Fiber Composition of a Neglected Wheat Species (*Triticum dicoccum* Schubler) As Determined by Pyrolysis/Gas Chromatography/Mass Spectrometry

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Two different emmer (*Triticum dicoccum* Schubler) landraces (spring and winter emmer) and durum wheat (*Triticum durum* Desf.) as a reference material were analyzed by pyrolysis/gas chromatography/mass spectrometry to characterize structural polymers present in kernel cell walls. A number of pyrolysis products which can be ascribed to lignin and polysaccharides were identified in the pyrograms. The comparison of *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) pyrolysis products, presumably derived from lignin, and polysaccharide markers in the three different samples showed significant differences which can be related to differences in the composition of structural polymers in spring and winter emmer and durum wheat. The relative yields of G-type and carbohydrate-derived pyrolysis products were significantly higher in durum wheat than in the emmer samples.

Keywords: *Emmer fiber; Triticum dicoccum; cereal analysis; dietary fiber; PY/GC/MS; pyrolysis*

INTRODUCTION

Emmer (*Triticum dicoccum* Schubler) belongs to the so-called hulled wheats. Findings of emmer kernels in archaeological sites in Sicily, Tuscany, and Latium witness the fact that emmer (Latin root "far", Italian name "farro") has been used as a staple food by prehistoric, Etruscan, and Roman populations until it was largely replaced by the modern bread wheat during the late Roman empire (D'Antuono, 1994).

Today, the increasing demand for traditional, natural, and fiber-rich food has stimulated a renewed interest in this ancient cereal (Blenford, 1994). Practically all products normally made of durum wheat are now available in emmer-based varieties, e.g. breakfast and soup products, cookies and other bakery products, pasta, and bread. Specialized centers exploit emmer as a health food to complement diets with the goal of losing weight. A matter of popular wisdom, yet largely appreciated by producers and consumers, is the fact that emmer acts as a gentle laxative, a regulator of intestine functions, and an energy supplier and has an apparent reduced allergenicity (Blenford, 1994; D'Antuono, 1995).

It is likely that many of the beneficial properties of emmer are due to the secondary components of its kernel, such as structural polymers (mainly cellulose, hemicellulose, and lignin), gums, and mucilages (D'Antuono, 1995). Actually, cell wall polymers are main constituents of the so-called "dietary fiber", an important nutritional factor which has been suggested as a protective agent against a variety of chronic diseases of the digestive tract (Trowell *et al*., 1976).

The molecular composition and structural arrangement of cell wall polymers play an important role from the standpoint of food texture, acceptability, nutritional value, and health. Lignin, a three-dimensional phenylpropanoid polymer, and the structural polysaccharides cellulose and hemicellulose are among the most important factors affecting such food qualities as taste and digestibility (Mellon *et al*., 1994).

Pyrolysis/gas chromatography/mass spectrometry (PY/GC/MS) is a useful tool for the characterization of lignin (Irwin, 1982), lignocellulosic and forage materials (Ralph and Hatfield, 1991; Galletti and Bocchini, 1995), and vegetable fiber and mucilage (Galletti *et al*., 1993; Mellon *et al*., 1994). PY/GC/MS is based upon the thermal degradation of the original and polymeric sample at temperatures in the 600−1000 °C range in an inert atmosphere. The pool of pyrolysis products, separated by gas chromatography and identified with the aid of mass spectrometry, is diagnostic of the starting material, which otherwise would be unsuitable for GC/MS analysis due to its high molecular weight.

The present paper reports on the PY/GC/MS characterization of the seed fiber of two emmer landraces from central Italy, namely winter and spring emmer, characterized by floury and vitreous endosperm, respectively, and of durum wheat for comparison. Data on the molecular composition of emmer fiber, particularly lignin, will be provided, and the potential of PY/GC/MS to bring to light the compositional differences characterizing emmer with respect to durum wheat will be discussed.

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Table 1. Tentative Identification of the Major Pyrolysis Products (Area %, Means of Three Replicates) from Spring and Winter Emmer and Durum Wheat

no.	name	MW	scan	winter emmer	spring emmer	durum wheat	LSD[a]	significance[b]
1	2-furaldehyde	96	310	13.2	12.8	13.6	–	ns
2	2-(hydroxymethyl)furan	98	375	5.5	7.1	5.0	0.6	***
3	cyclopent-1-ene-3,4-dione	96	432	1.2	0.8	0.9	–	ns
4	vinylbenzene	104	465	0.8	0.7	0.9	–	ns
5	2,3-dihydro-5-methylfuran-2-one	98	597	12.2	9.5	9.6	–	ns
6	5-methyl-2-furaldehyde	110	749	1.0	1.3	0.8	–	ns
7	3-methyl-5*H*-furan-2-one	98	782	0.4	0.2	0.6	–	ns
8	phenol	94	822	2.5	3.8	3.4	–	ns
9	1,5-anhydro-4-deoxypent-1-en-3-ulose	114	878	6.9	7.9	9.7	–	ns
10	2-hydroxy-3-methyl-2-cyclopenten-1-one	112	948	4.9	6.1	5.9	–	ns
11	2,3-dimethylcyclopenten-1-one	110	978	5.8	0.2	3.3	3.2	*
12	2,4-dihydropyran-3-one	98	1008	0.2	0.4	0.2	–	ns
13	2-methylphenol (*o*-cresol)	108	1039	0.6	0.2	0.5	0.3	*
14	2-methoxyphenol (guaiacol)	124	1116	3.0	3.1	3.8	–	ns
15	a dimethyldihydropyranone	126	1189	1.0	1.4	0.7	–	ns
16	4-vinylphenol	120	1416	0.4	0.0	0.5	–	ns
17	5-(hydroxymethyl)-2-furaldehyde	126	1441	7.6	19.8	3.8	10.2	*
18	2-methoxy-4-ethylphenol (4-ethylguaiacol)	152	1520	0.8	1.2	2.6	1.1	**
19	2-methoxy-4-vinylphenol (4-vinylguaiacol)	150	1589	19.0	13.6	21.9	–	ns
20	2,6-dimethoxyphenol (syringol)	154	1652	1.6	0.5	3.0	0.9	***
21	2-methoxy-4-propylphenol (4-propylguaiacol)	166	1679	1.0	0.3	0.8	–	ns
22	4-hydroxy-3-methoxybenzaldehyde (vanillin)	152	1747	0.3	0.0	1.1	0.7	*
23	2,6-dimethoxy-4-methylphenol (4-methylsyringol)	168	1812	0.2	0.0	0.4	–	ns
24	*trans*-2-methoxy-4-propenylphenol (*trans*-isoeugenol)	164	1822	0.5	0.3	1.0	0.6	*
25	4-hydroxy-3-methoxyacetophenone (acetovanillone)	166	1888	0.0	0.0	0.6	–	ns
26	1,6-anhydro-β-D-glucopyranose	162	1913	6.8	6.1	1.4	–	ns
27	4-hydroxy-3-(methoxyphenyl)acetone (guaiacylacetone)	180	1942	0.0	0.5	0.1	–	ns
28	2,6-dimethoxy-4-vinylphenol (4-vinylsyringol)	180	1996	2.6	1.7	3.3	–	ns
29	*trans*-2,6-dimethoxy-4-propenylphenol (*trans*-4-propenylsyringol)	194	2196	0.1	0.3	0.7	0.4	**
30	4-hydroxy-3-methoxycinnamaldehyde (*trans*-coniferyl aldehyde)	178	2260	0.1	0.0	0.0	0.1	*

[a] LSD = least significant difference at $P = 0.005$. [b] Analysis of variance: ns, not significant; *, significant at $P \leq 0.05$; **, significant at $P \leq 0.01$; and ***, significant at $P \leq 0.001$.

EXPERIMENTAL PROCEDURES

Samples. Spring and winter emmer landraces from Agnone and Amatrice, central Italy, and durum wheat, cv. Creso, were investigated. Kernel pericarp was sampled by means of a lancet under a microscope. Three kernels were analyzed for each sample.

PY/GC/MS. Pericarp aliquots (<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 model Magnum ion trap mass spectrometer. The gas chromatographic column was a Supelco SPB-5 model (30 m × 0.32 mm inside diameter, 0.25 μm 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* (one scan s^{-1}). Peak identification was based on mass spectral interpretation and on previous works using PY/GC/MS analysis of lignocellulosic materials (Ralph and Hatfield, 1991; Galletti and Bocchini, 1995). Peak areas were expressed as percentages of the total ion current (TIC) chromatogram. Analysis of variance was used to compare quantitative results.

RESULTS AND DISCUSSION

Figure 1 shows the PY/GC/MS profile (TIC) of spring emmer pericarp. Similar pyrograms were obtained for winter emmer and durum wheat. Thirty pyrolysis peaks were identified (Table 1). Essentially three classes of compounds are recognizable, i.e. furanic (peaks 1, 2, 5−7, and 17) and pyranic derivatives (9, 12, 15, and 26) and phenolics with various methoxy and alkyl substituents. The latter group can be subdivided into molecules with *p*-hydroxyphenyl (H) (peaks 8, 13, and 16), 2-methoxy-*p*-hydroxyphenyl (guaiacyl or G units) (peaks 14, 18, 19, 21, 22, 24, 25, 27, and 30), and

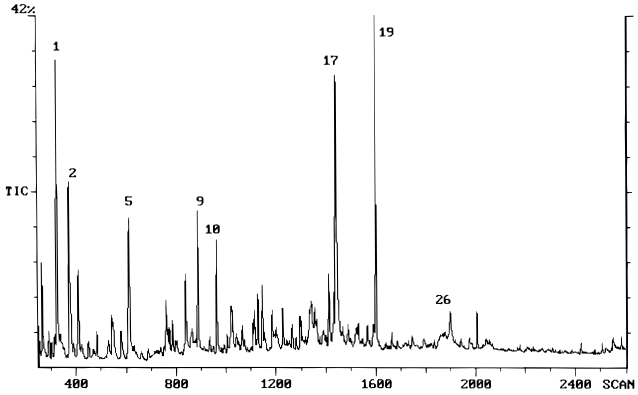

Figure 1. PY/GC/MS profile (TIC) of spring emmer kernel pericarp. Main peaks are numbered as in Table 1.

2,6-dimethoxy-*p*-hydroxyphenyl (syringyl or S units) aromatic moieties (peaks 20, 23, 28, and 29). While H, G, and S phenolic units are the monomeric constituents of the lignin macromolecule, the furanic and pyranic derivatives are the products of multiple dehydrations and rearrangements of carbohydrates under heating and can be considered as pyrolysis markers of the structural polysaccharides along with peaks 10 and 11 (Ralph and Hatfield, 1991; Galletti and Bocchini, 1995). The different alkyl substituents in the phenolic monomers can be neglected since they are the result of the thermal cleavage at different sites of the lignin propyl chain. Due to a certain variability of the experimental results, some apparently high differences in the relative abundance of some peaks did not differ significantly among samples. However, spring emmer resulted in rather clearly differentiated peaks for the composition of furanic and pyranic fractions (peaks 2, 11, and 17)

Table 2. Quantitative Analysis of the Main Classes of Pyrolysis Products, H-, G-, S-, and Carbohydrate-Derived Moieties

	spring emmer	winter emmer	durum wheat	LSD[a]	significance[b]
carbohydrate-derived fraction	78.7	67.9	58.8	8.2	**
H-derived fraction	3.4	1.7	4.6	1.9	*
G-derived fraction	15.7	25.5	29.6	5.1	**
S-derived fraction	2.2	4.9	7.0	3.4	*
S/G	0.14	0.19	0.24		

[a] LSD = least significant difference at $P = 0.05$. [b] Analysis of variance: *, significant at $P \leq 0.05$; and **, significant at $P \leq 0.01$.

and also for the percentage of some peaks of the lignin fraction (peaks 18, 20, 22, 24, 29, and 30).

The area percentages of the polysaccharide markers and of H, G, and S lignin units were summed to obtain the data presented in Table 2. The relative abundances of pyrolysis products may differ depending on the starting material. The pyrolysis of lignin results in more abundant and more easily identifiable products than that of polysaccharides. This is due to the stability of the aromatic moieties of lignin under the thermal energy supplied by the pyrolyzer and under the electron impact of the mass spectrometer, the latter resulting in mass spectra with abundant molecular ions and diagnostic fragmentation pathways. By contrast, polysaccharides undergo multiple dehydrations under pyrolysis and yield various positional isomers whose mass spectra can be difficult to interpret because of the small or lacking molecular ion (Ralph and Hatfield, 1991; Galletti and Bocchini, 1995).

Therefore, by no means is the relative distribution of polysaccharide and lignin markers shown in Table 2 truly representative of the actual fiber content of the material under investigation. However, such data offer a valuable tool for comparing the fiber composition of the two emmer landraces and of durum wheat.

The data of Table 2 show the relative amount of pyrolysis products which can be ascribed to polysaccharides, H, G, and S lignin in spring and winter emmer, and durum wheat. The most significant differences ($P < 0.01$) were found in polysaccharide fraction and G units, cellulose and hemicellulose pyrolysis products being less abundant in durum wheat than in spring and winter emmer (58.8 versus 67.9 and 78.7%). An opposite trend was observed for G units which were more abundant in durum wheat than in the two emmer landraces (29.6 versus 25.5 and 15.7%). S units showed a less significant difference ($P < 0.05$), but the same trend in the three samples, being minor components of structural cell wall polymers in cereals. As a whole, these results are consistent with those of Table 1 and confirm the differentiation of spring emmer with respect to the other two genotypes.

When the S/G ratio is considered as an index of the time elapsed during the deposition of the cell wall components (Terashima *et al.*, 1993), durum wheat should be considered at a later stage of the deposition process in comparison with winter and spring emmer (S/G of 0.24, 0.19, and 0.14, respectively, Table 2).

In conclusion, winter emmer seems to be intermediate compared to spring emmer and durum wheat, spring emmer being the most different from wheat in the relative abundance and composition of cell wall structural polymers.

PY/GC/MS proved to be a useful tool for the characterization of emmer landraces and durum wheat in terms of fiber composition. This kind of information could be of interest in studies aimed at comparing nutritional qualities of cereal fibers.

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