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Compositional analysis of neutral detergent, acid detergent, lignin and humus fractions of mushroom compost

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Abstract

The fibre present in mushroom compost samples was separated by a standard method of selective hydrolysis into three components: acid detergent fibre (ADF), neutral detergent fibre (NDF) and lignin. Results of differential thermogravimetric (DTG) analysis of these fractions suggest that with NDF the matrix polysaccharides were detected as a shoulder to the cellulose peak and that structural hemicelluloses pyrolysed before the lignin decomposition peak. However, in ADF, only cellulose and lignin pyrolysis peaks were detected and a hemicellulose decomposition peak or shoulder was not present. In the lignin there were two pyrolysis peaks which represented residual polysaccharides and lignin. Thermograms of humus which had been separated from the compost by ultrasonic treatment showed that microbial polysaccharides and phenolic compounds were the main components. Lignin and humus contained high concentrations of nitrogen compared with the other fractions. Comparison of thermograms of uncomposted wheat straw and straw from compost showed that straw cell-wall components were altered by fermentation.

Keywords: Acid detergent fibre (ADF); DTG; Hemicelluloses; Humus; Lignin; Mushroom compost; Neutral detergent fibre (NDF); Polysaccharides; Wheat straw

1. Introduction

One of the factors that determine mushroom yield is the proportion of fibre components present in pasteurised compost as determined by selective hydrolysis [1,2]. However, these components, cellulose, hemicelluloses and lignin, are inter-

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meshed with covalent cross-linkages. As a result the constituents cannot be extracted without causing degradation or structural change. The methods used for estimating detergent fibre and lignin fractions are based on a single extraction of soluble components to remove protein and other material. This allows a convenient means for studying the proportions of fibre components [3–5]. Neutral detergent fibre (NDF) represents cellulose, hemicellulose and lignin fractions of the plant cell-wall while ADF is characterised by the presence of cellulose and lignin only.

Many reports on the development of rapid methods for isolating the fibre components present in animal feeds have been published [3–7] and emphasis has been placed on the purity of prepared fractions, their speed, economy and versatility of application [5]. A composition study of ADF for carbon, nitrogen and ash content showed the fibre fraction to be relatively pure lignocellulose [5]. In addition, NDF and lignin were shown to contain residual protein and ash [3,5] thus fibre estimations need correcting for residuals.

Recent reports have shown that thermal methods, such as differential scanning calorimetry and differential thermogravimetry (DTG) can rapidly determine changes in fibre components during microbial degradation [8,9]. Elemental analysis (EA) can assess the proportions of carbon, hydrogen and nitrogen that occur during stages in the preparation of mushroom composts [1]. Thermogravimetric analyses of fibre components have shown that lignin is very thermally stable. Cellulose is intermediate and hemicelluloses least stable [10,11]. Fibre fractions of compost have not been analysed for their associated nitrogen or residual polysaccharides. However a few reports have suggested that humus present on composted straw is high in nitrogen [12–14].

The present study was aimed at evaluating the composition of the ADF, NDF, lignin, humus and degraded-straw fractions of mushroom compost by differential thermogravimetry and elemental analysis.

2. Materials and methods

2.1. Separation of fibre components

Samples used in this investigation were from a comparative trial on compost quality of pasteurised synthetic composts. All materials were dried at 85°C and ground to pass through a 0.5 mm mesh sieve. Fibre analysis by hydrolysis produced three fractions ADF, NDF and lignin [3–5]. These were filtered in sintered glass crucibles and analysed by differential thermogravimetry to assess their thermal stability. The proportions of carbon, hydrogen and nitrogen present in the above fractions were determined by elemental analysis.

The humus fraction adhering to straw compost which has undergone fermentation was separated by sonicating in a water bath for 30 min at 25°C. The resulting humus and straw fractions were filtered, dried, ground and analysed by DTG and EA.

2.2. Thermogravimetric analysis

Thermogravimetric (TG) analysis was determined in a Mettler furnace (TG 50) controlled by a TA processor connected to a computer for data handling. The

experimental conditions were as follows: initial temperature 25°C; final temperature 600°C; heating rate of 20°C min^{-1}; air flow 20 mm min^{-1}; sample size 3–4 mg. Each sample was tested in tripicate [1]. The differential thermogravimetric curve of the TG data was calculated by Graphware (Mettler Toledo). The primary peak (PP) band extended from 220–420°C, this was followed by a secondary peak (SP) from 420–580°C.

2.3. Analysis of reference samples

The following reference samples, obtained from Sigma Chemical Co, were analysed: alpha cellulose, levoglucosan, xylan from larchwood, sodium polypectate, lignosulphonic acid, indulin AT (alkali lignin), lignin isolated from wheat straw by sulfuric acid digestion [3], pullulan, chitin, chitosan and lipopolysaccharides (L-3012; L-3023).

2.4. Elemental analysis

The ADF, NDF and lignin fractions retained in aluminium vials were analysed by EA. This was achieved by combusting the samples (6–7 mg) in pure oxygen with an inert carrier gas (argon). The products, carbon dioxide, water and nitrogen dioxide were measured as carbon (C), hydrogen (H) and nitrogen (N), respectively, by gas chromatography using a PE 2400 CHN. Each measurement was in triplicate.

3. Results

3.1. C H N content of the fibre fractions

Comparative analysis of fibre for CHN revealed that ADF contained significantly ($P < 0.001$) the highest levels of carbon and hydrogen. Nitrogen in ADF and NDF fractions were lower compared to lignin or control (Table 1). Nitrogen levels in humus

Table 1
Comparison of carbon, hydrogen and nitrogen levels present in lignin, acid detergent fibre (ADF), neutral detergent fibre (NDF), and unextracted compost samples

Fibre fraction	Carbon/%	Hydrogen/%	Nitrogen/%
Lignin	41.3	4.1	2.3
ADF	43.1	5.8	1.9
NDF	40.2	5.5	1.7
unextracted	35.4	4.9	2.4
SEM[a]	0.691	0.081	0.066

[a] All SEMs significant at $P < 0.001$.

and straw fractions obtained by sonicating pasteurised compost were 2.7 and 1.2%, respectively.

3.2 DTG analysis of the fibre fractions

The mean ADF, NDF and lignin percentages of the three compost samples were 49.0, 53.7, and 20.3, respectively. The weight loss of lignin in the primary peak band was significantly ($P < 0.001$) lowest and this was associated with the highest peak decomposition temperature (357°C) compared with the other two fibre fractions. In the secondary decomposition peak, lignin showed maximum weight loss compared with other fractions but the highest peak decomposition temperature was associated with the ADF fraction (Table 2). The remaining inorganic material left in the crucible at 600°C was calculated as ash. Of the four fibre fractions, lignin contained significantly ($P < 0.001$) the highest proportion of ash.

The profiles of the thermograms were distinctly different in all fibre fractions. In ADF, a sharp primary peak at 305°C was followed by a minor secondary peak at near 510°C (Fig. 1). In contrast, the NDF fraction showed a broad pyrolysis peak at 322°C with shoulders at 250°C and 278°C. Another decomposition peak near 420°C with a shoulder at 410°C was detected, followed by a minor peak at 505°C (Fig. 1). The lignin isolated from mushroom compost exhibited a main decomposition peak at 500°C, which was preceded by a minor peak at 360°C with shoulders near 320°C (Fig. 1). The unextracted compost sample showed two decomposition peaks at 296°C and 485°C with shoulders near 360°C (Fig. 2).

Analysis of humus fraction showed the presence of microbial biomass and phenolic components with their respective peaks at 294°C and 476°C (Fig. 2). The percentage weight losses recorded for these two peaks were 35.3 and 17.3. Analysis of the straw fraction (SF) revealed two decomposition peaks at 329°C with 408°C and weight losses of 66.4% and 4%, respectively, were detected in the two pyrolysis bands. Comparison of the thermograms of the SF (Fig. 2) and uncomposted wheat straw (Fig. 3) revealed that fibre constituents such as cellulose and hemicellulose had been degraded in the SF, as shown by the sharpness of the primary peak and the higher peak decomposition

Table 2
Comparison of weight losses and peak decomposition temperatures of ADF, NDF, lignin fractions and unextracted compost samples by differential thermogravimetry

Sample	WLPP[a]	PT[b]	WLSP[c]	PT	ASH
ADF	55.3	308.4	22.9	510.8	19.3
NDF	62.2	320.6	15.5	428.4	19.5
Lignin	36.3	357.6	30.6	505.3	30.2
Unextracted	53.0	296.3	13.9	491.3	29.2
SEM[d]	0.673	4.32	0.300	2.58	0.717

[a] WLPP weight loss primary peak (200–420°C).
[b] PT peak temperatures of primary or secondary peaks.
[c] WLSP weight loss secondary peak (420–580°C).
[d] All SEMs significant at $P < 0.001$.

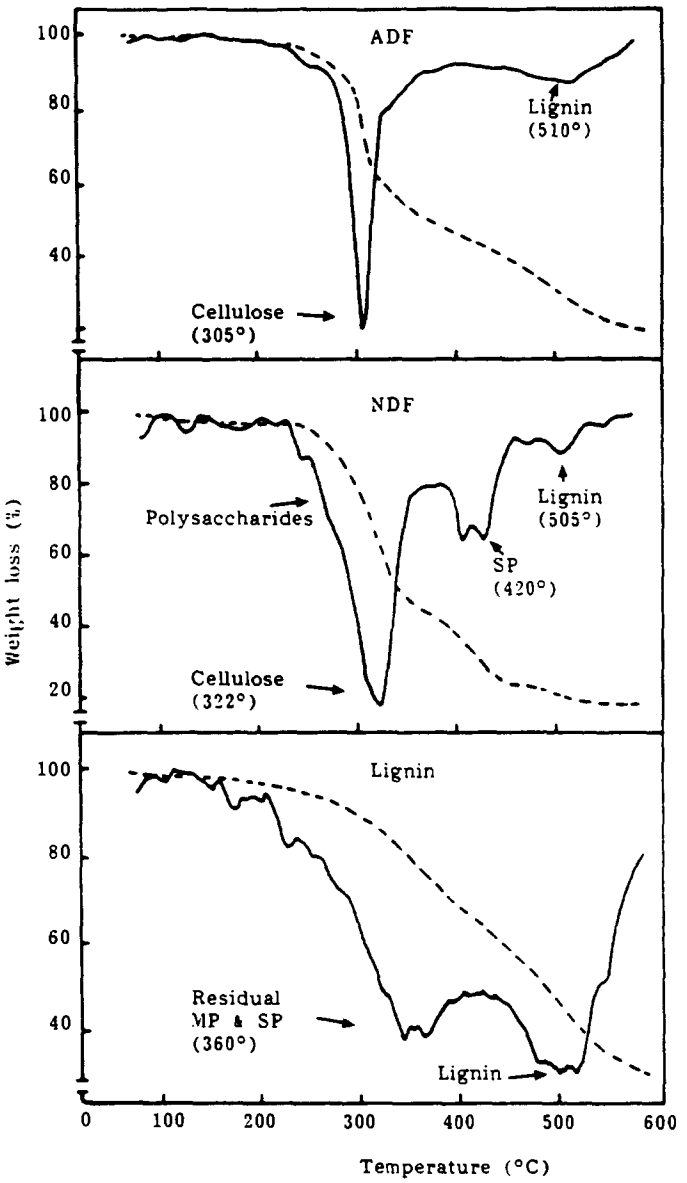

Fig. 1. Thermograms of ADF, NDF and lignin fractions separated from the compost samples showing the TG weight loss (--) and DTG profiles (—) of microbial polysaccharides (MP), structural polysaccharides (SP), cellulose and lignin present in the samples.

temperatures of the other peaks. In addition, the pyrolysis band representing lignin-type compounds in the straw fraction was present as a minor shoulder to the peak at 408°C. In the uncomposted straw, percentage weight losses for each peak of 78.2 and 8.5% were detected in the two pyrolysis bands.

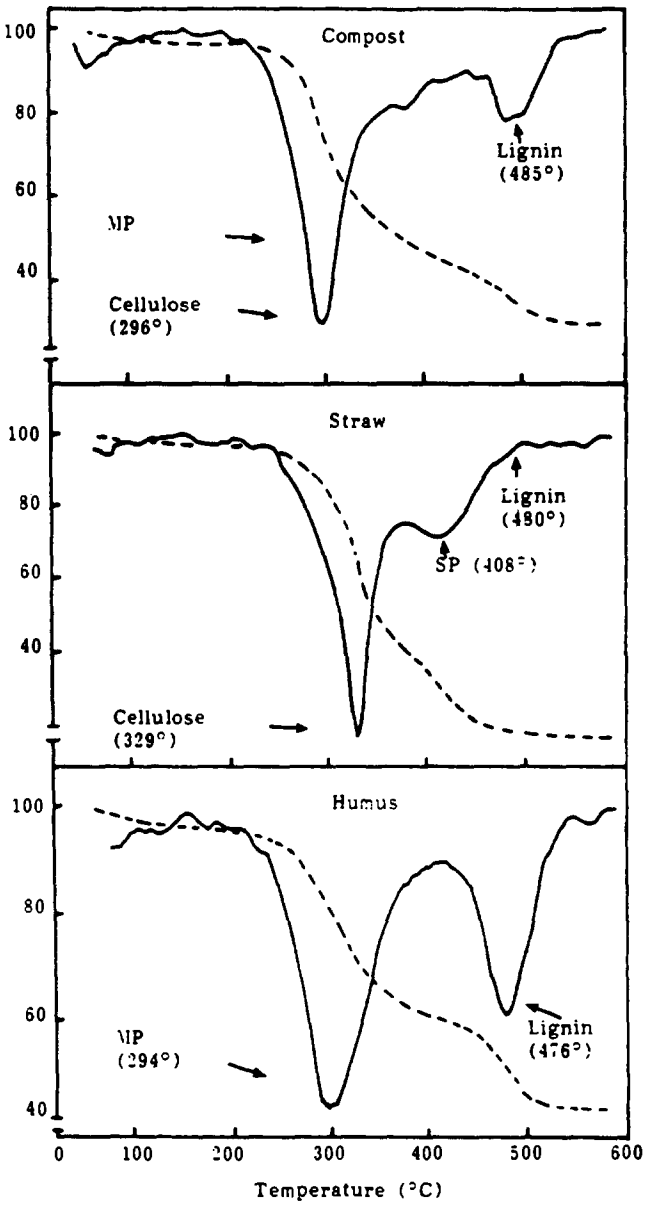

Fig. 2. Thermograms of compost and its fractions humus and straw obtained by ultrasonic treatment showing the TG weight loss (--) and DTG profiles (—) of microbial polysaccharides (MP), structural polysaccharides (SP), cellulose and lignin present in the samples.

3.3. *DTG of reference samples*

Assessment of the decomposition peaks of ADF, NDF and lignin can only be carried out after analysing the constituents, such as cellulose, xylan, lignin, microbial polysac-

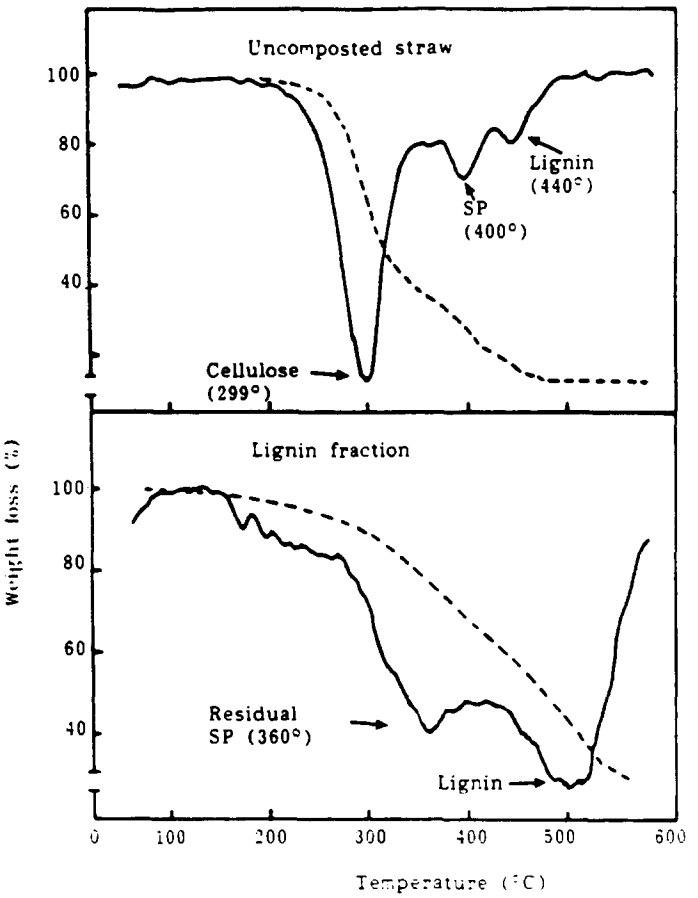

Fig. 3. Thermograms of uncomposted wheat straw and lignin separated from the straw showing the TG weight loss (--) and DTG profiles (—) of structural polysaccharides (SP), cellulose and lignin present in the samples.

charides and straw. Cellulose constitutes more than 30% of the dry weight of mushroom compost and its pyrolysis was associated with one major peak at 331°C (Table 3). The primary decomposition product of cellulose is reported to be levoglucosan [10] and with peak decomposition at 278°C. The xylan fraction representing non-structural hemicellulose present in compost pyrolysed at 282°C (Table 3) and structural hemicellulose, which exists in close association with lignin decomposed near 410°C [1].

Lignin contributes 19–25% to the dry weight of pasteurised composts (Sharma, unpublished data). Two reference samples of lignin prepared by different methods were compared. Indulin (alkali lignin) exhibited one major pyrosis peak at 436°C, while decomposition of lignosulphonic acid was associated with a major peak at near 450°C. A shoulder at ca 350°C to the main lignin peak could be due to residual polysacchar-

Table 3
DTG analysis of reference samples representing various components of mushroom compost

Sample	Temperature of active pyrolysis/°C			Volatilization/% at temperature (/°C):									
	Initial	Max.	Final	150	200	250	300	350	400	450	500	550	600
Alpha cellulose	240	331	360	2	12	14	20	75	80	85	96	97	97
Levoglucosan	160	278	360	–	8	25	86	99	–	–	–	–	–
Indulin AT	340	436	500	1	8	12	15	20	35	80	93	93	93
Lignosulphonic acid	416	450	600	8	8	13	16	30	42	58	68	75	75
Straw lignin	410	500	600	4	5	7	12	20	32	42	57	68	75
Xylan	243	282	305	5	6	13	62	73	85	89	91	91	91
Na polypectate	200	254	290	6	15	26	48	55	56	58	59	60	80
Microbial polysaccharides													
L-3012	200	278	320	2	5	8	48	60	66	71	71	74	74
L-3023	180	242	320	3	8	27	48	55	59	62	65	70	73
Pullulan	280	324	380	3	5	7	30	71	80	87	90	92	92
Chitin	200	320	360	2	4	10	20	58	67	70	75	85	91
Chitosan	220	303	400	5	4	7	25	47	55	57	67	75	83

ides. Analyses of a number of microbial polysaccharides have shown that pyrolysis of these cell-wall materials takes place between 240–320°C. Lignin fractionated from wheat straw was compared with the other reference samples (Table 3). The pyrolysis peaks of the straw lignin observed near 380 and 500°C were similar to those of compost lignin. Furthermore, the percentage weight loss in the 220–420°C decomposition band for the compost lignin was 36.3 compared with 29% in straw lignin. The higher weight loss in the former could be due to the presence of residual microbial polysaccharides (Fig. 3).

4. Discussion

During phase I and II of composting, recalcitrant materials, such as phenolics and microbial polymers, are concentrated where water solubles are mineralised [12,15]. Furthermore, polymerisation may occur by crosslinking nitrogen in ammonia to phenolic compounds, which is catalysed by Mn and Fe, producing quinones under fermentation heat. In addition, the functional groups of microbial cell-walls may be mediated by the basic pH of the compost thereby allowing these to react with nitrogen to form N-glucosamine or a nitrogen–lignin–humus complex [16–18].

The bacterial cells on the straw cuticle or parenchyma cells appear to be anchored by polysacharide fibres produced by microorganisms [1]. This could restrict the movement of bacterial cells to other straw fragments during compost mixing. Consequently, isolation of pure lignin from compost could be more difficult than from undecomposed plant material.

During acid digestion of compost for lignin determination, not all non-lignin constituents were removed as detected in a minor shoulder/peak before the main pyrolysis at 500°C. Thermograms of a number of reference compounds, such as xylan, microbial polysaccharides and lignin suggest that the minor shoulder/peak might be either microbial or plant polysaccharides. A similar shoulder was also detected in thermograms of lignin fractionated from undegraded wheat straw. Comparisons of the thermograms of ADF and NDF suggest that only cellulose and lignin are present in the former fraction. In the later, hemicellulose was observed as a shoulder to the main cellulose peak and possibly as a minor pyrolysis peak at near 410°C. This confirms that the acid detergent procedure is effective at removing hemicellulose.

Analyses of the phenolic fraction of compost confirmed that it is rich in N [14,19] and inorganic components. A distinct peak at 440°C for phenolic or lignin-type compounds was observed in the uncomposted straw. In contrast, its presence in the SF consisted of a shoulder at 480°C to the structural hemicellulose peak at 408°C. This suggests that lignin in wheat straw may have been depolymerised during fermentation. The resulting low molecular aromatic compounds diffused out of the straw to complex with carbohydrates and microbial cell-walls deposited on the surface during composting.

Since aromatic content of humus is high [2], much of the water-soluble polyphenols must have originated from chicken litter and new straw, which are rich in phenolics [1]. However, a previous report [14] suggests that phenolic compounds in humus are of

microbial origin rather than the result of straw breakdown. Wain [14] reported that humus contained 40% carbohydrate, 12% protein and 4% phenolics. Although the results of DTG and EA analyses of the compost humus fraction are not directly comparable with those in their report [14], broadly similar proportions of these three constituents were found.

A knowledge of the nutritive value of the polysaccharide and lignin fractions in mushroom compost is of importance for assessing compost quality [15,20]. Furthermore a comparison of the proportions of CHN, microbial polysaccharides and aromatic compounds in a range of composts may provide information concerning selectivity for *Agaricus bisporus* nutrition.

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