

FT-Raman Spectra of Unsoaked and NaOH-Soaked Wheat Kernels, Bran, and Ferulic Acid

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ABSTRACT

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The sodium hydroxide (NaOH) test for determining wheat color class depends on the observation that on soaking in NaOH, red wheat turns a darker red and white wheat turns straw yellow. To understand the mechanism of this test, Raman spectra of wheat bran, wheat starch, ferulic acid, and whole kernels of wheat, before and after NaOH soak, were studied. The major observable components in the whole kernel were that of starch, protein, and ferulic acid, perhaps esterified to arabinoxylan and sterols. When kernels are soaked in NaOH, spectral bands due to ferulic acid shift to lower energy and show a slightly reduced intensity

that is consistent with deprotonation of the phenolic group and extraction of a portion of the ferulic acid into solution. Other phenolic acids, alkyl resorcinols, and flavonoids observed in the NaOH extracts of wheat by HPLC were not observed in the Raman spectra. Wheat bran accounts for most of the ferulic acid in the whole kernel, as indicated by the increased intensity of the doublet at 1,631 and 1,600 cm^{-1} in the bran. The intense starch band at 480 cm^{-1} in whole kernel wheat was nearly absent in the wheat bran.

The sodium hydroxide (NaOH) test for determination of wheat color class depends on the observation that on soaking in NaOH, genetically red wheat turns a darker red and genetically white wheat turns straw yellow. This test works for all cultivars of wheat, including red wheat that appears visually white and white wheat that appears visually red (Ram et al 2002). The mechanism of this reaction is not understood. It is possible that NaOH deprotonates the phenolic compounds in wheat bran to cause the color changes.

Wheat contains several phenolic acids such as ferulic (4-hydroxy-3-methoxycinnamic), isoferulic (3-hydroxy-4-methoxycinnamic), coumaric (4-hydroxycinnamic), vanillic (4-hydroxy-3-methoxybenzoic), syringic (3,5-dimethoxy-4-hydroxybenzoic), caffeic (3,4-dihydroxycinnamic), and sinapinic (3,5-dimethoxy-4-hydroxycinnamic) acids (Rybka et al 1993; Hatcher and Kruger 1997). Ferulic acid, the main phenolic acid in wheat occurs in a concentration of $\approx 200 \mu\text{g/g}$ in wheat flour and $\approx 2,000 \mu\text{g/g}$ in bran (Pussayanawin et al 1988; Rybka et al 1993). Ferulic acid was determined a cell wall constituent by UV microscopy (Akin 1995), by HPLC analysis of bran extracts (Collins and D'Attilio 1996), and by fluorescence microscopy of wheat (Fulcher and Wong 1980). Wheat flour contains 2–3% pentosans (Geissmann and Neukom 1973; Petit-Benvegnen et al 1998). These are composed of arabinoxylans with a linear backbone of β -1,4-linked xyloses. The phenolic acids are generally esterified to arabinosyl residues at 2-O or 3-O branches of the xylan backbone (Izydorczyk and Billiaderis 1995). Phenolic acids esterified to plant sterols also have been reported (Seitz 1989). Arabinoxylan chains are cross-linked in the cell wall through diferulic bridges (Peyron et al 2001). Polymerized ferulic acid bound by ether bonds in lignin are alkali-resistant (Scalbert et al 1985).

Other phenolic compounds in wheat bran include apigenin and other flavonoids (Collins and D'Attilio 1996; Feng et al 1988; Feng and McDonald 1989). Miyamoto and Everson (1958) identified catechin and catechin tannin as the precursors of brown pigment and showed a correlation between kernel color. McCallum and Walker (1990) suggested that trace levels of proanthocyan-

dins in the bran contributed to seed coat color in wheat. Wheat also contains a major group of 5-*n*-alkylresorcinols (Seitz and Love 1987; Al-Ruqaie and Lorenz 1992) with odd-number side chains of C15-C25, 5-(2-oxoalkyl), and 5-(2-oxoalkenyl) resorcinols (Seitz 1992).

NIR spectroscopy has been used to distinguish red from white wheat before NaOH soak (Dowell 1998) and after NaOH soak (Dowell 1997; Ram et al 2002). However, identification of individual components is difficult and many times impossible. Aromatic compounds, particularly the cinnamic acid derivatives, exhibit characteristic and intense Raman spectra. Generally, the intensity of Raman bands for nonpolar or slightly polar groups is higher than for polar groups, and intensity of stretching vibrations is higher than that for deformation vibrations. Raman intensity is also usually higher for symmetric vibrations than for antisymmetric vibrations and enhanced for stretching vibrations of multiple bonds, such as that of $\nu_{\text{C}=\text{C}}$ (Baranska et al 1987; Grasseli and Bulkin 1991; Lewis and Edwards 2001).

Raman spectroscopy has been applied to determine protein and apparent amylose contents of milled rice (Himmelsbach et al 2001), amylose content in maize (Phillips et al 1999a), benzyl and acetyl modification of starches (Phillips et al 1999b, 2001), and for monitoring a bioprocess for ethanol production (Sivakesava et al 2001). Ma and Phillips (2002) have reviewed the applications of FT-Raman spectroscopy to cereal science. Wheat sections have been studied by confocal Raman microspectroscopy, and the spatial distribution of protein and phenolic compounds has been described (Piot et al 2000, 2002).

The objective of the research described here was to study the reaction of wheat soaked in NaOH by Raman spectroscopy.

MATERIALS AND METHODS

Samples. Hard red (R) and hard white (W) wheat samples were available from a previous study (Ram et al 2002). Raman spectra were obtained for whole kernels of eight samples of Heyne (W), seven samples of Tam 107 (R), six samples each of White Eagle (W) and NuPlains (W), five samples each of Larned (R) and Scout 66 (R), four samples each of Arlin (W) and Big Dawg (R), two samples each of Trego (W), 2174 (R), Ike (R), and Quantum (R), and one sample each of Betty (W), Oro Blanco (W), Rio Blanco (W), Jagger (R), Custer (R), and Akron (R). Hot and cold water-soaked samples were prepared by soaking kernels in $\approx 60^\circ\text{C}$ water for ≈ 20 min and water at ambient temperature ($\approx 22^\circ\text{C}$) for 30 min. Spectra of Betty (W), Rio Blanco (W), Scout66 (R), and Tam107 (R) soaked in hot and cold water were obtained. NaOH-soaked wheat was obtained by soaking whole wheat kernels according to

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a standard procedure (Ram et al 2002) and letting the kernels dry overnight on a filter paper. Spectra of NaOH soaked samples were obtained for two samples each of Trego (W) and 2174 (R), and one sample each of Betty (W), Oro Blanco (W), Rio Blanco (W), Heyne (W), Akron (R), Jagger (R), and Tam107 (R). Spectra of unsoaked bran were obtained for Betty (W), Rio Blanco (W), Tam107 (R), Scout66 (R), and Custer (R). Only one spectrum of bran soaked in NaOH was obtained, Betty (W), as this process ruined the Raman tube. Phenolic acids, apigenin, and pentadecyl resorcinol were obtained from Sigma-Aldrich (St. Louis, MO). Raman spectra of these chemicals were obtained from KBr pellets and also in 5M NaOH (presumably, the corresponding phenoxides were generated by dissolving the phenolic compounds in 5M NaOH).

Spectra. All spectra were obtained using a Nexus 870 FT-IR Continuum microscope attached to an FT-Raman system (Thermo Nicolet, Madison, WI). Data acquisition and analysis were performed using Thermo Nicolet software Omnic v 5.2. The FT-Raman system was equipped with a 1,064-nm Nd:YAG laser source and a CaF₂ beam splitter. The laser power available was 0–1.5W at the focus. For most samples, 0.32W was used to avoid overheating of the sample and to avoid fluorescence problems. Also, it was enough to obtain sufficiently strong signal-to-noise ratio for most peaks of interest. Overheating of the sample was particularly enhanced with red wheat and even more so after NaOH soak. The instrument had a fully motorized sample-positioning system that provided focus adjustment using the software. The instrument was equipped with two detectors, an InGaAS and a liquid nitrogen-cooled Ge detector. Only the Ge detector was used in these studies because it provided the best signal-to-noise ratio. Raman spectra of all samples were collected using 180° reflective geometry. The IR and Raman compartments were purged with compressed air that had been filtered free of organics, CO₂, and moisture. Sample holders were generally NMR tubes (7.8 × 5 mm) made of special thin, fluorescence-free glass (Thermo Nicolet). Raman spectra of phenolic compounds in KBr pellets were obtained by using a pellet holder accessory (Thermo Nicolet). FT-Raman spectra were obtained by placing each tube in front of the laser and focusing the Nd:YAG laser beam into the sample by maximizing the amplitude of the interferogram. Spectra were produced over the Raman shift 3,700–300 cm⁻¹, although the instrument could collect spectra down to 100 cm⁻¹. Typically, 256–512 interferograms were added at 4 cm⁻¹ resolution, with a sampling time of under 17 min. Because spectra obtained were slightly noisy, they were Fourier-smoothed

at 50% using Grams/32 AI v. 6.0 software (Thermo Galactic, Salem, NH). Even with the NIR laser, there was baseline drift in the spectra of whole kernels and bran owing to fluorescence from pericarp and seed coat material. Spectra were baseline-corrected using the Omnic v. 5.2 software to correct spectral drifts. The effect of baseline correction and smoothing on the Raman spectrum of a wheat kernel is shown in Fig. 1. Raman sample tubes were cleaned with soap, water, acetone, and methanol, making sure to remove any marks from charring and any residues from previous samples. Compounds were identified by matching sample spectrum with the literature (Baeten et al 1998; Himmelsbach and Akin 1998; Piot et al 2000), matching with library spectra (Thermo Nicolet), and by comparison of spectra obtained with samples of starch and ferulic acid.

Spectra were analyzed by visual inspection of overlaid spectra. Differences between spectra were obtained by using the spectral subtract procedure in the Omnic software.

RESULTS AND DISCUSSION

Red and white wheat. Approximately 29 Raman spectra of each red and white wheat were analyzed by inspecting overlaid spectra and by analysis of subtracted spectra. Spectra of multiple samples of several cultivars of each red and white wheat were essentially identical. The spectra of red and white wheat are not different visibly and by spectral subtraction. However, it appears that Neural Networks (NeuralWare, Pittsburgh, PA) can classify red and white wheat from their spectra in the wavelength range 1,550–1,700 cm⁻¹ (ferulic acid and gluten absorption region). Spectra of 40 unsoaked samples were used for training the network and eight samples were used for the test set. Software default values for momentum of 0.4 and learning rate of 0.5 were used. With these network parameters, classification was 100% correct for this limited set. The relative intensities of peaks due to CH₂, starch, protein, and ferulic acid varied slightly, but the position of the various spectral bands were identical. Significant differences were found only in the intensities of bands corresponding to starch and protein. Substantial random noise in the spectra interfered with accurate analysis of any minor spectral differences. This is probably due to the fact that we are using a 1,064 nm laser for excitation compared with visible lasers of 532 and 788 nm. Raman scattering intensity increases as the fourth-order of the (λ^4) excitation wavelength (Lewis and Edwards 2001). Thus, the Raman spectra of red and white wheat kernels, as represented in Fig. 2, were nearly identical. Also, FT-Raman is not

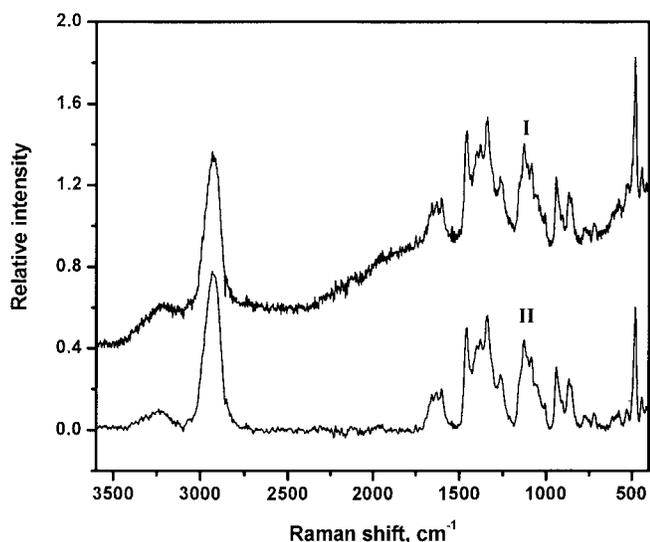


Fig. 1. Uncorrected Raman spectrum of a whole kernel of wheat (I), and after baseline correction for fluorescence and Fourier (50%) smoothing of the spectrum (II).

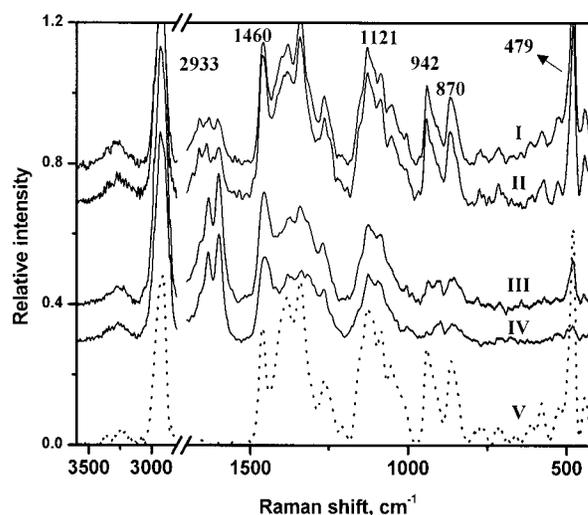


Fig. 2. Typical Raman spectra of unsoaked whole kernels of Tam107 (I) and Betty (II) wheat, bran of Tam107 (III) and Betty (IV) wheat, and wheat starch (V). Spectra of wheat kernels and bran (—); starch (---). Spectra baseline-corrected for residual fluorescence and Fourier-smoothed.

as sensitive as dispersive Raman. Similarly, spectra of bran from red and white wheat were also nearly identical. Soaking wheat kernels in cold water did not cause any significant change. Soaking in hot water changed the relative intensities of various bands, but did not cause any spectral shifts in any of the bands. It is possible that Raman spectra of some of the color components in red and white wheat are either weak or buried in some of the stronger bands of the endosperm components (starch and gluten).

Whole kernel, bran, and starch. A summary of the major Raman bands and their tentative assignments for whole wheat kernels (before and after NaOH soak), wheat bran, and wheat starch is provided in Table I. Starch and waxy hydrocarbons accounted for most of the peaks observed in the wheat kernel (Fig. 2, Table I) except the region 1,650–1,580 cm^{-1} . We inferred the presence of waxes and triglycerides from unpublished IR studies in which a peak at 1,735 cm^{-1} is observed in whole wheat and bran. This peak is weak in intensity in Raman (Baeten et al 1998). The presence of waxy materials was also indicated by the ratio of intensities of peaks at 2,931 cm^{-1} and 1,452 cm^{-1} in the whole kernel or bran compared with that for starch. However, the spectrum of the whole kernel can essentially be obtained by adding the spectra of

starch and bran (Fig. 2, Table I). The bands in the region 1,650–1,580 cm^{-1} were found in whole wheat kernels and wheat bran, but were absent in wheat starch. Gluten, the other major component of wheat flour, exhibited major peaks at 3,063(0.2), 2,934(1.3), 1,659(0.8), 1,450(0.8) cm^{-1} (numbers in parentheses are relative peak heights). Thus, the peak at 1,661 cm^{-1} found in the whole kernel may be attributed to amide I stretch in α -helix protein (Carter and Edwards 2001). This peak was of weaker intensity in the bran and appears only as a shoulder.

Ferulic acid in bran and whole kernels. The relative intensities of 1,631 and 1,600 cm^{-1} bands to the bands in the 1,460–1,380 cm^{-1} region are higher for bran than for the whole kernel. Ferulic acid has two bands of high intensity at 1,631 and 1,603 cm^{-1} (Table II) that match with two bands in wheat bran and kernel, as shown at the bottom of Fig. 3. From Tables I and II, it can be noticed that the bands in wheat bran are at 1,633 and 1,600 cm^{-1} . The slight shift in the ferulic acid frequencies in wheat was consistent with it being esterified to arabinoxylan (Fig. 4). Piot et al (2000) reported that these two frequencies shifted to lower energies, when ferulic acid was bound to arabinoxylan of 3 and 6 cm^{-1} , respectively. In HPLC analysis of acidified NaOH extracts of wheat, ferulic acid was the major phenolic constituent. Several other phenolic compounds, including *p*-coumaric, caffeic, and vanillic acids, some

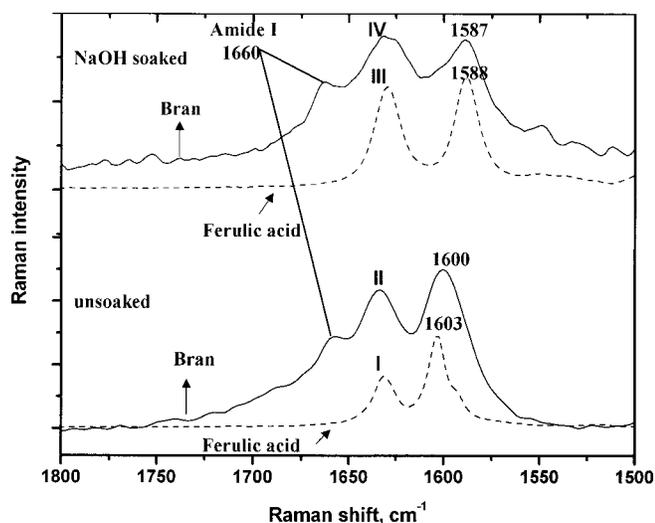


Fig. 3. Raman spectra of ferulic acid (I), wheat (Betty) bran (II), ferulic acid in NaOH (III) and wheat (Betty) bran soaked in NaOH.

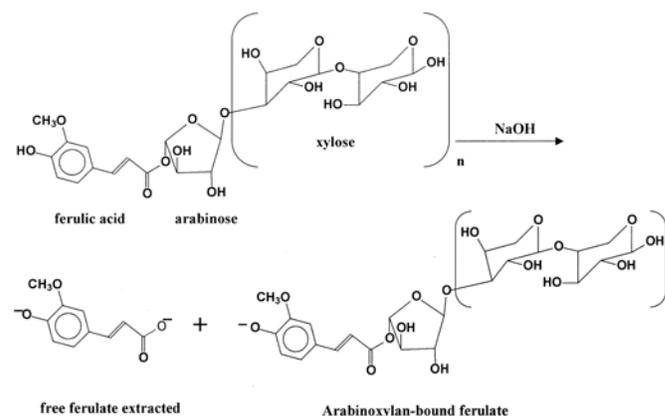


Fig. 4. Ferulic acid is bound to pentosans such as arabinoxylans in cell walls. NaOH can deprotonate the ferulic acid (or other phenolic acids) and can also hydrolyze the bound esters. Deprotonation of ferulic acid could cause a shift in Raman bands such as those shown in Fig 3.

TABLE I
Tentative Assignment of Major Raman Scattering Bands in Wheat^a

Whole Kernel		Bran	Starch	Vibration ^b	Compound ^b
Before NaOH soak	After NaOH soak				
2,932 (s)	2,925(s)	2,928 (s)	2,931 (s)	-CH ₂ -, ν asymmetric	Wax, hydrocarbon, fatty acid, starch
1,662 (m)	1,660(m)	1,661 (w)	...	-CO-NH-, Amide I	Protein
1,631(m), 1,604 (m)	1,628 (m), 1,585 (m)	1,633 (s), 1,600 (s)	...	ν -C=C-, benzene ring	Ferulic acid, lignin
1457, 1,382, 1,341 (s)	1,459, 1,380, 1,338 (s)	1,456, 1,378, 1,339 (s)	1,464, 1,380, 1,339 (s)	δ -CH ₂ -	Polysaccharides, arabinan, xylan, xyloglucan
1,264 (m)	1,262 (m)	1,268 (m)	1,262 (m)	=C-H in-plane deformation	Unsaturated fatty acid, ferulic acid
1,129, 1,084, 1,054 (s)	1,126, 1,084, 1,054 (s)	1,126, 1,084 (s)	1128, 1085, 1053 (s)	C-C, C-O	Cellulose, carbohydrate
942, 870 (m), 773 (w)	941, 867 (m), 769 (w)	936, 871 (m)	942, 864 (m), 772 (w)	C-O-H, C-C-H, O-C-H deformation	
578 (w), 527 (w), 479 (s), 443 (w), 359 (w)	574 (w), 521 (w), 480 (s), 443 (w), 357 (w)	573 (w), 479 (w), 443 (w)	579 (w), 528 (w), 478 (s), 442 (w), 357 (w)	ν C-H	Polysaccharides depends on dihedral angles and glycosidic linkages

^a S, strong; M, medium; W, weak.

^b Assignments based on Baeten et al (1998), Himmelsbach and Akin (1998), Piot et al (2000).

long-chain alkylresorcinols, and some flavonoids were also found (Collins and D'Attilio 1996; Hatcher and Kruger 1997). Raman spectra of several phenolic compounds found in wheat bran and the corresponding phenoxides are provided in Table III. Peaks representing phenolic acids other than ferulic acid were not distinct in the Raman spectra of wheat bran and whole kernels because they were present in lower amounts and had spectra overlapping with ferulic acid. The fact that phenolic compounds other than ferulic acid could not be detected in the Raman spectra was a probable reason why we were unable to observe any spectral differences between red and white wheat.

Cellulose, hemicellulosic polysaccharides, and starch. Most of the bands in the Raman spectra of the whole kernel were also found in starch. But the sharp intense band at 479 cm⁻¹ in starch and wheat kernel was nearly absent in wheat bran (Fig. 2). This was probably due to the fact that very little starch was present in wheat bran. Ferulic acid is reported to be bound to arabinoxylans (Fig. 4) in the bran layers, and arabinoxylans are characterized by Raman vibration frequencies 1,464, 1,369, 1,314, 1,123–1,090, 898, and 495 cm⁻¹ (Piot et al 2000; Peyron et al 2001). Studies of xylan-type polysaccharides and associated cell-wall components

by FT-IR and FT-Raman spectroscopies have been reported (Kacurakova et al 1999). Cellulose is characterized by Raman vibrations 1,120, 1,095, 900 (w), and 380 (w) cm⁻¹ (Himmelsbach and Akin 1998). Raman spectra of wheat bran indicated the presence of these bands. However, ferulic acid and other phenolic acids were found partly esterified to other alcohols and sterols (Seitz 1989; Collins and D'Attilio 1996), thus indicating that perhaps ferulic acid or any of the other phenolic acids was not exclusively bound to arabinoxylans.

NaOH-soaked kernels. Raman spectra of four cultivars of red and five cultivars of white wheat kernels, including two samples of one cultivar of each color class, soaked in NaOH were analyzed by visual comparison of the spectra to the spectra from corres-

TABLE II
Raman Spectra of Ferulic Acid and Disodium Ferulate

Raman Shift (cm ⁻¹)		Vibration
Ferulic Acid ^a	Disodium Ferulate ^b	
3,088, 3,061, 3,018	2,985	v Ar-H
2,983	2,860	v-CH ₃
1,631, 1,603	1,629, 1,588	v C=C, benzene ring
1,434	1,428, 1,389	δ CH ₃ , asym., sym.
1,274, 1,242, 1,178	1,268, 1,254 (m) 1,196	v C-C, C-O

^a Ferulic acid in KBr pellet.

^b Ferulic acid in aqueous NaOH at ≈pH 14.

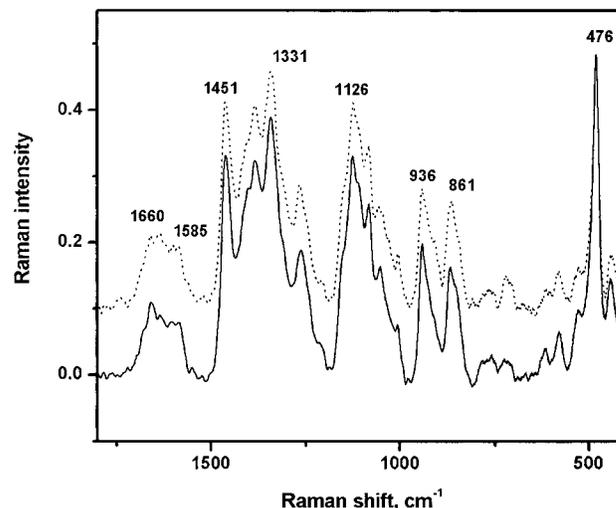


Fig. 5. Raman spectra of NaOH soaked wheat kernels Tam107 (—) and Betty (---).

TABLE III
Raman Spectra of Phenolic Compounds in Wheat Bran

Phenolic Compound	Raman Peaks (cm ⁻¹) (relative intensity)
Cinnamic acids	
Caffeic acid	3085(0.02), 3031(d)(0.02), 1642(0.51), 1616(1.0), 1534(0.11), 1355(0.14), 1301(0.81), 1188(0.25), 1109(0.1), 976(0.09), 854(0.08), 805(0.13), 448(0.08)
Caffeic acid and NaOH	1623(1.0), 1562(0.81), 1485 (0.33), 1387(0.39), 1300(0.43), 1273 (0.83), 1239(0.34), 1176(0.24), 1120(0.18)
<i>p</i> -Coumaric acid	3069(0.04), 3051(0.03), 3025(0.03), 1637(0.28), 1607(1.0), 1520(0.04), 1449(0.06), 1307(0.07), 1283(0.10), 1260(0.17), 1214(0.17), 1172(0.34), 978(0.04), 865(0.06), 801(0.05)
<i>p</i> -Coumaric acid and NaOH	1630(0.73), 1592(1.0), 1545(0.17), 1426(0.17), 1387(0.35), 1252(0.68), 1171(0.55)
Ferulic acid	Refer to Table II
Sinapinic acid	3023(0.06), 3004(0.05), 2965(0.02), 2938(0.03), 2839(0.03), 1646(0.50), 1597(1.0), 1518(0.15), 1461(0.11), 1426(0.11), 1337(0.25), 1306(0.70), 1273(0.16), 1161(0.37), 1118(0.09), 821(0.09)
Sinapinic acid/NaOH	1629(0.37), 1585(0.35), 1457(0.15), 1392(0.13), 1327(0.28), 1281(0.43), 1157(0.15)
Benzoic acids	
<i>p</i> -Hydroxybenzoic acid	3087(0.25), 3055(0.1) 1613(1.0), 1601(0.98), 1443(0.14), 1315(0.25), 1290(0.37), 1266(0.19), 1224(0.11), 1167(0.28), 1133(0.26), 844(0.34)
<i>p</i> -Hydroxybenzoic acid and NaOH	1590(0.15), 1381(0.61), 1296(0.14), 1142(0.25), 861(0.18)
Syringic acid	3080(0.16), 3035(0.338), 2983(0.55) 1699(1.0), 1594(0.82), 1469(0.24), 1446(0.23), 1372(0.28), 1323(0.28), 1200(0.60), 1181(0.31), 1103(0.23), 1033(0.27), 805(0.25)
Syringic acid and NaOH	1581(0.23), 1359(0.33)
Vanillic acid	3085(0.18), 3043(0.17), 2989(0.12), 2957(0.13), 2855(0.1), 1643(0.24), 1602(1.0), 1427(0.18), 1381(0.16), 1287(0.38), 1207(0.18), 1183(0.19), 920(0.15), 808(0.24), 742(0.45)
Vanillic acid and NaOH	1586(0.63), 1451(0.21), 1418(0.33), 1367(0.68), 1330(0.24), 1321(0.27), 1314(0.28), 930(0.21), 783(0.20), 775(0.20)
Flavonoids	
Apigenin	3078(0.11), 2984(0.10), 1655(0.16), 1608(0.53), 1590(0.95), 1501(0.30), 1446(0.22), 1404(0.19), 1357(0.21), 1270(0.39), 1247(1.0), 1184(0.37), 1119(0.11), 833(0.17), 744(0.10), 647(0.16), 581(0.20)
Catechin	3067(0.40), 1613(0.84), 1440(0.32), 1361(0.49), 1304(0.46), 1283(0.52), 1192(0.33) 1021(0.63), 768(1.0), 593(0.65), 555(0.42)
Catechin and NaOH	1578(0.28), 1547(0.11), 1321(0.11) 1302(0.18), 1169(0.09), 1021(0.16), 780(0.12), 759(0.12)
Quercetin	1609(1.0), 1593(0.62), 1550(0.65), 1440(0.60), 1401(0.57) 1373(0.50), 1330(0.64), 1321(0.56)
Quercetin/NaOH	1589(0.48), 1562(0.65), 1500(0.42), 1404(0.37), 1343(0.47), 1295(0.25)
5-Alkylresorcinol	
5-Pentadecylresorcinol	3052(0.13), 2933(0.14), 2984(1.0), 2848(0.61), 1609(0.10), 1466(0.22), 1441(0.35), 1421(0.18), 1354(0.12), 1297(0.37), 1131(0.26), 1065(0.24), 1003(0.60), 593(0.18)
5-Pentadecylresorcinol and NaOH	1559(0.15), 1452(0.2), 1441(0.22), 1302(0.18), 1078(0.14), 982(0.26)

ponding unsoaked kernels and by spectral subtraction. On soaking the wheat kernel in NaOH, there was a slight reduction in the intensities of certain bands accompanied by slight shifts in the band positions. The peak at 1,633 cm^{-1} shifted to 1,628 cm^{-1} , and the peak at 1,600 cm^{-1} shifted to 1,587 cm^{-1} (Fig. 3, Table I). The reduction in intensity perhaps corresponded to extraction of some ferulic acid into the solution, and the spectral shifts were consistent with deprotonation of the phenolic group in ferulic acid bound to arabinoxylan (Fig. 4). Unlike NaOH soaking, soaking red or white wheat kernels in hot or cold water did not cause spectral shifts in the ferulic acid bands. A comparison of Raman spectra of bran and ferulic acid, before and after NaOH soak, is shown in Fig. 3. Ferulic acid when deprotonated with aqueous NaOH (\approx pH 14) showed similar shifts in band positions, with the peak at 1,631 cm^{-1} shifted to 1629 cm^{-1} and the peak at 1,603 cm^{-1} shifted to 1,588 cm^{-1} (Fig. 3, Table II). At this pH, ferulic acid deprotonates both at the carboxylic acid and the phenolic groups, however, the spectral changes are caused by the deprotonation of only the phenolic group in ferulic acid as observed in NaOH-soaked wheat kernels and bran. There were no noticeable differences in Raman spectra from NaOH-soaked kernels of red and white wheat (Fig. 5). It is possible that Raman spectra of the color components in red and white wheat, even after NaOH soak, were weak or buried in some of the stronger bands that were observed.

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