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ESTIMATING THE LINOLEIC AND OLEIC ACID CONTENTS OF SUNFLOWER OIL BY REFRACTIVE INDEX[1]

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Abstract

Recent investigations showed that the refractive index (RI) of sunlfower (Helianthus annuus L.) oil could be used to estimate the content of linoleic and oleic acids. Fatty acid contents determined by gas-liquid chromatography served as standards. The absolute error distribution of linoleic and oleic acid estimates from relations developed in this study were calculated. The percent linoleic acid estimates were 66% within ±2.5, and 95% were within ±5.5 for oils that ranged from 37.5 to 75.1% acid. Of the percent oleic acid estimates, 66% were within ±3.0, and 95% within ±6.0 for oils that ranged from 11.4 to 51.2% acid. The most critical measurement was the temperature of the oil at the time the RI was determined. A 1-C error in the temperature measurement produced errors of 3.2 and 3.17% in the estimates of linoleic and oleic acid, respectively. The temperature-RI relation was not affected by acid content. Apparently only pressed oil can be used in the RI determination, since RI values measured on extracted oils were very inconsistent.

Introduction

Currently, the fatty acid composition of sunflower (Helianthus annuus L.) oil is determined by gas-liquid chromatography, which requires extraction with a solvent, usually petroleum ether. The method is slow, and the gas-liquid chromatograph is expensive. However, all the fatty acids can be relatively easily determined in the extract. Relations have been recently developed by Ermakov and Popova (4), Simpson (9), and the author that estimate the fatty acid content of oil from sunflower seeds from the refractive index of the oil. These relations were developed due to need for a rapid and inexpensive method for determining the linoleic and oleic acid contents of sunflower. The more rapid (RI) method could be used for classifying oils according to fatty acid composition for processing, for screening seeds in breeding programs, and for evaluating the seeds for research programs involving management practices to produce a specific fatty acid content. Examination of data (3,5,6,8) has shown that sunflower oil has a combined linoleic plus oleic acid content of 86 to 92%. As one acid content decreases, the other increases. It is well known that the formation of acids is affected by the termperature during seed development (1,6,7,8,9) and low temperatures tend to promote a high linoleic acid content. Therefore, a single cultivar can produce oils that range in linoleic acid content from 30 to 75%, depending on temperature.

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Comstock and Culbertson (2) devised a rapid method to determine the iodine value of flaxseed using the RI of the seed oil. The iodine value is related to the degree of unsaturation of the fatty acids, and thus, to the fatty acid composition. They found a high correlation (0.97) between the RI and the iodine value determined by other methods.

In working with sunflower oil, Ermakov and Popova (4) found a high correlation between the RI at 20 C of pressed sunflower oil and the linoleic acid content. Simpson (9) developed a method in which the RI of sunflower oils measured, at any known temperature, could be used to estimate the iodine value and linoleic and oleic acid contents. Simpson (9) points out that the use of a temperature coefficient for the conversion of RI measurements is open to some controversy.

The purpose of this paper is to confirm that linoleic and oleic acid contents can be estimated from RI measurements, the coefficient for temperature used in the RI estimates does not vary with fatty acid composition, and to show that the regression coefficients of Simpson (9), and Ermakov and Popova (4) are within experimental error.

Method

Thirty-six sunflower seed samples with known linoleic and oleic acid contents were acquired. The linoleic and oleic acid contents had been determined on an aliquot of these samples by gas-liquid chromatography (GLC) at the Barrow-Agee Laboratories [2] or at the USDA-ARS, Richard B. Russell Agricultural Research Center, at Athens, Georgia. The GLC results showed the linoleic acid contents ranged from 37.5 to 75.1% and the oleic acid contents from 11.4 to 51.2%.

For the refractive index (RI) determinations, 5 to 10 seeds were cold pressed in a laboratory press at 4.2×10^6 kg/m^2. The pressed oil was collected by vacuum in a small glass tube that had been drawn to a fine point on the collection end. The RI was determined on an Abbe refractometer which was marked in units of 0.001, and was estimated to the 0.0001 unit. The temperature of the water circulated through the refractometer was recorded as it entered the prism jacket and as it exited the prism jacket. The temperature was controlled in a reservoir from which the water was circulated. The RI of each oil was determined by two operators at different times and at 10 different prism jacket temperatures ranging from 10 to 40 C. The temperature of the water bath was adjusted by heating or cooling, and the instrument prisms allowed to equilibrate at that temperature. A third operator made three RI measurements on each oil, all at ambient temperature. Each operator made separate extractions.

In order to determine the effect of extraction procedures on the fatty acid contents of seed oil, seven of the samples were also crushed whole with mortar and pestle, washed into a flask with petroleum ether, shaken by hand for ten minutes, and filtered. The petroleum ether was evaporated and the RI of the extracted oils stored at room temperature and was determined several times during a period of two weeks.

[2] Trade names are used in this publication solely for the purpose of providing specific information. Mention of a trade name does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or an endorsement by the Department over other products not mentioned.

Regressions of the linoleic acid or oleic acid content as a function of RI and temperature, comparison of these regression coefficients, and Bartlett's test of residual variances were accomplished by procedures outlined by Snedecor and Cochran (10).

Results

The RI-temperature relation for each operator and each of the 36 samples was compared by calculating a simple linear regression of RI as a function of temperature for RI values measured at 10 temperatures. The slope of the RI-temperature relation varied from -3.11 to 10^{-4} to -3.53 to 10^{-4} for the 72 simple regressions. The differences in the residual variances between seeds for each operator were tested by Bartlett's test (10) using 36 estimates of the variance with 10 observations each. The Chi-square value for the residual variances among samples was 7.85 for Operator 1, and 0.38 for Operator 2, both well below the 5% level of significance, and indicated that the residual variances among samples was 7.85 for Operator 1, and 0.38 for Operator 2, both well below the 5% level of significance, and indicated that the residual variances among samples were homogeneous for each operator. The 36 regression coefficient slopes for each operator were then compared with an F-test. The F-values, 1.328 for Operator 1, and 1.334 for Operator 2, indicated that, for each operator, the slope of the regression lines did not differ markedly among samples. Therefore, the RI-temperature relation did not differ among the 36 samples. Since residual variances and regression slopes did not differ, the data for each operator were combined to test for differences between operators. The differences in the residual variances between operators were tested by a two-tailed F-test by use of two estimates of the variance with 360 observations each. The differences between all slopes were then tested with an F-test. The F-values of 0.78 for the residual variances and 0.28 for the differences in slope indicate that the residual variance was homogeneous and that the differences in slope were not significant. These analyses indicate that within the range of acid contents examined, the RI-temperature relation was stable and did not differ significantly between the two operators.

Multiple regression analysis of the linoleic and oleic acid content as functions of RI and temperature were made. The multiple correlation coefficient of RI and temperature (T) with linoleic acid was 0.959 with a standard error of estimate of ±2.75% linoleic acid. The equation was:

$$\text{LINOLEIC ACID \%} = 9601.0(\text{RI}) + 3.213(\text{T}) - 14159.5$$

The multiple correlation coefficient of RI and temperature with oleic acid was 0.953 with a standard error of estimate of ±2.93% oleic acid. The equation was:

$$\text{OLEIC ACID \%} = 9474.0(\text{RI}) - 3.170(\text{T}) + 14059.9$$

From the equations, it can be seen that accurate measurements of temperature are very important. A 1°C error in the temperature measurement would equal 3.213 and 3.170% in the estimates of linoleic and oleic acid, respectively. In Table 1 the regression coefficients of Goss, found in this study, were compared with those of Simpson (9) and Ermakov and Popova (4). The regression coefficients of Goss apparently agree closely with those of Ermakov and Popova (4).

TABLE 1. Comparison of the regression coefficients from Goss, Ermakov and Popova (4), and Simpson (9).

	Regression coefficients			
	Refractive Index	Temperature	Intercept 0 C	Intercept 20 C
Linoleic acid				
Goss	9601.0	3.213	-14159.5	-14095.2
Simpson	11665.0	4.126	-17219.5	-17137.0
Ermakov and Popova	9257.8	--	--	-.3589.7
Oleic acid				
Goss	-9474.0	-3.170	+14059.9	13996.5
Simpson	-12562.2	-4.443	+18037.7	18548.8

Statistical test of the error by the regression coefficients cannot be made without their data. By use of the 20 C intercept of the relation developed by Goss, and by Simpson (9), and of the equation of Ermakov and Popova (4) estimates of the linoleic and oleic acids were calculated (Table 2). The differences in linoleic acid percentages estimated by the Ermakov and Popova (4) equation, and the Goss equation are small and within experimental error. The equation developed by Simpson (9) results in linoleic acid estimates quite different than those of Goss or of Ermakov and Popova (4) particularly for the low levels. However, sunflower oils with levels of linoleic acid below 35% are rare. Calculated values for oleic acid also differeed between Simpson (9) and Goss, especially at high levels. However, sunflower oils with levels of oleic acid above 55% are rare. In another comparison between the equations of the Simpson (9) and Goss, the regression coefficients from each were used to estimate the oleic and linoleic acid contents from RI and temperature data collected by a third operator. The distribution of the absolute error between estimates from the RI and values determined by gas-liquid-chromatography (GLC) was determined for both sets of equations. For the percent linoleic acid estimates, 66% were within ±2.5 and ±3.0 of the values determined for GLC for the Goss and Simpson equations, respectively. For the percent oleic acid estimates, 66% were within ±3.0 and ±3.3 of the values determined by GLC for the Goss and Simpson equations, respectively. For 95% of the estimates of the percent linoleic acid, the Goss equation was within ±4.5 and the Simpson equation was within ±5.5 of the values determined by GLC. For 95% of the estimates of the percent oleic acid, the Goss equation was within ±4.8 and the Simpson equation was within ±5.5 of the values determined by GLC. The distribution of those errors suggest that the two estimates were much closer than was indicated by the calculated values in Table 2. The Goss equation would be expected to have the lowest errors in view of the fact that the oils from the same 36 samples of seed were used in the test described above and in development of the Goss equation. These findings support the use of the RI method of estimating fatty acids. Apparently, any one of the relations presented will produce estimates that would be adequate for general use.

Only the oil obtained by pressing the seeds had a reproducible RI. The relations presented in this paper by three separate investigators were all developed from pressed oils. In examination of the oils extracted with petroleum ether, it was found that the extracted oil RI was not always consistent with the pressed oil RI. In addition, the RI of the extracted oils varied with storage time, increasing and then decreasing or vice-versa. The reasons for the RI instability of the extracted oil is unknown.

TABLE 2. Comparison percent of linoleic and oleic acids calculated by equations of Goss, Simpson (9), and Ermakov and Popova (4).

RI - 20 C	Linoleic acid			Oleic acid	
	Goss %	Simpson %	Ermakov and Popova %	Goss %	Simpson %
1.4700	18.3	10.6	19.3	69.7	82.4
1.4710	27.9	22.2	28.6	60.2	69.8
1.4720	37.5	33.9	37.8	50.8	57.2
1.4730	47.1	45.5	47.0	41.3	44.7
1.4740	56.7	57.2	56.3	31.8	32.1
1.4750	66.3	68.9	65.6	22.4	19.6
1.4760	75.9	80.5	74.8	12.9	7.0

Summary

The RI can be used to estimate the linoleic and oleic acid contents of sunflower oil. The sunflower seeds are presssed at 4.2 x 10^6 kg/m^2 in a laboratory press and the RI of the oil is determined at known temperatures with a refractometer. The method, which is simple, fast, and inexpensive, requires only 5 to 10 seeds of the oil-producing varieties of sunflower. The absolute error of the linoleic and oleic acid estimates were within ±2.5 for linoleic acid and ±3.0 for oleic acid, and 95% of the percent acid estimates were within ±5.5 for linoleic acid and ±6.0 for oleic acid. Temperature was a critical factor in the measurements. An error of one degree centigrade was equivalent to errors of 3.23 and 3.17% in the linoleic and oleic acid estimates, respectively. Apparently, however, the accuracy would be adequate for classifying oils for processing, screening seeds in breeding programs, and evaluating seeds in research programs where an accuracy of ±3% of the percentage estimate would be adequate.

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