

Enrichment of Oil in Corn Fiber by Size Reduction and Floatation of Aleurone Cells

Vijay Singh^{1,2} and Robert A. Moreau³

Cereal Chem. 80(2):123–125

Corn fiber oil contains potentially valuable phytosterols that have nutraceutical properties (Moreau et al 1998). The amount of oil in corn fiber is very low, ≈ 1.5 to 3.0%. Due to the low concentration of oil in corn fiber, the extraction of the oil and its phytosterol compounds is inefficient and can be expensive. Singh et al (2001) showed that >90% of the corn fiber oil comes from the aleurone layer, which is $\approx 40\%$ of the coarse (pericarp) fiber fraction. Among the different classes of phytosterol compounds present in the corn fiber oil, >95% of the ferulate phytosterol esters (FPE), 60% of free phytosterols (St), and 90% of fatty acyl phytosterol esters (St:E) are present in the oil recovered from the aleurone layer (Singh et al 2001).

The aleurone layer in most of the yellow dent corn hybrids is a single layer of cells located directly beneath the pericarp tissue (Watson 1984). During conventional corn wet milling, the aleurone layer ends up in the fiber fraction. If the aleurone layer can be separated from the wet-milled fiber fraction, it should provide an enriched source of corn fiber oil and phytosterol compounds. Higher concentrations of oil and phytosterols could make the extraction more efficient and less expensive.

Oil in the aleurone layer is contained in the individual oil body cells. With size reduction, if the oil-containing cells were released into aqueous solution, they should float due to their low density and could then be recovered by floatation. This separation would be very similar to the separation of germ during the conventional corn wet-milling process. Corn germ, because of its oil, floats on the top of the ground corn slurry when the proper specific gravity is used (Watson 1984). Other processes have been developed to separate aleurone cells in ground wheat bran using sieves and an electrostatic separator (Stone and Minifie 1988). In this study, we attempted to increase the concentration of the oil and phytosterols in the fiber by floatation of the fiber tissue associated with the oil bodies (aleurone cells) in the fiber fraction.

MATERIALS AND METHODS

A yellow dent corn hybrid (Pioneer 33A13) grown during the 2000 crop season at the Agricultural Engineering Farm, University of Illinois at Urbana-Champaign, was field-dried to $\approx 15\%$ moisture content and combine harvested. Corn samples were hand-cleaned to remove broken corn and foreign material, packaged in plastic bags, and stored at 4°C until wet milling. The whole kernel moisture content of the samples was measured using the 103°C convection oven

method (Approved Method 44-15A, AACC 2000). The *n*-hexane was obtained from Burdick and Jackson and contained 85% *n*-hexane.

The wet milling of the samples for the fiber fraction was done using the 1-kg laboratory corn wet-milling procedure described by Eckhoff et al (1993). Fiber samples were dried in a convective oven and ground using a mixer (model 100, Magic Mill, Monsey, NY). Particle-size analysis of the ground fiber was done using standard sieves and a sieve shaker (model RX-86, W.S. Tyler, Mentor, OH) (Approved Method 20-10, AACC 2000). Ground fiber (5 g) was added to 400-mL water solutions with different specific gravities. The fiber was stirred in the solution and then added to a 500-mL separatory funnel. The solution in the separatory funnel was kept for 1 min and the bottom layer of the fiber that did not float was removed. The fiber fraction that floated was called floated material (FM) and the fiber fraction that did not float and settled down was called settled material (SM). Solutions with three different specific gravities (1.00, 1.04, and 1.09) were evaluated for separation of fiber. Sodium nitrate was used to achieve the desired specific gravity. After separation, all fiber samples were thoroughly rinsed with 1.0 L of distilled water and dried. Moisture content of fiber was measured by drying in a forced-air convection oven (Approved Method 44-18, AACC 2000). All of the separations were done in duplicate, and the samples were combined for oil and phytosterol analysis. Dried fiber samples were analyzed at least twice using HPLC. Results presented are the means of the duplicate analyses.

Based on the results of the first experiment, a second experiment was done in which commercial fiber fractions were obtained from two corn wet-milling plants in the Midwest. The same protocol was used for grinding and floatation of fiber as described above. However, in the second experiment, no sodium nitrate was added and a specific gravity of 1.00 was used for all experiments.

Recovered fiber samples were extracted using an accelerated solvent extractor (Dionex ASE 200) using hexane as the solvent. Ground fiber samples were placed in 11-cm³ sample-extraction cells. The extraction conditions in the cells were 1,000 psi pressure, 100°C, 5-min heating time, 10-min start time, three static cycles, 100% flush volume, and 60-sec purge time. Due to small sample size, the samples of pericarp and aleurone were extracted by placing in hexane (1 g of sample/10 mL of hexane) and homogenizing with a Polytron homogenizer. Homogenized samples were extracted by shaking for 1 hr in a wrist-action shaker at room temperature. After extraction, the hexane extracts were filtered with gentle vacuum through a GF/A glass fiber filter (Whatman Laboratory Products, Clifton, NJ) fitted in a Buchner funnel.

For HPLC analysis, part of the sample was removed from the extracted solvent as previously outlined by Moreau et al (1996). The lipid classes in samples were separated and quantified using a modified version of an HPLC technique developed by Moreau et al (1996). A modular ternary-gradient HPLC system was used (model 1050, Hewlett Packard, Avondale, PA). Two detectors were connected in series. The first was a fixed wavelength UV-visible detector set at 295 nm. The second was an evaporative light scattering detector (Alltec-Varex Mark III, Deerfield, IL) operated at 40°C with nitrogen as a nebulizing gas at a flow rate of 1.60 L (STP)/min. The column was a Chromsep Cartridge LiChrosorb DiOL, 5 μ m, 3 \times 100 mm (Chrompack, Raritan, NJ). The mobile-phase gradient of hexane, 2-propanol, and acetic acid was the same as used by Moreau et al

¹ Assistant professor, Department of Agricultural Engineering, University of Illinois, Urbana, IL 61801.

² Corresponding author. Phone: 217-333-9510; Fax: 217-244-0323; E-mail: vsingh@uiuc.edu.

³ Lead scientist, U.S. Department of Agriculture, Eastern Regional Research Center, Agricultural Research Service, 600 E. Mermaid Lane, Wyndmoor, PA 19038. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

TABLE I
Enrichment of Oil and Different Phytosterols in the Fiber Fraction by Floatation^{a,b}

Fiber Fraction	Sp. Gr. for Floatation	Fiber Recov. (%)	Oil in Fiber (%)	FPE in Fiber (%)	Increase in FPE Conc. in FM (%)	St in Fiber (%)	Increase in St Conc. in FM (%)	St:E in Fiber (%)	Increase in St:E Conc. in FM (%)	Total Phyt. Recov. in FM/100 g of Fiber (%)
Experiment 1 laboratory wet-milled fiber										
FM	1.00	34.0	3.3 ± 0.01	0.15 ± 0.00	37.5	0.07 ± 0.00	26.6	0.12 ± 0.01	25.8	44.5
SM		66.0	1.4 ± 0.02	0.07 ± 0.00		0.04 ± 0.00		0.06 ± 0.00		
Original			2.2 ± 0.15	0.11 ± 0.00		0.05 ± 0.00		0.10 ± 0.02		
FM	1.04	39.0	3.2 ± 0.01	0.13 ± 0.00	35.7	0.07 ± 0.00	19.0	0.11 ± 0.01	6.7	46.9
SM		61.0	1.5 ± 0.02	0.07 ± 0.00		0.04 ± 0.00		0.06 ± 0.00		
Original			2.3 ± 0.06	0.10 ± 0.00		0.06 ± 0.00		0.11 ± 0.00		
FM	1.09	39.0	2.6 ± 0.12	0.12 ± 0.00	24.9	0.06 ± 0.00	8.9	0.11 ± 0.00	17.9	46.3
SM		61.0	1.1 ± 0.03	0.06 ± 0.00		0.04 ± 0.00		0.05 ± 0.00		
Original			2.1 ± 0.07	0.10 ± 0.00		0.05 ± 0.00		0.10 ± 0.00		
Experiment 2 commercial wet-milled fiber										
Wet Mill 1										
Skimmed	1.00	19.0	3.2 ± 0.08	0.16 ± 0.00	69.8	0.06 ± 0.00	6.1	0.08 ± 0.00	33.5	27.4
Other		81.0	1.6 ± 0.32	0.07 ± 0.01		0.05 ± 0.00		0.05 ± 0.00		
Original			2.1 ± 0.12	0.09 ± 0.00		0.05 ± 0.00		0.06 ± 0.00		
Wet Mill 2										
Skimmed	1.00	18.0	5.8 ± 0.01	0.15 ± 0.00	76.3	0.11 ± 0.00	60.5	0.18 ± 0.00	70.5	30.5
Other		82.0	2.5 ± 0.35	0.07 ± 0.01		0.06 ± 0.00		0.09 ± 0.01		
Original			3.1 ± 0.05	0.09 ± 0.00		0.07 ± 0.00		0.10 ± 0.00		

^a Means ± standard deviation. All yields are means of two values.

^b FPE, ferulate phytosterol esters; FM, floated material (fiber fraction recovered by floatation); SM, settled material (fiber fraction that settled in bottom and did not float).

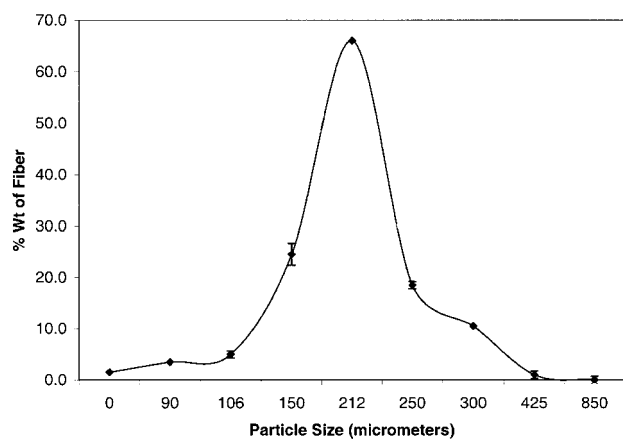


Fig. 1. Particle size distribution of fiber ground using a mixer.

(1996), and the flow rate was constant at 0.5 mL/min. The rest of the solvent sample was dried under nitrogen and heat using an N-EVAP analytical evaporator (Organomation, Berlin, MA).

RESULTS AND DISCUSSION

In the first experiment, ≈34.0 to 39.0% fiber was recovered as FM, depending on the specific gravity of the solution. No significant increase in FM was observed as the specific gravity increased from 1.04 to 1.09 (Table I). Depending on the specific gravity of the solution, the oil content in the FM increased by ≈24.5 to 51.1%, the FPE increased by ≈24.9 to 37.5%, the St increased from 8.9 to 26.6%, and the St:E increased from 6.7 to 25.8%, compared with oil, FPE, St, and St:E in the original fiber. The biggest increases in the oil, FPE, St, and St:E contents were achieved when the fiber was floated in only water (≈1.00 sp. gravity). A comparison between the total phytosterols recovered from FM and total phytosterol recovered in the original fiber, shows that FM only recovers ≈45 to 47% of total phytosterols recovered in the original fiber.

Based on the results of the first experiment, another experiment was done in which commercial fiber samples were obtained from two corn wet-milling plants. The samples were ground and floated only in water (1.00 sp. gravity). With these commercial fiber samples, ≈18% of the

fiber was recovered as FM. Significant enrichments of oil (≈53.3 to 86.4%) and individual phytosterols (≈6.1 to 76.3%) were observed in the FM compared with the original fiber samples (Table I). However, the total recovery of the phytosterols from the FM was only ≈27.4 to 30.1%, compared with the total recovery of phytosterols from the original fiber.

The results from this study suggest that there is a significant enrichment of oil and individual phytosterols compounds in the FM compared with the original fiber, but the total recovery of phytosterols from the FM is very poor. Poor recovery of phytosterols from the FM is probably because all the fiber (aleurone) cells that contain the oil and the phytosterol did not float in the solution and therefore were not recovered in the FM. Aleurone layer cells are estimated to be ≈7 to 70 μm, depending on the position in the kernel (Wolf et al 1952). However, the ground fiber produced from the Magic Mill mixer had particle size distribution ranges of ≈50 to 425 μm (Fig. 1). Most of the ground fiber particles (>65%) were ≈212 μm. The particles size analysis of the ground fiber suggest that most of the aleurone layer cells were probably stuck together with the other cell wall material, which could have increased the density of the fiber particles and prevented them from floating.

CONCLUSIONS

This study proves our initial hypothesis that floatation can be used to enrich the oil and the phytosterol compounds in the corn fiber fraction. However, to get enrichment as well as good total recovery of oil and phytosterols, the fiber particle size must be small (<100 μm). Achieving a particles size of <100 μm could be the limiting factor for this concept. Also, extractions done at high temperature and pressure (as with accelerated solvent extractor) would not be practical in commercial practice.

ACKNOWLEDGMENTS

We would like to thank Karen Kohout for technical assistance in preparing and analyzing the fiber samples.

LITERATURE CITED

American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th ed. Methods 20-10, 44-15A, 44-18. The Association: St. Paul, MN.

- Eckhoff, S. R., Rausch, K. D., Fox, E. J., Tso, C. C., Wu, X., Pan, Z., and Buriak, P. 1993. A laboratory wet-milling procedure to increase reproducibility and accuracy of product yields. *Cereal Chem.* 70:723-727.
- Moreau, R. A., Hicks, K. B., Nicolosi, R. J., and Norton, R. A. 1998. Corn fiber oil its preparation and use. U.S. patent 5,843,499.
- Moreau, R. A., Powell, M. J., and Hicks, K. B. 1996. Extraction and quantitative analysis of oil from commercial corn. *J. Agric. Food Chem.* 44:2149-2154.
- Singh, V., Moreau, R. A., and Cooke, P. H. 2001. Effect of corn milling practices on aleurone layer cells and their unique phytosterols. *Cereal Chem.* 78:436-441.
- Stone, B. A., and Minifie, J. 1988. Recovery of aleurone cells from wheat bran. U.S. patent 4,746,073.
- Wolf, M. J., Buzan, C. L., MacMasters, M. M., and Rist, C. E. 1952. Structure of mature corn kernel. III. Microscopic structure of the endosperm of dent corn. *Cereal Chem.* 29:349-36.
- Watson, S. A. 1984. Corn and sorghum starches: Production. Pages 417-468 in: *Starch: Chemistry and Technology*. R. L. Whistler, J. N. BeMiller, and E. F. Paschall, eds. Academic Press: Orlando, FL.

[Received March 6, 2002. Accepted August 5, 2002.]