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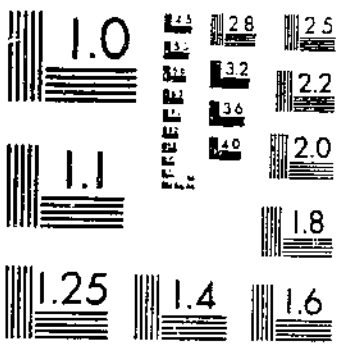

MICROCOPY RESOLUTION TEST CHART
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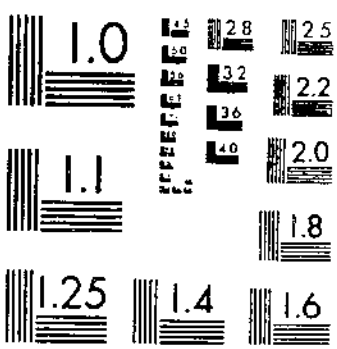

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Technical Bulletin No. 748 *November 1940*

UNITED STATES
DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

Rapid Determination of Soybean-Oil Content and of Iodine Number of Soybean Oil[1]

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NEED FOR RAPID OIL-TESTING METHODS

The rapid increase in domestic soybean production in recent years has been attended by an even more rapid increase in the quantity of soybeans used for crushing. For the crop year beginning October 1924 only 307,000 bushels, or about 6 percent of the domestic crop, were crushed, whereas in 1938 more than 44,000,000 bushels, or about 77 percent of the crop, were crushed in domestic mills and about 4,500,000 bushels, or about 8 percent, were exported.

Although a pound of soybean oil is worth from two to five times as much as a pound of the meal or cake left after removal of the bulk of the oil, yet, because soybeans have a relatively low oil content, the meal from a bushel of soybeans generally has a somewhat higher market value than the oil. The value of the meal, however, is not appreciably influenced by the oil content of the original soybeans. Oil quantity and quality, therefore, are important factors governing the intrinsic commercial values of soybeans.

Yellow varieties of soybeans are preferred in the United States for crushing. The oil is obtained either by the expeller method, the hydraulic-press method, or the solvent-extraction process. Commercial lots of yellow soybeans ordinarily contain from 17 to 22 percent of oil on a dry-matter basis. When soybeans are processed by either the expeller or hydraulic-press method the resulting meal or cake usually contains about 5 percent of oil. From these figures it can

[1] Submitted for publication Apr. 22, 1940.

readily be seen that soybeans containing 22 percent of oil will yield approximately 40 percent more oil by these methods than will soybeans containing 17 percent of oil. Thus a difference of 1 percent in oil content may account for a difference of as much as 8 percent in commercial oil yield by expeller or hydraulic-press methods. Oil content, therefore, is a much more important index of value than the actual oil-content percentages appear to indicate.

Soybean oil is used principally for food purposes and in the manufacture of paints and varnishes, and to a lesser extent for soap manufacture and other miscellaneous purposes. In the food industries oils of low iodine number are preferred because they may be more economically converted into hydrogenated fats and because of their possible greater stability in the liquid state. On the other hand, oils of high iodine number are much more desirable in the paint and varnish industry because of their more rapid drying properties and the more durable quality of film produced.

At present domestic commercial soybeans of the types used for crushing have oils with iodine numbers that generally fall between 128 and 138 (Wijs). Selected experimental samples of soybeans yield oils ranging from 117 to 147 in iodine number. In order better to meet the requirements of the two most important soybean-processing industries, efforts are now being made to develop new commercial varieties some of which yield oils with a lower and others with a higher iodine number than existing varieties. As a result of these efforts the range in iodine numbers encountered in oils from commercial soybeans may increase considerably, and the iodine number become a more important quality factor in evaluating this crop.

DETERMINATION OF OIL CONTENT

The Extraction Method

An extraction method for determining oil content by using petroleum ether as a solvent was used as a standard procedure against which the results obtained by the proposed rapid method were checked. The extraction method and its principal sources of error were studied in considerable detail by Zeleny and Coleman (*45*) [2] in connection with flaxseed. The following procedure, based largely on that study, was found to be satisfactory for soybeans:

(1) Reduce the weight of the clean, air-dry sample containing not more than 10 percent of moisture to approximately 50 gm. by use of a mechanical sampler or by hand quartering.

(2) Granulate the sample with a coffee-type mill adjusted for a fairly coarse grind.

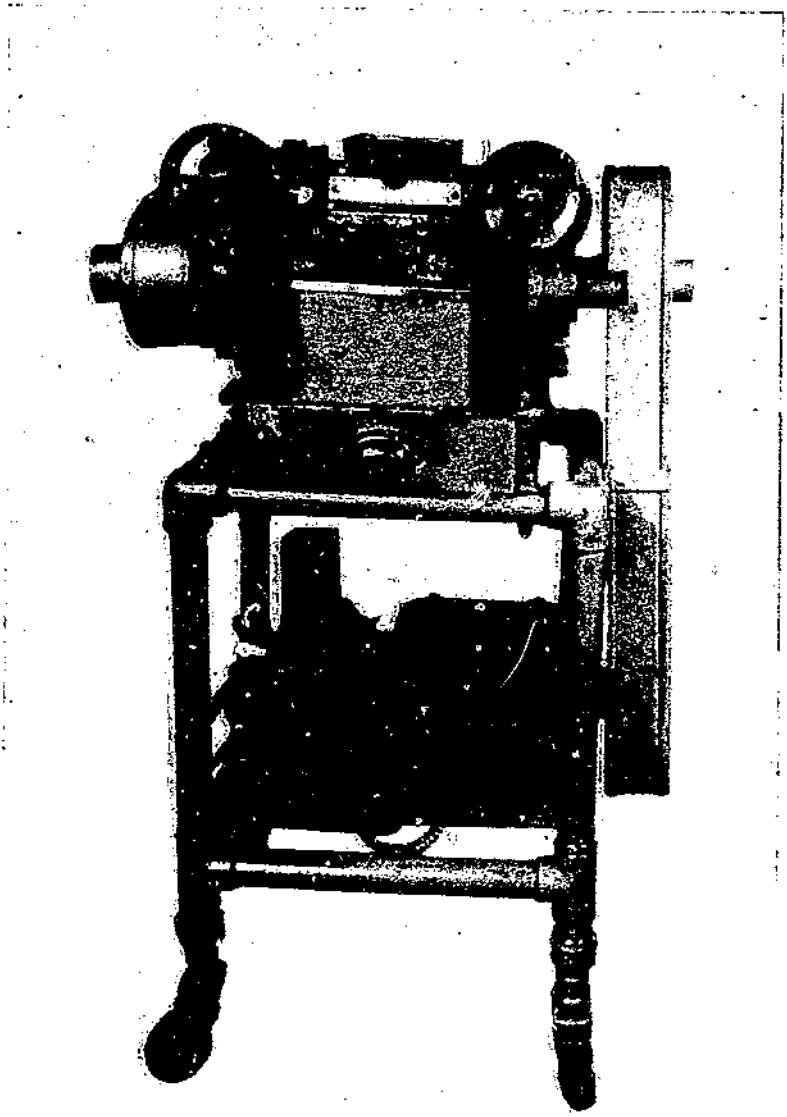
(3) For the final grinding a motor-driven experimental roller flour mill with 6- by 6-inch rolls, 40 corrugations to the inch, is recommended. The rolls should have a speed differential of about 9 to 7 and the faster roll should have a speed of about 900 revolutions per minute. Adjust the rolls to the minimum possible clearance but so that they do not make contact. Such a mill is shown in figure 1.

Pass from 10 to 20 gm. of the granulated sample through the mill, brush off any loose material from the stationary parts of the mill and discard the ground material. Then without cleaning the rolls or other moving parts of the mill, pass the remainder of the sample through, again brushing any loose material on the mill into the sample and again not cleaning the rolls or other moving parts. The

[2] Italic numbers in parenthese. .efer to Literature Cited, p. 20

sample should be passed through the roller mill three times, but no part of the sample need be discarded from the last two grindings.

(4) Samples should be weighed and extraction begun immediately after the grinding. In case any appreciable time elapses between these operations the



AMS 1275

FIGURE 1.—Motor-driven experimental roller-type mill.

ground samples should be kept in tightly stoppered glass bottles in a cool place and out of direct sunlight. After thoroughly mixing the ground sample weigh out accurately duplicate 2- to 10-gm. portions, transfer quantitatively to the extraction thimbles, and cover with wads of fat-free cotton. Dry the extraction flasks in an oven at 100° C., cool in a desiccator, and weigh.

(5) Extract with a petroleum ether conforming to the official specifications for petroleum ether for cottonseed extraction (*38*),[3] using any standard type of extraction apparatus. The extraction should be continued at least 18 hours with the solvent condensing at a rate of at least 100 drops a minute.

(6) After the extraction is complete, disconnect the flasks and evaporate the bulk of the solvent on the steam bath, removing the solvent vapors by suction or by carefully tipping the flasks from time to time. When no more vapor appears to be generated, remove the flasks from the steam bath and drive off the last traces of the solvent by one of the following methods: (*a*) Place the flasks in a vacuum oven at 95° to 100° C. for 30 minutes. (*b*) Place the flasks in a well-ventilated air oven at 95° to 100° for 1½ hours. (*c*) Leave the flasks on the steam bath for 1½ hours.

(7) Remove the flasks from the oven or steam bath and cool to room temperature in a desiccator. Weigh immediately on removal from the desiccator. If duplicates do not agree within 0.2 percent of oil, the determination should be repeated.

It should be noted that the usual procedure of regrinding the partially extracted sample in a mortar before continuing the extraction is dispensed with in the above extraction method. It has been shown that when the samples are ground with the roller-type mill as directed, usually less than 0.2 percent of additional extract is obtained by regrinding the samples, and evidence exists that this additional extract is made up in considerable part of material other than oil. It should be remembered, however, that in applying any extraction method for the determination of oil content to soybeans or any other oil seed, the sample should be reground in a mortar after partial extraction, unless it has been conclusively proved that with the procedure used no significant quantity of additional oil can be extracted as a result of such a regrinding.

Although the extraction method is considered the fundamental method for determining oil content in oil-bearing seeds, it leaves much to be desired for satisfying certain routine commercial demands that require a method for determining the oil content in a relatively short time. This is particularly true in the commercial inspection of carlots of soybeans, where it is impracticable to hold the cars "on track" for the length of time required to run oil analyses by the conventional extraction procedure.

The Refractometric Method

Development of the Method

Wesson (*39*) was able to determine the oil content of cottonseed meal and meats by determining the refractive index of a "halowax" (α-chloronaphthalene) extract. The method was based on the fact that the refractive index of a mixture of cottonseed oil with halowax bears an essentially linear relationship to the percentage of cottonseed oil in the mixture.

Coleman and Fellows used this principle in the development of a method for the determination of the oil content of flaxseed (*6*) and of other seeds and oil-bearing materials (*7*). The method consists of mixing definite quantities of halowax and of the finely ground seed in a mortar, thoroughly macerating the mixture with sand to extract

[3] These specifications are as follows. Initial boiling temperature, not less than 47° C., nor over 49° C.; dry-flask end point, not over 60° C., nor less than 56° C.; at least 95 percent distilling under 55° C. and not over 85 percent under 49° C.; specific gravity at 60° F., 0.650 to 0.675; color, water-white; evaporation residue not over 0.002 percent by weight; doctor test, sweet; copper-strip corrosion test, noncorrosive; unsaturated compounds, trace only, permitted.

the oil, filtering the mixture, and determining the refractive index of a small quantity of the clear filtrate. From a previously prepared conversion table the percentage of oil in the seed corresponding to the refractive index of the mixture may be read.

Geddes and Lehberg (*10*) have modified the method by employing a mixture consisting of approximately equal parts by volume of halowax and α-bromonaphthalene as a solvent rather than halowax alone. This makes it possible to adjust the refractive index of the solvent accurately to a predetermined value, thus eliminating the necessity of preparing a new conversion table for each new batch of halowax.

Modifications of the refractometric method have also been developed by Rasteryaev (*34*) for the determination of oil in various oil-bearing seeds, using chloroform as a solvent; by Groenhof (*11*) for the estimation of oil in copra, using benzyl alcohol and tetrahydronaphthalene as solvents; and by Illarionov and Demkovskiĭ (*13*), who found chlorobenzene a suitable solvent. Zander (*42*) and Ingraham and Simpson (*15*), in applying the method to oil-bearing seeds and their press cakes used halowax to extract the oil, while Ermakov (*8*) used α-bromonaphthalene. Leithe (*19, 20, 21, 22, 23, 24, 25*) and Leithe and Müller (*28*) have used the principle of the refractometric method in the determination of oil or fat in a variety of products including chocolate, dairy products, and soybeans. Leithe used benzine as a solvent in most of his work but later (*25*) found bromonaphthalene to be superior for this purpose.

Zeleny and Coleman (*45*) further perfected the method as it applies to flaxseed by taking into account the variation in refractive index of the oils from different lots of flaxseed. This variation in refractive index is sufficiently great to account in many cases for serious errors in the method when a flaxseed oil of average refractive index has been used to establish the relationship between oil content and refractive index of the solvent-oil mixture. A table of corrections was therefore prepared to supplement the conversion table based on a composite sample of flaxseed oil and a solvent consisting of a mixture of halowax and α-bromonaphthalene adjusted to a standard refractive index. This method has been adopted as official by the Association of Official Agricultural Chemists (*17, 40, 43*) and is being successfully applied in commercial practice.

More recently satisfactory results with various modifications of the refractometric method have been reported by Leithe and Lamel (*26, 27*), Bartstra (*4*), Rubinskiĭ (*35*), Rubinskiĭ and Barmicheva (*36*), Kluin (*16*), Cleve (*5*), Frahm and Koolhaas (*9*), and Wittka (*41*) for determining the oil content of various products including castorbeans, peanuts, rapeseed, palm kernels, cacao beans, corn, and tung nuts. Scharrer and Lamel (*37*) have presented evidence indicating that in cases where higher results are obtained by gravimetric extraction procedures than by the refractometric method, the higher values are often due to the presence of nonfatty substances in the extracts and therefore the refractometric results are more likely to be correct.

ADAPTATION OF THE REFRACTOMETRIC OIL-CONTENT METHOD TO SOYBEANS

Believing that the domestic soybean industry might well benefit from a suitable rapid refractometric procedure for determining oil

content, similar to that previously recommended for flaxseed (*45*) and now in practical use, the Agricultural Marketing Service has undertaken the adaptation of the method to soybean analysis in a form that should prove suitable for routine inspection procedures. A preliminary report on this subject has been made by Zeleny and Neustadt (*46*).

Using a mixture of halowax (α-chloronaphthalene) and α-bromonaphthalene adjusted to a refractive index of 1.63940 at 25° C. as a standard solvent, the refractive indices of mixtures of this solvent in various proportions with a composite sample of freshly prepared soybean oil having a refractive index, $n_D^{25}=1.47302$ were determined (table 1).

TABLE 1.—*Refractive indices at 25° C. of known mixtures of the halowax, α-bromonaphthalene solvent with a composite sample of soybean oil*

Oil in mixture	n_D^{25}	Oil in mixture	n_D^{25}
Percent		*Percent*	
0.000	1.63940	9.154	1.61891
3.961	1.63031	12.091	1.61078
4.893	1.62817	16.647	1.60311
5.705	1.62646	20.186	1.59612
6.569	1.62468	88.879	1.48675
7.266	1.62297	100.000	1.47302

The percentage of oil in the mixture obtained in the actual analysis of soybeans may be calculated from the formula:

$$\frac{100\ Wx}{W'+Wx}$$

where W=weight of ground soybeans in grams,
W'=weight of solvent in grams, and
x=weight of oil in grams in 1 gram of the ground soybeans.

It may be shown with this formula that when 2 gm. of ground soybeans are mixed with 5 cc. of the standard solvent, a range in soybean oil content of 10 to 26 percent will correspond approximately to a range in oil content of the solvent-oil mixture of 3 to 7 percent. Thus, by plotting the values shown in table 1, the refractive index of the solvent extract corresponding to any value for oil content of soybeans may be determined. In this manner a conversion table was prepared for converting refractive-index readings into soybean oil content percentages (table 2).

Obviously this table will be strictly valid only for the analysis of soybeans whose oil has the refractive index, $n_D^{25}=1.47302$, since an oil having that value was used in the preparation of the table. A correction table (table 3) was therefore prepared to indicate the values to be added or subtracted from the values obtained by the conversion table for soybeans whose oils have refractive indices other than the index used as a standard. In actual practice, however, soybeans that have oils with refractive indices differing sufficiently from this standard to cause a significant error in the uncorrected oil content value taken from the conversion table, are seldom found in commercial channels.

TABLE 2.—*Conversion table for determining the percentage of oil in soybeans from the refractive index of the halowax, α-bromonaphthalene extract*

Oil	n_D^{25}	Oil	n_D^{25}	Oil	n_D^{25}	Oil	n_D^{25}	Oil	n_D^{25}	Oil	n_D^{25}
Percent		*Percent*		*Percent*		*Percent*		*Percent*		*Percent*	
10.0	1.63250	12.7	1.63069	15.4	1.62895	18.1	1.62727	20.8	1.62563	23.5	1.62402
.1	1.63243	.8	1.63062	.5	1.62889	.2	1.62721	.9	1.62557	.6	1.62396
.2	1.63236	.9	1.63056	.6	1.62882	.3	1.62715	21.0	1.62551	.7	1.62390
.3	1.63230	13.0	1.63049	.7	1.62876	.4	1.62709	.1	1.62545	.8	1.62384
.4	1.63223	.1	1.63043	.8	1.62870	.5	1.62703	.2	1.62539	.9	1.62378
.5	1.63216	.2	1.63036	.9	1.62863	.6	1.62696	.3	1.62533	24.0	1.62372
.6	1.63209	.3	1.63030	16.0	1.62857	.7	1.62690	.4	1.62527	.1	1.62366
.7	1.63202	.4	1.63023	.1	1.62851	.8	1.62684	.5	1.62521	.2	1.62360
.8	1.63196	.5	1.63017	.2	1.62845	.9	1.62678	.6	1.62515	.3	1.62354
.9	1.63189	.6	1.63010	.3	1.62838	19.0	1.62672	.7	1.62509	.4	1.62348
11.0	1.63182	.7	1.63004	.4	1.62832	.1	1.62666	.8	1.62503	.5	1.62343
.1	1.63175	.8	1.62997	.5	1.62826	.2	1.62660	.9	1.62497	.6	1.62337
.2	1.63169	.9	1.62991	.6	1.62820	.3	1.62654	22.0	1.62491	.7	1.62331
.3	1.63162	14.0	1.62984	.7	1.62814	.4	1.62648	.1	1.62485	.8	1.62325
.4	1.63155	.1	1.62978	.8	1.62807	.5	1.62642	.2	1.62479	.9	1.62319
.5	1.63149	.2	1.62971	.9	1.62801	.6	1.62635	.3	1.62473	25.0	1.62313
.6	1.63142	.3	1.62965	17.0	1.62795	.7	1.62629	.4	1.62467	.1	1.62307
.7	1.63135	.4	1.62958	.1	1.62789	.8	1.62623	.5	1.62462	.2	1.62301
.8	1.63128	.5	1.62952	.2	1.62783	.9	1.62617	.6	1.62456	.3	1.62296
.9	1.63122	.6	1.62946	.3	1.62776	20.0	1.62611	.7	1.62450	.4	1.62290
12.0	1.63115	.7	1.62939	.4	1.62770	.1	1.62605	.8	1.62444	.5	1.62284
.1	1.63108	.8	1.62933	.5	1.62764	.2	1.62599	.9	1.62438	.6	1.62278
.2	1.63102	.9	1.62926	.6	1.62758	.3	1.62593	23.0	1.62432	.7	1.62272
.3	1.63095	15.0	1.62920	.7	1.62752	.4	1.62587	.1	1.62426	.8	1.62267
.4	1.63089	.1	1.62914	.8	1.62745	.5	1.62581	.2	1.62420	.9	1.62261
.5	1.63082	.2	1.62907	.9	1.62739	.6	1.62575	.3	1.62414	26.0	1.62255
.6	1.63075	.3	1.62901	18.0	1.62733	.7	1.62569	.4	1.62408		

TABLE 3.—*Corrections* [1] *to be applied to results obtained from conversion table*

[Corrections in terms of percent of oil]

n_D^{25}–1.4730	10 [2]	11 [2]	12 [2]	13 [2]	14 [2]	15 [2]	16 [2]	17 [2]	18 [2]	19 [2]	20 [2]	21 [2]	22 [2]	23 [2]	24 [2]	25 [2]	26 [2]
0.0001	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
.0002	.01	.01	.01	.01	.01	.01	.02	.02	.02	.02	.02	.02	.02	.02	.02	.02	.03
.0003	.01	.02	.02	.02	.02	.02	.02	.02	.03	.03	.03	.03	.03	.03	.01	.01	.01
.0004	.02	.02	.02	.02	.03	.03	.03	.03	.04	.04	.04	.04	.04	.05	.05	.05	.05
.0005	.02	.03	.03	.03	.03	.04	.04	.04	.04	.05	.05	.05	.05	.06	.06	.06	.06
.0006	.03	.03	.03	.04	.04	.05	.05	.05	.06	.06	.06	.06	.07	.07	.07	.07	.08
.0007	.03	.04	.04	.04	.05	.05	.05	.06	.06	.06	.07	.07	.08	.08	.08	.09	.09
.0008	.04	.04	.05	.05	.05	.06	.06	.07	.07	.07	.08	.08	.09	.09	.09	.10	.10
.0009	.04	.05	.05	.06	.06	.06	.07	.07	.08	.08	.09	.09	.10	.10	.11	.11	.12
.0010	.05	.05	.06	.06	.07	.07	.08	.08	.09	.09	.10	.10	.11	.11	.12	.12	.13
.0011	.05	.06	.06	.07	.07	.08	.08	.09	.10	.10	.11	.11	.12	.12	.13	.14	.14
.0012	.06	.06	.07	.07	.08	.09	.09	.10	.10	.11	.12	.12	.13	.14	.14	.15	.15
.0013	.06	.07	.07	.08	.09	.09	.10	.11	.11	.12	.13	.13	.14	.15	.15	.16	.17
.0014	.07	.07	.08	.09	.09	.10	.11	.12	.12	.13	.14	.14	.15	.16	.16	.17	.18
.0015	.07	.08	.09	.09	.10	.11	.12	.12	.13	.14	.15	.15	.16	.17	.18	.18	.19
.0016	.07	.08	.09	.10	.11	.12	.12	.13	.14	.15	.16	.16	.17	.18	.19	.20	.20
.0017	.08	.09	.10	.11	.11	.12	.13	.14	.15	.16	.17	.17	.18	.19	.20	.21	.22
.0018	.08	.09	.10	.11	.12	.13	.14	.15	.16	.17	.18	.18	.19	.20	.21	.22	.23
.0019	.09	.10	.11	.12	.13	.14	.15	.16	.17	.18	.19	.19	.20	.21	.22	.23	.24
.0020	.09	.10	.11	.12	.13	.14	.15	.17	.18	.19	.20	.21	.22	.23	.24	.25	.26
.0021	.10	.11	.12	.13	.14	.15	.16	.17	.18	.19	.20	.22	.23	.24	.25	.26	.27
.0022	.10	.11	.12	.13	.15	.16	.17	.18	.19	.20	.21	.23	.24	.25	.26	.27	.28
.0023	.11	.12	.13	.14	.15	.17	.18	.19	.20	.21	.22	.24	.25	.26	.27	.28	.29
.0024	.11	.12	.14	.15	.16	.17	.19	.20	.21	.22	.23	.25	.26	.27	.28	.29	.31
.0025	.12	.13	.14	.15	.17	.18	.19	.21	.22	.23	.24	.26	.27	.28	.29	.31	.32

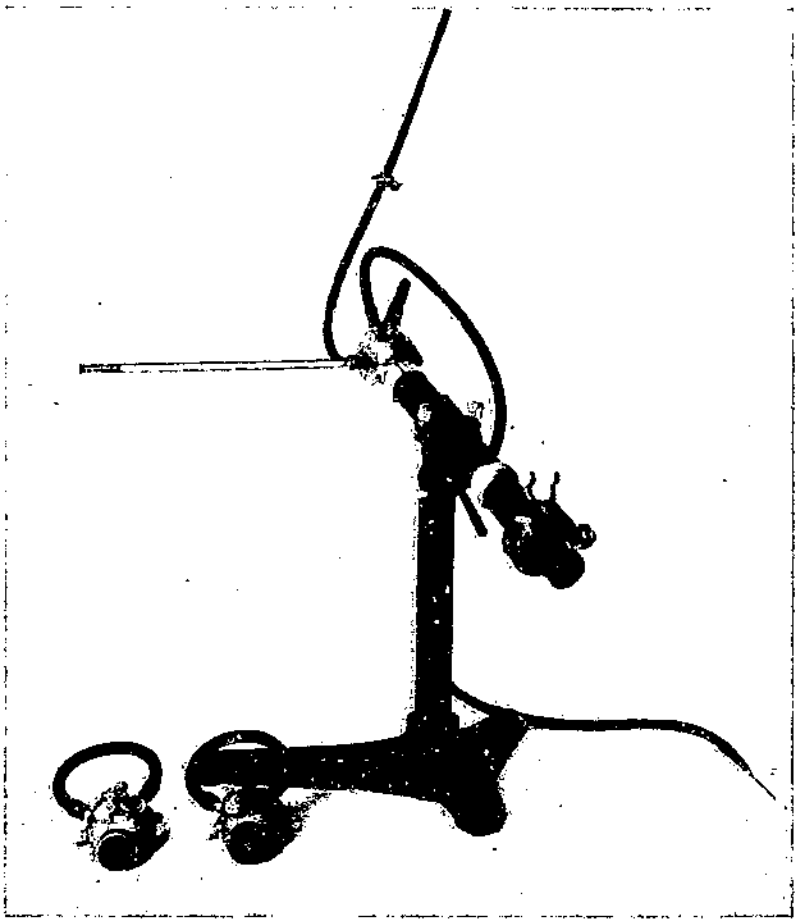
[1] Values to be added when (n_D^{25}–1.4730) is positive; subtracted when (n_D^{25}–1.4730) is negative.
[2] Percent oil as determined from table 2.

DETAILS OF THE METHOD

EQUIPMENT

The necessary equipment and supplies required are as follows:

A mill (or mills) capable of grinding the soybeans to such a degree of fineness that after extraction with ether or petroleum ether, at least 90 percent of the meal will pass through a 40-mesh bolting cloth. For this purpose a coffee-type mill is recommended to granulate the soybeans and a motor-driven experimental roller



AMS 1276

FIGURE 2.—Dipping-type refractometer with interchangeable double-prism heads.

flour mill with 6- by 6-inch steel rolls, corrugated 40 to the inch, for the final grinding (fig. 1). The rolls of the roller mill should have a speed differential of about 9 to 7, and a speed of about 900 r. p. m. for the faster roll.

An analytical balance.

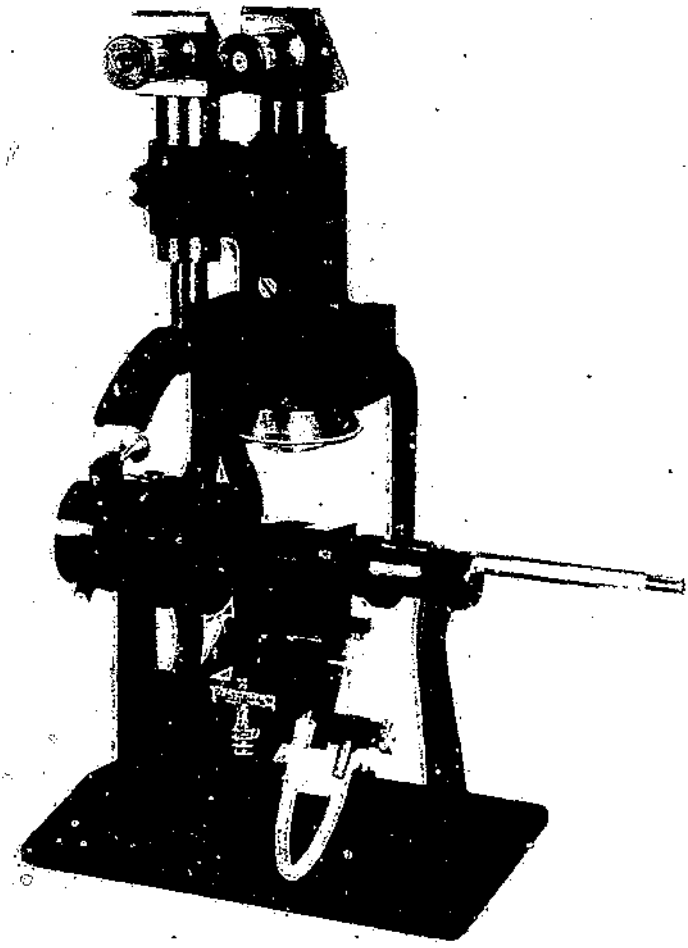
An electric hot plate.

A refractometer having an accuracy of $n = \pm 0.00002$ within the ranges of $n_D^{25} = 1.470$ to 1.477 and $n_D^{25} = 1.622$ to 1.640. Three types of refractometers having sufficiently high precision for this work are now available, a dipping-type instrument equipped with interchangeable water-jacketed prism heads for use with small quantities of liquid (fig. 2), a modified precision Abbé-type instrument

(fig. 3), and a precision refractometer designed and equipped to use monochromatic light (fig. 4).

A temperature-regulating device for controlling the temperature of the water flowing through the water jackets of the refractometer. (Optional.)

An electric oven.



AMS 1132

FIGURE 3. Precision Abbé-type refractometer.

An accurately calibrated 5-ml. pipette.

Halowax.

α-bromonaphthalene.

Ethyl alcohol for cleaning prisms.

3-inch porcelain mortars with pestles.

Reagent-quality sharp sea sand (fine-grain) or equivalent.

Supply of test tubes, 1½-inch glass funnels, retentive filter papers (preferably folded), and absorbent cotton.

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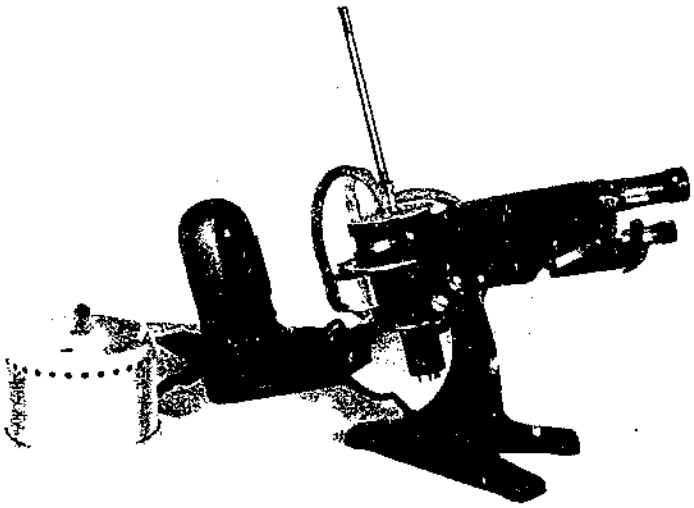
PREPARATION OF STANDARD SOLVENT

Prepare a mixture of halowax and α-bromonaphthalene having a refractive index $n_D^{25}=1.63940\pm2$. Such a mixture contains approximately 74 percent of halowax and 26 percent of α-bromonaphthalene by weight but must be carefully adjusted so that the desired refractive index is attained. If a temperature-regulating device is available the determination of refractive index is simplified by passing water at exactly 25° C. through the water jacket of the refractometer. Equally satisfactory results may be obtained, however, by using water at room temperature and making the necessary temperature correction. For the above mixture this correction in refractive index is 0.00045 for each 1°, to be added to the reading if the temperature



AMS 1447

FIGURE 4.—Precision refractometer designed and equipped to use monochromatic light.

is above 25° and subtracted if the temperature is below that point. It is important that all readings of water-jacket temperatures be made to the nearest 0.1°.

This solution should keep for a considerable period of time without perceptible change in refractive index, but it is advisable to check it periodically. The solution should be kept in a dark bottle provided with a glass stopper or molded plastic screw cap and should be kept away from direct sunlight.

ANALYTICAL PROCEDURE

(1) Obtain a representative sample of about 50 gm. of the soybeans either by hand-quartering or by use of a mechanical sampling device.

(2) Grind the beans as fine as practicable with the equipment available. The experimental roller flour mill described above is recommended for this purpose. It is usually best to crack the beans in a coarse mill before grinding. Follow the procedure outlined in step (3) of the extraction method (p. 2).

(3) Weigh out accurately 2 gm. of the well-mixed meal and transfer the weighed sample to a 3-inch porcelain mortar that has been warmed to about 60° C.

(4) Add about 1.5 gm. of reagent-quality sea sand and exactly 5 ml. of a halowax, α-bromonaphthalene mixture having a refractive index of $n_D^{25}=1.63940\pm2$. Use the utmost care in the measurement of this solution. (It is best accomplished by using an accurately calibrated pipette having a delivery time of not less than 15 seconds.)

(5) Grind the mixture in the mortar vigorously for 3 minutes, constantly scraping into the bottom the particles of meal that are thrown against the sides of the mortar. Vigorous grinding is necessary for complete extraction of the oil. The analyst using the method for the first time should make a number of experimental replicate determinations for the purpose of establishing the proper mortar-grinding technique. A properly ground sample will yield the maximum possible quantity of oil.

(6) Filter the mixture into a test tube through folded filter paper that is of good quality, fat-free, and of sufficiently fine porosity to yield a clear filtrate.

(7) Determine the refractive index of the filtrate at 25° C. to an accuracy of ±0.00002. If the reading is made at any temperature other than 25°, add 0.00043 for each degree above 25°. (It is important that all temperature readings be made to the nearest 0.1°.)

(8) Using table 2, note the percentage of oil corresponding to the refractive-index reading obtained in (7). This is the uncorrected value for oil content.

(9)[4] In a flask shake for a minute or two about 5 gm. of the meal with about 25 ml. of a good grade of low-boiling petroleum ether and filter into a small shallow evaporating dish. Carefully evaporate off the solvent on a steam bath or hot plate at low heat, and place the dish in an oven at 105° C. for 20 minutes. Cool the oil thus prepared to room temperature and determine its refractive index at 25°. (The refractive-index correction for temperature for soybean oil is 0.000364 per 1°, to be added if the temperature at which the reading is taken is above 25°, and subtracted if below that temperature. If preferred, the sample of oil may be prepared by pressing a small sample of the ground seed in a laboratory hydraulic press and filtering the oil so obtained if it is not entirely clear.)

(10)[4] From the refractive index of the oil as determined in (9), subtract the value 1.4730 (refractive index at 25° C. of the composite sample of soybean oil used in obtaining data for the conversion table). Using this difference, determine from table 3 the correction to be applied to the uncorrected value for oil content as determined in (8). If the difference is positive, add the correction; if negative, subtract it.

RELIABILITY OF THE REFRACTOMETRIC METHOD

To check the reliability of the refractometric method for determining oil content in soybeans, 76 samples of soybeans were analyzed for oil content by both the refractometric method and the petroleum-ether extraction method. This series of samples ranged in oil content from 15.39 percent to 23.91 percent on a dry-matter basis and included 42 individual varieties of soybeans grown in different parts of the country as well as samples of commercial soybeans from the 1937 and 1938 crops. A few samples of split-soybean separations also were included in the series. The results of these analyses are listed in table 4 in the order of increasing oil content. The average oil content for the entire series as determined by the two methods differed by only 0.03 percent of oil, and the average of the individual discrepancies was 0.18 percent of oil. Thus the results obtained by the refractometric method are in good agreement with those obtained by the petroleum-ether extraction method.

[4] At the present time (1940) steps 9 and 10 may be omitted in the analysis of the general run of commercial yellow soybeans, as they add very little to the accuracy of the determination. However, with the probable advent in the near future of new commercial varieties, some with oil of a much higher iodine number and others with oil of a much lower number than the present commercial varieties, the correction described in steps 9 and 10 may become quite important.

TABLE 4.—*Comparison of the oil content (dry-matter basis) of 76 samples of soybeans as determined by the petroleum-ether extraction method and by the refractometric method*

Sample designation	Description and source of samples	Oil content by method—		Difference B—A
		A, petroleum ether extraction	B, refractometric	
		Percent	*Percent*	*Percent*
A–5	Laredo	15.39	15.23	−0.16
B–19	Laredo, 1937; Monetta, S. C	15.47	15.17	−.30
B–14	Wilson-Five, 1935; Holgate, Ohio	15.99	15.99	.00
B–4	Haberlandt	16.20	16.20	.00
B–21	Monetta, 1937; Monetta, S. C	16.38	16.36	−.02
V–49	Laredo	16.60	16.18	−.42
V–46	Medium green	16.68	16.64	−.04
B–34	Peking, 1936; Ohio	16.69	17.07	+.38
A–4	Monetta	16.76	16.39	−.37
85A	Whole sound soybeans only	16.95	17.02	+.07
85	Commercial, 1938	17.19	17.26	+.07
B–42	Avoyelles, 1937; Monetta, S. C	17.33	17.09	−.24
A–6	Mammoth yellow	17.39	17.18	−.21
C–44	Commercial, 1937	17.48	17.60	+.12
B–41	Biloxi, 1937; Monetta, S. C	17.64	17.47	−.17
V–50	Otootan	17.65	17.57	−.08
V–47	Biloxi	17.73	17.46	−.27
B–5	Mammoth yellow	17.73	17.58	−.15
B–1	Virginia	17.94	18.13	+.19
B–36	Otootan, 1937; Monetta, S. C	18.01	18.03	+.02
B–37	Georgian, 1937; Monetta, S. C	18.05	18.04	−.01
B–3	Easycook	18.15	18.00	−.15
B–38	Creole, 1937; Monetta, S. C	18.19	18.19	.00
B–20	Cayuga, 1937; Ithaca, N. Y	18.42	18.09	−.33
B–10	Tarheel, 1937; North Carolina	18.51	18.44	−.07
B–6	Tokyo	18.57	18.61	+.04
B–11	Mandarin, 1937; Ames, Iowa	18.79	18.81	+.02
B–29	Palmetto, 1936; Monetta, S. C	18.80	18.38	−.42
C–47	Commercial, 1937	18.82	18.82	.00
B–35	Mandarin, 1936; Ohio	18.94	19.17	+.23
B–27	White Biloxi, 1936; Monetta, S. C	18.97	18.97	.00
B–8	Spooner Mandarin, 1937; Spooner, Wis	19.03	19.27	+.24
B–40	Charlee, 1937; Monetta, S. C	19.06	19.12	+.06
B–2	Hollybrook	19.17	19.23	+.06
V–45	Tarheel	19.17	19.39	+.22
V–48	Virginia	19.41	19.21	−.20
B–26	Mamloxi, 1937; Stoneville, Miss	19.50	19.38	−.12
B–16	Mukden, 1936; Iowa	19.70	20.00	+.30
B–15	Mandell, 1936; Illinois	19.86	20.10	+.24
B–28	Mamredo, 1937; Stoneville, Miss	19.94	19.64	−.30
A–2	Commercial, 1937	20.12	19.95	−.17
B–30	Hayseed, 1937; Monetta, S. C	20.13	19.95	−.18
C–43	Commercial, 1937	20.17	20.41	+.24
B–25	Delsta, 1937; Stoneville, Miss	20.21	19.90	−.31
C–48	Commercial, 1937	20.22	20.41	+.19
B–18	Missoy, 1937; West Point, Miss	20.24	20.47	+.23
B–9	Harbinsoy, 1937; Urbana, Ill	20.26	20.32	+.06
B–13	Habaro, 1937; North Dakota	20.29	20.47	+.18
84A	Whole sound soybeans only	20.43	20.48	+.05
86A	do	20.48	20.67	+.19
B–24	Wisconsin Early Black, 1937; Ames, Iowa	20.50	20.51	+.01
B–23	Manchuria (13177), 1935; Holgate, Ohio	20.57	20.54	−.03
B–17	Macoupin, 1935; Holgate, Ohio	20.64	20.94	+.30
83A	Whole sound soybeans only	20.73	20.46	−.27
86	Commercial, 1938	20.76	20.76	.00
B–22	Scioto, 1936; Scioto, Ohio	20.79	20.99	+.20
84	Commercial, 1938	20.82	20.59	−.23
10A	Whole sound soybeans only	21.03	20.87	−.16
8A	do	21.14	21.84	+.70
B–7	Minsoy, 1937; Ames, Iowa	21.24	21.07	−.17
8	Commercial, 1938	21.31	21.73	+.42
B–31	Illini, 1936; Ohio	21.38	21.08	−.30
B–39	A. K., 1936; Arlington, Va	21.43	21.26	−.17
A–1	Commercial, 1937	21.51	21.22	−.29
A–3	do	21.74	21.50	−.24
B–32	Dunfield, 1936; Ohio	21.77	22.02	+.25
D–53	Commercial, 1937	21.80	21.78	−.02
E–58	do	21.83	21.45	−.38
B–12	Hudson Manchu, 1937; Vermont	21.89	21.86	−.03
84B	Split soybeans only	21.90	21.58	−.32
C–49	Commercial, 1937	22.07	22.08	+.01
86B	Split soybeans only	22.19	22.29	+.10
83B	do	22.23	22.07	−.16
82D	Small, through 19/64-inch sieve	22.26	22.11	−.15
8B	Split soybeans only	22.95	23.21	+.26
B–33	Manchu, 1936; Ohio	23.91	23.75	−.16
	Average	19.49	19.46	[1] 0.18

[1] Without regard to sign.

REFRACTOMETRIC DETERMINATION OF IODINE NUMBER

History of the Method

It has long been known that a positive correlation exists between the refractive index and the iodine number of animal and vegetable oils in general. Lewkowitsch (*29, v. 1, p. 338*), however, after accumulating data on a large number of different oils, concluded that no definite relationship existed between these two factors. Niegemann and Kayser (*32*), on the other hand, reported such a relationship in the case of oils from flaxseed samples grown in a given region. Arnold (*1*) and Backer (*3*) have demonstrated a relationship between iodine number, saponification number, and refractive index.

Pickering and Cowlishaw (*33*) have developed the following mathematical equation to show the relationship between refractive index, iodine number, saponification number, and acid number:

$$n_D^{40}=1.4643-0.000066S-\frac{0.0096A}{S}+0.000117I$$

where S=saponification number
A=acid number
I=iodine number

This equation was shown to apply to freshly prepared fats or oils from various kinds of oil-bearing seeds. The relationship does not hold, however, for oils that have been prepared for an appreciable length of time, since the oxidation and polymerization that take place when the oil stands cause a marked increase in the refractive index.

Zeleny and Coleman (*44, 45*) determined refractive indices and iodine numbers (Wijs) on freshly prepared oils from 96 samples of flaxseed covering a range in iodine numbers from 155.4 to 197.3. The coefficient of correlation between refractive index and iodine number was found to be +0.9965 with a standard error of prediction for the iodine number of ±0.82. The regression equation was expressed as:

$$I=8584.966\ n_D^{25}-12513.827$$

Hopper and Nesbitt (*12*) and Lehberg and Geddes (*18*) have since reported similar studies on a much larger series of flaxseed samples and have obtained the following regression equations:

$$n_D^{25}=1.45723+0.00011846\ I \text{ (Hopper and Nesbitt)}$$

$$I=266.18-1.7980\ Z \text{ (Lehberg and Geddes)}$$

where I=iodine number (Wijs)
and Z=Zeiss refractometric-scale reading at 25° C.

The results obtained by these three different laboratories working independently are in good agreement, thus indicating that the refractive index may be used, under properly controlled conditions, as a reliable measure of the iodine number of flaxseed oils.

More recently Illarionov and Torchinskiĭ (*14*) have reported a relationship between refractive index and iodine number derived from a study of 24 different animal and vegetable oils free from oxyacids, cyclic acids, and conjugated double bonds. This relationship is expressed by the equation:

$$n_D^{20}=1.4595+0.000118\ I$$

Maruta and Teruyama (*31*) found the following relationships between iodine number and refractive index in partially hydrogenated soybean oils:

$$n_D^{50}=1.4484+0.000118\ I \text{ (for iodine numbers greater than 85)}$$

and $n_D^{50}=1.4494+0.000103\ I$ (for iodine numbers less than 85)

Majors and Milner (*30*) have determined refractive indices and iodine numbers on freshly prepared oils from numerous varieties of soybeans in connection with agronomic experiments at the United States Regional Soybean Industrial Products Laboratory. Using their data from soybeans of the 1937 and 1938 crops, combined with the data obtained by Hopper and Nesbitt (*12*) on 1,485 samples of

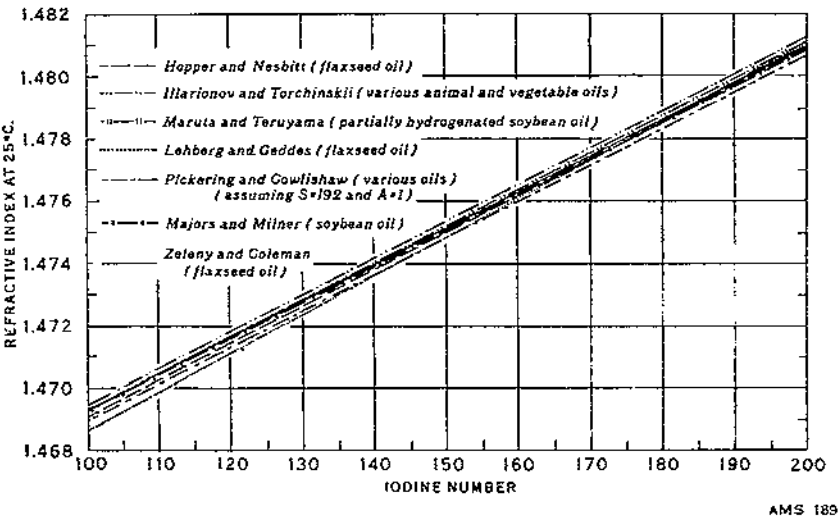


FIGURE 5.—The relationship between iodine number and refractive index of various fatty oils as determined by seven investigators. The curves are extrapolated beyond the range investigated in most cases.

flaxseed, they derived the following regression equation when iodine number was considered as the dependent variable:

$$I=8626.877\ n_D^{25}-12575.226$$

The regression lines for estimating iodine number in the range between 100 and 200 from refractive index as determined in seven laboratories are shown in figure 5.

The lines shown are in most cases extrapolated beyond the range of values actually used in calculating the regression equations, but serve to show a reasonably good agreement among different investigators even though different oils were used.

APPLICATION OF THE METHOD TO SOYBEANS

Refractive indices and iodine numbers (Wijs) were determined for the oils from 140 samples of soybeans covering a range in iodine

numbers of from 116.5 to 142.7. The oils were obtained by petroleum ether extraction, Soxhlet extractors being used. The last traces of solvent were removed from the oils in all cases by heating them in the extraction flasks in the vacuum oven at 100° C. for 30 minutes. Iodine numbers were determined according to the official Wijs method of the Association of Official Agricultural Chemists (*2, p. 412*). The iodine numbers and refractive indices are listed in columns 2 and 3 of table 6. The correlation coefficient obtained between these 2 sets of values is +0.963 with a standard error of estimate for iodine number of 0.87 units.

Of the 140 samples of oil included in this investigation all but 9 fell within the relatively narrow iodine number range between 129 and 138. With such a distribution of samples it is not possible to obtain a wholly reliable regression equation for predicting iodine

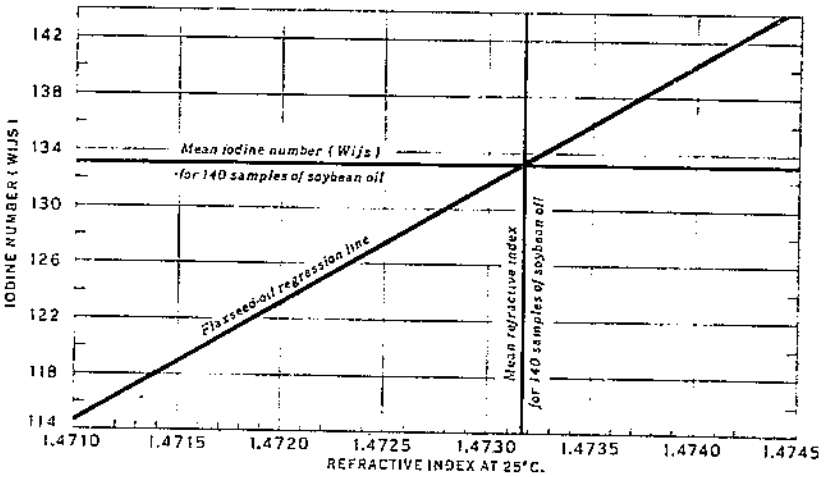


FIGURE 6.—Flaxseed-oil regression line in relation to mean values for soybean-oil iodine numbers and refractive indices.

numbers of oils falling considerably outside this range. In the flaxseed investigation of Zeleny and Coleman (*45*) previously referred to the regression equation was based on samples fairly well distributed over a relatively wide range in iodine numbers (155.4 to 197.5). The slope of the regression line in this case, therefore, was rather accurately established.

Considerable evidence exists to indicate that the relationship between the refractive index and iodine number of freshly prepared vegetable oils is essentially identical for all or most oils that contain only traces of unsaturated fatty acids other than oleic, linolic, and linolenic acids. Both flaxseed and soybean oils fall in this category, and the mean values for refractive index and iodine number in the series of soybean oils investigated, when plotted on coordinate paper, intersect at a point falling almost exactly on the flaxseed regression line as shown in figure 6. Thus the mean value of 1.47317 for n_D^{45} corresponds to an iodine number of 133.29 calculated from the flaxseed

regression equation, whereas the actual mean value is 133.20. It also may be shown that the average error in estimating iodine number from the flaxseed equation for the 9 soybean-oil samples falling farthest from the mean (those outside the range of 129–138) is only 0.5 unit as compared with an average error of 0.74 unit for the entire series of samples. Furthermore, the plus and minus errors of these 9 extreme samples almost exactly balance; thus the average iodine number of these samples as determined differs by only 0.15 unit from the average iodine number as calculated from the flaxseed regression equation. The same relationship between refractive index and iodine number, therefore, appears to hold for both soybean oil and flaxseed oil. A conversion table (table 5) has been prepared for determining the Wijs iodine numbers of freshly prepared soybean oils from their refractive indices.

Iodine-number values determined refractometrically as compared with the values determined by the conventional Wijs procedure on the series of samples under investigation are shown in table 6.

TABLE 5.—*Conversion table for determining Wijs iodine number of freshly prepared soybean oil from refractive index*

[Data calculated from flaxseed-oil regression equation: $I=8584.966\ n_D^{25}-12513.827$]

n_D^{25}	Iodine number	n_D^{25}	Iodine number	n_D^{25}	Iodine number	n_D^{25}	Iodine number	n_D^{25}	Iodine number
1.4693	100.1	1.4708	112.9	1.4722	125.0	1.4736	137.0	1.4750	149.0
1.4694	100.9	1.4709	113.8	1.4723	125.8	1.4737	137.8	1.4751	149.9
1.4695	101.8	1.4710	114.7	1.4724	126.7	1.4738	138.7	1.4752	150.7
1.4696	102.6	1.4711	115.5	1.4725	127.5	1.4739	139.6	1.4753	151.6
1.4697	103.5	1.4712	116.4	1.4726	128.4	1.4740	140.4	1.4754	152.4
1.4698	104.4	1.4713	117.2	1.4727	129.3	1.4741	141.3	1.4755	153.3
1.4699	105.2	1.4714	118.1	1.4728	130.1	1.4742	142.1	1.4756	154.1
1.4700	106.1	1.4715	119.0	1.4729	131.0	1.4743	143.0	1.4757	155.0
1.4701	106.9	1.4716	119.8	1.4730	131.8	1.4744	143.8	1.4758	155.9
1.4702	107.8	1.4717	120.7	1.4731	132.7	1.4745	144.7	1.4759	156.7
1.4703	108.6	1.4718	121.6	1.4732	133.5	1.4746	145.6	1.4760	157.6
1.4704	109.5	1.4719	122.4	1.4733	134.4	1.4747	146.4	1.4761	158.4
1.4705	110.4	1.4720	123.2	1.4734	135.3	1.4748	147.3	1.4762	159.3
1.4706	111.2	1.4721	124.1	1.4735	136.1	1.4749	148.1	1.4763	160.2
1.4707	112.1								

TABLE 6.—*Iodine number (Wijs) and refractive index of oils from 140 samples of soybeans*

Sample designation	Iodine number (Wijs) (A)	n_D^{25}	Iodine number from refractive index (B)	Difference, B—A	Sample designation	Iodine number (Wijs) (A)	n_D^{25}	Iodine number from refractive index (B)	Difference, B—A
803	116.5	1.47127	117.0	+0.5	7-C-7	130.7	1.47289	130.9	+0.2
700	118.3	1.47132	117.4	−.9	7-C-25	130.7	1.47303	132.1	+1.4
166	119.6	1.47151	119.1	−.5	7-C-30	130.8	1.47273	129.5	−1.3
206-Z 1	127.4	1.47247	127.3	−.1	8-Ark-17B	130.8	1.47307	132.4	+1.6
8-Ind-13B	127.9	1.47254	127.9	.0	8-Ark-17A	130.9	1.47302	132.0	+1.1
8-Ind-13	129.2	1.47264	128.8	−.4	7-C-29	131.2	1.47296	131.5	+.3
7-C-8	129.6	1.47270	129.3	−.3	7-C-33	131.3	1.47289	130.9	−.4
7-C-11	129.9	1.47268	128.2	−1.7	7-C-42	131.5	1.47276	129.8	−1.7
7-C-34	129.9	1.47287	130.7	+.8	7-C-4	131.7	1.47293	131.2	−.5
7-C-35	129.9	1.47275	129.7	−.2	7-C-9	131.7	1.47288	130.8	−.9
7-C-26	130.1	1.47273	129.5	−.6	7-KC-41	131.7	1.47293	131.2	−.5
7-KC-38	130.2	1.47290	131.0	+.8	8-Ind-16B	131.7	1.47310	132.7	+1.0
8-Ind-13A	130.2	1.47278	129.0	−.3	7-C-5	131.8	1.47301	131.9	+.1
7-C-28	130.4	1.47287	130.7	+.3	7-C-16	131.8	1.47297	131.6	−.2
7-C-15	130.6	1.47290	131.0	+.4	BPI-48	131.9	1.47308	132.5	+.6

[1] Brown soybeans.

TABLE 6.—*Iodine number (Wijs) and refractive index of oils from 140 samples of soybeans*—Continued

Sample designation	Iodine number (Wijs) (A)	n_D^{25}	Iodine number from refractive index (B)	Difference, B−A	Sample designation	Iodine number (Wijs) (A)	n_D^{25}	Iodine number from refractive index (B)	Difference, B−A
S-Ark-17	131.9	1.47289	130.9	−1.0	S-S-2A	133.3	1.47320	133.5	−0.3
7-C-19	132.0	1.47309	132.6	+.6	S-Ind-11A	133.8	1.47326	134.0	+.2
7-C-32	132.0	1.47292	131.2	−.8	S-Ind-15B	133.8	1.47317	133.3	−.5
7-KC-37	132.2	1.47299	131.7	−.5	S-In-24B	133.8	1.47324	133.9	+.1
S-Ind-12A	132.3	1.47306	132.3	.0	S-S-2	133.9	1.47318	133.3	−.6
7-C-6	132.4	1.47296	131.5	−.9	S-O-7	133.9	1.47332	134.6	+.7
7-C-18	132.4	1.47305	132.3	−.1	S-O-7B	133.9	1.47346	135.8	+1.9
7-C-24	132.4	1.47305	132.3	−.1	S-Ind-16	133.9	1.47312	132.9	−1.0
S-O-2B	132.4	1.47302	132.0	−.4	S-In-23B	133.9	1.47333	134.7	+.8
S-O-8	132.4	1.47330	135.2	+2.8	S-In-24	133.9	1.47333	134.6	+.7
S-O-2	132.5	1.47325	134.0	+1.5	7-C-21	134.0	1.47313	132.9	−1.1
S-O-8B	132.5	1.47321	133.6	+1.1	S-O-7A	134.0	1.47334	134.8	+.8
S-Mo-18B	132.5	1.47311	132.8	+.3	BPI-25	134.1	1.47320	133.6	−.5
S-Mo-19B	132.5	1.47313	132.9	+.4	S-Ind-11	134.2	1.47330	134.4	+.2
S-In-22	132.5	1.47334	134.8	+2.3	S-Ind-15A	134.2	1.47320	133.5	−.7
7-C-3	132.6	1.47305	132.3	−.3	205-Z [1]	134.3	1.47328	134.2	−.1
7-C-23	132.6	1.47314	133.0	+.4	207-Z [1]	134.3	1.47335	134.9	+.6
S-O-3B	132.6	1.47322	133.7	+1.1	S-O-6B	134.3	1.47330	134.4	+.1
5223	132.7	1.47312	132.9	+.2	S-In-21	134.4	1.47337	135.0	+.6
BPI-5	132.7	1.47321	133.6	+.9	S-Ind-15	134.5	1.47320	133.5	−1.0
S-O-3	132.7	1.47336	134.9	+2.2	S-Ind-21A	134.5	1.47341	135.4	+.9
S-O-8A	132.7	1.47328	134.3	+1.6	S-Chi-25G	134.6	1.47343	135.5	+.9
BPI-45	132.8	1.47307	132.4	−.4	5193	134.7	1.47334	134.7	.0
7-C-2, 2C	132.8	1.47304	132.2	−.6	S-In-23A	134.7	1.47341	135.4	+.7
7-C-13	132.8	1.47297	131.6	−1.2	S-O-9B	134.8	1.47344	135.7	+.9
7-C-22	132.8	1.47317	133.3	+.5	S-O-4	134.9	1.47338	135.1	+.2
S-O-2A	132.8	1.47327	134.1	+1.3	S-O-4A	134.9	1.47355	136.6	+1.7
S-Ind-12	132.8	1.47309	132.6	−.2	S-In-24A	134.9	1.47338	135.0	+.1
S-In-22A	132.8	1.47332	134.6	+1.8	S-O-5	135.1	1.47339	135.2	+.1
BPI-30	133.0	1.47322	133.8	+.8	S-Ind-14	135.2	1.47329	134.3	−.9
7-C-36	133.0	1.47321	133.6	+.6	S-Ind-14A	135.4	1.47327	134.1	−1.3
S-S-1B	133.0	1.47317	133.3	+.3	S-NY-26	135.6	1.47362	137.2	+1.6
S-S-2B	133.0	1.47310	132.7	−.3	S-O-9A	135.7	1.47351	136.2	+.5
S-Mo-18	133.0	1.47311	132.8	−.2	S-In-23	135.7	1.47339	135.2	−.5
7-C-2, 2D	133.1	1.47317	133.3	+.2	S-Chi-25	135.7	1.47329	134.3	−1.4
S-Mo-19A	133.1	1.47318	133.2	+.1	S-Ind-14B	135.8	1.47315	133.1	−2.7
BPI-4	133.2	1.47332	134.6	+1.4	S-Chi-25B	135.8	1.47329	134.3	−1.5
7-C-2, D	133.2	1.47310	132.7	−.5	S-O-9	136.0	1.47362	137.2	+1.2
S-O-1B	133.2	1.47324	133.9	+1.7	S-O-1	136.5	1.47373	138.1	+1.6
S-O-5A	133.2	1.47311	132.8	−.4	S-Chi-20B	136.5	1.47358	136.8	+.3
S-O-6	133.3	1.47328	134.2	+.9	BPI-41	136.6	1.47358	136.8	+.2
S-Ind-16A	133.3	1.47312	132.9	−.4	S-Chi-20A	136.6	1.47362	137.2	+.6
S-In-21B	133.3	1.47320	133.5	+.2	S-Chi-25A	136.8	1.47338	135.0	−1.8
7-C-17	133.4	1.47303	132.1	−1.3	BPI-6	136.9	1.47363	137.2	+.3
7-C-20	133.4	1.47311	132.8	−.6	S-O-10B	136.9	1.47356	136.6	−.3
S-O-3A	133.5	1.47337	135.0	+1.5	S-O-1A	137.0	1.47373	138.1	+1.1
S-Mo-18A	133.5	1.47316	133.2	−.3	S-O-10	137.2	1.47371	137.9	+.7
7-C-2, C	133.6	1.47306	132.3	−1.3	S-O-10A	137.3	1.47366	137.5	+.2
S-O-6A	133.6	1.47321	133.6	.0	BPI-47	137.4	1.47356	136.6	−.8
S-S-1A	133.7	1.47329	134.3	+.6	BPI-40	137.7	1.47375	138.2	+.5
S-Mo-19	133.7	1.47322	133.7	.0	BPI-42	139.5	1.47399	140.3	+.8
S-In-22B	133.7	1.47327	134.1	+.4	44	140.2	1.47395	140.0	−.2
7-C-12	133.8	1.47317	133.3	−.5	BPI-46	142.5	1.47425	142.6	+.1
7-C-14	133.8	1.47305	132.3	−1.5	BPI-3	142.7	1.47415	141.7	−1.0
7-C-18	133.8	1.47317	133.3	−.5	Average	133.2	1.47317	133.3	
S-S-1	133.8	1.47344	135.7	+1.9					

[1] Black soybeans.

To determine the effect the method of preparing the oil sample has on its refractive index, oils were prepared from four samples of soybeans by each of the following methods:

(1) COLD PRESSING.—The freshly ground soybeans were pressed at room temperature in a laboratory hydraulic press at a pressure of about 20,000 pounds per square inch.

(2) HOT PRESSING.—Pressing was accomplished in the same manner as above except that the plates of the press were heated to 110° C.

(3) RAPID PARTIAL EXTRACTION.—About 5 gm. of the freshly ground soybeans were shaken with about 25 ml. of petroleum ether for a minute or two. The mixture was filtered, the solvent evaporated, and the extracted oil heated in a shallow dish for 20 minutes at 105° C.

(4) COMPLETE EXTRACTION.—Extraction was accomplished by the method described on page 2, using Soxhlet extractors.

The refractive indices of the oil samples prepared by these four methods are listed in table 7. There appears to be a slight tendency for the refractive index (and therefore presumably the iodine number) to increase with increasingly complete extraction of the oil. The differences observed among the four methods of extraction investigated, however, are small and are of little practical significance.

TABLE 7.—*Refractive indices at 25° C. of oils prepared by four different methods from four samples of soybeans*

Soybean sample designation	Cold-pressed oil	Hot-pressed oil	Oil by rapid partial extraction	Completely extracted oil
	n_D^{25}	n_D^{25}	n_D^{25}	n_D^{25}
S-III-58	1.47290	1.47287	1.47295	1.47307
S-III-58 A	1.47287	1.47290	1.47293	1.47290
S-O-61 A	1.47308	1.47314	1.47317	1.47325
S-NO-62 A	1.47298	1.47307	1.47301	1.47306
Average	1.47296	1.47300	1.47302	1.47307

When the oil content of soybeans has been determined by the refractometric method (p. 8) including the correction for actual refractive index of the oil, no additional labor is required to determine the iodine number, since the refractive index of the oil itself has been determined and may be converted into iodine number by using table 5. In case the iodine number of the oil is to be determined independently the following procedure may be used.

PROCEDURE FOR DETERMINING IODINE NUMBER REFRACTOMETRICALLY

The procedure for determining iodine number refractometrically is as follows:

(1) Grind a representative sample of the clean soybeans with a suitable type of mill.

(2) Prepare a small sample of oil from the freshly ground seed by one of the methods listed on pages 17 and 18.

(3) Determine the refractive index of the freshly prepared oil at 25° C. If the reading is taken at any other temperature, add 0.000364 for each 1° above 25° and subtract that value for each 1° below that temperature.

(4) Convert the refractive index value into iodine number (Wijs) by using table 5.

LIMITATIONS OF THE METHOD

It should be distinctly understood that the refractometric method for determining the iodine number is intended to apply only to samples of oil freshly prepared from essentially sound soybeans in such a way that no significant amount of hydrolysis, oxidation, or polymerization has occurred, and no solvent residues remain in the oil. The method is not entirely reliable when applied to oils from soy-

bean samples containing high percentages of damaged or split beans, or which are more than 1 year old.

The method is not intended for the direct determination of the iodine number of commercial soybean oils since the processing the oil undergoes tends to alter its refractive index.

The principal value of the method should be for determining in advance the iodine number of the oil that a given lot of soybeans will produce. The method should be of considerable value to the soybean processor because determinations can be made in a small fraction of the time required for the conventional iodine-number determinations, and because the use of high-priced chemical reagents is eliminated.

The method should also be useful in connection with plant-breeding work, since the determination requires only 1 or 2 drops of oil and is thus applicable to very small samples of seed.

SPECIAL PRECAUTIONS IN USING THE REFRACTOMETER

The refractometer should be carefully checked for accuracy before being used. For this purpose auxiliary testing prisms of known refractive index are generally supplied with the instrument. If possible, several such auxiliary prisms should be used with the instrument to insure accuracy in all parts of the range. If errors are detected that cannot be eliminated by readjustment of the instrument, a correction table should be prepared. It is a good practice to have the instrument periodically inspected and checked by someone who is thoroughly familiar with optical instruments.

Before the instrument is read, sufficient time must be allowed for the liquid to acquire the temperature of the prisms. The reading will remain constant only when this equilibrium is established.

Clean the faces of the prisms thoroughly between determinations. This is best accomplished by wiping with dry absorbent cotton, then with cotton dipped in ethyl alcohol, and finally again with dry cotton. Solutions containing the halowax, α-bromonaphthalene solvent should not be left on the prism longer than necessary to make the reading, since in some refractometers the cement holding the prisms in place is corroded by this solvent.

SUMMARY

Oil content is an important factor in determining the intrinsic commercial value of soybeans, and is subject to relatively wide variations. Iodine number, another important quality factor, does not at present (1940) vary widely in the commercial crop, but with the probable advent of new commercial varieties bred for high and for low iodine numbers to meet the demands for specific industrial uses this factor will probably assume greater commercial importance.

Methods now in common use for determining oil content and iodine number are too time-consuming for commercial inspection procedures. The rapid refractometric methods previously developed for the routine determination of both these factors in flaxseed have therefore been adapted to the anaylsis of soybeans. These methods have been compared with the fundamental methods for determining

oil content and iodine number, and have been found to give reliable results in a much shorter time than that required by the conventional procedures.

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