A Simple Iodine-Number Refractometer for Testing Flaxseed and Soybeans[1]

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IODINE number of the oil is one of the most important indexes of the intrinsic commercial value of flaxseed and, to a lesser extent, soybeans. Linseed and soybean oils of high iodine number are preferred for paint and varnish manufacture because of their superior drying properties; and soybean oil of low iodine number is preferred for food purposes, particularly when the oil is not hydrogenated, because of its greater stability. The iodine number of linseed oil varies over a wide range, depending partly upon the flaxseed variety and, to a greater extent, upon the climatic conditions under which the flaxseed is grown. The normal range in linseed-oil iodine number is from about 150 to about 200 although flaxseed grown under exceptionally hot and dry climatic conditions has been known to contain oils with iodine numbers as low as 132 and flaxseed grown experimentally in Alaska has shown oil iodine numbers of as high as 212.

Soybean-oil iodine numbers usually fall within the comparatively narrow range of 125 to 145. Efforts may eventually be made however to breed soybeans having oils of higher iodine number to meet the needs of the paint and varnish industries, and of lower iodine numbers for edible purposes. It is anticipated therefore that iodine number may become an increasingly important factor in the evaluation of soybeans.

Refractometric Determination

Arnold (1) appears to be the first investigator to have shown a relationship between the refractive index and iodine number of oils. His observations were later confirmed by Backer (2). Niegemann and Kayser (6) found a relationship between refractive index and iodine number in the case of flaxseed samples grown in a given region, and Pickering and Cowlishaw (7) found a complex relationship to exist between the refractive index, iodine number, saponification number, and acid number of various prepared vegetable oils.

The practicability of using the refractive index of a freshly prepared vegetable oil from sound plant material as a relatively accurate measure of its iodine number was first demonstrated by Zeleny and Coleman (8) working with flaxseed. This work was very shortly confirmed by the work of Hopper and Nesbitt (4) and of Lehberg and Geddes (5). The refractometric method for determining oil iodine numbers was later applied to soybeans by Zeleny and Neustadt (10), who found that the relationship between refractive index and iodine number appeared to be identical for the freshly prepared oils of both flaxseed and soybeans. Some evidence exists to indicate that the relationship between the refractive index and the iodine number of freshly prepared vegetable oils is essentially identical for all or most oils that contain not more than traces of glycerides of unsaturated fatty acids other than oleic, linoleic, and linolenic acids.

The refractometric method for determining the iodine numbers of the oils in flaxseed and soybeans is now rather commonly used by processors because of its convenience and the rapidity with which tests can be made. Determinations may be made with freshly extracted oils from which all traces of solvent have been removed or, more simply, with oils freshly expressed from the whole or freshly ground seed by means of a small laboratory-type hydraulic press. Only a few drops of oil are needed. For accurate work the refractive index of the oil should be determined to an accuracy of $\pm$ 0.00002. The refractive index is commonly determined at 25.0°C., either by controlling the temperature of the refractometer prisms to that temperature or by determining accurately the actual temperature of the prisms and correcting the value obtained to a temperature of 25.0°C. The refractive index temperature correction factor for flaxseed oil is 0.000357 and for soybean oil 0.000364 per degree C. These corrections are added when refractive index readings are taken at temperatures above 25.0°C. and subtracted when readings are taken below that temperature. The iodine number may then be calculated from the refractive index by means of the equation:

$$\text{Iodine number (Wijs)} = 8584.97\, n_D^{25} - 12513.83$$

or by means of the published conversion tables for flaxseed oil (8, 9) and soybean oil (10).

Development of the Refractometer

For routine use in the testing of flaxseed and soybeans for oil iodine number, a simple, relatively inexpensive, direct-reading refractometer that could be operated by anyone with a minimum of instruction would have many obvious advantages over the rather complex and very expensive, precision refractometers usually required for iodine-number determinations. Small compact "hand" refractometers that have found widespread application for certain industrial purposes have been available for some time (U. S. Patent 2,319,889). These instruments however cover a rather wide range in refractive index and have a sensibility lower than that required for the determination of iodine number.

It seemed completely feasible that a compact, portable, hand-held refractometer of adequate sensibility could be developed which would:

a) Cover the range 1.4693 to 1.4817 in refractive index, this range being equivalent to iodine numbers of 100.1 to 206.5;
b) Have an accuracy of at least $\pm$ 0.0001 in terms of refractive index, this being equivalent to less than $\pm$ 1.0 in iodine number;
c) Have a scale designed to read directly in terms of iodine number; and
d) Have a thermometer imbedded in the body of the instrument as close as possible to the prism and calibrated to read directly in iodine-number corrections.

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[1] This study was made under authority of the Research and Marketing Act of 1946. (Presented at the spring meeting, American Oil Chemists' Society, May 1-3, 1950, Atlanta, Ga.)

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The possibility of developing such an instrument was discussed in detail with engineers of an optical instrument manufacturing company. The idea was considered practicable, and such an instrument was designed and developed (Figures 1 and 2).

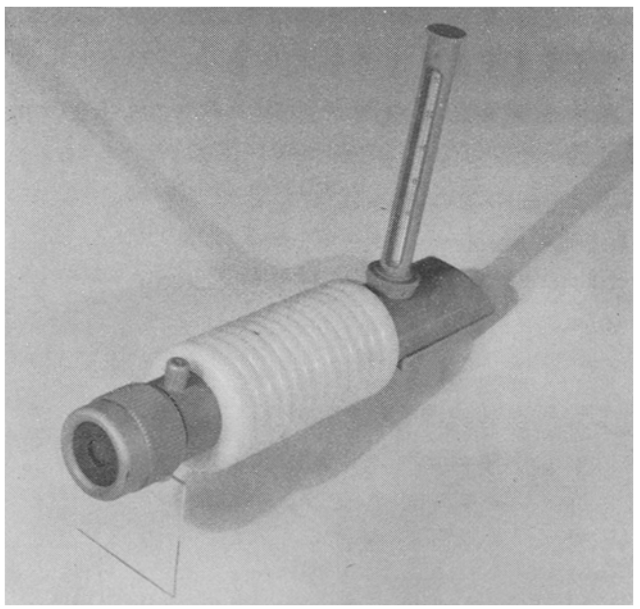

FIG. 1. Iodine-number refractometer.

This instrument is a simple, yet accurate hand refractometer which reads directly in iodine-number units. It is a modern adaptation of the Abbe principle. The oil sample is placed upon the prism face and is illuminated by any light—daylight or Mazda light—through a small port at the front of the instrument. This light is reflected at the interface between the oil film and prism. Following the laws of optics, the angle of this reflected light cannot be less than the so-called *critical angle* (3).

The critical angle of total reflection is that at which total reflection occurs when a beam of light passes from a denser to a lighter medium; in this case, when a beam of light passes from the prism to the oil film. Thus when this beam of light, which has been reflected, passes into a telescope, there will be angular directions, less than the critical angle, from which no light can come, and in the objective field of the telescope there will be seen a sharp line of demarcation between illuminated and unilluminated parts of the field. The position of this borderline is directly related to the refractive index of the sample, in this case, the oil film. Accordingly, a scale (Figure 3) is placed in the focal plane of the objective lens of the telescope. Since the relationship between refractive index and iodine number has been established, the scale is made to read iodine number directly without recourse to conversion tables.

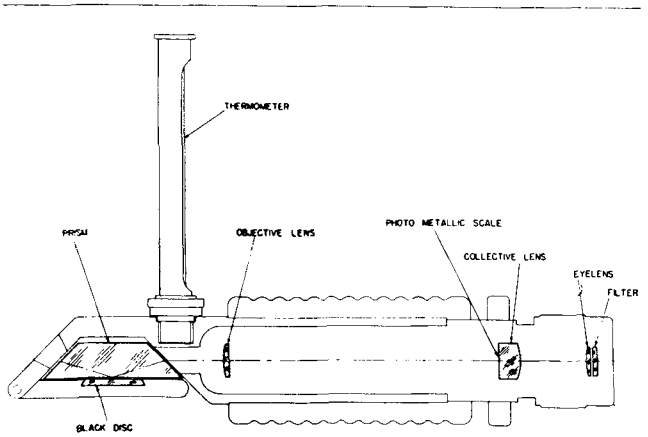

FIG. 2. Diagrammatic sketch showing principal functional parts of iodine-number refractometer.

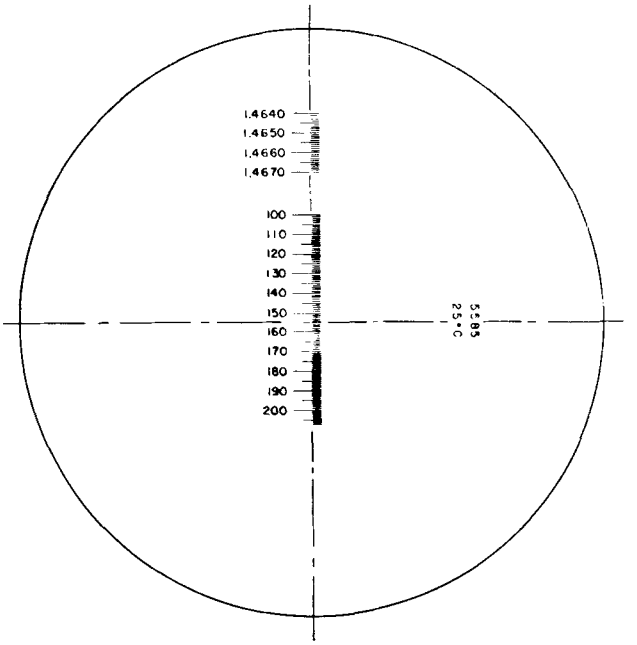

FIG. 3. Visual field of iodine-number refractometer.

If the source of light illuminating the refractometer prism is "white light" in which radiation of all visible wave lengths is present, the line of demarcation—the borderline—will be drawn out into a spectrum from red through orange, yellow, green, and blue since all refractive materials exhibit a refractive index that is different for each wave length. If a correcting element like an Amici compensator prism, as used on Abbe refractometers, is introduced into the dispersed beam, it can be adjusted to return all colors to parallel, angular paths, and the borderline will again be sharp and devoid of color.

In the iodine-number refractometer (Figure 2) an Amici compensator is not used. Instead the back angle is so chosen that practically all dispersion caused by soybean or flaxseed oil is compensated. Whatever vestigial dispersion remains is corrected by a filter in the eyepiece. The temperature coefficient of refractive index of these oils is high enough (approximately 0.00036 per degree Centigrade) so that trouble may be encountered because of it unless proper corrective measures are taken. Without thermal insulation the temperature of the instrument, and hence the oil, can be changed by the mere act of handling the instrument. To provide such thermal insulation a shield of nylon covers most of the body tube. Investigation of the properties of nylon has shown that it excels in all of the desired properties: thermal insulation, chemical resistivity, and machinability. This sleeve is undercut on its inner side so that very little contact

exists between it and the body of the instrument and there is a space between the two. The exterior of the sleeve is corrugated to prevent slippage from the hand.

The instrument does not rely upon temperature control since an attempt to control temperature would remove the desired feature of portability. Instead of attempting to control the temperature a special thermometer is imbedded in the prism housing in as close contact as possible with the prism and the bulb is inserted in a bed of copper foil. The thermometer is scaled in iodine number corrections instead of degrees of temperature.

The first experimental model of this instrument was tested with 89 samples of oil, consisting of flaxseed oil, soybean oil, and various mixtures of flaxseed and soybean oils, covering a relatively wide range in iodine numbers. The readings were compared with the corresponding iodine numbers calculated from the refractive indices on the same samples, obtained with a precision refractometer that had been carefully checked for accuracy. The mean deviation between the results obtained with the two instruments was 0.28 iodine-number units and the maximum deviation was 0.7 units. Thus the experimental hand refractometer was shown to be in satisfactory agreement with the precision refractometer. Readings with the hand refractometer were made using different sources of light, including daylight, fluorescent light, and light from various types of incandescent bulbs. All types of light used were found to be equally effective, provided that proper light intensities were used.

Three of the first production lot of these hand refractometers were also tested in comparison with the precision refractometer. All three instruments were found to be satisfactorily accurate. The results obtained are shown in Table I.

The iodine-number scale in the hand refractometer is graduated in whole units but can readily be read to the nearest one-half unit. With a little experience one-tenth iodine-number units may be estimated. Iodine-number readings on a series of oil samples covering a considerable range in iodine numbers were made by 10 persons, most of whom had no previous experience with refractometers. Satisfactory agreement was obtained among the different operators, indicating that no particular skill is required in using the instrument.

From the studies conducted it may be concluded that a person with little technical training, using the new, iodine-number refractometer and a simple, hand-operated laboratory hydraulic press, can determine routinely and with satisfactory accuracy oil iodine numbers of flaxseed or soybeans at the rate of about one test every five minutes, starting with the original unground flaxseed or soybean samples. Such a test therefore would appear to meet the requirements for speed and practicability needed for use in routine testing.

TABLE I

Comparison of Three Hand Refractometers (H38, H56, and H59)[a] With a Five-Place Precision Refractometer (PR)

Oil Sample	Iodine Number, Wijs			
	PR	H38	H56	H59
Soybean	134.9	134.5	135.0	135.5
Soybean	134.9	135.5	135.5	135.0
Soybean	133.9	133.5	133.7	134.0
Soybean	138.2	138.0	138.7	138.5
Soybean	139.8	140.3	140.0	140.0
Soybean	131.2	131.6	132.0	132.3
Soybean	133.4	133.0	133.5	133.5
Soybean	131.7	131.8	131.7	132.0
Soybean	131.4	131.5	131.0	131.2
Soybean	131.2	131.0	131.0	131.0
Soybean	131.2	131.0	131.0	131.5
Soybean	131.2	131.0	131.3	131.3
Soybean	130.0	129.5	130.3	130.0
Soybean	130.9	131.0	131.0	131.0
Soybean	130.9	130.5	130.5	130.8
Linseed	184.2	184.8	183.5	184.0
Linseed	179.2	180.0	180.0	179.3
Linseed	185.5	186.0	186.0	186.0
Linseed	183.7	184.5	185.0	183.8
Linseed	178.7	179.3	179.5	179.0
Linseed	178.4	179.0	178.5	178.5
Linseed	189.4	189.5	189.8	189.0
Linseed	180.8	181.2	181.0	180.5
Linseed	188.4	189.0	189.0	188.0
Mixture	172.3	172.7	172.8	172.0
Mixture	172.3	172.5	172.5	172.8
Mixture	148.7	149.3	149.5	149.5
Mixture	147.9	148.0	148.0	148.0
Mixture	153.0	153.0	153.0	153.0
Mixture	143.0	143.2	143.2	143.5
Mixture	151.2	151.2	151.2	151.5
Mixture	148.2	148.5	148.5	148.5
Mixture	154.2	154.5	154.5	154.5
Mixture	149.3	149.7	149.8	149.7
Average deviation		0.36	0.37	0.29

[a] The hand refractometers were read to the nearest 0.5 iodine number and the thermometer corrections were estimated to the nearest 0.1 iodine number.

Precautions

In testing flaxseed or soybeans for oil iodine number by use of the new iodine-number refractometer, the following precautions should be observed:

a) Since the method appears to be applicable only to relatively sound flaxseed and soybeans, oils from badly damaged, sour, or musty flaxseed or soybeans should be tested chemically by the Wijs method.

b) When flaxseed or soybeans are ground prior to extraction or pressing, the extraction or the pressing of th oil should be started immediately after grinding. Hydrolysis, oxidation, and possibly polymerization of the oil progress rapidly in the ground material. All of these changes cause changes in the refractive index of the oil and alter the normal relationship between refractive index and iodine number.

c) Refractometer readings should be made the same day the oil is extracted or pressed to avoid errors that might result from hydrolysis, oxidation, or polymerization of the oil on standing.

d) If the oils are prepared by solvent extraction, all traces of solvent must be removed before refractometer readings are made. Petroleum solvents having high-boiling-point fractions should not be used because of the difficulty in removing these fractions. Normal hexane or "Skellysolve F" are acceptable solvents for this purpose.

e) Oils to be tested must be clear, and, if necessary, should be filtered. Extracted oils should be clear without additional filtration if proper extraction thimbles are used. Filter pads may be used in hydraulic presses, if necessary, to obtain clear oils.

f) The refractometer should be at the same temperature as the room in which the tests are made, and the room should be maintained at a reasonably uniform temperature. The refractometer should never be operated in a cold draft, as from an open window, or close to a hot radiator or other source of radiant heat.

g) After each reading the refractometer prism should be thoroughly washed with alcohol or petroleum ether to remove the oil. All traces of the solvent should be removed before the next reading is made. Cotton saturated with solvent is suitable for cleaning the prism and dry cotton for removing the solvent.

Summary

A simple, relatively inexpensive refractometer has been developed for use in testing flaxseed and soybeans for oil iodine number. The refractometer reads directly in iodine-number units and the temperature correction thermometer is likewise calibrated to read

directly in iodine-number units. With this refractometer and a simple, hand-operated laboratory hydraulic press, an operator with little technical training can test flaxseed or soybean samples for oil iodine numbers at the rate of about one test every five minutes. It is anticipated that this instrument will prove to be very useful in the routine testing of flaxseed, soybeans, and possibly certain other oil-bearing seeds.

Acknowledgment

The excellent work of J. W. Forrest and H. W. Straat of the Bausch and Lomb Optical Company in the physical design of the iodine-number refractometer is acknowledged.

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Determination of Total Gossypol Pigments in Cottonseed Oils

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THE presence of gossypol and gossypol-like pigments has been indicated (1, 2, 4, 11, 12) in crude cottonseed oils obtained by hydraulic-pressing, screw-pressing, and solvent extraction methods of cottonseed processing. Several gravimetric methods for their determination have been offered (3, 7, 8, 9), all of which require several days for the complete precipitation of dianilino-gossypol. Two spectrophotometric methods have also been reported (1, 10). One, based on the dianilino-gossypol reaction (10), indicates that the reaction product in the case of the gossypol pigments present in hydraulic-pressed oil have an absorption spectra different from the spectra of the reaction products for the pigments in screw-pressed oil and for pure gossypol. This was attributed to a slight modification of the molecular structure of the gossypol by the conditions of hydraulic pressing although no experimental evidence was given. The antimony trichloride reaction applied to alkaline extracts of screw-pressed oils gave reaction products which were not characteristic for gossypol and no reaction product was obtained with alkaline extracts of hydraulic-pressed oils (1).

The recently published method for the determination of free gossypol pigments in cottonseed materials (5) is not applicable to cottonseed oils due to the insolubility of oils in aqueous acetone and isopropanol. A method is now proposed for the application of the *p*-anisidine-gossypol reaction to the analysis of cottonseed oils for total gossypol pigments.

Evidence is presented that a number of gossypol-like pigments are present in different types of crude cottonseed oils and that *p*-anisidine reacts with these pigments, under the conditions of the method, to give reaction products spectrophotometrically identical with the product for the reaction of pure gossypol with the same reagent.

[1] One of the laboratories of the Bureau of Industrial and Agricultural Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

Reagents

a) Hexane-isopropanol solvent: 794 ml. of commercial hexane [2] and 206 ml. of reagent grade isopropanol.

b) Glacial acetic acid: A.C.S. reagent grade.

c) *p*-anisidine: prepare a saturated solution of technical grade *p*-anisidine in hot water and filter through paper. Upon cooling in a water bath, with stirring, to room temperature, the black oxidation products settle out on the sides of the beaker. Decant the supernatant liquid into a clean beaker and keep overnight in a refrigerator. The crystalline product is usually pure. If it is slightly yellow, recrystallize. Dry it in a desiccator over phosphorus pentoxide and store in a brown bottle. The reagent thus prepared is stable.

d) *p*-anisidine solution: dissolve 0.500 g. of recrystallized *p*-anisidine in the hexane-isopropanol solvent, add 1-ml. of glacial acetic acid and dilute to 50-ml. volume with the solvent. Store in a brown bottle and prepare fresh daily.

e) Acetic acid solution: dilute 1-ml. of glacial acetic acid to 50-ml. volume with the hexane-isopropanol solvent.

f) Standard gossypol solution: dissolve 25 mg. of pure gossypol in the hexane-isopropanol solvent and dilute to 200-ml. volume with the solvent. This stock solution contains 0.125 mg. of gossypol per ml. Dilute 2, 5, 10, 15, 20, 25, 30, 35, and 40-ml. of the stock solution to 50-ml. volumes with the hexane-isopropanol solvent to provide the standard gossypol solutions for use in preparing the calibration curve.

Analytical Procedure

Weigh a sample of the oil containing from 0.6 to 1.8 mg. of gossypol into a 25-ml. volumetric flask and dilute to volume with the hexane-isopropanol solvent. If the oil solution is turbid, filter through paper (S&S 589 Blue Ribbon or equivalent). Pipette dupli-

[2] Skellysolve B was used as the commercial hexane. The mention of trade products does not imply that they are endorsed or recommended by the Department of Agriculture over similar products not mentioned.