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A PRACTICAL ENZYMATIC METHOD TO ESTIMATE WHEAT STRAW QUALITY AS RAW MATERIAL FOR MUSHROOM CULTIVATION

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Abstract

Wheat straw is the principal raw material for edible mushroom substrate preparation in Europe and America. The knowledge of its biodegradability by saprotrophic microorganisms is useful. By combining an enzymatic method with simple chemical analyses 29 samples of straw were distinguished in a principal component analysis. The effects of genetical variations, of cultivation practices and of sites of cultivation were observed. The measurement of the in vitro *degradability of polysaccharides was the most discriminative variate with the two first principal components. Correlations between the measurements were determined with 52 straw samples and a practical simplified method was proposed. It includes ash content, water soluble compounds and the* in vitro *degradability of polysaccharides measured with a mixture of polysaccharidases. The significance of these measurements was investigated and discussed.*

Key words: Wheat straw, biodegradability, cellulases, mushroom.

INTRODUCTION

Raw materials for edible mushroom substrate preparation are agricultural lignocellulosic by-products, mostly cereal straws. A change in the quality of straw is often considered the main reason for various problems during composting for cultivation of *Agaricus bisporus* (Lange) Sing. and for variations in yields of *Pleurotus* species or *Lentinula edodes* (Ber.) Pegler which are traditionally cultivated on straw-based logs in Europe. In cereal production interest is centred mainly on grain quantity and quality but straw value has received less attention. It is, however, inevitable that the improvement of varieties and changes in the cultivation techniques have consequences on straw quality which can change within years. The consequences of variations in straw quality are documented in the case of the use of straw for cattle feed, but changes in straw value as a substrate for mushroom cultivation have received little attention. The effects of growth retardent and fungicide applications on wheat straw chemical composition and the consequences on straw biodegradation by pure cultures of edible fungi or natural microbial communities were investigated with a limited number of straw samples (Savoie *et al.*, 1992; Savoie *et al.*, 1994; Savoie *et al.*, 1995a). During these tests means to estimate straw quality with the specific objective of its use for edible mushroom production were investigated. For feeding values, chemical analyses and *in vitro* and *in vivo* digestibility tests were used. The aim of the present investigation was to define a practical method based on an *in vitro* test for the measurement of straw biodegradability. The efficiency and the significance of this method are discussed.

METHODS

Samples of wheat straw harvested in 1993 were collected from straw suppliers and identified as having different origins or were collected in experimental plots in randomized block designs. For 29 samples among 52 analyzed, the cultivar of wheat and the site of cultivation were identified. Straw was oven-dried for 18 h at 80°C and ground through a 0·5 mm sieve. The ash content was determined after combustion at 550°C for 3 h. Neutral detergent fibre (NDF) content was determined by using a forage-fibre analysis method (Van Soest & Wine, 1967). Hot ethanol-soluble (HES) dry matter was obtained by two successive extractions with 95% ethanol and 70% ethanol at 85°C under reflux (Savoie *et al.*, 1994). The weight lost by the straw was defined as HES component. Before estimation of water-soluble (WS) dry matter, straw was sieved to give particle sizes between 0·1 and 0·2 mm. Sieved straw (0·5 g)

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was placed in a 100 ml flask with 50 ml of water containing 100 mg Thimerozal/l as a protectant. The flask was rotated end-over-end at 45 r.p.m. for 2 h at room temperature. After centrifugation at 12000 **g** for 15 min, the supernatant was removed and another 2 h water extraction was carried out. After extraction the straw residue was collected on a pre-weighed sintered glass filter and rinsed with 150 ml of water. After drying at 80°C for 24 h, the weight lost was calculated to determine the WS component. This water-extracted straw was used to estimate the *in vitro* degradability of cell-wall polysaccharides (PD) by enzymatic hydrolysis. One litre of enzyme solution in 0·1 M acetate pH 4·8 with 100 mg Thimerozal/l contained 123 IU of xylanase activity (EC. 3·2·1·8) and 51 IU of cellulase activity (EC. 3·2·1·4) at 45°C, pH 5·0. The mixture of enzymes was from N.P.P. (Pau, France). Incubation of 20 mg of water-extracted straw with 5 ml of enzyme solution was performed at 40°C for 24 h. After centrifugation at 19500 **g** for 15 min, 4°C, the supernatant was assayed for soluble sugars as anthrone-reactive-carbohydrates (Seaman *et al.*, 1963). The weight of WS in the initial straw was used to calculate the weight of non-extracted straw corresponding to the 20 mg of extracted straw. The results were expressed as mg equivalent glucose per g of initial straw. For each measurement, five replicates per straw samples were analyzed.

The consequences of the enzymatic hydrolysis during PD measurements were identified by incubating 150 mg of water-extracted straw with 25 ml of the enzymatic solution. Periodically, 3 ml of supernatant were taken off, centrifuged at 19500 **g**, 4°C, for 15 min and assayed for soluble sugars and cellulase activities. Soluble sugars were assayed both as anthrone-reactive-carbohydrates and as reducing sugars (Miller, 1959). Cellulase activities were assayed as FPases (Mandels *et al.*, 1976). One FP U/ml was one μmole of glucose released from 50 mg of Whatman No. 1 filter paper in 1 ml of supernatant in 1 h. The incubations and the measurements were triplicated.

The sugars released from the straw by the enzyme solution were identified by thin-layer chromatography (TLC). After incubation of straw in the enzyme solution for 7, 16 and 24 h, the supernatant was collected by centrifugation, concentrated by freeze-drying and stored at −18°C. The samples were diluted into 0·5 ml of water before being spotted on silica-gel plates. The plates were developed twice in an ascending manner for 1 h 30 min with a 15 min drying time between developments. The solvent was isopropanol–deionized water–ethyl acetate (6·5:3·5:10; Brown & Andersson, 1971). After development the plates were sprayed with a detection reagent (100 mg of naphthoresorcinol + 10 ml of ethanol + 1 ml of phosphoric acid) and dried for 15 min at 120°C. Standards were used to identify the sugars.

Means of the replicates of measurements for each straw sample were used to characterize straw quality with a principal component analysis. Linear regression analysis by the least squares method was used to determine correlations between the parameters measured.

RESULTS

Variability in straw quality

A principal component analysis indicated the overall relationships between the five parameters estimating straw biodegradability for 29 samples identified by the cultivar and the site of cultivation. The samples were coded with a capital letter defining the cultivar, a number defining a geographic area in France with homogeneous climatic conditions and a letter to identify different sites of cultivation in the same geographic area (Table 1). On the co-variance matrix calculated with the standardized data (Table 2), the first component which explained 39% of the total variation opposed ash and PD to NDF and WS. Opposition between HES and PD defined the second component accounting for 26% of the total variation. The samples from area 3 came from different blocks in the same experimental plots and were harvested at the same time. The cultivars were more separated by component 2 than by the first component (Fig. 1). The difference between $S3^+$ and $S3^-$ was due to the absence of fungicide treatment during growth of $S3^-$. The samples from area 4 are examples of the differences due to the site of cultivation, which corresponded to differences in soil quality and in cultivation practices. There were great differences between these samples but most of them were characterized by high values for PD. For the cultivar Soisson (S) the differences between samples

Table 1. Definition of the straw samples used for the principal-component analysis

Cultivars	A=Artaban, B=Baroudeur, D=Delph, E=Eureka, F=Festival, G=Goelent, H=Hardi, I=Ixas, Pa=Pactol, P=Pistou, R=Rossini, S=Soisson, Ta=Talent, T=Théséee
Geographical area in France[a]	1=Bourgogne (east) 2=Poitou + Aquitaine (west) 3=Midi-Peñeés (south-west) 4=Centre

[a] Number plus letter defining particular collection area in main area (see text).

Table 2. Co-variance matrix of a principal-component analysis obtained with the measurements on 29 straw samples characterized for the cultivar and origin

	Principal component 1	Principal component 2
Ash[a]	−0·77	0·38
NDF	0·66	−0·47
HES	0·18	0·90
WS	0·61	0·06
PD	−0·71	−0·58

Correlation coefficients (r).
[a] See text for abbreviations.

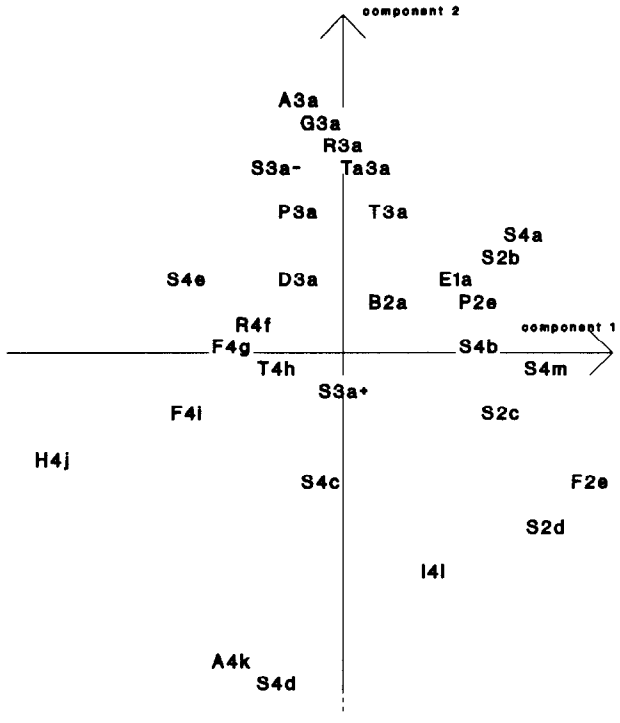

Fig. 1. Discrimination of 29 straw samples by the two first components of a principal component analysis obtained with five parameters estimating straw biodegradability.

cultivated in the same geographic areas but in different plots were as high as differences between samples cultivated in different geographic areas.

Correlation between the measurements

Correlations between the five parameters estimating straw biodegradability were calculated with the data of analyses from 52 different straw samples (Table 3). The strong relationships between NDF and ash, and between HES and WS, and the absence of significant correlation between PD and the other parameters suggested that the major part of the information on straw quality could be obtained in a simplified method by measuring only ash, WS and PD. The significant relationships between HES and ash or NDF are in agreement with the elimination of this parameter in the simplified method.

Table 3. Relationships between measurements of straw composition for 52 different straw samples

	Ash	NFD	HES	WS	PD
Ash	1				
NDF	**−0·81**	1			
HES	**0·40**	**−0·58**	1		
WS	0·09	−0·39	**0·50**	1	
PD	0·24	−0·08	−0·35	−0·29	1

Correlation coefficients (r). Bold figures are r significantly different from 0 at $P<0·01$.

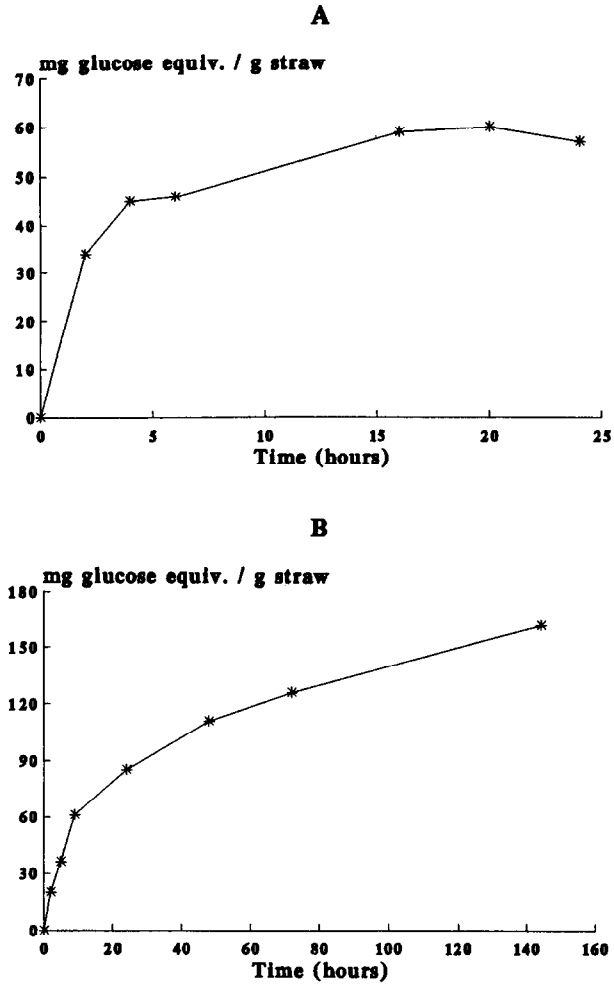

Fig. 2. Release of water-soluble sugars from straw in the presence of a mixture of polysaccharidases: (A) anthrone-reactive sugars; (B) reducing sugars measured with the DNS reagent.

Significance of the PD measurement

Anthrone-reactive-carbohydrates represent the hexoses from mono- and oligo-saccharides but not the pentoses, whereas monomers of both hexose and pentose polysaccharides are measured as reducing sugars with the DNS-reagent.

A plateau of release of anthrone-reactive-carbohydrates from straw by action of polysaccharidases was obtained after 16 h of incubation, whereas the release of reducing sugars still increased significantly after several days (Fig. 2). The results of TLC indi-

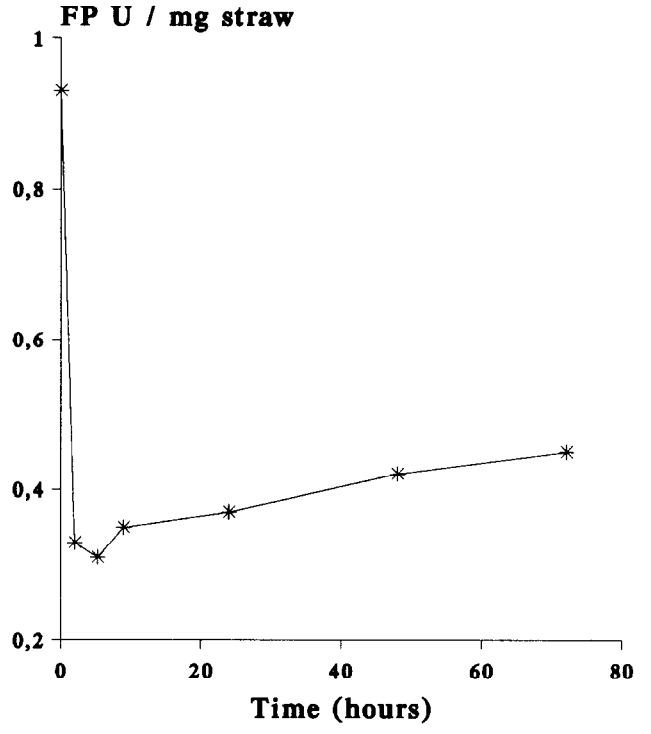

Fig. 3. Free cellulase activity not adsorbed on straw during incubation of straw with a mixture of polysaccharidases.

cated that after 24 h of hydrolysis the most important sugar released was glucose, whereas xylose seemed to be present in smaller quantities and only traces of cellobiose and cellodextrins were observed (results not shown). After 7 or 16 h of hydrolysis no monosaccharide was observed, but cellobiose and cellotrilose were identified. FP U in the filtrates decreased rapidly at the beginning of the contact with straw and then increased slowly (Fig. 3).

DISCUSSION

Conventional analyses of straw used for mushroom production include ash, total nitrogen, cellulose, hemicellulose and lignin contents. The information given by these analyses is difficult to use. Lignin is generally considered to be the major biodegradation obstacle, but the findings obtained with wood are rarely valid with cereal straw. Either no or significantly positive correlations between lignin content and biological or enzymatic degradation of straw have been observed by several authors (Sharma *et al.*, 1986; Miron & Ben-Ghedalia, 1992; Savoie *et al.*, 1992; Savoie *et al.*, 1994). In fact the chemical nature of lignins and how they are linked with other cell wall polymers is as important as the total amount of lignin present (Cornu *et al.*, 1994). Only analysis by multiple-instrumental-techniques, such as ^{13}C NMR procedures, enables a good evaluation of plant-residue quality and degradation, but they need expensive apparatus and are not practical for routine analysis (Reeves & Schmidt, 1994). Estimations of the degradability of straw cell-wall polysaccharides by enzymatic methods could be performed by most of the agronomical laboratories without new investments. By combining an enzymatic method with simple chemical analyses we were able to distinguish 29 samples of straw in a principal-component analysis. Effects of genetical variations, of cultivation practices and of sites of cultivation were also observed.

The measure of the *in vitro* degradability of polysaccharides (PD) was among the most discriminative variates with the two first principal components. During conventional composting in a commercial farm, about two-thirds of the initial wall polysaccharides were consumed by compost microorganisms and only 17% of the total polysaccharides were used during mushroom production (Iiyama *et al.*, 1994). Cheshire *et al.* (1979) observed that the rates of decomposition of straw polysaccharides in soil were better expressed by a double exponential function than by a single one. PD could be an estimate of the part of the straw tissues which is decomposed at a faster rate than other parts. The consequences of the enzymatic treatment during PD measurement were determined. Most of the available cellulose was converted into glucose within 24–48 h, whereas the hydrolysis of non-glucose polysaccharides was slower. The changes in cellulase activities remaining in the filtrate showed that the decrease in the rate of straw cellulose hydrolysis was not due to non-competitive inhibition (Lee & Fan, 1983). The progressive increase of soluble cellulases after adsorption onto the straw was probably the result of a decrease in the number of adsorption sites following the solubilization of the more available fractions of the cellulose (Lee & Fan, 1982; 1983).

During composting of 12 different wheat straw samples it was observed that the overall degradation of fibres and the rapid development of microbial activities during the first days of the process were higher when the PD of initial straw was higher (Savoie *et al.*, 1995a). A high production of cellulases after two days of composting was correlated with high quantities of soluble sugars, whereas when cellulose was less degradable the maximum of cellulase activities was observed later in the process than when PD was high (Savoie *et al.*, 1995a). Otherwise significant positive correlations between PD and *in situ* degradation of NDF by *P. ostreatus* or *L. edodes* were observed (Savoie *et al.*, 1994). PD appears then as an efficient means to characterize the biodegradability of straw but it was also useful to follow the changes in plant cell-wall polysaccharides during mushroom (*A. bisporus*) composting. After 5 days of environmentally controlled composting of wheat straw plus poultry manure, PD measures were 7–14% of the value at the beginning of the process (Savoie & Libmond, 1994), whereas some increases in PD could be observed during high-temperature

phases (Savoie *et al.*, 1995b). The low value of PD after the first days of the process should correspond to the slow rate of cellulose decomposition observed by Cheshire *et al.* (1979) corresponding to the less available part of the straw polysaccharides.

PD was the principal variate of the methodology used in the present work but the other variates were also responsible for the differences between the straw samples.

Hot ethanol solubility was shown to provide an estimate of plant residue degradability in forest soils (Spalding, 1979). The HES fraction consists largely of available components, including lipids and waxes, whereas WS compounds correspond to the readily available soluble C pool described by Reinertsen *et al.* (1984). However, the strong positive correlation between HES and WS permits the use of only WS in a simplified method to estimate straw biodegradability. The weight of WS can be used directly, but it is also possible to determine specific components, such as sugars in water extracts. With the 52 samples analyzed the mean of NDF content was 0·80 g g^{-1}, whereas the mean of the ash contents was 0·08 g g^{-1}. NDF estimates the quantity of straw cell wall, which, in turn, represents about 87% of the total organic matter in straw. Thus, strong negative relationships between ash and NDF contents result. NDF measurement is then not kept in the simplified method. We propose a practical method to estimate straw biodegradability which is based on the measurement of the *in vitro* degradability of polysaccharides by using an enzymatic hydrolysis which is an estimate of C resources which will be relatively available to saprotrophic microorganisms producing polysaccharidases. This information is completed by the quantity of WS, which consists of readily available compounds of importance during the first hours of straw biodecomposition, and by the ash content, which estimates the overall organic resources in the straw. Some specific measurements, such as total N or total P, which could be useful to define the quantities of additives necessary to obtain a balanced medium, may be added to the measurements of straw biodegradability.

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