Fat Content in Corn Grits: Effects of Grinding, Extraction Solvents, and Analytical Methods

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ABSTRACT

Yellow brewers' grits ground to different particle sizes were extracted from 2 to 16 hr. with pentane-hexane, diethyl ether, or carbon tetrachloride. For comparative purposes, fat content of the grits was determined also by acid hydrolysis and by an extraction-transesterification procedure wherein the extract was analyzed by gas-liquid chromatography. Particle size strongly influenced the reported fat content regardless of method of analysis or extraction solvent used. Standardization of the grinding and analytical procedure would provide a basis for direct comparison of reported fat contents of brewers' grits.

Rapid and reasonably accurate determination of the fat content of their finished products is essential to cereal millers. Many solvent-extraction procedures have been proposed for analysis of cereal grains and their milled products for fat content, but only a few are in current use. Among these are Soxhlet, Butt-type, and Goldfisch extractors (1-3), each operable with various solvents; the Omnimixer method of Haas and Fleischman (3), which involves simultaneously grinding the brewers' grits and extracting the fat; and the procedure of Tsen et al. (4) utilizing a mixture consisting of chloroform, methanol, and water. Other solvents and solvent combinations tried are listed in a review by Mecham (5).

Instrumentation has brought other methods of fat analysis to the fore. Among them is nuclear magnetic resonance (NMR), which is being used for both research and process control (6-8). While NMR can analyze samples quickly, certain disadvantages exist. Common problems involving NMR analysis for fat content include initial equipment cost and a need for partial drying if water content of a sample exceeds 3%. Furthermore, accuracy is poor at low levels (less than 3%) and results to date by wide-line NMR are ±0.3% absolute (6-9).

The gas-liquid chromatographic (GLC) method of Black et al. (10) has become routine at the Northern Laboratory to determine fat in endosperm fractions from dry-milled corn. However, their method includes an overnight extraction-transesterification step.

Although many laboratories are equipped with GLC and a few with NMR equipment, solvent-extraction methods are used more because necessary equipment and materials are readily available, easy to use, and inexpensive. However, as we shall show, conventional solvent extraction removes the fat to a varying degree from corn grits. The reliability and speed of these methods are poor, unfortunately, owing to inherent weaknesses that we have found which include the following: a) incomplete and sometimes inconsistent extraction of the fat; b) contamination of the extract with nonfat solubles, such as low-molecular-weight carbohydrates extracted by alcohol-containing solvents or by water in the solvent; and c)

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contamination by foreign material including either paper or cotton fibers, or both, as well as fines from the sample. Consequently, we evaluated several variables for solvent extraction and compared the results with those obtained by GLC and acid hydrolysis. This report describes the influence of such factors as particle size, analytical method, extraction time, and the extractive ability of various solvents upon the quantity of fat found in a sample of corn grits.

MATERIALS AND METHODS

Commercially prepared brewers' grits (-16+20 mesh) from yellow dent corn containing 13% moisture were ground to three different size ranges. The finest particles came from grinding grits in a Pallmann pulverizer equipped with a 60-, 80-, or 100-mm. diameter retaining ring. Grits ground in a Udy mill (hammer mill with cyclone collector) having a 0.012-in. slotted screen or in an Alpine pin mill operating at 18,000 r.p.m. produced particles of intermediate size, whereas grits ground in a Mikro Samplmill with a 0.046-in., round-hole perforated screen produced the largest particles.

Particle-size distribution of the ground grit samples was determined by sieving 100-g. samples for 5 min. on a Rotap shaker to separate particles retained on 40- and 80-mesh U.S. standard test sieves, and by differential settling of approximately 0.25-g. samples of the -80-mesh particles in a Sharples Micromerograph, which includes an air-sedimentation apparatus and an electronic weighing mechanism (11).

Fat content in the ground grits was determined by several procedures: Butt extraction using pentane-hexane, diethyl ether, or carbon tetrachloride; the fat-acid hydrolysis method of the Association of Official Analytical Chemists (12); and the GLC method of Black et al. (10). In the GLC method, fat in the ground sample is extracted and transesterified for 16 hr. at room temperature with a mixture of benzene, methanol (containing anhydrous hydrogen chloride gas), and

TABLE I. PARTICLE SIZE ANALYSIS OF YELLOW BREWERS' GRITS (-16+20) GROUND IN DIFFERENT MILLS

		Grinding Mill and Setting					
		Pallmann pulverizer, mm.			Udy mill, 0.012-in. slotted screen	Alpine pin mill, one pass, 18,000 r.p.m.	Mikro Samplmill, 0.046-in. screen
U.S. Mesh	Mesh Opening μ	60[a]	80	100			
		% of product finer than size indicated[b]					
40	420	100	100	100	100	98	80
80	177	100	100	100	61	70	33
140	101	94	95	83	41	36	17
200	72	88	89	73	30	19	12
325	42	80	81	64	3	0	1
...	20	38	41	34	0	0	0
		MMD[c] μ					
		26	25	32	124	125	275

[a] Diameter of retainer ring.
[b] As determined by sieve analysis for +80 mesh particles and by sedimentation column (Sharples Micromerograph) for particles finer than 80 mesh.
[c] Mass median diameter.

dimethoxypropane. A portion of the transesterified mixture is injected directly into a gas chromatograph equipped with an SE-30 column (Applied Science Laboratories) that separates methyl esters into saturated C_{16} (methyl palmitate), saturated C_{18}, and unsaturated C_{18} groups. Fat content of the sample is calculated by comparing the total peak weight of its fatty ester peaks with those from the known weight of refined corn oil used as a standard.

RESULTS AND DISCUSSION

Data on particle-size distribution and mass median diameter (MMD) of samples for six different grinds are given in Table I. Values represent percentages of the sample finer than the size indicated. The MMD (i.e., the size where half the particles by weight are larger and half smaller than this diameter) of the six grinds fell into three groups; namely, 25 to 32 μ for the Pallmann samples, about 125 μ for the Udy and pin mill samples, and 275 μ for the Mikro Samplmill samples. A comparison of the percentage of particles of a given size over the range involved shows overall agreement with the MMD, although there are individual differences. These differences reflect the difficulty of accurately determining the particle-size distribution of the samples because of the small quantity analyzed by sedimentation.

Comparative data are given in Table II for quantity of fat extracted from the most finely ground grits and from -16+20 mesh unground grits by three common solvents in Butt-type extractors. With a 2-hr. extraction of either coarse or finely ground grits, carbon tetrachloride removed fat more readily than did either diethyl ether or pentane-hexane. Usually 40 to 50% more fat was extracted by all three solvents when the grits were finely ground. Significant variation was associated with particle size and method for data in Table II. There was about a 0.10 increase in fat in going from 2 to 16 hr. extraction. Carbon tetrachloride extracts were

TABLE II. FAT DETERMINATION[a] OF BREWERS' GRITS VARIES WITH EXTRACTION SOLVENT' EXTRACTION TIME, AND PARTICLE SIZE

Solvent	Extraction Time hr.	Fat Content, % d.b.	
		Coarse[b]	Fine[c]
Pentane-hexane	2	0.62	1.07
	4	0.68	1.10
	8	0.80	1.15
	16	0.81	1.16
Diethyl ether	2	0.72	1.04
	4	0.76	1.06
	8	0.78	1.12
	16	0.80	1.14
Carbon tetrachloride	2	0.79	1.14
	4	0.77	1.18
	8	0.80	1.18
	16	0.81	1.25

[a] Relative standard deviation = 3.4%.
[b] -16+20 mesh grits, unground.
[c] -16+20 mesh grits, ground in Pallmann pulverizer with 60-mm. ring.

TABLE III. EFFECT OF EXTRACTION SOLVENT, PARTICLE SIZE, AND ANALYTICAL METHOD ON FAT DETERMINATION[a] OF BREWERS' GRITS

Mill Used for Grinding Grits	Mass Median Diameter μ	Fat Content, % d.b.					Reanalysis of Acid-Hydrolysis Fat by GLC % d.b.	Mean[d] (LSD = 0.05)
		Extraction solvent[b]						
		Pentane-hexane	Diethyl ether	Carbon tetrachloride	GLC[c]	Acid hydrolysis		
Pailmann pulverizer								
60-mm. ring	26	1.17	1.16	1.22	1.25	1.60	1.16	1.19
80-mm. ring	25	0.98	0.96	1.05	1.14	1.42	0.99	1.02
100-mm. ring	32	0.94	0.89	0.96	1.12	1.45	0.99	0.98
Udy mill	125	0.75	0.78	0.82	0.86	1.21	0.84	0.81
Alpine pin mill	124	0.86	0.77	0.84	0.93	1.12	0.80	0.84
Mikro Samplmill	275	0.64	0.69	0.74	0.80	1.07	0.77	0.73
Unground	---	0.65	0.73	0.75	0.79	1.07	0.79	0.74
Mean (LSD = 0.04)[e]		0.86	0.85	0.91	0.98	1.28	0.91	

[a] Relative standard deviation = 4.0%.
[b] Six-hour extraction.
[c] Sixteen-hour extraction-transesterification step. GLC, gas-liquid chromatography.
[d] Omitting acid hydrolysis values. LSD = least significant difference.
[e] LSD at 5% level.

significantly higher than the other two solvents by 0.06. Fat was not extracted completely from unground grits by any of the solvents, even after 16 hr., as is evident from results with finely ground grits. Analyses of variance showed significant effects (at the 1% level) associated with extraction time, solvent, and particle size. None of the interactions between solvent, time, and particle size were significant (Table III). The relative standard deviation for all data shown in both tables is 3.7%.

In addition to extractable fat, "bound" fat has been reported present in cereal grains by several workers (5, 13). Difference between total fat content as determined by acid hydrolysis and extractable fat is generally considered as representing the amount of bound fat (13).

Data given in Table III further indicate the variation in extractable fat content and in total fat content by acid hydrolysis. Variation was due to the following: a) particle size as affected by grinding procedure; b) solvent used; and c) method of analysis. Degree of fat removal increased with fineness of grind regardless of type of extraction solvent or analytical method. For any given MMD, fat content also varied over a smaller range depending on the solvent and method of analysis. Initial values obtained by acid hydrolysis were higher than those by GLC. However, subsequent GLC analysis of fat obtained by acid hydrolysis proved that nonfat material was present and that the corrected glyceride values were less than or equivalent to those from direct GLC analysis (Table III). These results demonstrate that the GLC method consistently gave the highest true fat value. Twenty residue samples from pentane-hexane extractions (values ranged from 0.48 to 1.51% fat, the average being 0.76%) were reanalyzed by the GLC method. The average value obtained was an additional 0.04% triglyceride fat.

Nonfat material, such as fiber or lint from cotton and filter paper, often contributes to the error in solvent-extraction procedures. Other nonfat material may be finely ground particles from the extracted sample or material such as extracted carbohydrates, the latter depending on the solvent polarity.

Because amount of fat that was extracted varied with particle size and time, the question arises, is the variation in quantity due in part or entirely to variation in composition of the extracted fat, as reported for soybeans and corn endosperm (14,15). In his studies with inbred lines of corn, Jellum (15) noted an increase in palmitic and a decrease in oleic acid contents of the endosperm fat as methanol content of his solvent mixture increased. In our routine determination of fat content of dry-milled endosperm fractions by the GLC method, which uses a solvent containing some methanol, we have consistently found an enrichment in palmitic acid to 15 to 18% for the endosperm fat, similar to that reported by Jellum, but no variation attributable to effect of particle size. We know that the GLC method determines only methyl esters derived from triglycerides, and our results were always compared against corn oil used as a standard. In extractions made with pentane-hexane, the fatty acid composition was exactly the same for identical samples extracted for 30 min. or 6 hr. Previous to the work reported herein, we found the fatty acid composition remained constant, regardless of the fineness of grind or identity of the corn fraction. Blessin[2] also found no unusual change in

[2]Blessin, Charles W., Research Chemist, Cereal Properties Laboratory, Agricultural Research Service, U.S. Department of Agriculture. Personal communication.

chemical composition of the fat even when varying amounts were extracted from corn endosperm fractions, as determined by thin-layer chromatography even when exhaustive extraction was used.

Our work clearly illustrates the pronounced influence of particle size on the quantity of extractable fat found in brewers' grits, as well as the influence of extraction solvent and time. In view of these demonstrated effects we recommend, for routine control and trading purposes, standardization of the methods for grinding the grits and analyzing their fat content. In lieu of such standardization, an accurate description of particle-size range of the sample as extracted and of the analytical method selected should always be reported with fat content of the grit fraction.

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