Fatty Acid Composition of Corn Endosperm and Germ Oils as Influenced by Different Extraction Procedures[1]

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ABSTRACT

Gas liquid chromatography was used to determine fatty acid composition of oil obtained from corn (*Zea mays* L.) inbred lines by different extraction procedures. Kernels were separated into endosperm and germ fractions for nine inbreds which had a considerable range in fatty acid composition of oil. Oil obtained from the endosperm by different extraction procedures was significantly different in composition for all fatty acids. Oil obtained from the germ by different extraction procedures was similar in fatty acid composition. The differences in response of endosperm and germ oils to extraction procedures were attributed to the type of lipid found in each fraction. It was concluded that choice of extraction procedures was of little importance in studies of oil from the corn germ, but that specific extraction procedures must be recognized as a factor when studying oil composition of the endosperm.

INTRODUCTION

Breeding and development of agronomic crops with improved chemical composition has received increasing emphasis in recent years. Recent reviews (1-3) have shown a large amount of genetic diversity available for oil composition of rapeseed, sunflower and safflower. New varieties of rapeseed and safflower have been developed which have oils of specific fatty acid composition for particular industrial uses. Corn is low in oil content as compared with oilseed crops and less emphasis has been placed on developing corn with oil of specific fatty acid composition. However, previous studies (4-6) have shown large differences in oil composition in various corn inbreds and hybrids.

Results of future studies concerning the oil composition of agronomic crops may be influenced by such methods and techniques as the selection of extraction solvents. The total amount of lipid extracted by hexane or by petroleum ether is considerably less than that extracted by chloroform-methanol, benzene-ethanol-water, or water saturated butanol (7,8). This is especially true for tissues which contain a large amount of bound or polar lipids. Lough et al. (9) described an improved double extraction procedure for obtaining complete lipid extraction from foods by using a mixture of chloroform-methanol. Several studies by Arnold and Choudhury (10-13) with cottonseed, peanut and soybean oils showed that the rate, amount, color and quality of total oil was influenced by the solvent used in the extraction procedure. Successive extraction of cottonseed by petroleum ether resulted in oil with different fatty acid composition, especially in the amount of cyclopropenoid and linoleic acid contents of the total oil (14). Tsen et al. (15) used a mixture of ethyl alcohol-chloroform-water to obtain a rapid and complete extraction of lipids from various wheat products. They also showed the amount of lipids was considerably greater than obtained with petroleum ether. A recent report by Rogols et al. (16) showed the amount and type of lipid extracted from wheat starch, corn starch and tapioca starch was dependent on the solvents used for extraction.

The endosperm of corn is low in total lipid content and the lipids are not readily extracted with solvents such as hexane and petroleum ether. In contrast, the germ lipids are nonpolar in nature and are generally extracted by hexane or petroleum ether. In a previous study (17), endosperm and germ lipids were extracted by a mixture of methanol-petroleum ether and were shown to be quite different in fatty acid composition. The present study was initiated to determine the fatty acid composition of the oil obtained by different extraction procedures on the endosperm and germ fractions of the corn kernel.

EXPERIMENTAL PROCEDURES

Corn Inbred Lines

Nine inbred lines with a wide range in oil composition for each of the major fatty acids were grown in 1967 and 1968. Three self-pollinated ears of each inbred line was used for study in each of the two years. The original experiment station source of each inbred was: SH258 and GE82 (Georgia), CI-84B and CI-90A (USDA), NY140 and X-187 (New York), N31 (Nebraska), Pa36 (Pennsylvania), and W9 (Wisconsin). CI-90A, N31, Pa36 and W9 have been released to the seed industry by their respective stations and the other inbreds are unreleased lines.

Extraction Procedures

Approximately 20 to 30 kernels from each ear of each inbred were separated by hand into endosperm and germ fractions. The endosperm fraction included the pericarp and the germ fraction consisted of the scutellum and embryo axis. The area between the two kernel fractions was discarded to make sure there was no mixture of the fractions. Extraction procedures were:

1. Overnight extraction in a 2:1 mixture (4 to 5 ml) of petroleum ether (Skellysolve F)-absolute methanol. Further extraction was obtained by adding 10 ml of 3% sulfuric acid in anhydrous methanol and heating in a hot water bath

TABLE I

Variance for Each Fatty Acid of Each Kernel Fraction

Source of variation	Degrees of freedom	Expected mean square[a]
Replication	2	
Year	1	$\sigma_a^2 + r i p \sigma_y^2$
Error (a)	2	σ_a^2
Inbred	8	$\sigma_b^2 + r p \sigma_{yi}^2 + r y p \theta_I^2$
Year X inbred	8	$\sigma_b^2 + r p \sigma_{yi}^2$
Error (b)	32	σ_b^2
Procedure	2	$\sigma_c^2 + r i \sigma_{yp}^2 + r y i \theta_P^2$
Year X procedure	2	$\sigma_c^2 + r i \sigma_{yp}^2$
Inbred X procedure	16	$\sigma_c^2 + r \sigma_{yip}^2 + r y \sigma_{Ip}^2$
Error (c)	88	$\sigma_c^2 + r \sigma_{yip}^2$

[a]Year was considered a random variable (σ^2) and inbreds and procedures as fixed variables (θ^2). Error (a) mean square was used to test the significance of years, year X inbred interaction mean square for inbreds, etc. The threeway interaction mean square (year X inbred X procedure) was pooled with error (c) and used to test the significance of inbred X procedure and year X procedure. Three ears of each inbred line were used and were considered as replications in the analysis of variance.

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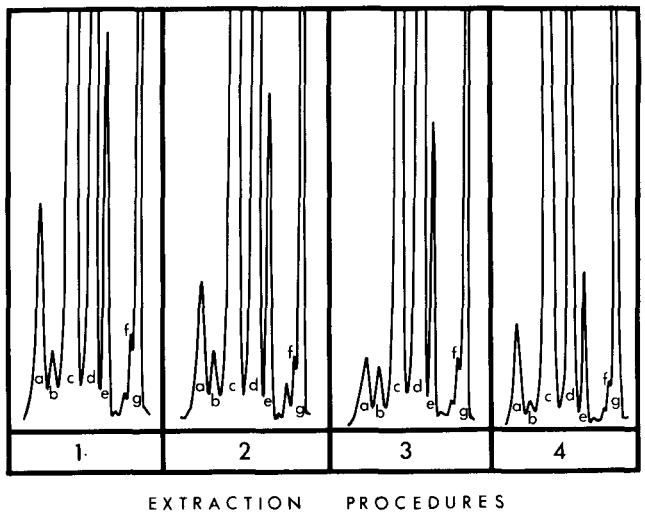

FIG. 1. Chromatograms of oil obtained from the endosperm of corn inbred SH258 by four different extraction procedures (see Experimental Procedures). Identification of fatty acid peaks (a) linolenic, (b) arachidic, (c) linoleic, (d) oleic, (e) stearic, (f) palmitoleic and (g) palmitic. Several short chain fatty acids and solvent peak are not shown on these chromatograms. Retention time for linolenic acid was 5 min.

at 63 C for 5 hr (procedure used for obtaining fatty acid methyl esters).

2. Overnight extraction in a 1:1 mixture (4 to 5 ml) of chloroform-methanol. The extracting solution was decanted from the kernel fractions before the addition of sulfuric acid-methanol solution for the formation of methyl esters of the fatty acids.

3. Overnight extraction in petroleum ether (Skellysolve F). The petroleum ether was decanted and the samples handled as described in procedure 2.

After methylation of oil samples, the flasks were cooled and 3 ml of Skellysolve F were added and the flasks were shaken. Distilled water was added to bring the Skellysolve F layer into the neck of the flask. The solvent layer was filtered through anhydrous Na_2SO_4 into small 1 dram vials (15 x 45 mm) and were left at room temperature overnight for evaporation of the solvent. Vials containing the methyl esters were stored at 0 C.

Analysis by Gas Liquid Chromatography

Analyses were made with a Varian Aerograph Model 1200-2 gas chromatograph (flame ionization detector) and an Infotronics Model CRS-11HSB digital integrator. In 1967, the methyl esters were separated on a 2.74 m by 3.18 mm copper column packed with 8% (by weight) of

TABLE II

Mean Squares From the Analysis of Variance of Fatty Acids in the Oil Extracted From the Endosperm and Germ Fractions of Corn

Source of variation	Degrees of freedom	Endosperm					Germ				
		16:0	18:0	18:1	18:2	18:3	16:0	18:0	18:1	18:2	18:3
Replication	2	5.28	.04	11.75	4.59	.83	.95	.03	1.23	3.37	.00
Year	1	.83	14.92[a]	125.80[a]	232.05[a]	1.08	141.19[b]	10.97[a]	.25	230.98[b]	.27[b]
Error (a)	2	.66	.12	.40	.95	.63	2.07	.10	6.20	5.53	.01
Inbred	8	96.62[a]	4.07[a]	1536.36[a]	1517.36[a]	5.55[b]	146.88[a]	5.63[a]	3457.61[a]	3705.49[a]	2.40[a]
Year X inbred	8	4.45[a]	.13	31.75[a]	14.00[a]	1.34[a]	.56	.08	24.88[a]	25.24[a]	.12[a]
Error (b)	32	1.20	.13	4.25	2.45	.13	.51	.07	3.39	3.18	.01
Procedure	2	737.23[a]	2.21[b]	2303.77[a]	224.06[b]	37.33[a]	7.33[b]	.05	.59	6.38	.08
Year X procedure	2	4.69[a]	.09	20.21[a]	5.57	.25	.27	.06[a]	5.82[a]	4.61[a]	.09[a]
Inbred X procedure	16	2.97[a]	.23[a]	104.43[a]	106.95[a]	.42[a]	.31[b]	.01	.68	.55	.03[a]
Error (c)	88	.93	.05	2.10	1.83	.09	.17	.01	.43	.44	.01

[a]Mean square was significant at 1% level.
[b]Mean square was significant at 5% level.

TABLE III

Averages of Fatty Acid Composition of Oil Extracted From the Endosperm and Germ Fractions of Corn

	Endosperm					Germ				
	16:0	18:0	18:1	18:2	18:3	16:0	18:0	18:1	18:2	18:3
Average of inbreds and procedures										
Year										
1967	15.5	2.3	25.8	53.2	2.3	10.6	1.5	35.2	51.5	0.6
1968	15.6	2.9	27.5	50.8	2.1	12.5	2.1	35.2	49.1	0.7
Average of years and procedures										
Inbred										
SH258	14.3	2.7	39.2	41.0	1.7	10.7	2.3	56.3	29.8	0.4
GE82	14.2	2.6	38.5	41.7	1.8	10.7	2.5	56.4	29.5	0.4
CI-84B	19.5	2.8	31.5	43.5	1.8	18.2	1.1	36.9	42.8	0.6
CI-90A	15.0	2.2	34.0	44.9	3.2	12.1	1.3	41.4	44.4	0.5
NY140	14.9	3.2	24.1	54.2	2.4	9.2	2.2	32.7	54.5	0.7
N31	18.9	2.8	17.0	57.3	2.8	13.3	1.8	25.0	58.4	0.9
W9	16.1	1.9	18.2	61.1	1.9	11.3	1.2	26.2	60.2	0.7
X-187	12.3	3.3	20.6	61.4	1.6	9.3	2.4	22.0	65.4	0.4
Pa36	14.7	2.2	16.7	63.1	2.5	9.2	1.4	19.9	67.5	1.5
Average of years and inbreds										
Procedure										
1	19.3	2.7	20.4	53.9	2.9	11.7	1.8	35.2	50.1	0.7
2	15.5	2.8	26.2	52.3	2.4	11.8	1.8	35.1	50.1	0.7
3	11.9	2.4	33.4	49.9	1.3	11.1	1.8	35.3	50.7	0.6

stabilized DEGS on 80/100 mesh Aeropak 30 solid support. In 1968, a 2.44 m by 6.35 mm copper column was used packed with 10% DEGS on Aeropak 30. Injection port, column oven, and detector were operated at 270, 220 and 300 C, respectively. Helium was used as the carrier gas. Column performance and detector response were checked with National Heart Institute type fatty acid standards and found to be within acceptable range of error.

Experimental Design and Statistical Analysis

Since previous studies (17,18) have shown differences in oil composition of the endosperm and germ, the results of this study were based on an analysis of variance for each fatty acid of each kernel fraction as shown in Table I.

RESULTS

Typical chromatograms of the oil obtained by four extraction procedures on the endosperm of inbred SH258 in 1968 are shown in Figure 1. Differences in oil composition due to extraction procedure are especially evident by the ratio of peak heights for stearic, arachidic and linolenic acids. Differences among extraction procedures were also observed for short chain fatty acids which eluted before palmitic acid and for the 17-carbon fatty acids. Quantitative results were obtained for palmitoleic acid of the endosperm oil and for arachidic acid of the endosperm and germ oils. Although differences in palmitoleic and arachidic acids were observed among inbred lines, these fatty acids occur in small amounts and further results are not given in this paper.

Analysis of variance of the data showed that fatty acid composition was significantly different beween 1967 and 1968 for all fatty acids except palmitic and linolenic acids of the endosperm oil and oleic acid of the germ oil (Table II). However, differences in oil composition between years were not great and is similar to results previously published (19) for corn oil. Inbred lines were significantly different for all fatty acids of endosperm and germ oils (Table II). From the endosperm, oils obtained by different extraction procedures were different for all fatty acids, but with greatest effects on palmitic and oleic acids. Skellysolve F (procedure 3) extraction of the endosperm resulted in a small amount of oil which was similar in composition to the germ oil. Extraction procedures influenced (5% level) the amount of palmitic acid extracted from the germ oil and had no influence on the other fatty acids. The inbred X procedure interaction was significant for all fatty acids of the endosperm oil and this indicates that all inbreds did not react similarly to the different extraction procedures used on the endosperm.

Averages of the fatty acid composition for endosperm and germ oils are given in Table III for different years, inbreds and procedures. The endosperm oil was higher than the germ oil in palmitic, stearic and linolenic acids and lower in oleic acid. The germ oil exhibited a greater range from low to high amounts of linoleic acid as compared to the endosperm oil. Although not measured, it was evident that the greatest amount of lipid material was extracted by procedure 1 followed in order by procedures 2 and 3.

DISCUSSION

Numerous reports have been made in recent years concerning the fatty acid composition of oil in various agronomic crops. Different solvents and extraction procedures have been used in these studies by different investigators. Although the amount and type of lipid extracted by various procedures have been reported (7-16), little information is available concerning the influence of different extraction procedures on the oil obtained from the endosperm and germ fractions of the corn kernel. The use of different extraction procedures in the present study showed that procedures greatly affected the fatty acid composition of the oil obtained from the endosperm, but had little effect on the germ oil. Leng (20) and Curtis et al. (21) have reported that approximately 90% of the total oil in the corn kernel at maturity is located in the germ. Weber (22) reported the per cent range in triglycerides of total oil to be 74.9 to 92.4 for three different corn strains. The different reaction of endosperm and germ to extraction procedures is apparently due to the type of lipid present in each of these two kernel fractions. Since the predominant type of lipid in the germ is triglycerides, any of the commonly used solvents or extraction procedures are effective in the total extraction of the oil from the germ. However, the endosperm lipids are composed of more complexed polar lipids which are quite dependent as to the amount and type of lipid extracted by various extraction procedures.

Future studies concerning the fatty acid composition of the oil in the corn germ need not be concerned with different extraction procedures. Also, results reported in the literature obtained with different extraction procedures may be compared for germ oil composition. However, any study designed to study oil of the corn endosperm or of total kernel oil should recognize the possible influence on composition of the oil obtained by different extraction procedures.

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