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NMR and DFRC Characterization of Lignins Isolated from Fruit, Vegetable and Cereal Dietary Fiber

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Summary

Information about lignin in dietary fiber and in food in general is rare and usually based on the unspecific Klason lignin methodology to measure an acid-insoluble residue. The aim of our studies was to prove the existence of lignin according its definition in different dietary fibers and to structurally describe those lignins. Dietary fibers partially enriched in their lignins contents were analyzed by using the derivatization followed by reductive cleavage (DFRC) methodology to cleave diagnostic lignin β-aryl ethers in lignins and lignin-like materials thus liberating acetylated monolignols. Acetylated monolignols were liberated, often in small quantities, from all 14 cereal, fruit and vegetable fibers; yields were referred to acetyl bromide soluble "lignin" (ABSL) contents. Fiber lignins showed a wide range of syringyl/guaiacyl (S/G) ratios and released unusual monomers such as aldehydes. Dioxane/water milled lignins (DL) and acidolysis lignins (AL) were isolated from kiwi, pear, rhubarb and wheat fibers, showing interesting lignin compositions or being reported as highly lignified. Clean pear and kiwi lignin isolates allowed for substantive profiling by 2D-NMR (HSQC, HMBC), but it is suggested that the significance of lignin in wheat has been overestimated. S/G measures from volume-integration of NMR contours in 2D ^{13}C–^{1}H correlation spectra showed that pear lignin and wheat "lignin" have comparable G and S contributions, that kiwi lignins are particularly G-rich, and suggest that rhubarb lignins are S-rich. These data correlate well with the data from the DFRC method applied to whole lignin fractions. Typical lignin structures, including the newly discovered ones such as glycerols, spirodienones, and dibenzodioxocins, are demonstrated via their NMR correlations in the fruit and vegetable sample spectra.

Introduction

Lignins are polymeric natural products from three primary precursors (*p*-coumaryl, coniferyl (CA), and sinapyl (SA) alcohols) arising from an enzyme-catalyzed dehydrogenative polymerization [1,2] (Figure 1). Phenolics other than the three primary monolignols may also be incorporated into lignins [2], though often in much smaller quantities. Although some dietary fiber methodologies exclude the determination of lignins, they are generally regarded as dietary fiber components [3]. The incorporation of lignins into the definition of dietary fiber is justified by their properties: they are plant-derived, not absorbed in the small intestine,

probably partly degraded in the gut [4], and they exhibit potential health benefits. Lignins were shown to be efficient antioxidants [5-7] and structure-activity relationships were proposed [7,8]. As part of the cell wall complex, lignins are efficient adsorbers of mutagenic heterocyclic aromatic amines [9,10], thus preventing their absorption in the small intestine and their activation in the liver. Lignin abilities to adsorb heterocyclic aromatic amines are dependent on the lignin composition [9,10]. Although lignin was regarded as non-degradable, it was recently demonstrated that lignins are partly converted into mammalian lignans in rats [4].

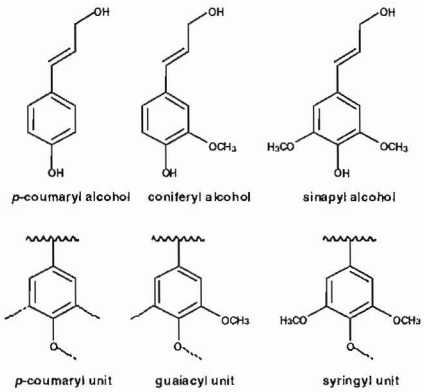


Figure 1. Primary lignin monomers (monolignols) and generic lignin units deriving from monolignols incorporated into the lignin polymer during lignification.

Knowledge about food-derived lignins is cursory at best. Most information about lignin contents is based on the application of the Klason lignin methodology, a method that non-specifically measures an acid-insoluble residue. Although it works fine for highly lignified tissues like in wood or grass stems, its application to low-lignin materials like food is questionable, probably severely overestimating lignin contents [11] by including residual structural proteins, tannin-protein complexes, cutin, waxes, etc. It was demonstrated that it is possible to create Klason lignin in samples that do not contain lignin at all [11]. Compositional data regarding food-derived lignins are even rarer and the available data are quite confusing. Therefore the aim of our studies was to establish the existence of lignins or lignin-like materials in a range of cereals, fruits and vegetables and to describe their compositions and structures [12-14].

Material and Methods

Fruits and vegetables were obtained from a local German supplier, cereal samples were from a local mill or were purchased from a local supermarket. Cultivars and origins were confirmed by the supplier. Their stages of maturity, textures, etc. met the requirements for human consumption.

Methods to isolate dietary fibers, to enrich the lignin contents in fibers, to separate low-molecular weight components possibly confused with polymeric lignin, isolation of DL and AL, application of the DFRC method [15] and NMR experiments are summarized in Figure 2 and detailed in Bunzel *et al.* 2004, 2005 [12, 13], and Bunzel & Ralph 2006 [14].

Results and Discussion

Using the DFRC method, coniferyl and sinapyl acetates were produced from all fruit, vegetable and cereal fibers investigated (Figure 3b). These results prove the existence of oligo- or polymeric phenolic compounds incorporating β-O-4-linked CA and SA as G or S units into their structures [12,13]. Analyzing the cereal fibers, interfering peaks, which possibly stem from polysaccharide degradation products not separated in our clean-up procedure, impeded the identification of *p*-coumaryl acetates. In some fruit and vegetable fibers the identification of *p*-coumaryl acetates was possible although, with the exception of small radish fiber, only in trace amounts. Fruit and vegetable fibers were enhanced in their lignin contents by partial enzymatic degradation of fiber polysaccharides, also minimizing the formation of interfering peaks during the DFRC procedure. In cereals, yields of the liberated monomers on a dietary fiber and especially on an ABSL basis were surprisingly low. The molar sum of liberated acetylated monomers CA and SA ranged between 5 and 15 μmol/g of

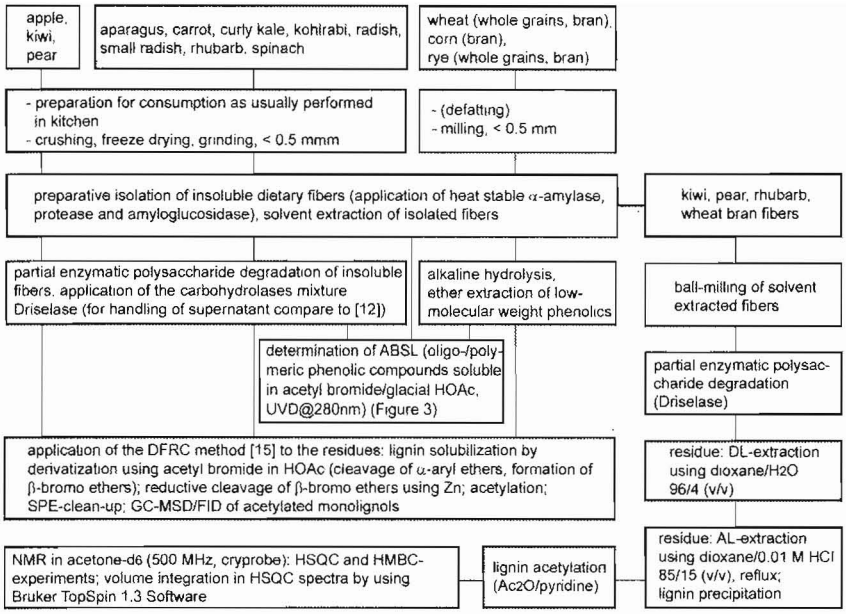


Figure 2. Overview about the applied methodologies to characterize lignin structures in insoluble dietary fibers from fruits, vegetables and cereals. ABSL – acetyl bromide soluble lignin; AL – acidolysis lignin; DL – dioxane water milled lignin; DFRC – derivatization followed by reductive cleavage.

dietary fiber and between 50 and 250 µmol/g of ABSL [13]. In grass stems, for example values about 350 µmol/g of lignin would be expected whereas from wood even higher yields (800 µmol/g of lignin and more) are achieved. Overestimation of ABSL would result in such low yields but it is also possible that "lignins" from cereal grains are not completely solubilized in the reaction medium due to particular matrix features or due to structural characteristics of cereal grain "lignins". The molar sum of liberated acetylated monomers CA and SA from fruit and vegetable fibers ranged between 20 and 514 µmol/g of ABSL. Low yields were obtained from radish and spinach fibers, whereas pear fiber showed the highest yield. Compositional information about the fiber lignins is available from the analyzed G/S ratios. These are shown in Figure 3, highlighting a remarkably high S-content in rhubarb lignins, whereas kiwi, spinach and carrot have extremely high G-levels.

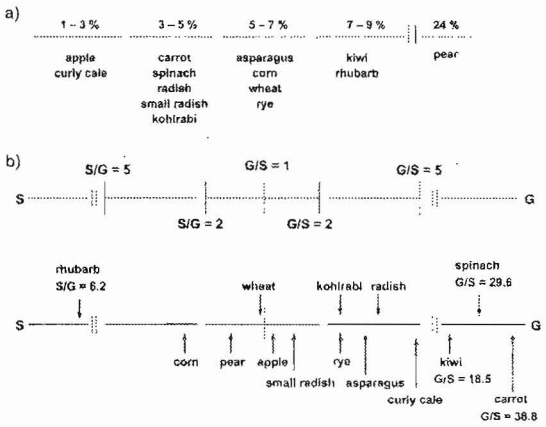
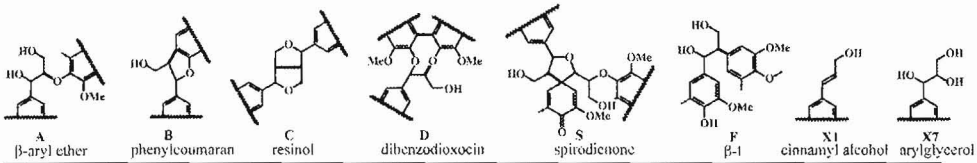


Figure 3. a) Acetyl bromide soluble lignin contents of different insoluble dietary fibers. b) Guaiacyl/syringyl (G/S) ratios of different pre-treated insoluble fibers as determined by application of the DFRC method.

DL and AL were isolated from pear, kiwi and rhubarb, whereas extraction of wheat resulted in highly impure DL and only tiny amounts of AL [14]. Applying 2D-NMR to acetylated DL and AL enabled us to evaluate the S/G-nature of the isolated lignins by volume integration of specific correlation peaks in the HSQC spectrum without the restrictions of the DFRC method that only measures β-O-4-linked G- and S-

units. In addition we identified typical interunit linkages in these lignins and, again by volume integration of specific correlation signals, we were able to determine their ratios (Table 1). For details concerning NMR profiling of the isolated lignins, especially using the HMBC experiment to identify the monomers that are involved in the various linkage types, we refer to the original paper [14]. Finally it has to be noted that lignin-like structures are also considered part of the (poly)phenolic domain of suberin, a polymer class differing considerably from lignins. Existence of suberin has to be considered especially for those fibers where low acetylated CA/SA-liberation yields (on an ABSL basis) were obtained and where the isolation of lignins was less successful, e.g. for wheat bran. However, diagnostic suberin long chain hydroxylated fatty acids were found only in trace amounts in the external layers of wheat grains [16].

Table 1. NMR-derived guaiacyl/syringyl (G/S) data and interunit linkage data for lignins from kiwi, pear, rhubarb, and wheat (interunit linkages A –X7 are shown below).



Sample	Yield [mg/g]§	%G	%S	S/G	%A	%B	%C	%D	%S+F	%X1	%X7
Kiwi Ac-DL#	26	93.6	6.4	0.07	68.3	12.0	3.9	8.2	2.6	3.7	1.2
Kiwi Ac-AL*	24	98.4	1.6	0.02	73.0	12.8	3.4	7.0	1.0	2.3	0.4
Pear Ac-DL	29	45.1	54.9	1.2	77.9	4.5	10.6	2.1	2.3	1.7	0.9
Pear Ac-AL	26	41.8	58.2	1.4	82.1	4.3	9.7	1.5	1.2	0.7	0.5
Rhubarb Ac-DL	15	3.6	96.4	27	93.0		5.8		1.3		
Rhubarb Ac-AL	7	2.2	97.8	45	95.5		4.5				
Wheat Ac-DL	8	44.6	55.4	1.2	90.5		9.5				

§ Yield is on a total insoluble fiber basis; # Ac-DL – acetylated dioxane/water milled lignin; Ac-AL – acetylated acidolysis lignin

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