# Changes in the Phenolic Composition of Maize Stovers after Blooming

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The phenolic composition of 10 samples of maize stovers collected every 7 days after blooming was determined by high-performance liquid chromatography with electrochemical detection (HPLC-ED) and pyrolysis/gas chromatography/mass spectrometry (PY/GC/MS). An increase of the *p*-hydroxycinnamic acids (*i.e.*, *p*-coumaric acid and ferulic acid) with maturation was observed by HPLC-ED of mild alkaline extracts. Likewise, vanillin and syringaldehyde, *i.e.*, the typical aldehydes constituting lignin, showed an increase with maturation in the alkali-soluble lignin fraction, as determined by HPLC-ED after nitrobenzene oxidation. As to PY/GC/MS, a less marked variation in the typical lignin units was observed with ripening. The ratios between syringyl and guaiacyl lignin units as determined by both analytical techniques were higher, but of the same order, than those determined previously in a set of maize stovers sampled over a wider range of maturation.

**Keywords:** *Maize stover; phenolics; lignin; high-performance liquid chromatography (HPLC); pyrolysis/gas chromatography/mass spectrometry (PY/GC/MS)*

## INTRODUCTION

Plant phenolics play an important role in lignification processes and in the degradability of cell wall polysaccharides by rumen microorganisms (Jung and Deetz, 1993). The presence of cinnamic acids ester−ether linked between cell wall polymers has been demonstrated (Lam *et al.*, 1992), as well as the negative impact of such phenolics on forage quality (Jung and Deetz, 1993; Hartley, 1972). Lignin composition in terms of syringyl and guaiacyl units has been correlated to degradability by many authors (Reeves, 1985; Buxton and Russell, 1988; Jung and Casler, 1991). Recently, Barrière *et al.* (1994) and Argillier *et al.* (1996) investigated the lignin monomeric composition of silage maize with brown-midrib genes by thioacidolysis, a procedure for lignin depolymerization, and subsequent derivatization and gas chromatographic analysis (Lapierre *et al.*, 1986; Lapierre, 1993), in order to determine feeding value and biochemical characteristics. Among other results, the content of the alkali-labile hydroxycinnamic acids *p*-coumaric and ferulic acids and of guaiacyl and syringyl units was provided for the hybrids under study.

Similar data can be conveniently obtained by high-performance liquid chromatography with electrochemical detection (HPLC-ED) and pyrolysis/gas chromatography/mass spectrometry (PY/GC/MS). Such techniques were recently shown to be practical tools for the monitoring of phenolic and lignin composition in maize stovers during maturation (Galletti *et al.*, 1996). The main advantage of the former technique is its sensitivity and selectivity toward oxidizable compounds, whereas the latter technique is particularly attractive because it is very rapid and requires samples of a few micrograms. PY/GC/MS is particularly suited for screening experiments in which lignin monomeric composition and its relative amount in the cell wall are evaluated. Little or no information about the lignin alkyl chain and structure is obtained by PY/GC/MS. By contrast, thioacidolysis, in its various evolutions, can provide data on the type and amount of uncondensed $\beta$-O-4 units and their free phenolic groups and on carbon−carbon and diphenyl ether bonds (Lapierre, 1993). Its main disadvantages with respect to PY/GC/MS are the sample workup, the formation of isomers which complicate the chromatogram, and the use of nasty chemicals such as diazomethane, boron trifluoride etherate, and ethanethiol.

In the present paper, we report on the phenolic composition of maize stovers at various stages of maturity after blooming, when maize is ready for feeding purposes in Italy. The aims of such an experiment were (1) to check the validity of the above-mentioned techniques in order to show compositional differences of maize stover phenolics and (2) to determine a pool of phenolic markers which might be used by animal producers as lignification indexes in order to choose the most appropriate harvesting time.

## EXPERIMENTAL PROCEDURES

**Samples.** The agronomic trial was performed at the farm Terreni Eredità Pallotti of the University of Bologna, Carpi (Modena) (44° 4′ north, 13° 3′ east, 30 m above sea level), using a commercial hybrid (Costanza, Pioneer, class 600). Samples were collected every 7 days starting July 13, 1993 (female blooming, >70% bloomed plants, 75 days after emergency), and ending September 14, 1993. Each sample was made of five plants random collected over a 3.5 $m^2$ field with a cultivation density of 8.9 plants $m^{-2}$. Stovers were separated from the rest of the plant and stored at −20 °C. Just before analysis, the samples were roughly ground in the presence of dry ice and then oven-dried at 60 °C and finally ground to pass a 1 mm screen.

**HPLC-ED.** Sample aliquots (100 mg) were extracted in thick glass, screw cap 10-mL tubes with 0.1 M sodium hydroxide (5 mL, 110 °C, 10 min, magnetic stirring). The slurry was cooled and centrifuged at 3000 rpm, and the supernatant was decanted. The residue was washed three times with water.

Extracts and their washings were combined and diluted to 25 mL with water. An aliquot (10 mL) was filtered through a 0.22 $\mu$m cartridge (A). Sodium hydroxide (0.4 g) and nitroben-

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**Table 1. Esterified *p*-Coumaric and Ferulic Acids ($\mu$mol g$^{-1}$) after 0.1 M NaOH Extraction and HPLC-ED Analysis**

| | no. of days after blooming | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 | 42 | 49 | 56 | 63 |
| *p*-coumaric acid | 43.98 | 47.36 | 45.56 | 47.59 | 49.96 | 48.8 | 52.3 | 48.51 | 57.05 | 53.22 |
| ferulic acid | 18.88 | 20.29 | 18.1 | 17.25 | 19.09 | 20.46 | 22.43 | 20.25 | 22.29 | 26.17 |
| total | 62.85 | 67.64 | 63.66 | 64.94 | 68.05 | 69.25 | 74.73 | 68.76 | 79.34 | 79.39 |

**Table 2. Phenolic ($\mu$mol g$^{-1}$) Composition and S/G Ratio of the Alkali-Soluble Lignin after Strong Nitrobenzene Oxidation As Determined by HPLC-ED**

| | no. of days after blooming | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 | 42 | 49 | 56 | 63 |
| *p*-hydroxybenzaldehyde | 3.425 | 4.844 | 6.294 | 4.252 | 4.822 | 2.469 | 7.112 | 8.094 | 10.14 | 6.085 |
| vanillin | 26.87 | 27.85 | 32.78 | 33.82 | 36.52 | 33.95 | 43.54 | 33.89 | 42.81 | 37.58 |
| syringic acid | 4.482 | 5.153 | 0.994 | 3.726 | 1.529 | 4.844 | 0.300 | 4.017 | 8.252 | 5.614 |
| syringaldehyde | 36.24 | 39.85 | 44.67 | 45.22 | 45.02 | 51.55 | 55.48 | 52.20 | 58.60 | 51.93 |
| total | 71.02 | 77.70 | 84.75 | 87.01 | 87.89 | 92.81 | 106.4 | 98.2 | 119.8 | 101.2 |
| S/G | 1.35 | 1.43 | 1.36 | 1.34 | 1.23 | 1.52 | 1.27 | 1.54 | 1.37 | 1.38 |

zene (100 $\mu$L) were added to 5 mL of A (thus obtaining a 2 M NaOH solution) in a thick glass, screw cap 10-mL tube and kept at 160 °C for 2 h under magnetic stirring (CAUTION: nitrobenzene is highly toxic by inhalation, contact with the skin, and ingestion and can cause cumulative effects; handle it under a fume cupboard with protective clothes and gloves; do not use tubes whose glass is deteriorated after several extractions). After cooling, the reaction mixture was filtered through a 0.22 $\mu$m cartridge (B). Aliquots (1 mL) of A and B were acidified with 20 and 160 $\mu$L of 37% HCl, respectively, diluted to 25 mL with water, and injected (20 $\mu$L) into HPLC.

A reversed-phase column (150 × 4.6 mm, 5 $\mu$m) Spherisorb hexyl was operated in isocratic conditions with methanol/0.1% perchloric acid in water (15/85, v/v; 1 mL min$^{-1}$). A Waters M45 pump, a Rheodyne 7725i, and an ESA Coulochem model 5100 A (analytical cell set at 0.80 V) were used. Peaks were quantified by calibration curves with standard solutions in the $(1 \times 10^{-6})$–$(1 \times 10^{-5})$ M concentration range. Results are the average of duplicate extractions and duplicate HPLC analyses.

**PY/GC/MS.** Sample aliquots (approximately 0.5 mg) were pyrolyzed in a quartz sample holder using a Chemical Data System Pyroprobe 1000 heated filament pyrolyzer at 600 °C for 5 s. The pyrolyzer was connected to a Varian 3400 gas chromatograph which, in turn, was coupled to a Finnigan mat magnum ion trap mass spectrometer. The gas chromatographic column was a Supelco SPB-5 (30 m × 0.32 mm i.d., 0.25 $\mu$m film thickness) operated from 50 to 290 °C at 5 °C min$^{-1}$ holding the initial temperature for 10 min. The injector was at 250 °C in the split mode (1/100 split ratio). The PY/GC interface was at 200 °C. Mass spectra were recorded under electron impact at 70 eV from 40 to 400 *m*/*z* (1 scan s$^{-1}$). Peak identification was based on mass spectral interpretation and on previous works on PY/GC/MS analysis of lignocellulosic materials (Galletti and Bocchini, 1995; Ralph and Hatfield, 1991). Peak areas (duplicate analysis) were calculated on the base peak in the mass spectrum and are expressed as percentages.

## RESULTS AND DISCUSSION

**HPLC-ED.** This analysis was carried out on mild alkaline extracts which solubilize phenolic monomers and ester-linked *p*-hydroxycinnamic acids, as well as some lignin (Hartley, 1972; Galletti *et al*., 1996). Monomers and *p*-hydroxycinnamic acids were directly determined by HPLC-ED without any further treatment, whereas the alkali-soluble lignin fraction was analyzed in the form of phenolic acids and aldehydes after oxidative hydrolysis with nitrobenzene in strong alkali. The nutritional relevance of alkali-extractable *p*-hydroxycinnamic acids has been discussed by Hartley (1972), who reported a positive correlation between in vivo digestibility of neutral detergent fiber in perennial ryegrass herbage and the ratio of ferulic acid to *p*-coumaric acid extractable in alkali. The chromatographic conditions and separations of phenolics have been described and discussed elsewhere (Galletti *et al*., 1990, 1996).

Table 1 shows the content of *p*-coumaric and ferulic acids of the 10 samples under investigation as obtained after mild alkaline extraction. These acids were the main phenolic constituent of the alkaline extract totaling to about 80% of the chromatographic area. They are the primary components of the so-called "non-core" lignin fraction. *p*-Coumaric acid ranged from about 44 to 57 $\mu$mol g$^{-1}$ from the first to the last sample, respectively, and ferulic acid ranged from about 19 to 26 $\mu$mol g$^{-1}$. In comparison to other reports on the concentrations of alkali-soluble *p*-hydroxycinnamic acids in maize stems (Jung and Deetz, 1993; Galletti *et al*., 1996), the data of ferulic acid are comparable whereas those of *p*-coumaric acid are lower. In spite of some fluctuation from sample to sample, a general increase in the concentration of both hydroxycinnamic acids was observed with maturation. This trend was better represented by the following linear regression equations, where *x* ranges from 0 to 63 and represents the number of days corresponding to samples from 1 to 10:

$$p\text{-coumaric acid } (\mu\text{mol g}^{-1}) = 0.15(\pm 7.77 \times 10^{-2})x + 44.55(\pm 2.95)$$

$$R^2 = 0.72,\ P = 0.05$$

$$\text{ferulic acid } (\mu\text{mol g}^{-1}) = 9.35 \times 10^{-2}(\pm 6.78 \times 10^{-2})x + 17.48(\pm 2.53)$$

$$R^2 = 0.56,\ P = 0.05$$

The increase of ester-bound *p*-coumaric acid with maturation with a better correlation coefficient than that of ferulic acid is consistent with previous reports (Galletti *et al*., 1996; Terashima *et al*., 1993) and is attributable to the deposition of most ester-linked *p*-coumaric acids in the wall during lignification of the secondary wall (Jung and Deetz, 1993; Ralph *et al*., 1994).

Table 2 shows the content of *p*-hydroxybenzaldehyde, vanillin, syringic acid, and syringaldehyde after strong alkaline nitrobenzene oxidation of the previous extract. These results describe the composition of the alkali-soluble lignin, which has been shown previously to form

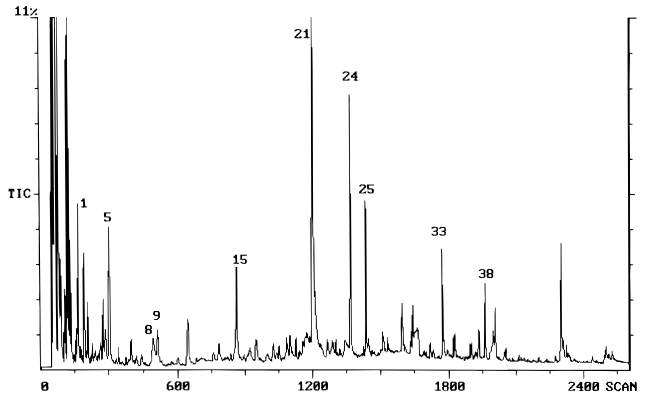


**Figure 1.** PY/GC/MS profile (total ion chromatogram) of a representative maize stover sample (main peaks are numbered as in Table 3).

a large part of the maize lignin (Galletti *et al.*, 1996). Vanillin and syringaldehyde were the main constituents and represent guaiacyl (G) and syringyl (S) lignin units. Their increase with maturation is better described by the following linear regression equations:

$$\text{vanillin } (\mu\text{mol g}^{-1}) = 0.20(\pm 0.13)x + 28.60(\pm 4.89)$$

$$R^2 = 0.61,\ P = 0.05$$

$$\text{syringaldehyde } (\mu\text{mol g}^{-1}) = 0.30(\pm 0.12)x + 38.60(\pm 4.30)$$

$$R^2 = 0.82,\ P = 0.05$$

However, the ratio S/G or syringaldehyde/vanillin was practically constant in the 1.23−1.54 range (average 1.38), a value slightly higher than that previously found (approximately 1.2) for the highest degree of maturation (Galletti *et al.*, 1996). The total amount of phenolics of this fraction showed a good correlation with maturation, better represented by the following linear regression equation:

$$\text{total phenolics } (\mu\text{mol g}^{-1}) = 0.60(\pm 0.25)x + 73.68(\pm 9.22)$$

$$R^2 = 0.80,\ P = 0.05$$

Minor amounts of *p*-coumaric and ferulic acids were found in this fraction (data not shown). Such values were variable and apparently not correlated with maturation. They represented only a minor percentage of the quantities found in the mild alkaline extract, namely, 15% and 5%, respectively.

**PY/GC/MS.** The pyrogram of one sample is reported in Figure 1 as an example of the pyrolytic profiles obtained from maize stovers. It should be mentioned again that the quantitative analysis of the pyrolysis fragments was carried out using the intensity of the base peak in their mass spectra. This allowed a quantitation free from chromatographic interferences due to coeluting peaks, which, though small, might have been a problem in samples characterized by small quantitative differences. The PY/GC/MS quantitative data of the present work might be slightly different from, yet comparable to, those presented previously (Galletti *et al.*, 1996), in which the areas of the pyrolysis peaks were calculated using the intensity of all ions (or total ion current). In accordance with previous findings (Galletti and Reeves, 1991; Galletti *et al.*, 1996), 4-vinylphenol, 4-vinylguaiacol, and 2,6-dimethoxyphenol (peaks 21, 24, and 25, respectively) were among the most abundant pyrolysis fragments, representing the three typical *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) lignin units. Structural polysaccharides gave rise to several fragments of various intensity, the main products being peaks 4, 5, 9, and 30. Table 3 collects the relative percentages of all pyrolysis products. Furaldehyde and 4-vinylphenol (markers for carbohydrates and H lignin units, respectively) showed slight and opposite variations, the former decreasing and the latter increasing with maturation.

Table 4 shows the total amounts of pyrolysis fragments originating from polysaccharides and *p*-hydroxyphenyl, guaiacyl, and syringyl lignin units. There was a relative decrease of the polysaccharide markers and an increase of the lignin markers with maturation. The relative amount of polysaccharide pyrolysis fragments showed a good negative correlation with maturation, better represented by the following linear regression equation:

$$\text{polysaccharide pyrolysis fragments (relative \%)} = -0.16(\pm 5.58 \times 10^{-2})x + 31.09(\pm 2.09)$$

$$R^2 = 0.85,\ P = 0.05$$

However, the decrease was less evident than that found in the previous similar experiment on maize stovers (Galletti *et al.*, 1996). This occurrence was not unexpected because sampling in the previous experiment started when plants were very immature and the main changes in the content of lignin and polysaccharide markers were observed only in the first three/four samples.

The ratio between syringyl and guaiacyl lignin units (S/G) (Table 4) showed a weak correlation with maturation, represented by the following linear regression equation:

$$\text{S/G} = 2.84 \times 10^{-3}\ (\pm 2.71 \times 10^{-3})x + 0.63(\pm 0.10)$$

$$R^2 = 0.42,\ P = 0.05$$

This indicates a less marked variation than that found in the previous experiment (Galletti *et al.*, 1996), where $R^2 = 0.86$. The S/G ratio ranged from 0.58 to 0.91 (average 0.72), a value slightly higher than that found in the previous work for the most mature maize stovers (approximately 0.5) (Galletti *et al.*, 1996). The relatively poor $R^2$ value for the S/G ratio is mostly due to the low correlation coefficient of G units with maturation ($R^2 = 0.05$), as opposed to the higher $R^2$ value for S and H units ($R^2 = 0.58$ and 0.59, respectively). It is also interesting to note that the difference in S/G ratios as obtained by the two techniques (*i.e.*, HPLC and PY/GC/MS) is consistent with the previous report (Galletti *et al.*, 1996), with HPLC yielding the higher ratio. Finally, the relatively high percentages of H units confirm previous findings by PY/GC/MS of maize stems (Galletti *et al.*, 1996). They reflect the global H unit content of the cell wall including lignin and *p*-hydroxycinnamic acids as determined by direct pyrolysis of the whole sample. These data do not contradict the HPLC-ED data, which, by contrast, regard only the *p*-hydroxycinnamic acids and the lignin fraction extractable in mild alkali.

In conclusion, changes in phenolic composition and in the relative amounts of lignin and polysaccharide

**Table 3. Peak Identification, Molecular Weight, Origin (C, Carbohydrates; H, *p*-Hydroxyphenyl Lignin Units; G, Guaiacyl Lignin Units; S, Syringyl Lignin Units), Scan Numbers (s), and Area in the PY/GC/MS Chromatograms of Maize Stovers (Peak Numbers as in Figure 1)**

| | | | | | no. of days after blooming | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| no. | name | MW | origin | scan | 0 | 7 | 14 | 21 | 28 | 35 | 42 | 49 | 56 | 63 |
| **1** | 2-furaldehyde | 96 | C | 165 | 2.92 | 3.04 | 2.61 | 2.96 | 2.89 | 2.65 | 2.33 | 2.22 | 1.77 | 2.42 |
| **2** | cyclopent-1-ene-3,4-dione | 96 | C | 228 | 1.15 | 0.64 | 0.83 | 0.73 | 0.57 | 0.65 | 0.58 | 0.58 | 0.37 | 0.42 |
| **3** | 2-methyl-2-cyclopenten-1-one | 96 | C | 265 | 1.33 | 1.01 | 1.03 | 1.00 | 0.80 | 1.03 | 0.87 | 0.85 | 0.68 | 0.70 |
| **4** | (5*H*)-furan-2-one | 84 | C | 275 | 5.78 | 5.59 | 5.76 | 4.81 | 4.75 | 5.41 | 4.29 | 4.13 | 3.56 | 3.04 |
| **5** | 2,3-dihydro-5-methylfuran-2-one | 98 | C | 301 | 6.78 | 9.61 | 7.04 | 6.38 | 5.35 | 6.36 | 4.99 | 4.91 | 4.50 | 4.49 |
| **6** | 5-methyl-2-furaldehyde | 110 | C | 400 | 1.50 | 0.80 | 1.46 | 1.87 | 1.25 | 0.73 | 1.17 | 1.14 | 0.47 | 0.97 |
| **7** | 2,4-dihydropyran-3-one | 98 | C | 441 | 0.46 | 0.49 | 0.47 | 0.42 | 0.38 | 0.39 | 0.36 | 0.38 | 0.30 | 0.26 |
| **8** | phenol | 94 | H | 493 | 3.79 | 2.74 | 2.59 | 2.87 | 3.12 | 2.76 | 2.99 | 2.74 | 2.86 | 2.92 |
| **9** | 1,5-anhydro-4-deoxypent-1-en-3-ulose | 114 | C | 513 | 3.09 | 3.40 | 4.37 | 6.48 | 6.70 | 3.83 | 4.54 | 4.63 | 2.78 | 3.76 |
| **10** | 3-hydroxy-2-methyl-2-cyclopenten-1-one | 112 | C | 574 | 0.61 | 0.68 | 0.59 | 0.53 | 0.74 | 0.70 | 0.49 | 0.47 | 0.33 | 0.35 |
| **11** | 2-hydroxy-3-methyl-2-cyclopenten-1-one | 112 | C | 644 | 1.43 | 0.74 | 1.28 | 1.45 | 1.21 | 1.45 | 1.28 | 1.23 | 0.77 | 0.91 |
| **12** | 2,3-dimethylcyclopenten-1-one | 110 | C | 685 | 0.34 | 0.24 | 0.27 | 0.27 | 0.21 | 0.25 | 0.22 | 0.22 | 0.19 | 0.19 |
| **13** | 5-ethylfuraldehyde | 124 | C | 688 | 0.07 | 0.05 | 0.06 | 0.06 | 0.07 | 0.04 | 0.04 | 0.04 | 0.03 | 0.04 |
| **14** | 4-methylphenol | 108 | H | 853 | 1.90 | 1.26 | 1.36 | 1.43 | 1.50 | 1.32 | 1.43 | 1.33 | 1.24 | 1.26 |
| **15** | guaiacol | 124 | G | 867 | 2.85 | 2.63 | 2.48 | 2.67 | 2.10 | 2.05 | 2.62 | 2.37 | 2.86 | 3.00 |
| **16** | 3-hydroxy-2-methyl-(4*H*)-pyran-4-one | 126 | C | 923 | 0.55 | 0.63 | 0.85 | 0.74 | 0.58 | 0.52 | 0.57 | 0.72 | 0.43 | 0.37 |
| **17** | 3,4-dihydroxybenzaldehyde | 138 | | 1054 | 0.64 | 0.79 | 0.62 | 0.59 | 0.62 | 0.59 | 0.61 | 0.84 | 0.59 | 0.48 |
| **18** | 4-ethylphenol | 122 | H | 1086 | 2.24 | 1.68 | 1.52 | 1.66 | 2.02 | 1.57 | 1.73 | 1.78 | 1.63 | 2.92 |
| **19** | 4-methylguaiacol | 138 | G | 1128 | 0.57 | 0.62 | 0.64 | 0.64 | 0.51 | 0.38 | 0.64 | 0.57 | 0.60 | 0.73 |
| **20** | catechol | 110 | | 1168 | 2.09 | 2.62 | 2.22 | 1.79 | 1.72 | 2.13 | 1.48 | 1.98 | 1.63 | 1.76 |
| **21** | 4-vinylphenol | 120 | H | 1200 | 28.19 | 28.25 | 28.27 | 27.52 | 31.77 | 34.73 | 30.87 | 32.49 | 33.04 | 33.40 |
| **22** | 3-methoxycatechol | 140 | | 1266 | 0.74 | 1.03 | 1.15 | 0.96 | 0.90 | 0.78 | 0.96 | 1.01 | 1.05 | 1.34 |
| **23** | 4-ethylguaiacol | 152 | G | 1304 | 0.72 | 0.71 | 0.72 | 0.73 | 0.53 | 0.39 | 0.73 | 0.61 | 0.67 | 0.93 |
| **24** | 4-vinylguaiacol | 150 | G | 1368 | 11.87 | 10.29 | 11.90 | 12.76 | 11.37 | 10.49 | 12.78 | 11.84 | 11.22 | 12.56 |
| **25** | 2,6-dimethoxyphenol | 154 | S | 1433 | 4.57 | 4.68 | 4.75 | 4.09 | 4.57 | 4.03 | 5.69 | 5.24 | 6.63 | 5.98 |
| **26** | vanillin | 152 | G | 1509 | 1.21 | 0.97 | 1.04 | 1.04 | 1.31 | 1.63 | 1.25 | 1.16 | 0.89 | 0.97 |
| **27** | 2,6-dimethoxy-4-methylphenol | 168 | S | 1594 | 0.83 | 1.06 | 1.03 | 0.93 | 0.93 | 0.76 | 1.29 | 1.13 | 1.31 | 1.22 |
| **28** | *trans*-isoeugenol | 164 | G | 1597 | 1.01 | 0.96 | 1.18 | 1.04 | 0.84 | 0.72 | 0.93 | 0.99 | 1.06 | 1.28 |
| **29** | homovanillin | 166 | G | 1609 | 0.26 | 0.34 | 0.31 | 0.28 | 0.36 | 0.34 | 0.31 | 0.28 | 0.30 | 0.34 |
| **30** | 1,6-anhydro-$\beta$-D-glucopyranose | 162 | C | 1646 | 2.53 | 3.14 | 2.12 | 2.64 | 1.79 | 1.92 | 1.64 | 2.50 | 4.86 | 1.67 |
| **31** | 4-ethyl-2,6-dimethoxyphenol | 182 | S | 1717 | 0.30 | 0.37 | 0.35 | 0.30 | 0.28 | 0.22 | 0.47 | 0.39 | 0.49 | 0.48 |
| **32** | guaiacylacetone | 180 | G | 1719 | 0.59 | 0.63 | 0.54 | 0.53 | 0.47 | 0.46 | 0.49 | 0.49 | 0.65 | 0.63 |
| **33** | 2,6-dimethoxy-4-vinylphenol | 180 | S | 1773 | 2.96 | 3.12 | 2.94 | 2.77 | 3.12 | 3.49 | 3.61 | 3.41 | 4.08 | 3.32 |
| **34** | 4-allyl-2,6-dimethoxyphenol | 194 | S | 1830 | 0.41 | 0.39 | 0.49 | 0.40 | 0.43 | 0.41 | 0.54 | 0.50 | 0.55 | 0.50 |
| **35** | *cis*-2,6-dimethoxy-4-propenylphenol | 194 | S | 1898 | 0.25 | 0.26 | 0.33 | 0.26 | 0.27 | 0.25 | 0.34 | 0.32 | 0.36 | 0.35 |
| **36** | syringaldehyde | 182 | S | 1904 | 0.26 | 0.34 | 0.32 | 0.32 | 0.37 | 0.35 | 0.38 | 0.37 | 0.43 | 0.30 |
| **37** | 1-(3,5-dimethoxy-4-hydroxyphenyl)propyne | 192 | S | 1927 | 0.11 | 0.12 | 0.13 | 0.11 | 0.13 | 0.14 | 0.13 | 0.13 | 0.11 | 0.10 |
| **38** | *trans*-2,6-dimethoxy-4-propenylphenol | 194 | S | 1965 | 1.58 | 1.62 | 1.92 | 1.65 | 1.79 | 1.62 | 2.23 | 2.13 | 2.26 | 2.00 |
| **39** | *trans*-conyferaldehyde | 178 | G | 2001 | 0.17 | 0.30 | 0.20 | 0.26 | 0.14 | 0.23 | 0.26 | 0.19 | 0.21 | 0.19 |
| **40** | acetosyringone | 196 | S | 2005 | 0.40 | 0.66 | 0.54 | 0.57 | 0.48 | 0.55 | 0.53 | 0.51 | 0.58 | 0.51 |
| **41** | *trans*-coniferyl alcohol | 180 | G | 2008 | 0.46 | 0.81 | 0.89 | 0.85 | 0.46 | 0.97 | 0.70 | 0.53 | 0.81 | 0.43 |
| **42** | syringylacetone | 210 | S | 2056 | 0.34 | 0.49 | 0.40 | 0.44 | 0.42 | 0.53 | 0.45 | 0.45 | 0.59 | 0.37 |
| **43** | propiosyringone | 210 | S | 2126 | 0.13 | 0.18 | 0.17 | 0.18 | 0.16 | 0.22 | 0.19 | 0.17 | 0.27 | 0.13 |

**Table 4. Total Amounts of the Pyrolysis Products Ascribed to Carbohydrates and H, G, and S Lignin Units and S/G Ratio**

| | no. of days after blooming | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 | 42 | 49 | 56 | 63 |
| polysaccharide fraction | 28.55 | 30.07 | 28.73 | 30.35 | 27.52 | 25.91 | 23.37 | 24.04 | 21.04 | 19.59 |
| H units | 36.12 | 33.94 | 33.74 | 33.49 | 38.29 | 40.38 | 37.02 | 38.34 | 38.76 | 40.51 |
| G units | 19.71 | 18.27 | 19.91 | 20.79 | 18.04 | 17.66 | 20.71 | 19.04 | 19.27 | 21.06 |
| S units | 12.14 | 13.29 | 13.36 | 12.02 | 12.91 | 12.55 | 15.85 | 14.74 | 17.66 | 15.25 |
| S/G | 0.62 | 0.73 | 0.67 | 0.58 | 0.72 | 0.71 | 0.77 | 0.77 | 0.92 | 0.72 |

pyrolysis markers were observed in spite of the relatively late maturation period. Not surprisingly, such changes were less sharp than those found when plants were sampled starting at a very immature stage (Galletti *et al*., 1996). However, the high coefficient of linear correlation found for *p*-hydroxycinnamic acids as determined by HPLC in mild alkaline extracts suggests that the amount of ester links between phenolics and cell wall structural polymers is subjected to changes after blooming. Likewise, a marked increase in vanillin and syringaldehyde with maturation as determined by HPLC after hydrolysis of the alkali-soluble lignin fraction and an increase in some lignin markers, such as 4-vinylphenol (peak 21), 2,6-dimethoxyphenol (peak 25), 2,6-dimethoxy-4-vinylphenol (peak 33), and *trans*-2,6-dimethoxy-4-propenylphenol (peak 38), as determined by PY/GC/MS, show that the cell wall structural polymers undergo processes of modification of their composition during the considered maturation interval.

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