Detection of Sterol, Stanol, Lipid and Carbohydrate Components in Corn Fiber Products. ^{13}C NMR and Chromatographic Methods

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Introduction. Archer Daniels Midland, PNNL, and the National Corn Growers Association have collaborated in this study to develop the enhanced recovery of components of wet-milled corn fiber. The components include hemicellulose, and high value sterol and stanol neutraceutical components. The study augmented conventional analytical methods for glycerides and saccharides with the development of ^{13}C NMR spectroscopic data allowing the *direct* detection, in the raw oil, of a suite of free sterols and stanols, together with fatty acid, ferulate, and coumarate derivatives of sterols and stanols. ^{13}C NMR data for the ferulate and coumarate sterol and stanol esters have not been published, and thus were developed in this work. We present new spectroscopic data for the sterol substances and illustrate approaches to analysis of neutraceutical product distributions.

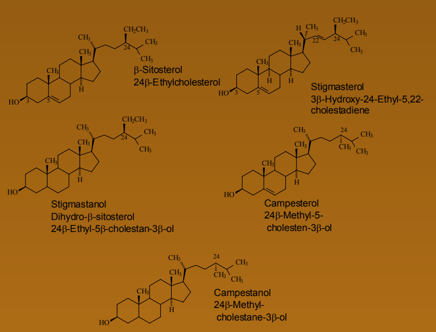


Figure 1. Major Sterol Components of Wet Milled Corn Fiber Oil These components constitute the bulk of fiber associated sterols

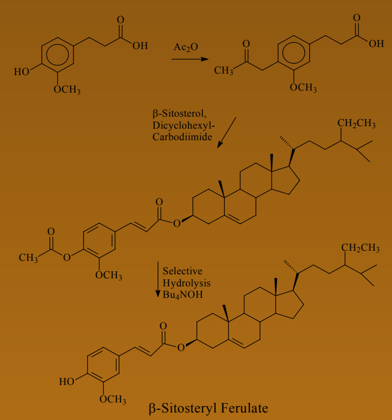


Figure 2. The primary ester derivatives of wet milled corn fiber oil are ferulate and fatty acid sterol and stanol esters. Coumarate esters are found to be trace components, at less than 1/7th the ferulate content.

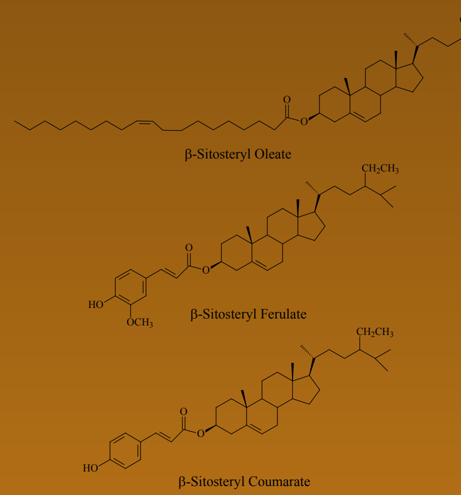
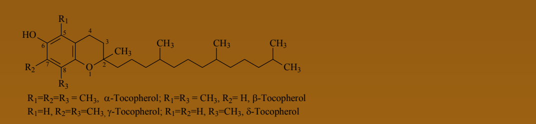


Figure 3. Authentic ferulate and coumarate esters prepared by acetylation, esterification, and selective hydrolysis steps



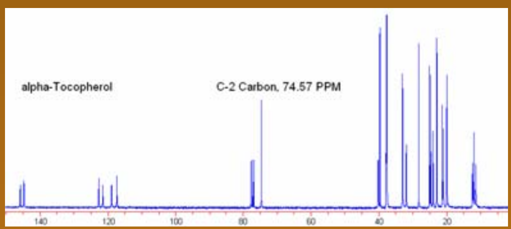


Figure 4. Tocopherols and tocotrienols provide useful diagnostic peaks in the aromatic region and for the C-2 resonance. Note the *absence* of tocopherols in the corn fiber oil (Figure 5, below).

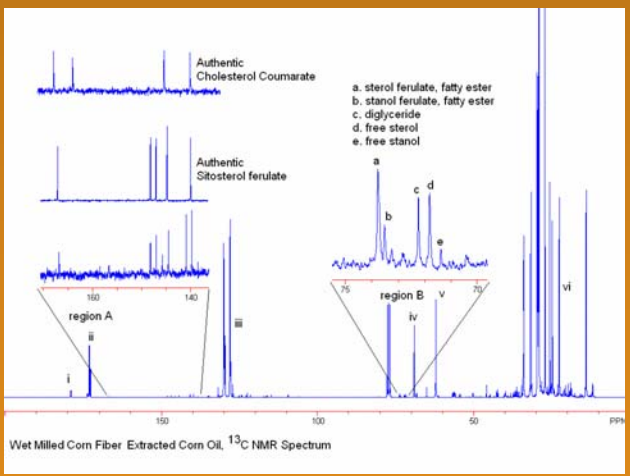


Figure 5. The 3-carbon of the sterol and stanol ring system and the aromatic carbons of ferulate and coumarate rings are useful diagnostic resonances for quantifying ratios of ester/free sterol and stanol/sterol ratios. The spectrum above illustrates the dominance of ferulate over coumarate esters (ratio > 7:1). Major product groupings are shown above: (i), free fatty acid carboxyl, (ii), glyceride and sterol/stanol fatty ester carboxylate, (iii), olefinic resonances, primarily of linoleic and oleic acid functions, (iv) quaternary triglyceride C-O, (v) outer triglyceride C-O's, and (vi) alkane carbon of glycerides. The resonances provide direct measure of classes of sterols, stanols, esters, and primary components in corn oil. Fumarate aromatic resonances remain unchanged as the sterol/fatty component is varied. Similarly, all classes of esters give identical chemical shifts for the C3 sterol carbon, within 0.1 ppm. Thus total fumarate, coumarate, content is directly available. The spectrum (region B) shows the absence of tocopherols in the corn fiber extracts (aliphatic C-O resonance of the C2 carbon at 74.6 ppm).

Finally, fatty acid esters and sterol ferulate making up peak a, region B can be decomposed into fatty ester and ferulate by comparison with the aromatic ferulate resonances. This exercise reveals that fatty esters occur in excess of ferulates by a ratio of 1.5-2:1, in agreement with independent analysis

Table. ^{13}C Chemical Shifts of C-3 Carbon of Sterols, Stanols, and Esters.

The data show separation of free sterols and stanols, allowing measurement of total alcohols and sterol/stanol ratio. The data show convenient separation of esters and free sterols, allowing the total of all sterol/stanol nuclei to be measured, and the ratio ester/free sterol to be measured

	Free Sterol/ Stanol	Ester			
		Ferulate	Coumarate	Acetate	Oleate
Cholesterol	71.81	74.126	73.908		73.858
Cholestanol	71.32				
Sitosterol	72.028	74.139		74.231	
Sitostanol	71.524	73.913			73.657
Campesterol	72.041	74.149			
Stigmasterol	72.030	74.143	74.028	74.162	73.861

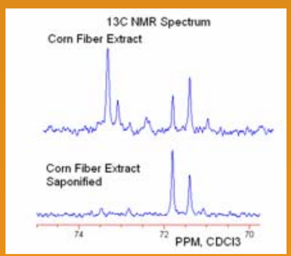


Figure 6. Figure (left) illustrates saponification of ferulate and fatty acid esters to free sterols and stanols. Total free sterol and sterol/stanol ratio determined.

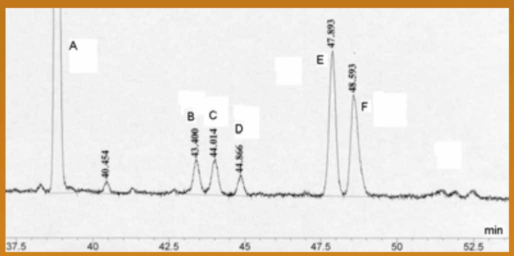


Figure 7. GC Analysis of Silylated Sterol Derivatives: A,cholesterol (standard), B, Campesterol; C, Campestanol; D, Stigmasterol, E, β-Sitosterol; F, β-Sitostanol. The GC analysis is carried out after saponification and Trimethylsilylation. Peak at 40.54 is brassicasterol.

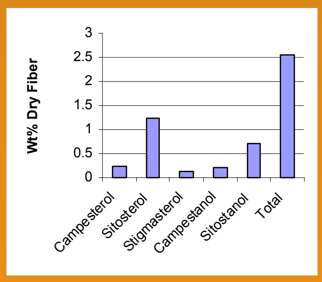


Figure 8 . GC analysis reveals 2.5 wt% sterols and stanols, and a sterol/stanol ratio of 1.6. The relative yields of sterols and stanols are essentially constant for the corn fiber extract oil for a given hydrolysis condition. NMR analysis reveals 2.6% yield for this material and a sterol/stanol ratio of 1.5.

Summary. With the availability of authentic sterol ester derivatives, this study provides data supporting the direct analysis of sterol/stanol content in grain oils. The spectroscopic methods are particularly useful for components that are difficult to analyze by gas chromatographic methods, or for which inadequate selectivity and structural uniqueness exists for liquid chromatographic methods. The superior structural information available from ^{13}C NMR spectroscopy together with detailed chemical shift information for constituents allows NMR to be conveniently used for analysis of minor organic components in grain byproduct streams.

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