were manually removed from the clams in plant B.

It can be concluded that clam bellies may be used in the fermentation of agricultural wastes (as wheat straw and corn cobs) and shellfish wastes (crabs, shrimp, crawfish) for the production of ruminant feeds. They may also be used as a source for the production of various specific and nonspecific industrial carbohydrases. Since clam bellies have high protein and low ash contents, they may be further studied for the production of clam-flavored pet foods. Maximum recovery of clam bellies can be achieved by installing self-cleaning screens on the debellying process flume. The use of a subsequent compacting (pressing) operation to remove excess water and concentrate belly material may be considered. The solid materials should be rapidly chilled or frozen to prevent degradation in enzyme activity. Installation of the equipment and operational procedures can be achieved with relatively minor equipment cost and plant modification.

Registry No. Phosphorus, 7723-14-0; calcium, 7440-70-2; magnesium, 7439-95-4; potassium, 7440-09-7; sodium, 7440-23-5; iron, 7439-89-6; copper, 7440-50-8; zinc, 7440-66-6; α-glycosidase, 74315-95-0; β-glycosidase, 39346-29-7; α-1,6-glucosidase, 37288-48-5; α-1,4-glucosidase, 9001-42-7; β-1,6-glucosidase, 55326-47-1; β-1,4-glucosidase, 37288-52-1; laminarinase, 9025-37-0.

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Soluble Phenolic Monomers in Forage Crops

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The proportion of alkali-labile phenolic monomers soluble in neutral detergent, methanol, water, or rumen buffer was determined in mature alfalfa and corn stem material by HPLC. The determination of soluble proportion was influenced by treatment, with neutral detergent generally extracting the highest proportion of phenolics. Phenolic monomers were quantified in stems and buffer-treated stem residues of a wide variety of forage crop species. A large range in the concentrations of the major alkali-labile phenolics, *p*-coumaric acid (PCA) and ferulic acid (FA), was found with much higher concentrations in grasses than legumes. The rumen buffer soluble proportion of alkali-labile PCA and FA in grasses (9 and 9%, respectively) was much lower than in legumes (84 and 90%, respectively). The high solubility of PCA and FA indicated that most of the alkali-labile PCA and FA in legumes was not bound to hemicellulose or lignin. Caffeic acid, not previously reported to be a major alkali-labile component of forages, was detected and confirmed to comprise approximately 6 g kg^{-1} of the dry weight of immature limpograss stems.

Phenolic monomers have been implicated in inhibition of structural carbohydrate digestion. Ferulic acid (FA) and *p*-coumaric acid (PCA) were toxic to cellulolytic bacteria (Chesson et al., 1982). Herald and Davidson (1983) observed bacterial inhibition due to hydroxycinnamic acids, with PCA being the most effective inhibitor tested. In another study, PCA and *p*-hydroxybenzaldehyde (PHBAL) were toxic to cellulolytic bacteria but syringic acid (SYA), *p*-hydroxybenzoic acid (PHBA), and hydrocinnamic acid stimulated growth of these bacteria (Borneman et al., 1986). Vanillin (VAN) depressed both cellulose and xylan digestion (Varel and Jung, 1986), while benzoic, cinnamic, and caffeic acids depressed digestion of cellulose (Jung, 1985).

The presence of free or nonesterified phenolic monomers in plant tissues is not well documented. Jung et al. (1983a) detected no free, ether-soluble phenolic compounds in alfalfa hay, soybean stover, smooth bromegrass hay, or corn stalklage samples. *p*-Hydroxybenzoic, vanillic, *p*-coumaric, and ferulic acids were found in water extracts of alfalfa

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Table I. Fiber Composition (Gram per Kilogram Dry Weight) of Mature Stems of Eight Grass and Five Legume Species[a]

	neutral detergent fiber	cellulose[b]	hemicellulose[b]	lignin[b]
Grass				
corn (*Zea mays* L.)	609	312	244	53
sorghum (*Sorghum bicolor* (L.) Moench.)	695	328	308	60
smooth bromegrass (*Bromus inermis* Leyss.)	660	337	241	83
reed canarygrass (*Phalaris arundinacea* L.)	761	417	260	84
Bermuda grass (*Cynodon dactylon* (L.) Pers.)	814	350	367	98
limpograss (*Hemarthria altissima* (Poir.) Staph & Hubb. var. Floralta)	800	336	401	64
oats (*Avena sativa* L.)	796	409	268	116
wheat (*Triticum aestivum* L.)	788	385	305	94
Legume				
Alfalfa (*Medicago sativa* L.)	627	336	153	138
birdsfoot trefoil (*Lotus corniculatus* L.)	594	322	142	130
American jointvetch (*Aeschynomene americana* L.)	660	405	132	125
rhizoma peanut (*Arachis glabrata* Benth.)	597	331	144	122
kudzu (*Pueraria phaseoloides* (Roxb.) Benth.)	619	341	168	108
BLSD[c]	42	24	16	7

[a] Means of three replicates. [b] Estimated by a sequential neutral detergent, acid detergent, and permanganate lignin procedure. [c] Bayes least significant difference (k = 100, approximately P = 0.05).

(Newby et al., 1980). The stem of jute plants contained more free phenolic acids than the bark (Mosihuzzaman et al., 1988). Free ferulic and caffeic acids were detected in methanol extracts of alfalfa and cabbage, and the problems associated with isolating intact caffeic acid (CA) were discussed (Huang et al., 1986). Caffeic acid was very sensitive to air oxidation in alkaline solution, and recovery of CA following base extraction was very low.

Our objectives were to (1) determine the proportion of alkali-labile phenolic monomers in alfalfa and corn stems soluble in boiling neutral detergent, boiling methanol, boiling distilled water, and rumen buffer at 40 °C and (2) determine the rumen buffer soluble portion of alkali-labile phenolic monomers extracted from stems of a wide range of forage crop species.

MATERIALS AND METHODS

Plant Materials. Three replicates of mature stems of eight grass and five legume species were collected, dried (60 °C), and ground to pass a 1-mm screen (Table I). Bermuda grass, limpograss, American jointvetch, rhizoma peanut, and kudzu were collected near Gainesville, FL. The remaining species were collected near West Lafayette, IN. Fiber components in samples were estimated by a sequential neutral detergent fiber (NDF), acid detergent fiber (ADF), permanganate lignin (PL), and acid-insoluble ash procedure (Cherney et al., 1985). Hemicellulose was estimated as the difference between ADF and NDF values. Cellulose was estimated as the difference between PL and acid insoluble ash values.

Extraction of Plant Materials. Residues were prepared from dried, ground plant samples (0.5 g) by incubation for 4 h in rumen buffer at 40 °C (Marten and Barnes, 1980) or by refluxing for 1 h in neutral detergent (Goering and Van Soest, 1970), 100% methanol, or deionized water. The resulting residues and unextracted stem samples (0.1 g) were hydrolyzed under nitrogen in the absence of light in 10 mL of 1 M NaOH for 24 h at 25 °C to yield alkali-labile phenolics. Samples were acidified to pH 2.5 with 6 M HCl, vacuum filtered, and brought to a volume of 50 mL with water. A portion of this solution was passed through a 0.45-μm Nylon 66 membrane filter. In all cases, sample preparation immediately preceded injection.

Analytical Methods. Filtered solution (100 μL) was injected into a high-performance liquid chromatograph (Millipore Corp., Waters Chromatography Division). The analytical column (Hewlett-Packard, 200 mm × 4.6 mm) contained 5-μm particles of Lichrosorb C-8 and was protected by a guard column packed with Perisorb C-8. Solvents and gradient conditions were similar to those used by Torres et al. (1987). The two solvents used were 0.7% aqueous acetic acid (A) and 50% aqueous acetonitrile (B). The gradient was 10–20% B over the first 10 min, 20–50% B over the next 15 min, and 50% B for 5 min, followed by reequilibration of the system prior to the next injection. Phenolic monomers were detected with a Millipore/Waters 490 programmable multiwavelength UV detector. The recorder was a Hewlett-Packard 3393A computing integrator. The conditions described allowed simultaneous separation of the compounds in Table II as well as gallic acid, protocatechuic acid, gentistic acid, protocatechualdehyde, syringaldehyde, and *trans*-cinnamic acid.

Subjecting a mixture standard of phenolic monomers to the base extraction procedure produced consistent, but different, recoveries for each compound. Gallic, gentisic, and caffeic acids did not survive base extraction and were not detected in base-treated standards. Recovery of other compounds ranged from 60 to 100%. The external method of calibration was accomplished for reported compounds, except CA, with use of standards treated according to the base extraction procedure used for the plant samples. The quantity of CA in plant extracts was estimated from the response factor for CA obtained by injection of standard CA that had not been subjected to the sample preparation procedure.

Responses for all compounds were linear in the concentration range used for calibration and detection. Solubility was calculated as the ratio of soluble quantity to total quantity of alkali-labile phenolic monomers and expressed on a percentage basis. Soluble quantity was calculated as the difference between the quantity of alkali-labile phenolics in untreated stems and treated stem residues. All concentrations were expressed on a dry weight basis (105 °C).

The experiment was analyzed as a randomized complete block design with three replicates. Statistical significance was determined by analysis of variance procedures and F-tests. Where F-tests for species were significant, a Bayes least significant difference (BLSD) was determined for mean separation (Smith, 1978).

Caffeic Acid Analysis. Caffeic acid appeared to be present in base extracts in relatively large quantities in some species, although CA in the standard mixture had

Table II. Alkali-Labile Phenolic Monomers (Milligrams per Kilogram Dry Weight) in Mature Stems of Eight Grass and Five Legume Species[a]

	PHBA[b]	PHBAL	CA	SYA	VAN	VA	PCA	FA
			Grass					
corn	26	472	114	132	106	120	29110	5242
sorghum	59	450	34	57	153	99	25921	5055
smooth bromegrass	22	105	32	37	265	74	5832	2452
reed canarygrass	51	515	27	66	312	66	21803	4932
Bermuda grass	39	297	19	75	305	155	11384	4861
limpograss	101	183	913	22	119	60	6666	3192
oats	29	219	ND[c]	107	211	84	10446	3945
wheat	37	77	7	79	175	111	5451	4444
			Legume					
alfalfa	46	38	59	97	96	248	254	680
birdsfoot trefoil	50	46	67	141	82	297	394	555
American jointvetch	34	17	40	76	63	418	79	185
rhizoma peanut	48	17	132	31	71	114	779	1121
kudzu	102	26	103	202	44	57	460	1051
BLSD[d]	11	31	207	48	68	53	1546	280

[a] Means of three replicates. [b] Key: PHBA = *p*-hydroxybenzoic acid; PHBAL = *p*-hydroxybenzaldehyde; CA = caffeic acid; SYA = syringic acid, VAN = vanillin; VA = vanillic acid; PCA = *p*-coumaric acid; FA = ferulic acid. [c] Not detected. [d] Bayes least significant difference (k = 100, approximately P = 0.05).

not survived base treatment. Mass spectral analysis was used to confirm the presence of CA in base extracts of plants. The compound having the same elution time as CA was collected from the HPLC column, extracted into ether, and evaporated to dryness. The mass spectrum of the compound was obtained with the direct insertion probe (heated to 200 °C) of a Finnigan 4000 mass spectrometer. This compound also was derivatized by adding 0.1 mL of bis(trimethylsilyl)trifluoroacetamide (BSTFA) to the dried compound collected from the HPLC column and heating at 110 °C for 30 min. The mass spectrum of the derivatized compound was obtained by GC–MS. The column conditions were as follows: 30-m DB-1 column (J & W Scientific) held at 100 °C for 2 min and then programmed to 240 °C at 10 °C min^{-1}. The TMS derivative of CA eluted in approximately 15 min. Both electron impact (EI) and isobutane chemical ionization were used in both MS analyses.

In the extraction of plant materials procedure described above, phenolics were extracted under nitrogen but NaOH was not purged of oxygen prior to the extraction. Since CA is highly unstable and undergoes rapid oxidative degradation, limpograss samples were extracted with vacuum-degassed NaOH, in order to estimate extent of CA degradation due to dissolved oxygen in NaOH.

The survival of NaOH-extracted CA was determined by extracting limpograss for 0.25, 0.5, 1, 3, 6, 9, 12, 15, 18, 21, and 24 h in degassed NaOH. Immature limpograss stems (3-week regrowth) were used instead of the mature stems (7-week regrowth) in the previous portion of this study.

RESULTS AND DISCUSSION

Fiber concentrations (Table I) were similar to those previously reported in the literature (Van Soest, 1982). Neutral detergent fiber concentrations ranged from 594 g kg^{-1} birdsfoot trefoil stems to 814 g kg^{-1} in Bermuda grass stems. Grasses tended to have similar concentrations of cellulose and hemicellulose, while cellulose concentration in legumes was approximately twice that of hemicellulose. Permanganate lignin concentration ranged from 53 kg^{-1} in corn to 138 g kg^{-1} in alfalfa.

Alkali-Labile Phenolics. Since samples were oven-dried, concentrations may be more indicative of forage crops stored as hay, compared to fresh-harvested material. Gallic acid, protocatachuic acid, gentistic acid, protocatachualdehyde, syringaldehyde, sinapic acid, and *trans*-cinnamic acid either were not detected or were present in base extracts in amounts too small to be quantified. As noted by several researchers (Jung et al., 1983a,b), grasses contained substantially more alkali-labile PCA and FA than legumes (Table II). There also was a large range in PCA and FA concentrations within grass and legume groups. *p*-Coumaric and ferulic acids have been considered to serve as cross-linkages in plant cell walls (Hartley, 1972). The remaining alkali-labile phenolic monomers were present in much smaller concentrations that varied greatly among species. The PHBA and syringic acid (SYA) concentrations were in the same range for grass and legume species. The PHBAL concentrations in grasses were consistently much higher than those in legumes. Highest concentrations of vanillic acid (VA) were found in legumes. Limpograss contained a relatively large amount of CA that survived base extraction.

Extraction Treatments. Corn and alfalfa, representing grass and legume species, were used to determine the effect of extraction procedure on the soluble proportion of alkali-labile phenolic monomers (Table III). The difference between concentrations of alkali-labile phenolics in untreated stem tissue and treated stem residues was used to estimate solubility. Phenolic monomers in alfalfa were generally more soluble than those in corn. This indicated that most base-extracted phenolic monomers in alfalfa were not covalently bound to the cell wall but were associated with cell solubles. In alfalfa, methanol solubility of several compounds, particularly PCA and FA, was lower than the other extraction procedures. Huang et al. (1986) estimated free FA to be 37% of the total alkali-labile FA in alfalfa, based on an 80% methanol extraction. Our estimate also was 37%, based on a 100% methanol extraction.

Phenolic monomers varied in solubility, depending on the treatment and species. In both corn and alfalfa, neutral detergent removed significantly more PHBAL than the other three treatments. In alfalfa, solubility of PHBA and VA was influenced by extraction treatment, but not in corn. Boiling water and rumen buffer treatments produced similar results in most cases. Essentially no PHBAL, VAN, or PCA was soluble in rumen buffer in corn. The average of three replicates in corn resulted in a negative (−2%) solubility for VAN but was not statistically different from deionized water (1%) or methanol (9%). Concentrations of CA in solution are not necessarily reflective of the reported CA solubilities, due to the unstable nature of CA.

Table III. Proportion of Alkali-Labile Phenolic Monomers (Percent Soluble) in Corn and Alfalfa Soluble in Rumen Buffer at 40 °C, Boiling Neutral Detergent, Boiling Methanol, and Boiling Deionized Water[a]

	PHBA[b]	PHBAL	CA	SYA	VAN	VA	PCA	FA
			Corn					
rumen buffer	38	1	78	80	−2	69	3	11
neutral detergent	41	65	91	86	19	69	27	21
100% methanol	38	9	100[c]	85	9	72	14	10
deionized water	38	8	100[c]	80	1	72	8	15
BLSD[d]	NS	3	3	5	13	NS	10	9
			Alfalfa					
rumen buffer	52	38	100[c]	85	29	86	88	96
neutral detergent	81	70	96	90	69	90	87	98
100% methanol	55	29	59	70	57	70	30	37
deionized water	62	40	97	86	47	87	87	98
BLSD	5	13	10	10	14	7	14	6

[a] Means of three replicates, with solubility calculated as the ratio of soluble quantity to total quantity of alkali-labile phenolic monomers. Soluble quantity was calculated as the difference between the quantity of alkali-labile phenolics in untreated stems and treated stem residues. [b] Key: PHBA = *p*-hydroxybenzoic acid; PHBAL = *p*-hydroxybenzaldehyde; CA = caffeic acid; SYA = syringic acid; VAN = vanillin; VA = vanillic acid; PCA = *p*-coumaric acid; FA = ferulic acid. [c] Not detected in treated residue. [d] Bayes least significant difference ($k = 100$, approximately $P = 0.05$).

Table IV. Proportion of Alkali-Labile Phenolic Monomers (Percent Soluble) in Eight Grass and Five Legume Species Soluble in Rumen Buffer at 40 °C[a]

	PHBA[b]	PHBAL	CA	SYA	VAN	VA	PCA	FA
			Grass					
corn	38	1	78	80	−2	69	5	11
sorghum	53	15	70	61	0	50	5	7
smooth bromegrass	43	3	85	44	27	33	1	5
reed canarygrass	37	1	87	51	12	17	3	5
Bermuda grass	27	3	81	62	18	50	4	5
limpograss	82	20	98	50	11	42	18	17
oats	33	21	ND[c]	66	16	32	24	16
wheat	51	1	38	65	15	45	13	8
			Legume					
alfalfa	52	38	100[d]	85	29	86	88	96
birdsfoot trefoil	13	58	100[d]	93	39	90	84	96
American jointvetch	31	36	100[d]	82	22	84	57	63
rhizoma peanut	75	27	100[d]	59	12	74	96	96
kudzu	87	52	100[d]	93	0	62	94	99
BLSD[e]	9	16	30	13	NS	10	14	8

[a] Means of three replicates, with solubility calculated as the ratio of soluble quantity to total quantity of alkali-labile phenolic monomers. Soluble quantity was calculated as the difference between the quantity of alkali-labile phenolics in untreated stems and buffer-treated stem residues. [b] Key: PHBA = *p*-hydroxybenzoic acid; PHBAL = *p*-hydroxybenzaldehyde; CA = caffeic acid; SYA = syringic acid, VAN = vanillin; VA = vanillic acid; PCA = *p*-coumaric acid; FA = ferulic acid. [c] Not detected in untreated stem or buffer-treated residue. [d] Not detected in buffer-treated residue. [e] Bayes least significant difference ($k = 100$, approximately $P = 0.05$).

Rumen Buffer Soluble Phenolics. A range of species were surveyed for solubility with the rumen buffer treatment, since it is likely the most reflective of actual rumen conditions of the four treatments studied. Large differences in solubilities were found between species and between phenolic monomers (Table IV). As noted for alfalfa and corn, most legumes surveyed had higher solubilities than grasses, particularly in the case of the major phenolic monomers PCA and FA. Solubilities of PCA and FA were 9 and 9%, respectively, in grasses and 84 and 90%, respectively, in legumes. Solubility of PHBA varied greatly, ranging from 27 to 82% in grasses and 13 to 87% in legumes. Solubilities of PCA and FA in American jointvetch were significantly lower than other legume species.

Treatment of grass forage crops with NaOH results in a large increase in the digestibility of the crop (Theander, 1985), while treatment of legumes with NaOH has much less of an effect on digestibility (Van Soest, 1981). Improved digestibility with base treatment is presumably due to breaking esterified linkages of PCA and FA with hemicellulose and core lignin, thus opening up the cell wall structure to bacterial enzymes. The difference in response to base treatment between grasses and legumes can be related to concentrations of PCA and FA and their solubility in rumen buffer. Grasses have large concentrations of buffer-insoluble PCA and FA, corresponding to a large number of esterified linkages, accounting for the large increase in digestibility following base treatment. Legumes have relatively smaller concentrations of PCA and FA than grasses that are largely rumen-buffer soluble. In legumes there are very few ester linkages of PCA and FA to cell wall material that can be influenced by base treatment. Thus, base treatment of legumes would not be expected to influence digestibility as it does in grasses.

Quantity of Soluble Phenolics. Phenolic monomers soluble in rumen buffer may impact plant digestion in ruminant animals (Borneman et al., 1986; Chesson et al., 1982; Varel and Jung, 1986). Their effect will depend on the soluble quantity initially released in the rumen and on phenolics released during digestion. Values in Table V should be indicative of soluble quantities of phenolics initially released in the rumen. Largest quantities of soluble PCA were found in grasses, with oat stems exceeding 2.6 g kg^{-1}. Concentrations of soluble FA in stems of rhizoma peanut and kudzu exceeded those in all other legumes and grasses. Quantities of soluble phenolic mo-

Table V. Quantity of Alkali-Labile Phenolic Monomers (Milligrams per Kilogram Dry Weight) in Eight Grass and Five Legume Species Soluble in Rumen Buffer at 40 °C[a]

	PHBA[b]	PHBAL	CA	SYA	VAN	VA	PCA	FA
			Grass					
corn	10	7	31	106	0	83	1563	584
sorghum	31	71	24	35	16	50	1172	348
smooth bromegrass	10	8	33	17	73	24	1073	132
reed canarygrass	19	31	23	35	39	11	1973	410
Bermuda grass	11	9	8	47	59	77	1426	126
limpograss	83	38	894	11	29	25	1253	554
oats	9	48	0	75	78	27	2629	664
wheat	19	5	3	52	26	50	698	378
			Legume					
alfalfa	24	14	59	83	29	214	224	652
birdsfoot trefoil	7	29	67	132	37	268	332	531
American jointvetch	11	7	40	64	40	352	47	118
rhizoma peanut	36	5	132	18	11	84	749	1077
kudzu	89	14	103	189	1	36	433	1037
BLSD[c]	10	39	216	47	NS	52	1494	338

[a] Means of three replicates, with soluble quantity calculated as the difference between the quantity of alkali-labile phenolics in untreated stems and treated stem residues. [b] Key: PHBA = *p*-hydroxybenzoic acid; PHBAL = *p*-hydroxybenzaldehyde; CA = caffeic acid; SYA = syringic acid, VAN = vanillin; VA = vanillic acid; PCA = *p*-coumaric acid; FA = ferulic acid. [c] Bayes least significant difference (k = 100, approximately p = 0.05).

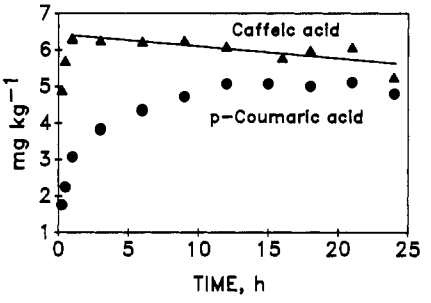

Figure 1. Release and stability of caffeic acid and *p*-coumaric acid extracted from immature limpograss stems with degassed 1 M NaOH.

nomers other than PCA or FA were present in much smaller concentrations, with the exception of CA in limpograss.

Caffeic Acid. Mass spectral analysis confirmed the presence of CA. Both the direct probe of the underivatized compound with an elution time matching CA and the GC–MS analysis of trimethylsilyl derivatives of the compound produced mass spectra of authentic CA. The EI mass spectrum of CA was m/z 180 (100%), 163 (28%), 136 (29%), 135 (20%), and 134 (44%), and the EI mass spectrum of TMS-CA was m/z 396 (55%), 381 (13%), 219 (71%), and 73 (100%). Base extraction of limpograss, rhizoma peanut, and corn samples indicated that 70–80% of CA extracted with degassed NaOH was destroyed if NaOH was not degassed prior to extraction. Caffeic acid concentrations in Tables II and V would, therefore, be much larger if degassed NaOH had been used.

Immature limpograss stems were used to determine the stability of base-extracted CA in degassed NaOH (Figure 1). Immature stems were used because they were found to contain high concentrations of CA. Caffeic acid (99% of the total) was released during the first hour of base extraction, with a small linear decline in CA concentration as duration of extraction increased. Huang et al. (1986) noted that when CA was added to 1% NaOH for 24 h, there was a total loss of the CA peak. We also found a total loss of CA in standards if the NaOH was not degassed. Some caffeic acid extracted from plants did survive, however, in nondegassed NaOH in this experiment. *p*-Coumaric acid was included in Figure 1 because it is normally released in largest quantities from alkali-extracted grasses. The PCA data were transformed by the Lineweaver–Burk equation (double-reciprocal plot) (Lehninger, 1975) to estimate the maximum amount of PCA released. On the basis of that analysis, 99% of the total PCA was released after 9 h of extraction with base and remained relatively constant up to 24 h. Immature limpograss stems were very unusual in that CA was present in larger quantities than PCA. Since almost all alkali-labile CA was soluble in rumen buffer, immature limpograss stems could release up to 6 g of CA kg^{-1} dry weight in the rumen.

Results indicate very large differences in alkali-labile phenolic monomers among species. Solubility of alkali-labile phenolics in rumen buffer was highly dependent on the individual compound and on the plant species. Whether or not the concentrations of soluble phenolics found here are high enough to affect cellulolytic bacteria is not clear. As noted by Varel and Jung (1986), cellulolytic bacteria are closely associated with the plant material being degraded, and concentrations of phenolics in this microenvironment may be higher than in rumen fluid. Neutral detergent, methanol, water, and rumen buffer extractions generally did not result in similar solubility values, suggesting that solubilities obtained by different extraction procedures may not be comparable, depending on the plant species and phenolic compounds in question. Caffeic acid was the phenolic monomer present in the largest quantity in immature limpograss stems. Although limpograss was proven to contain large quantities of CA, improved methodologies are needed to estimate exact quantities of CA in limpograss and other species.

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Registry No. CA, 331-39-5; PCA, 7400-08-0; FA, 1135-24-6; PHBA, 99-96-7; PHBAL, 123-08-0; SYA, 530-57-4; VA, 121-34-6; VAN, 121-33-5; cellulose, 9004-34-6; hemicellulose, 9034-32-6; lignin, 9005-53-2.

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Studies on the Carotenoid Pigments of Paprika (*Capsicum annuum* L.var Sz-20)

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Fatty acid carotenoid esters and unesterified hypophasic and epiphasic carotenoids were extracted from paprika fruit at different stages of ripening and processing. The pigments were separated by high-performance liquid chromatography (HPLC) on Chromsil C_{18} reversed-phase column with 59:57:4 (v/v/v) isocratic conditions and without prior saponificiation of the samples. Monoesters of capsanthin were found to contain mostly unsaturated fatty acids ($C_{18:2}$) while diesters of both capsanthin and capsorubin contained saturated fatty acids such as C_{12}, C_{14}, and C_{16}. The carotenoid esters were more stable, toward lipoxygenase (LOX) catalyzed linoleic acid oxidation, than free pigments. Furthermore, capsanthin esters containing saturated fatty acids resisted the enzymatic oxidation better than the others did.

Paprika (*Capsicum annuum*) is one of the oldest and most important food colors. Its products are the sources of natural carotenoid concentrates. The total red or yellow pigment content of paprika was determined by measuring the extinction of the benzene extract (Benedek, 1958; Fekete et al., 1976). TLC and open-column chromatography (OCC) were used for the separation of carotenoid pigments from paprika products (Vinkler and Richter, 1972; Buckle and Rahman 1979). Recently, a system of HPLC and supercritical fluid chromatography (SFC) were elaborated and developed for the separation, identification, and determination of paprika oleoresins and associated carotenoids (Baranyai et al., 1982; Gere, 1983). However, the analyses done by these methods are still carried out by gradient systems with or without resorting to saponification of the pigment samples.

Spectrophotometric methods, based on determining the decrease in the absorbance at 460 nm, were used for the measurement of carotenoid destruction through a coupled oxidation with LOX and linoleic acid (Ben Aziz et al., 1971; Nicolas et al., 1982; Hsieh and McDonald, 1984; Edwards and Lee, 1986). These methods are not suitable for the simultaneous determination of several pigments. The HPLC method was first applied by Hoschke et al. (1984) to study the changes occurring in the cartenoids of paprika pigment incubated with LOX and linoleic acid. In the method, ethanol was used in up to 5% of the reaction mixture to solubilize the pigments before the addition of the enzyme.

The purpose of this investigation was the separation and

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