive. The points for 1935 and 1936 represent one and two samples, while 65 and 10 samples were averaged for 1937 and 1938, respectively. Examination of the acid number of oil from a 1925 soybean sample showed that this very wide deviation could not be attributed to the free fatty acid content. Slow enzymatic changes may have occurred in the soybean oil upon storage to account for the effect here noted. The apparent reversal in trend between 1937 and 1938 cannot be explained at this time, but confirms results obtained for Butt-extracted oils for these two years. All samples for this investigation of age effects were analyzed within one month's time, and the differences between 1937 and 1938 cold-pressed oil samples must be regarded as peculiar to those two years.

The instrument used for the determination of refractive indices in this investigation is an expensive and rather uncommon one. However, since a butyro refractometer is comparatively cheap and readily available, four soybean oils were examined in a Zeiss butyro refractometer. This instrument can be calibrated with glass prisms as described for the dripping type. The temperature control remains the same, and the vernier scale can be estimated to within 0.02 division. For the four soybean oils, the average deviation of the butyro refractometer readings from those of the dipping refractometer was 0.00003 units of refractive index.

The calculation of the iodine number of a normal soybean oil from an observed refractive index reading is a simple matter if maximum accuracy is not essential. The refractive index value is substituted in the equation representing Relation I for the combined data of soybean and linseed oils, and the iodine number calculated directly. In case results are desired which are more accurate than those obtained from the above correlation, the other equations derived in this investigation for the specific crop years may be used. Similar relationships may be formulated for crop years more recent than those examined to date. The use of either of these methods for determining the iodine number offers considerable saving in both time and chemicals when compared to usual chemical methods, especially if the work of a laboratory involves the the analysis of oils from a large number of soybean samples.

Because the form of the equations relating the iodine number and refractive index is cumbersome for repeated use unless a calculating machine is available, a simplified form is suggested which involves the multiplication of terms containing no more than four significant digits. Such a revision makes possible the use of five-place logarithms. The coefficient of the refractive index term is factored out of the right-hand side of the equation, and the coefficient is then

rounded off to four digits. To illustrate, Relation I for the combined data:

$$\text{I. No.} = 8626.877n_D^{25} - 12{,}575.226,$$

becomes

$$\text{I. No.} = 8627.\ (n_D^{25} - 1.45768).$$

A constant effort is made by the U. S. Regional Soybean Industrial Products Laboratory to find and use methods which will furnish additional information about the many unique soybean varieties analyzed in connection with the agronomic program. As a means of finding soybean oils whose properties are different from those obtained from the usual commercial varieties, and at the same time reducing the analytical work necessary, only one determination each has been made, in the past few months, of the iodine number and the refractive index on oil obtained in the course of routine analysis. In case these values did not check with the equation, the sample was subjected to further examination. Of some 400 samples of soybeans, embracing 185 varieties, so far examined, only 3 have given oils whose iodine number and index of refraction did not conform statistically to the relationships given above. These samples are being studied in greater detail.

Conclusions

The iodine number of soybean oil can be determined by calculation from the measurement of refractive index with an accuracy comparable to any chemical method, provided that the instrument used is sufficiently accurate, the measurements are carefully made, and, for any given crop year, a correlation curve is established.

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Molecular Distillation of Soybean and Corn Oils*

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THE process of molecular distillation is now well known through its application in concentrating vitamins A and D from certain fish liver oils (1). In the present paper, data obtained from a study of two American vegetable oils, soybean and corn, are presented.

A batch of 1000 gallons of alkali refined soybean oil was molecularly distilled at an average absolute pressure of 0.002 mm. of Hg. and at a rate of 7 to 8 gallons per hour. The oil was passed through 4 still units operating at temperatures shown in Table 1. The residue was redistilled at a higher temperature range, but since no fraction distilled from the first unit only three fractions and a final residue were obtained. The nine fractions and the original refined oil were analyzed with the results given in Table 1.

A batch of 700 gallons of corn oil was distilled in substantially the same way except that the residue was not redistilled. The five fractions and the original refined oil were analyzed with the results given in Table 2.

Methods of Analysis

The free fatty acid and saponification values were determined by the official A.O.C.S. methods and the iodine value determinations were carried out by the Wijs method. The thiocyanogen value determinations and the calculation of the percentage of the unsaturated acid constituents were made in accordance with the method of Kaufmann and co-workers as described by Jamieson (2) in the case of the corn oil and by the A.O.C.S. procedure in the case of the soybean oil.

The determination of unsaponifiable matter was carried out in accordance with the procedure often used for vitamin extraction in the laboratories of Distillation Products, Inc. This method employs a five-fold ethyl ether extraction of the saponified sample following which the fractions are combined and washed twice with water, once with N/4 KOH, and twice more with water. The ethereal extract was then evaporated and the residue dried to constant weight in a tared flask. The data given in the

TABLE 1
MOLECULARLY DISTILLED SOYBEAN OIL

CHARACTERISTIC	Orig. Oil Refined	Frac. 1	Frac. 2	Frac. 3	Frac. 4	Res.	Frac. R_2	Frac. R_3	Frac. R_4	Final Residue
Per cent Distilled		0.02	3.4	4.4	4.4		23.2	20.4	28.4	
Distill'n Temp.		170-8°C.	255-65	265-70	275		280	295	295	
Color — Evelyn L4400	1.35	too dark		1.28	1.28	1.22	1.10	0.33	0.40	1.28
Color — Evelyn L5200	.716	too dark		0.328	0.172	0.301	0.059	0.019	0.023	0.810
Lovibond — Yellow	70a			35b	35b	130b	25b	18b	20b	95c
Lovibond — Red	14.1a			11.2b	6.2b	10.9b	3.0b	1.6b	1.7b	66c
Free Fatty Acid (Oleic), percent	0.04	5.0	0.5	0.2	0.15	0.03	0.10	0.06	0.04	0.09
Unsaponified Matter, percent	0.8	74.2	10.7	1.5	0.7	0.04	0.28	—	—	—
Saponification Number	193	50	175	193	193.5	194	193	193	194	193
Sap. Number of Glycerides	194	194	196	196	195	194	194	193	194	193
Refractive Index 40°C.	1.4682			1.4673	1.4672	1.4680	1.4673	1.4676	1.4674	1.4682
Iodine Val. (Wij's)	134.5	150.8	131.2	127.2	127.8	134.4	129.9	131.2	132.0	137.6
Thiocyan. Value	83.7			79.7	79.2	83.8	82.3	83.0	80.6	87.1
Iod. Val. of Fatty Acids	139.2		136.3	134.7	132.8	140.1	136.8	136.8	137.0	143.2
Thiocyan. Val. of Fatty Acids	86.8		81.3	83.1	82.8	87.3	85.8	86.3	85.8	90.1
Solid Fatty Acids, percent	12.5		16.0	14.9	15.8	11.5	12.9	12.6	12.2	11.0
Unsaturated (by difference), percent	87.5		84.0	85.1	84.2	88.5	87.1	87.4	87.8	89.0
Oleic Acid, percent	29.7		23.2	28.2	29.2	30.1	30.9	31.7	31.3	30.4
Linoleic Acid, percent	49.4		54.8	50.1	47.7	50.4	48.4	47.7	49.4	48.0
Linolenic Acid, percent	8.4		6.0	6.8	7.3	8.0	7.8	8.0	7.1	10.6

a — 2.5 inch column. b — 5.25 inch column. c — 1 inch column.

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