DETERMINATION OF OIL CONTENTS OF DRY-MILLED CORN FRACTIONS BY GAS-LIQUID CHROMATOGRAPHY[1]

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ABSTRACT

A method was developed to determine the oil content of dry-milled corn fractions by gas-liquid chromatography (GLC). Oil in ground corn was extracted and transesterified to the methyl esters and the transesterification mixture analyzed directly by GLC. Corn oil samples treated identically (transesterified) served as standards for the determination. The transesterification reagents were a mixture of methanol containing hydrogen chloride gas with dimethoxypropane and benzene. The liquid phase for the gas-chromatographic analysis was silicone gum rubber SE-30. The precision for the method is high, especially below the 1% oil level, the relative standard deviation being ± 3.6%. The GLC determination is not the limiting factor, because oil can be detected at a level less than 0.001%. The extraction-transesterification step removes at least 98% of the oil. Typical nonoil foreign matter will not interfere with the determination, since the analysis is specific for glyceride oils. The original oil content of samples containing oxidized or possibly bound oil can be determined from the methyl palmitate content. This method has also been applied to the determination of oil in other oilseed materials.

Oil content of various fractions is an important criterion in the dry-milling of corn. For many years the petroleum ether extraction method (1) has been used as a measure of oil content. This method is reasonably accurate for high oil contents, but at levels below 1% especially, results can be quite unreliable owing to incomplete extraction, inconsistent extraction due to channeling, or contamination of the extract with nonoil foreign matter.

Broad-band nuclear magnetic resonance has been applied to the determination of oil content of milled corn fractions (2); however, the equipment is expensive and is available in only a few laboratories.

Gas-liquid chromatography (GLC) has greatly simplified the analysis of oils and their derivatives.

This paper describes a gas-chromatographic method for determination of oil content based on quantitative preparation and elution of the methyl esters derived from oil in a ground corn fraction. Corn oil may be chromatographed in three basic forms as triglycerides (3,4), fatty acids (5,6), or methyl esters of the fatty acids (7,8). The third form was selected because triglycerides require extremely high oven temperatures for analysis and because fatty acids take longer to prepare. Methyl esters can be made easily by transesterification.

A number of reagents for converting fatty acids or triglycerides to their methyl esters have been reported. A quantitative method of detection of component fatty acids in milk fat has been developed by Gehrke and Goerlitz (9). The esterification step involves the reaction of iodomethane with the silver salt

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of the acid. Schlenk and Gellerman (10) described the esterification of fatty acids with diazomethane. This reagent is toxic and hazardous, and also it may subject the oils to side reactions; in addition, the reagent must be freshly prepared each day. Methanol-potassium methoxide has been reported by Luddy and co-workers (11) as a means of transesterification of triglycerides. The presence of ground corn and of 10% or more moisture in the corn would probably prevent the reaction from nearing completion because of the adverse effects starch and water have on potassium methoxide. Wynne and associates (12) have shown that perchloric acid catalyzes the methylation of fatty acids, but perchloric acid in the presence of ground corn could have detrimental effects on the oil analysis. Boron trifluoride (13,14) and boron trichloride (15) are excellent transesterification catalysts and produce limited side effects when used properly. However, because of their extremely corrosive nature, boron catalysts cannot be injected directly into a GLC thermal conductivity cell. The most satisfactory reagents are those that do not require elevated temperatures. A low temperature minimizes uneven evaporation losses during refluxing or passage of gas through the reaction mixture and reduces any concurrent effect on the sample or standard.

Mason and Waller (16) have shown that triglycerides may be converted quantitatively at room temperature to their respective methyl esters; methanol-HCl is used with dimethoxypropane (DMP) to remove interfering water, and benzene as a solvent. Their procedure has been adapted to the determination of oil content of milled corn fractions by using the reaction mixture as an extraction solvent. The method was modified by omitting the neutralization step, which caused uneven solvent loss by evaporation and interfered with quantitation. The transesterified triglyceride-solvent mixture is chromatographed and compared with standards of corn oil treated in an identical manner. As an extraction mixture, these solvents have two major advantages: the reagents will transesterify oil without refluxing or heating, and presumably the oil can be transesterified *in situ*.

Materials and Methods

The sample was ground in a Wiley mill through a 60-mesh screen or its equivalent, and the approximate moisture content was determined. If the sample contained more than 10% moisture, the excess was removed in a vacuum oven at room temperature for 2–3 hr. This treatment usually left the sample with 6–8% moisture. Sample size depended on the oil content. For grits with less than 2% oil, a 5-g. sample was used. Higher oil contents required less sample. The sample was weighed on a direct-reading, single-pan balance to three significant figures. Smaller samples had to be weighed more accurately. The extraction container was a 2-oz. narrow-mouth bottle with a screw cap whose paper liner had been replaced with a tight-fitting Teflon disk, 1/16-in. thick.

The following reagents were added to the contents of the bottle in order: 15 ml. of dry benzene, 5 ml. of 10% w./v. anhydrous hydrogen chloride gas dissolved in anhydrous methanol, and 5 ml. of DMP. The contents of the bottle were swirled to obtain a thorough mixture.

All samples were mixed for 16 hr. or overnight on a Palo laboratory shaker, which transmitted a swirling action to the contents of the bottle. Samples of refined corn oil were used as standards. The standards were prepared by weighing 25, 50, and 75 mg. of oil into reaction bottles and adding the same reagents as above. These amounts represented 0.5, 1.00, and 1.5% oil in a 5-g. sample.

At the end of the reaction period, the samples and standards were allowed to settle and the supernatant was chromatographed on an F&M Model 720 gas chromatograph operated at 240°C. with detector temperature of 320°C. and injector temperature of 300°C. The chromatograph was equipped with a thermal conductivity detector operated at 150 ma. and a 6-ft. stainless-steel column packed with 10% silicone gum rubber SE-30 coated on 80/100-mesh Gas-Chrom P. The attenuator was set at 1 and the helium flow was adjusted to 75 cc./min.

After the chromatograph oven had attained thermal equilibrium, a 50-μl. sample of the solvent mixture was injected into the instrument with a Hamilton microsyringe equipped with a mechanical stop to ensure reproducibility. This size of sample was injected regardless of oil content. The volatile solvents were immediately detected and their peaks recorded by a 1-sec. full-scale recorder operated at a chart speed of ½ in./min. Approximately 5 min. after injection, the methyl palmitate (C_{16} saturate) began to elute, followed in 3 min. by a peak containing the methyl stearate (C_{18} saturate) and the unsaturated C_{18} esters ($C_{18:1}$, $C_{18:2}$, and $C_{18:3}$). The entire sample required approximately 10 min. for elution. After a base line was ruled in under each peak, the peaks were cut out and weighed to $\pm$ 0.05 mg. Before any unknowns were analyzed, successive 50-μl. portions of a solution containing the corn oil standard were injected until the column became sufficiently saturated with methyl esters to give reproducible results.

A slope value (b) was calculated for each set of standardization data as follows: $b = (Y_1/0.5 + Y_2/1.0 + Y_3/1.5)/3$, where Y is the weight of the peaks (cut out paper) in mg. for the standards representing 0.50, 1.00, and 1.50% oil, respectively. The percentage of oil present in a sample can be calculated from the following equation: $X = (Y)/(b)(W)$, where X = % oil (as-is basis), Y = total peak weight in mg., W = sample weight in grams/5, and b = slope value.

Results and Discussion

Before the nonpolar silicone SE-30 column was selected for the gas-chromatographic analysis, several other columns were tried, including diethylene glycol succinate (DEGS), Apiezon L, and SF-96—all 10% liquid phase. As shown in Fig. 1, a, the reagents and a representative corn oil standard containing 0.5% unsaponifiables gave no unknown peaks in the range of corn oil methyl esters with the DEGS column; however, when a milled corn sample replaced the standard corn oil, many unknown peaks appeared (Fig. 1, b). Several of these peaks obscured the corn oil methyl ester peaks. Another disadvantage of the DEGS was its ability to separate the C_{18} unsaturates. This

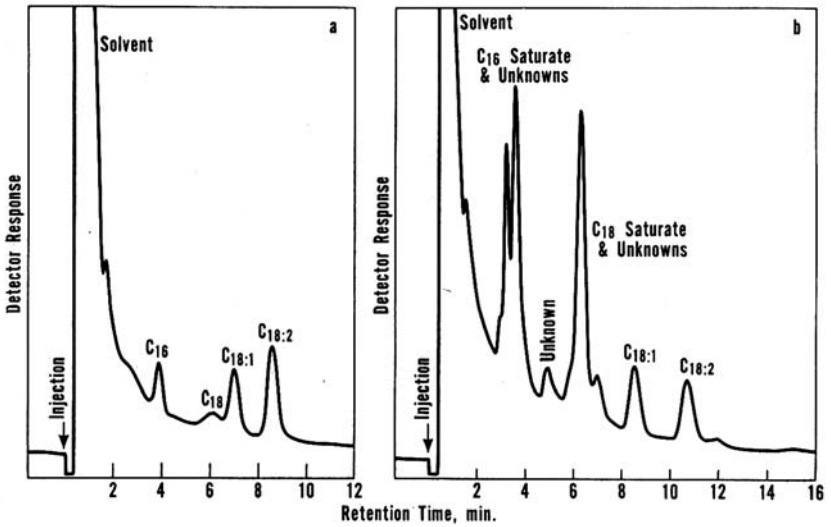

Fig. 1. a, Chromatogram of 51 mg. of a refined corn oil standard run on a diethylene glycol succinate (DEGS) column. b, Chromatogram of dry-milled corn hull fraction run on a DEGS column.

made integration of the area more tedious and less accurate, since there were more peaks to cut out and weigh.

The less polar Apiezon L was tried because it did not separate the C_{18} unsaturates and apparently yielded no unknown peaks (Fig. 2, a). All samples

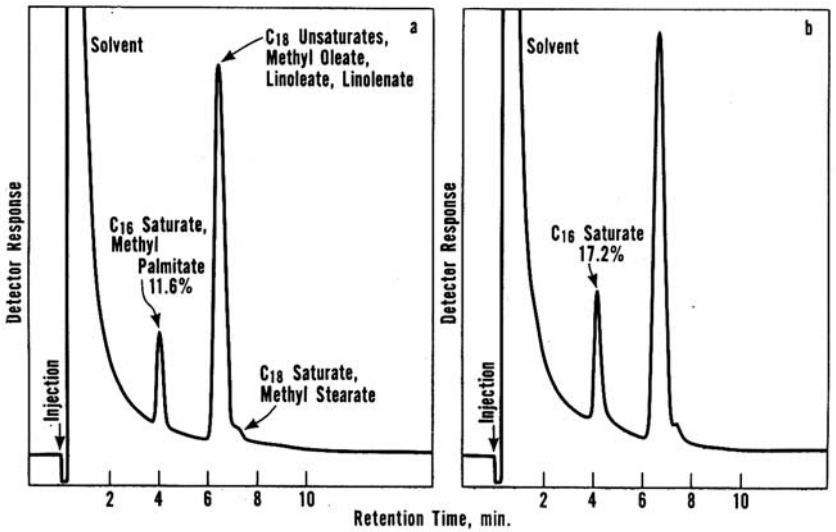

Fig. 2. a, Chromatogram of 88 mg. of a refined corn oil standard run on an Apiezon L column. b, Chromatogram of a corn hull fraction run on an Apiezon L column.

yielded 12–13% methyl palmitate when analyzed on the Apiezon L, with one exception: the hull sample appeared to contain between 15 and 40% methyl palmitate (Fig. 2, b). An identical hull sample, when analyzed on a silicone gum rubber column (SE-30), contained 12.1% methyl palmitate plus an unknown (Fig. 3, a). The SE-30 gave a separation not detected by the Apiezon L column between the C_{16} saturate (palmitate) and the unknown. This unknown was produced by a reaction between the reagents and some unidentified compound, possibly a wax, present only in the hull fraction. Although the silicone SF-96 column gave a slightly better separation between C_{16} and

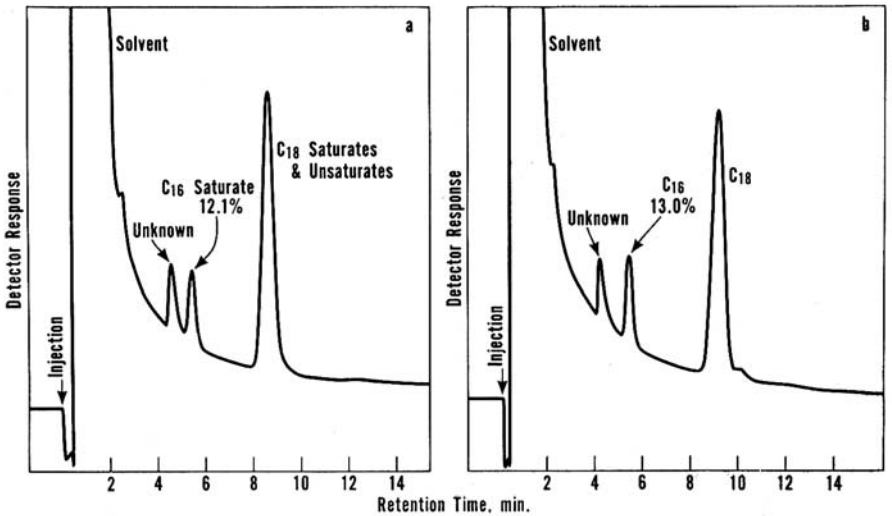

Fig. 3. a, Chromatogram of a hull fraction identical with that of Fig. 2, b, run on a silicone gum rubber SE-30 column. b, Chromatogram of a hull fraction similar to that in Fig. 3, a, run on a silicone gum rubber SF-96 column.

the unknown (Fig. 3, b), the SE-30 column was preferred to the SF-96 because of the higher vapor pressure of the SE-30.

Peak-weight response values were determined for a number of standards prepared from several different samples of refined, bleached, and deodorized corn oil containing less than 0.5% unsaponifiable matter. The peak-weight response values compared favorably for standards containing various amounts of oil. The 11–13% yield of C_{16} from the standards agrees with the known content of C_{16} in corn oil (17). High values for the C_{16} component indicate that not all of the C_{18} unsaturates were chromatographed (reached the detector). This might be due to any of several causes, e.g. oxidation or polymerization of unsaturated fatty acids, or incomplete extraction or esterification.

Such factors as solvent temperature, chromatographic column characteristics, and other uncontrollable conditions which affect the day-to-day standardization values were significant. These effects made it necessary to standardize

the method each time that samples were run. On the basis of statistical evaluation of the data from 42 standardizations, each covering the three levels of 0.5, 1.0, and 1.5% for oil content, a relative standard deviation of ± 3.6% was established for the slope value (b) regardless of the percentage used. Because of the direct relation between absolute error and oil content, samples containing 5% or more oil can be analyzed with greater precision by the Butt extraction procedure.

Water must be removed to prevent interferences with the transesterification reagents. Water cannot be removed by heat, however, because when ground corn is heated to temperatures in excess of 80°C. the oil analysis gives low values. This problem is circumvented by adding additional DMP, which combines with the glycerol of the reaction and moisture in the corn to form isopropylidene glycerol, acetone, and methanol. Approximately 0.5 ml. of DMP is required to react with glycerol produced from the maximum amount of fat that might be encountered. The remaining 4.5 ml. of DMP will react with about 12% sample moisture. The amount of benzene was chosen that yielded total immersion of bulky samples. Mason and Waller (16) have shown that at least an 8% HCl concentration in methanol was required to yield complete transesterification of triglycerides in 16 hr. at room temperature. After

TABLE I

PRECISION OF CORN OIL ANALYSIS BY GAS-LIQUID CHROMATOGRAPHY

SAMPLE	OIL CONTENT[a]			RELATIVE STANDARD DEVIATION
	1st Day	2nd Day	3rd Day	
	%	%	%	%
—3½ + 4 Grits	0.30	0.30	0.28	± 3.5
	0.29	0.31	0.31	
	0.98	0.98	0.95	± 2.7
	0.96	0.96	0.91	
	0.98	0.98	0.98	
	1.04	1.06	1.03	± 1.9
	1.06	1.01	1.03	
	1.12	1.13	1.12	± 0.5
	1.28	1.24	1.28	± 1.8
—4 + 6 Grits	0.28	0.28	0.29	± 1.8
	0.29	0.28	0.28	
	0.63	0.64	0.64	± 0.4
	0.73	0.70	0.70	± 1.1
	1.11	1.11	1.09	± 0.5
—6 + 8 Grits	0.62	0.63	0.64	± 2.4
	0.65	0.63	0.64	
Hull fraction	1.67	1.65	1.64	± 0.4
	2.40	2.45	2.47	± 0.7
	3.14	2.92	3.07	± 3.0
	3.00	3.00	2.92	
—25 + Pan fines	6.40	6.44	6.31	± 1.6
	6.25	6.33	6.48	

[a] Moisture-free basis.

a 16-hr. extraction of the corn samples, the percentage of HCl was still above 8. The relative standard deviation for GLC ranged between 0.4 and 3.5% for a series of samples (Table I). Several samples were re-extracted after being reground in a mortar. The additional oil from re-extraction indicated that the original extraction was at least 98% complete.

This method possesses several advantages over the Butt extraction method. In the Butt analysis, channeling probably has the most detrimental effect on precision. The greatest disadvantage of the Butt method, however, is its inability to extract all the oil. Extraction would be better with samples ground finer, but fine grinding tends to increase channeling. After samples had been ground in a laboratory mill fitted with a 0.027-in. slotted screen and extracted for 16 hr. by the Butt method, the residues were air-dried, reground in a mortar, and reanalyzed by the GLC procedure reported herein. Additional oil was found by GLC. For example, a sample containing 2% oil by Butt extraction yielded 2.4% oil by GLC. This increase by GLC represents 20% more oil. Samples run by both the Butt method and GLC invariably yielded higher values by GLC. When the Butt-extracted material was rerun by GLC, the amount of additional oil found when added to the Butt oil analysis yielded values that correlated well with the original analysis from GLC.

The GLC procedure is specific for glycerides, fatty acids, and sterol esters of corn oil; thus, foreign matter, such as fines and paper fiber from the Butt extraction, is not a problem. The detection system of GLC is highly sensitive, the thermal conductivity detector responding to a level of about 0.01–0.03% oil; however, by substituting a hydrogen flame ionization detector, a level of oil less than 0.001% can be analyzed.

Another advantage of GLC is its ability to determine the original oil content of a sample that contains oxidized or possibly bound oil. Beadle and co-workers (17) have shown the methyl palmitate (C_{16}) content of corn oil methyl esters to be 11–12%, with only slight differences between samples. GLC yields a value for C_{16} on every sample. If the C_{16} is much higher than 11–12%, it can be assumed that the C_{18} unsaturates have either become oxidized or possibly bound in the matrix of the meal, as pointed out earlier. By correcting the C_{16} value to the proper percentage for corn oil, a higher figure will be obtained from the C_{18} and thus a larger value is obtained for the total oil. These C_{16} corrections on oxidized samples correlate well with the original oil content of corn samples.

Also tested by GLC were wheat, soybean, rice, mustard, crambe, and rapeseed meals with standards prepared from the appropriate oil of each meal. The slope values for these oils agreed closely with those of the corn oil values. This agreement would be expected, since the standards were all pure oil.

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