Variation in the Extractability of Esterified *p*-Coumaric and Ferulic Acids from Forage Cell Walls

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Extraction conditions that may affect yield of esterified phenolics from forage cell walls were examined. Esterified *p*-coumaric and ferulic acids were extracted from alfalfa, smooth bromegrass, and switchgrass samples with various concentrations of NaOH. Forage fiber samples were prepared prior to alkaline extraction by enzymatic removal of starch and alcohol extraction (AIR), extraction with neutral detergent (NDF), or extraction with neutral detergent followed by ball-milling to reduce particle size (BM-NDF). The forage species had different phenolic acid contents, with alfalfa having the lowest and switchgrass the greatest. An interaction was observed for sample preparation method and alkali concentration. The AIR and NDF gave maximal yields of *p*-coumaric and ferulic acids from alfalfa with use of 2 M NaOH for extraction, whereas the yield from grasses peaked at 1 M. Recovery of *p*-coumaric and ferulic acids was greater from the AIR preparation than NDF for all forages. NaOH at 4 M concentration was needed for maximal yield from BM-NDF of grasses, and 6 M concentration was best for alfalfa BM-NDF. Immature and mature growth stage samples of forages behaved differently in response to extraction conditions. The molar ratio of *p*-coumaric to ferulic acids extracted from forage samples changed in response to different preparation conditions and alkali concentrations. Recommendations are given for maximizing yields of extractable phenolic acid esters from forage cell walls.

p-Coumaric and ferulic acids have been found to be esterified to the cell walls of grass and legume species (Jung et al., 1983a). Concentrations of these esterified phenolic acids are greater in grasses than legumes, but the distribution of phenolic acids is different between anatomical fractions of these forages. In corn (*Zea mays*) herbage, stem concentrations of *p*-coumaric and ferulic acids were greater than those in leaves (Hartley and Haverkamp, 1984); however, the opposite result was observed in alfalfa (*Medicago sativa*) (Bittner, 1984). Physiological maturity of forages is known to alter the concentrations of esterified *p*-coumaric and ferulic acids (Jung et al., 1983b; Burritt et al., 1984). These esterified phenolic acids have been implicated as inhibitors of ruminal fermentation of cell wall polysaccharides based on negative correlations observed between in vitro digestibility of grasses and the *p*-coumaric to ferulic acid ratio of the forage (Hartley, 1972; Burritt et al., 1984). As the ratio of these phenolics increased, digestibility was observed to decline. Concentration of *p*-coumaric acid, but not ferulic acid, also was negatively correlated with ruminal digestibility of grasses (Burritt et al., 1984). The ability of these cinnamic acids to inhibit ruminal fermentation of polysaccharides has been corroborated with model systems using free and ester-linked *p*-coumaric and ferulic acids (Akin, 1982; Jung and Sahlu, 1986a).

Virtually all the data on esterified cinnamic acid concentrations in forage cell walls were derived by extraction of neutral detergent fiber (Goering and Van Soest, 1970) with 1 M NaOH. The purpose of this study was to evaluate the effect of sample preparation method to remove non cell wall components, sample particle size, and alkali concentration on yield of *p*-coumaric and ferulic acids from various forages.

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MATERIALS AND METHODS

Forage Sample Preparation. Alfalfa, smooth bromegrass (*Bromus inermis*), and switchgrass (*Panicum virgatum*) were chosen as forage sources as they represent the three major taxa of forages (C_3 legume, C_3 grass, and C_4 grass, respectively) normally fed to ruminants and are known to differ in cell wall chemistry (Akin et al., 1984). A single sample of each forage species was harvested at a vegetative, immature growth stage and again after development of mature seed. Total herbage was collected by clipping the plants 2 cm above ground level. Forage samples were frozen, lyophilized, and ground through a 1-mm screen in a cyclone mill (Tecator AB, Höganäs, Sweden). Non cell wall forage components were removed by extraction with neutral detergent (Goering and Van Soest, 1970), minus sodium sulfite, or by treatment with amylase to remove starch and extraction with 80% ethanol to remove alcohol-soluble phenolic glycosides. The neutral detergent fiber (NDF) was prepared by refluxing the forage samples with neutral detergent (100 mL/g sample) for 60 min. The NDF was then filtered and washed 4× with large volumes of hot water and 2× with acetone. The NDF samples were allowed to air-dry in a hood. The starch-free alcohol-insoluble residue (AIR) was prepared according to Theander and Westerlund (1986). Acetate buffer (25 mL, 0.1 M, pH 5.0) and 0.5 mL of heat-stable α-amylase (Sigma Chemical Co., St. Louis, MO) were added per gram of forage sample and heated at 90 °C for 60 min. After the mixture was cooled to 50 °C, 1 mL of amyloglucosidase (Sigma) was added per gram of sample and the resultant mixture heated for 3 h at 60 °C. Sufficient 95% ethanol was added to achieve a final concentration of 80% ethanol, and the sample was held at 4 °C overnight. The AIR was recovered by centrifugation, washed 2× with 80% ethanol and once with acetone, and allowed to air-dry under a hood. A third sample preparation treatment with a smaller particle size was prepared by ball-milling NDF (BM-NDF) for 1 min at 4 °C in a shaker mill (Pica Blender Mill Model 2601; Hankison Co., Canonsburg, PA). Dry matter was determined at 100 °C, and organic matter was calculated after ashing at 450 °C for 4 h.

Extraction of Esterified Cinnamic Acids. Prepared forage samples were extracted with 0.5, 1, 2, 4, or 6 M NaOH by a modification of the procedure of Jung et al. (1983a). Samples (200 mg) were placed in 25 × 150 mm glass culture tubes

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Table I. Recovery of Organic Matter from Forages after Sample Preparation

	recovery,[a] mg/g OM			
	AIR		NDF	
forage	immature	mature	immature	mature
alfalfa	766	1007	308	667
smooth bromegrass	879	991	552	702
switchgrass	940	1008	647	768

[a] Results are expressed relative to the organic matter content of the original sample.

with Teflon-line caps. The appropriate anaerobic NaOH solution (10 mL) was added to each tube under a N_2 stream. Samples were extracted at 39 °C for 24 h in the dark with occasional shaking. Samples were subsequently filtered and washed with 5 mL of water. The combined filtrate and wash was acidified to pH 2.6 with concentrated phosphoric acid. The acidified sample solution was loaded on a C_{18} solid-phase extraction column (Supelco, Inc., Bellefonte, PA), the column was washed with 2 mL of NaOH–phosphoric acid solution (pH 2.6), and the cinnamic acids were eluted with two 2.5-mL washes of 50% methanol. The eluted samples were brought to a final volume of 10 mL, filtered through a 0.2-μm filter, and stored at −20 °C until analyzed. All extractions were performed in duplicate.

Cinnamic Acid Analysis. Identification and quantification of phenolic acids was done by LC (Jung et al., 1983a). A Gilson gradient autoanalytical system (Gilson Medical Electronics, Inc., Middleton, WI) with a programmable dual-wavelength UV detector (Gilson Model 116) was utilized. Samples (20 μL) were injected and separated by isocratic elution through a Spherisorb-ODS, C_{18}, 5-μm column (Supelco). The solvent (97.7% water–0.3% glacial acetic acid–2% butanol) was pumped at 3 mL/min. Column temperature was maintained at 50 °C. The chromatographic separation was developed to detect 10 different phenolic acids and aldehydes, and both 254 and 320 nm were monitored simultaneously. *p*-Coumaric and ferulic acids were quantified at 320 nm, and the ratio of absorbances at 254 vs 320 nm was used to check compound identification.

Statistical Analysis. The forage species were known to differ greatly in phenolic acid content, and therefore, the data were analyzed separately for each forage. The statistical model employed was a 3 × 2 × 5 factorial of sample preparation method, forage maturity, and alkali concentration. Individual means were tested by the F-protected least significant difference method (Steel and Torrie, 1960).

RESULTS

The recovery of forage organic matter in the AIR and NDF prepared samples is shown in Table I. The recovery of organic matter from BM-NDF was assumed to be the same as NDF. The virtually complete recovery of organic matter by the AIR preparation suggests that residual protein and lipid material remain in the AIR, as observed previously (Selvendran, 1975). Treatment of α-cellulose and oatspelts xylan (Sigma) with the AIR procedure resulted in organic matter recoveries of 104–117%. This suggests that partial acetylation of the cell wall polysaccharides occurs due to heating in acetate buffer, which would add organic matter to the samples. As expected, NDF content of the legume was least and the C_4 grass, switchgrass, contained the highest concentration of NDF. Forage NDF content was also increased with physiological maturity of the sample. Similar general trends were seen for the AIR. Although not quantified, the leaf to stem ratio of the forages declined with advanced maturity. The forage samples provided a broad spectrum of both forage types and fiber content. Because organic matter recovery relative to the original plant material was different among the sample preparation methods, the yields of phenolic acids reported were corrected to the initial organic matter content of the forage samples.

Degradation of *p*-coumaric and ferulic acids at the different NaOH concentrations was determined by treating a mixture of pure phenolic standards, mixed with glucose as a carrier to facilitate handling, with each alkali concentration in the same manner as the forage samples. Phenolic standards were not mixed with forage samples to avoid matrix interactions that might limit recovery of the free acids. Since free phenolic acids are present in extremely small amounts, such matrix–phenolic interactions were not considered valid. Degradability of phenolic acids in alkali was relatively consistent. The recovery of *p*-coumaric acid averaged 74.6 ± 0.7% and did not differ ($P > 0.05$) among the NaOH concentrations. Ferulic acid recovery was greater ($P < 0.05$) at 0.5 M NaOH (79.7%) than at the other concentrations (75.0 ± 0.8%), which were not different ($P > 0.05$). The data on yield of *p*-coumaric and ferulic acids from forages were corrected for their recovery at each NaOH concentration.

Statistical analysis of the esterified phenolic acid yield data from the different sample preparation methods, alkali concentrations, and forage maturity stages revealed a very complex situation. For every forage species the three main effects (alkali concentration, sample preparation method, and forage maturity) were always significant ($P < 0.05$) as were the two-way interactions, except for alkali concentration × forage maturity interaction, which was significant in half the cases (Table II). Ferulic acid yield even exhibited a significant three-way interaction of preparation method × alkali concentrations × forage maturity for both alfalfa and smooth bromegrass. The alkali concentration × forage maturity interactions resulted from a smaller difference in phenolic yield, but still significant, between maturity stages for the AIR preparation method than seen for the other two procedures. The observed three-way interactions were more difficult to interpret but appeared to be related to small differences as to which alkali concentration provided maximal phenolic acid yields relative to forage maturity in each of the preparation methods. Further discussion will be limited to the greater differences associated with main effects and the alkali concentration interactions with preparation method and forage maturity.

The yields of *p*-coumaric and ferulic acids from the different sample preparation procedures, after extraction by various NaOH concentrations, are shown in Figures 1–3. Mean values, across maturity stages, are given because differences were consistent for both immature and mature forage samples. For alfalfa, alkali concentrations had relatively little effect on release of phenolic acids from AIR or NDF; however, reduction of particle size resulted in greater yields with increasing alkali strength from BM-NDF (Figure 1). The AIR sample preparation method resulted in consistently greater ($P < 0.05$) yields of both *p*-coumaric and ferulic acids than obtained from NDF. *p*-Coumaric acid yield from alfalfa was greater ($P < 0.05$) from NDF than BM-NDF at 0.5, 1, and 2 M NaOH concentrations, but similar at higher alkali levels (Figure 1a). Release of ferulic acid from alfalfa was greater ($P < 0.05$) from NDF than the BM-NDF preparation at all NaOH concentrations (Figure 1b).

A different pattern of response was seen for smooth bromegrass. For both AIR and NDF, release of phenolic acids increased as alkali strength changed from 0.5 to 1 M NaOH, but yields declined with further increases in NaOH concentration (Figure 2). In contrast, *p*-coumaric and ferulic acid yields increased from BM-NDF

Table II. Summary of Statistical Analysis for the Extractability of Phenolic Acids due to the Variables Studied[a]

		main effects			interaction			
forage species	phenolic acid	NaOH concn (A)	prep method (B)	forage maturity (C)	A × B	A × C	B × C	A × B × C
alfalfa	*p*-coumaric	**	**	**	**	**	*	NS
	ferulic	**	**	*	**	**	NS	*
smooth bromegrass	*p*-coumaric	**	**	**	**	**	NS	NS
	ferulic	**	**	**	**	**	NS	**
switchgrass	*p*-coumaric	**	**	**	**	**	*	NS
	ferulic	**	**	**	**	**	**	NS

[a] Key: *, **, significant effect at $P < 0.05$ and 0.01, respectively; NS, nonsignificant ($P > 0.05$).

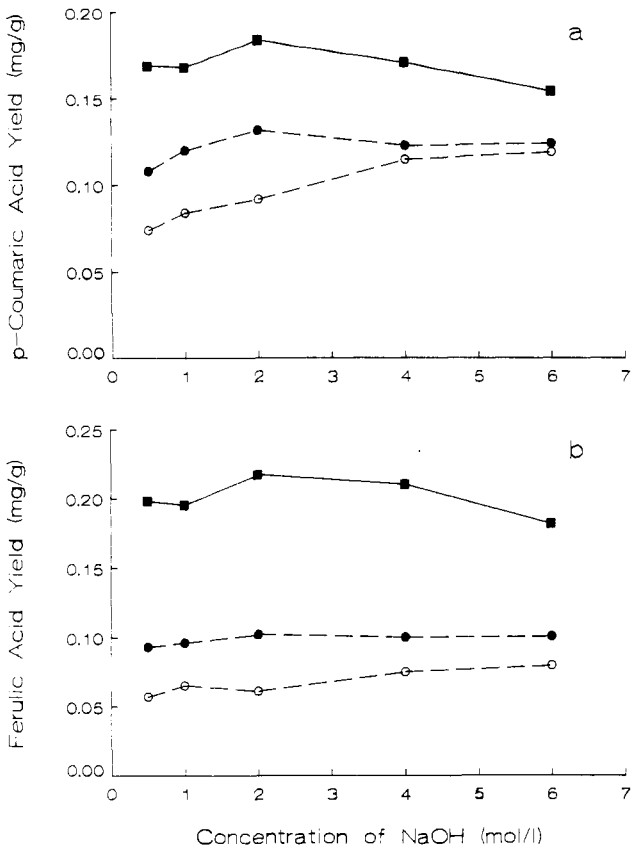

Figure 1. Yields of *p*-coumaric (a) and ferulic (b) acids from alfalfa samples extracted with alkali. Values are averages of AIR (■), NDF (●), and BM-NDF (○) from immature and mature forage samples. Standard errors of the mean were 0.006 and 0.005 mg/g of OM for *p*-coumaric and ferulic acids, respectively.

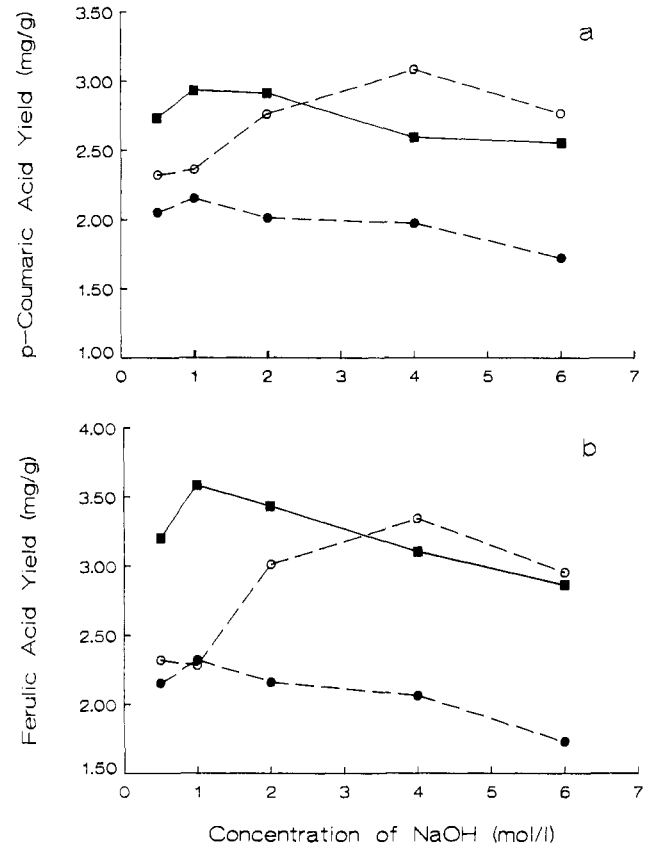

Figure 2. Yields of *p*-coumaric (a) and ferulic (b) acids from smooth bromegrass samples extracted with alkali. Values are averages of AIR (■), NDF (●), and BM-NDF (○) from immature and mature forage samples. Standard errors of the mean were 0.08 and 0.09 mg/g of OM for *p*-coumaric and ferulic acids, respectively.

smooth bromegrass as alkali strength increased from 0.5 to 4 M, which was followed by a decline at 6 M NaOH. Yields of phenolic acids were greater ($P < 0.05$) from the AIR than the NDF preparation at all NaOH concentrations, as observed for alfalfa. Unlike alfalfa, extraction of *p*-coumaric and ferulic acids from smooth bromegrass was greater ($P < 0.05$) from the BM-NDF than found for the NDF at 2, 4, and 6 M NaOH. *p*-Coumaric acid yield also was greater ($P < 0.05$) at 0.5 M NaOH from BM-NDF than NDF (Figure 2a).

The response of switchgrass to sample preparation method and NaOH concentration was more similar to smooth bromegrass than alfalfa. Maximum yields of both *p*-coumaric and ferulic acids from switchgrass AIR and NDF preparations were found with 1 M NaOH, and yields declined at higher alkali concentrations (Figure 3). As in smooth bromegrass, phenolic acid release from switchgrass BM-NDF peaked at 4 M NaOH. Yields of both phenolic acids were greater ($P < 0.05$) from AIR than NDF of switchgrass. Unlike alfalfa or smooth bromegrass, *p*-coumaric acid release from BM-NDF was consistently greater ($P < 0.05$) than from NDF in the case of switchgrass.

Degree of forage physiological maturity appeared to influence the content of phenolic acids in different sample preparations. For all forages and sample preparation methods, yields of *p*-coumaric and ferulic acids were different ($P < 0.05$) between immature and mature forage material when averaged across alkali concentrations (Table III). Release of *p*-coumaric was always greater ($P < 0.05$) from mature forage than immature samples. In contrast, ferulic acid yield from mature alfalfa was greater ($P < 0.05$) then from immature, but the opposite was seen for both smooth bromegrass and switchgrass. Sample preparation method also influenced yield of phenolic acids. Both *p*-coumaric and ferulic acid yields from immature and mature forage samples were greater ($P < 0.05$) from the AIR than from NDF (Table III). Reduction of particle size resulted in greater ($P < 0.05$) phenolic acid yields from BM-NDF than seen with the NDF preparation for the grass samples, but the comparative

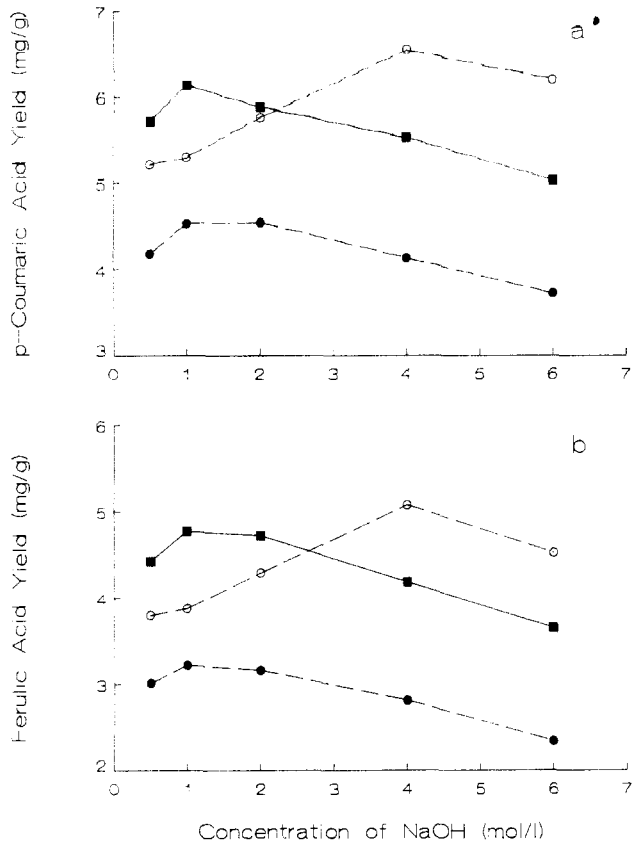

Figure 3. Yields of *p*-coumaric (a) and ferulic (b) acids from switchgrass samples extracted with alkali. Values are averages of AIR (■), NDF (●), and BM-NDF (○) from immature and mature forage samples. Standard errors of the mean were 0.17 and 0.09 mg/g of OM for *p*-coumaric and ferulic acids, respectively.

yields from alfalfa were variable between the sample preparation methods.

Not only are yield of *p*-coumaric and ferulic acids different among sample preparations and alkali strengths, but the relative proportions of the phenolic products also vary. Table IV illustrates the differences among extraction conditions for *p*-coumaric to ferulic acid molar ratio when the highest yielding alkali concentration for each forage and preparation method was used. Except for BM-NDF of alfalfa, all immature and mature forage samples treated the same were different ($P < 0.05$) in phenolic acid ratio, with proportionately more *p*-coumaric acid in the mature samples. Within a forage sample, the AIR provided the lowest ($P < 0.05$) ratio or overlapped with that for BM-NDF.

DISCUSSION

The differences noted in *p*-coumaric and ferulic acid contents of alfalfa compared to the grass species have been observed previously (Jung et al., 1983a,b). Changes in yield of phenolic acids due to physiological maturity of the forage also agreed with previous work (Burritt et al., 1984). Both ferulic and *p*-coumaric acids are esterified to hemicellulosic components and core lignin in the cell wall, but ferulic acid is predominantly associated with the polysaccharide fraction while *p*-coumaric acid is primarily attached to core lignin (Atsushi et al., 1984; Azuma et al., 1985). The increase in the *p*-coumaric to ferulic acid ratio with forage maturation (Table III) can be explained by the reduction in hemicellulose synthesis or concentration and increase of core lignin in the cell wall that occurs during maturation (Jung and Vogel, 1986b).

The yield differences of phenolic acids from NDF compared to AIR (30–40% less from NDF) were striking. It appears that the neutral detergent procedure results in loss of forage cell wall material that contains appreciable amounts of esterified phenolic acids. It has been shown that forage cell wall content estimated by NDF is up to 25% less than the sum of cell wall neutral sugars, uronic acids, and Klason lignin (Theander and Aman, 1980). The loss of cell wall by the NDF procedure was greatest from legumes, and across forage species most of the loss was from uronic acids, arabinose, rhamnose, and galactose fractions (Theander and Aman, 1980). Fry (1982, 1983) has shown that ferulic acid, and some *p*-coumaric acid, is esterified to arabinose and galactose units of spinach (*Spinacia oleracea*) pectic substances. Feruloylated pectins also have been reported from sugar beet (*Beta saccharifera*) pulp (Rombouts and Thibault, 1986). Although the pectins in forages have not been well studied, it appears that loss of pectic substances from the plant cell wall by neutral detergent extraction could account for some of the difference in yield of phenolic acids, especially ferulic acid (Table III), between the AIR and NDF preparations.

It has previously been found that intact forage samples contain more phenolic acids than the NDF fraction (Jung et al., 1983a). From these data it was concluded that 17–80% of the esterified *p*-coumaric and ferulic acids in forages were in the cell soluble fraction rather than the cell wall. Cherney et al. (1989) have recently reported similar results for eight grass and five legume species. However, the loss of cell wall polysaccharides containing esterified phenolic components by neutral detergent extraction would indicate that NDF-based estimates of cell soluble phenolics are too high. This leaves the question of whether the AIR preparation of Theander and Westerlund (1986) solubilizes all of the non cell wall bound esterified phenolics. Mosihuzzaman et al. (1988) found 36 and 6% of total *p*-coumaric and ferulic acids, respectively, were in the cell-soluble fraction using 80% ethanol extraction in one species of jute (*Corchorus capsularis*), but <1 and 11% of total *p*-coumaric and ferulic acids, respectively, in the cell solubles in another jute species (*Corchorus olitorius*). How data on jute, a member of the Tiliaceae, extracted with 80% ethanol under reflux relate to grasses and legumes extracted at 4 °C is not known. Julkunen-Tiitto (1985) found that 80% methanol was more effective than 80% acetone, water, or diethyl ether for extraction of total leaf phenolics from willow (*Salix spp.*). Both free phenolic acids and coniferin, a glycoside of glucose and the alcohol derivative of ferlic acid, are soluble in aqueous alcohol (Windholz et al., 1983). If extraction with 80% ethanol does remove all non cell wall bound phenolic acids, then previous conclusions regarding estimates of cell soluble phenolics are incorrect and probably most *p*-coumaric and ferulic acids are cell wall bound.

The yield profiles of the phenolic acids from extraction of AIR and NDF with different alkali concentrations indicate that 1 M NaOH provides maximal yields from the grasses (Figures 2 and 3), but 2 M NaOH is required for a legume such as alfalfa (Figure 1). Extraction of a hybrid digitgrass (*Digitaria spp.*) with 0.5, 1.0, or 1.5 M NaOH resulted in no differences in yield of *p*-coumaric or ferulic acids, nor did extraction time (12–36 h) influence yield (Chaves et al., 1982). Further increase in alkali strength did not substantially affect phenolic acid yield from alfalfa in the current study but did result in declining recovery from the grasses. This suggests that

Table III. Effect of Forage Maturity and Sample Preparation Method on Phenolic Acid Extraction across Alkali Concentrations

phenolic acid	sample prepn[a]	phenolic acid yield, mg/g OM					
		alfalfa		smooth bromegrass		switchgrass	
		immature	mature	immature	mature	immature	mature
p-coumaric[b]	AIR	0.12[d]	0.21[d]	1.67[d]	3.81[d]	4.13[d]	7.19[d]
	NDF	0.06[e]	0.18[e]	1.31[e]	2.65[e]	3.24[e]	5.20[e]
	BM-NDF	0.04[f]	0.15[f]	1.52[f]	3.80[d]	4.57[f]	7.03[d]
	SEM[c]	0.004		0.05		0.11	
ferulic[b]	AIR	0.18[d]	0.22[d]	3.08[d]	2.49[d]	5.36[d]	3.34[d]
	NDF	0.05[e]	0.14[e]	2.53[e]	1.64[e]	3.57[e]	2.24[e]
	BM-NDF	0.06[f]	0.13[f]	3.35[f]	2.21[f]	5.62[f]	3.00[f]
	SEM	0.003		0.06		0.05	

[a] Key: starch-free, alcohol-insoluble residue, AIR; neutral detergent fiber, NDF; ball-milled neutral detergent fiber, BM-NDF. [b] For all forages and sample preparation methods yield of phenolic acid was different ($P < 0.05$) between immature and mature forages. [c] Standard error of the mean, SEM. (d–f) Means in the same column, for the same phenolic acid, not sharing a common superscript are different ($P < 0.05$).

Table IV. Molar Ratio of *p*-Coumaric to Ferulic Acid for Each Sample Preparation Method Extracted with Alkali Concentration Giving Maximal Yield

method[a]	molar ratio (PCA/FA)[b]					
	alfalfa		smooth bromegrass		switchgrass	
	immature	mature	immature	mature	immature	mature
AIR[c]	0.92[e] (2)	1.19[e] (2)	0.51[e] (1)	1.76[e] (1)	0.91[e] (1)	2.49[e] (1)
NDF[c]	1.26[f] (2)	1.43[f] (2)	0.61[f] (1)	1.86[f] (1)	1.05[f] (1)	2.60[f] (1)
BM-NDF[d]	1.49[g] (6)	1.42[f] (6)	0.54[e] (4)	1.94[g] (4)	0.89[e] (4)	2.57[f] (4)
SEM	0.05		0.02		0.01	

[a] Key to abbreviations is given in Table II. [b] Concentration of NaOH (M) used for extraction of each forage sample preparation method is given in parentheses. [c] For all forages, immature and mature samples differ ($P < 0.05$). [d] Immature and mature smooth bromegrass and switchgrass samples differ ($P < 0.05$). (e–g) Means in the same column not sharing a common superscript differ ($P < 0.05$).

high concentrations of alkali cause *p*-coumaric and ferulic acids to interact with the cell wall matrix in some, as yet unexplained, manner reducing yield.

Generally, reduction of particle size will result in greater extraction of components from an insoluble matrix. For the grass samples this was generally true for BM-NDF phenolic acid yield compared to NDF. However, the opposite was generally true for alfalfa. Chaves et al. (1982) also found slightly greater yields of ferulic, but not *p*-coumaric, acid from 1 vs 0.25 mm ground forage (5.7 vs 5.1 mg/g NDF). It also was unexpected that increasing alkali concentrations would give a different yield profile for BM-NDF than the NDF preparation for grasses (i.e., the maximum at 4 vs 1 M NaOH). These yields even exceeded those from AIR in some cases. The reduction in particle size by ball-milling, and possible depolymerization of core lignin (Sarkanen and Ludwig, 1971), may be causing release of etherified (Scalbert et al., 1985) or other linkage forms of *p*-coumaric and ferulic acids.

As a result of this study, several modifications have been made in the standard procedure employed in our laboratory for extraction of esterified phenolic acids from forage cell walls, and the following recommendations are offered for consideration by others. It appears that use of 1 M NaOH is appropriate for extraction of *p*-coumaric and ferulic acids from grass species but 2 M NaOH should be considered for legume species. The use of a starch-free alcohol-insoluble preparation appears to be preferable to NDF for quantification of total esterified phenolics in forage cell walls. Until more information is available about the chemical changes induced by ball-milling, reduction of particle size beyond a 1-mm grind is not encouraged. Complex and numerous interacting properties influence analysis of esterified phenolics in forage cell wall; thus, care should be given to the choice of analysis method when interpretation of the role of these compounds in ruminal polysaccharide fermentation is to be made.

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Registry No. *p*-Coumaric acid, 7400-08-0; ferulic acid, 1135-24-6.

Determination of Several Pesticides with a Chemical Ionization Ion Trap Detector

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A total of one hundred (twenty five each) apple, peach, tomato, and potato samples were analyzed for twelve pesticides and two pesticide metabolites with a slightly modified Luke multiresidue extraction procedure, separation by capillary column gas chromatography with cold on-column injection, and detection by mass chromatography with an ion trap mass spectrometer in the chemical ionization mode (GC/CIMS). Residues of carbaryl, captan, dichloran, dimethoate, methamidophos, phosmet, and tetrahydrophthalimide were found in several samples, with peaches containing the most residues. None of the residues found were above legal tolerances. Recovery studies were performed at the 0.5 ppm fortification level of each pesticide and metabolite at least three times in each of the four crops. Recoveries were between 73 and 120%, with an average coefficient of variation of 11%. Because the computer can be programmed to search for several hundred targeted ions, the use of capillary column GC/CIMS is a promising method that should be explored by regulatory agencies for the analysis of pesticide residues.

The Luke extraction procedure (Luke et al., 1981; AOAC, 1985) is, at last count, capable of extracting 234 pesticides and pesticide metabolites (Luke et al., 1988) and is, consequently, extensively used by regulatory agencies for multiresidue analyses. This method requires no column chromatography cleanup step as interfering chromatographic peaks are minimized by the use of specific detectors such as the flame photometric and the Hall electrolytic conductivity detectors. Current methodology makes use of packed gas chromatography columns of various polarities. Thus, an analyst determining all the possible pesticide residues extracted by the Luke extraction procedure needs to make from four to six separate determinations for each sample.

We believe that these analyses could be vastly improved by the use of a single capillary gas chromatographic column whose effluent is detected and quantified by mass chromatography after chemical ionization mass spectrometry. We now report our initial results using this system for the analysis of twelve selected pesticides and two pesticide metabolites in four commonly consumed commodities.

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