Solid-State ^{13}C NMR of Cell Walls in Wheat Bran

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Cell walls from wheat bran were examined intact by cross-polarization, magic-angle spinning ^{13}C NMR to determine their polymer composition. The carbohydrate part of the ^{13}C spectrum was typical of graminaceous cell walls having cellulose and arabinoxylans as their main components. Only a little lignin was observed in the spectrum, but signals from the hydrocarbon chains of cutin were particularly obvious. It is suggested that cutin, rather than lignin, plays an important role in protecting wheat bran from microbial degradation in the rumen and in the human gut and that the hydrophobic properties of the cutin are likely to be responsible for some of its nutritional properties.

Keywords: *Bran; cell walls; lignin; cutin; solid-state NMR*

INTRODUCTION

While wheat bran is generally thought of as a lignified fiber material, its Klason lignin content of ca. 3% (U.S. Subcommittee on Feed Composition, 1982) is much lower than that of other lignified fibers such as straw. Bran in the strict anatomical sense is rather resistant to degradation in the rumen and in the human digestive tract, although commercial bran samples contain substantial amounts of adhering starchy endosperm and the starch is rapidly digested. The Klason lignin that is present seems therefore to be unusually effective in inhibiting degradation of the bran cell walls.

Solid-state ^{13}C NMR methods have proved useful in the investigation of lignified plant materials (e.g., Maciel et al., 1985; Leary et al., 1986; Love et al., 1992; Newman, 1992) since no solubilization is required and it is possible to look directly at the structure of components such as lignin that cannot be solubilized without degradation. Here we report a solid-state NMR study of wheat bran using the conventional CP-MAS (cross-polarization−magic angle spinning) method. It is possible to obtain more accurate quantitation of aromatic components such as lignin by avoiding the use of CP (Love et al., 1992), but this approach is less sensitive and hence less suitable for materials of low lignin content (Love et al., 1994).

EXPERIMENTAL PROCEDURES

Cell Wall Preparation. Wheat bran, purchased locally, was ground under liquid nitrogen for 1 min and added to 100 cm^3 of a boiling salt solution for 30 s to gelatinize starch. The sample was decanted into 750 cm^3 of chilled salt solution containing 10 mM sodium acetate, 3 mM KCl, 2 mM $MgCl_2$, and 1 mM $CaCl_2$. This solution is similar in ionic concentration to the solution surrounding the plant cell in vivo (Jarvis, 1991). The excess solution was poured off, and pancreatic α-amylase and pullulanase (Sigma) were added. The bran was stirred overnight in the enzyme solution to break down the starch. The cell walls were defatted and sequentially dried in acetone (50%, 75%, and 100%). Starch was shown to be absent by colorimetric determination with I_2/KI.

NMR Spectrometry. Water (0.25 cm^3/g) was added to improve the spectral resolution. The CP-MAS spectrum was measured on a Varian VXR-300 spectrometer with a contact time of 0.5 ms, a proton field ca. 40 kHz during both CP and data acquisition, and a relaxation delay of 1 s. The spectrum shown is derived from 8160 scans.

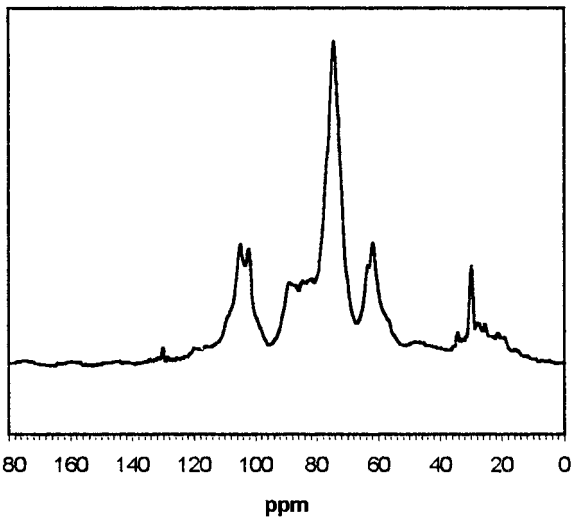

Figure 1. Solid-state ^{13}C NMR spectrum of cell walls from wheat bran.

RESULTS

The ^{13}C solid-state spectrum of cell walls from wheat bran is shown in Figure 1. Signal assignments are in Table 1. The carbohydrate portion of the spectrum is typical of graminaceous cell walls with secondary thickening (Maciel et al., 1985; Wallace et al., 1995), containing cellulose of moderately high crystallinity and acetylated arabinoxylans. The signal intensity from aromatic carbons (120−160 ppm) was unexpectedly low, and the characteristic signal at 153 ppm from 4-O-etherified syringyl residues in lignin was not detected. Reduced signal intensity from aromatic carbon nuclei is sometimes observed in CP-MAS spectra due to their low CP efficiency, particularly when the CP contact time is <1 ms. However this problem does not affect the methoxyl carbon peak at 56 ppm, and its low intensity suggests that phenylpropanoid residues similar to those found in lignin were present at a level of only about 2% of the bran cell walls.

An unexpected feature of the spectrum is the large contribution from the hydrocarbon chains of cutin (29 ppm) (Zlotnik-Mazori and Stark, 1988; Pacchiano et al.,

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Table 1. Resonance Assignments

chemical shift, ppm	assignment
173	carboxyl of acetyl
131	C-1 of phenolic acids and guaiacyl units in lignin and cutin
109	C-1 of arabinofuranose in arabinoxylans
105	C-1 of cellulose
102	C-1 of xylose in arabinoxylans
88	C-4 of crystalline cellulose
84	C-4 of crystallite-surface cellulose
82	C-4 of xylose in arabinoxylans
75	general carbohydrate C-2, C-3, C-5
65	C-6 of crystalline cellulose
62	C-6 of crystallite-surface cellulose, C-5 of xylan
56	aromatic methoxyl
41	protein
30, 28	CH_2 in cutin
21	CH_3 of acetyl

1993; Wallace et al., 1995). The aromatic peak at 130 ppm may also be assigned to phenolic residues in cutin. The high molecular mobility of some of the aliphatic components of cutinized cell walls (Zlotnik-Mazori and Stark, 1988) makes exact quantitation uncertain, but a delayed-contact experiment (data not shown) suggested that the relaxation behavior of the hydrocarbon signals observed did not differ greatly from that of the carbohydrates. It is possible that a certain amount of much more mobile aliphatic material (e.g., cuticular waxes) remained unobserved due to inefficient CP (Zlotnik-Mazori and Stark, 1988). While proteins also show some spectral features around 29−31 ppm, their intensity is normally less than at 41 ppm, so it is not likely that protein makes a significant contribution to the methylene signal at 29 ppm. The cutin content is therefore unlikely to be overestimated from the recorded intensity of the 29 ppm peak.

DISCUSSION

The CP-MAS spectrum makes it clear that wheat bran contains more cutin than lignin. Cutin is highly resistant to chemical as well as enzymic degradation and remains in the acid residue that is conventionally termed Klason "lignin". Cutin, however, is much more hydrophobic than lignin, and this may alter the biological properties of the material.

Wheat bran is a complex, multilayered structure (Kent, 1983), and cell walls from all its layers contribute to the NMR spectrum. The testa—the middle layer between the papery outer "beeswing" layers and the thick-walled aleurone layer next to the endosperm—has been shown by histological examination to contain a double cuticular layer, slightly different in ultrastructure from the cuticles of plant parts exposed to the air (Stenvert and Moss, 1974; Morrison, 1975). Waxes and hydroxylated fatty acids typical of cutin were extracted from the testa of barley (Briggs, 1974). Removal of the wheat testa by dissection greatly increased the rate of water permeation into the grain (Hinton, 1955). The presence of cutin in the testa is therefore likely to be crucial in the conditioning process (Kent, 1983), where a carefully controlled moisture gradient from the outer bran layers to the endosperm is used to control the fracture properties of the wheat grain during milling (Moss, 1973).

Many of the nutritional effects described for wheat bran supplementation will be due to the cutin content. Cutin has not been previously thought to play an important part in the human diet (Southgate, 1995). However, because cutin is more hydrophobic than lignin, this will have a critical effect on the nutritional effects of wheat bran.

In humans, wheat bran supplementation decreases constipation and the symptoms associated with constipation. Supplementation does not alter the proportions of bacteria in the colon although the total amount of bacteria excreted is increased (McLean-Baird et al., 1977; Stephen et al., 1986). A supplement of wheat bran decreases the risk of developing colonic cancer. Bile acids are bound in vitro (Kritchevsky and Story, 1993) and in vivo (Spiller et al., 1984) by wheat bran. However, when it is given as a supplement, cholesterol levels rise, but the rise in cholesterol may be due to the oil associated with the aleurone layer.

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LITERATURE CITED

Briggs, D. E. Hydrocarbons, phenols and sterols of the testa and pigment strand in the grain of *Hordeum distichon*. *Phytochemistry* **1974**, *13*, 987−996.

Hinton, J. J. C. Resistance of the testa to entry of water into the wheat kernel. *Cereal Chem*. **1955**, *32*, 296−306.

Jarvis, M. C. Solid state ^{13}C-n.m.r. spectra of *Vigna* primary cell walls and their polysaccharide components *Carbohydr. Res*. **1990**, *201*, 327−333.

Kritchevsky, D.; Story, J. A. Influence of dietary fiber on cholesterol metabolism in experimental animals. In *CRC Handbook of Dietary Fibre in Human Nutrition*, 2nd ed.; Spiller, G. A., Ed.; CRC: Boca Raton, FL, 1993; pp 163−178.

Leary, G. J.; Newman, R. H.; Morgan, K. R. A carbon-13 nuclear magnetic resonance study of chemical processes involved in the isolation of Klason lignin. *Holzforschung* **1986**, *41*, 13−18.

Love, G. D.; Snape, C. E.; Jarvis, M. C. Determination of the aromatic lignin content in oak wood by quantitative solid state ^{13}C NMR. *Biopolymers* **1992**, *32*, 1187−1192.

Love, G. D.; Snape, C. E.; Jarvis, M. C.; Morrison, I. R. Determination of phenolic structures in flax fibre by solid state ^{13}C NMR spectroscopy. *Phytochemistry* **1994**, *35*, 489−491.

Maciel, G. E.; Haw, J. F.; Smith, D. H.; Gabrielsen, B. C.; Hatfield, G. R. Carbon-13 nuclear magnetic resonance of herbaceous plants and their components, using cross polarization and magic-angle spinning. *J. Agric. Food Chem*. **1985**, *33*, 185−191.

McLean-Baird, I.; Walters, R. L.; Davies, P. S.; Hill, M. J.; Drasar, B. S.; Southgate, D. A. T. The effect of two dietary fibre supplements on gastrointestinal transit, stool weight and frequency, and bacterial flora, and fecal bile acids in normal subjects. *Metabolism* **1977**, *26*, 117−128.

Morrison, I. N. Ultrastructure of the cuticular membranes of the developing wheat grain. *Can. J. Bot*. **1975**, *53*, 2077−2087.

Moss, R. Conditioning studies on Australian wheat. II. Morphology of wheat and its relationship to conditioning. *J. Sci. Food Agric*. **1973**, *24*, 1067−1076.

Newman, R. H. Nuclear magnetic resonance study of spatial relationships between chemical components in wood cell walls. *Holzforschung* **1992**, *46*, 205−210.

Pacciano, R. A.; Sohn, W.; Chandla, V. L.; Garbow, J. R.; Stark, R. E. Isolation and spectral characterization of plant-cuticle polyesters. *J. Agric. Food Chem*. **1993**, *41*, 78−83.

Southgate, D. A. T. *Dietary Fibre Analysis*; Royal Society of Chemistry; Cambridge, U.K.,1995.

Spiller, G. A.; Wong, L. G.; Nunes, J. D.; Story, J. A.; Petro, M. S.; Furumoto, E. J.; Alton-Spiller, M.; Whittam, J. H.; Scala, J. Effect of four levels of hard wheatbran on fecal

composition and transit time in healthy young women. *Fed. Proc.* **1984**, *43*, 392.

Stenvert, N. L.; Moss, R. The separation and technological significance of the outer layers of the wheat grain. *J. Sci. Food Agric.* **1974**, *25*, 629−635.

Stephen, A. M.; Wiggins, H. S.; Englyst, H. N.; Cole, T. J.; Wayman, B. J.; Cummings, J. H. The effect of age, sex and level of intake of dietary fibre from wheat on large-bowel function in thirty healthy adults. *Br. J. Nutr.* **1986**, *56*, 3 49−361.

U.S. Subcommittee on Feed Composition. *US-Canadian Tables of Feed Composition*; National Academy Press: Washington, DC, 1982.

Wallace, G.; Russell, W. R.; Lomax, J. A.; Jarvis, M. C.; Lapierre, C.; Chesson, A. Extraction of phenolic-carbohydrate complexes from graminaceous cell walls. *Carbohydr. Res.* **1995**, *272*, 41−53.

Zlotnik-Mazori, T.; Stark, R. E. Nuclear magnetic resonance studies of cutin, an insoluble plant polyester. *Macromolecules* **1988**, *21*, 2412−2417.

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