

Influence of wheat cultivars on straw quality and *Pleurotus ostreatus* cultivation

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Abstract

The main raw material for *Pleurotus ostreatus* (oyster mushroom) cultivation is wheat straw. Estimation of straw biodegradability from 15 different spring wheat cultivars under irrigation in South Africa was determined using linear discriminant analysis to discriminate or group the 15 cultivars by combining chemical analysis and in vitro enzymatic hydrolysis. Significant differences ($P < 0.01$) were found between ash, nitrogen, reducing sugars, anthrone reactive-carbohydrates, water-soluble dry matter, and oyster mushroom yields. The significance of these measurements was investigated and discussed. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Wheat (*Triticum aestivum* L.) production under irrigation in the summer rainfall region in South Africa entails the production of spring wheat types planted in the late fall and winter (Small Grain Institute, 1997). Crops like these generate a considerable amount of wheat straw every year. This is an enormous under-utilised energy resource, but of great potential as feed for ruminants, and as raw materials for the cultivation of edible mushrooms (Zadrazil et al., 1996). The mushroom industry is probably the only significant one, which converts straw directly into a protein source for humans. Sources of variability in straw could include cereal variety, several factors related to cultural practice, the weather, factors related to harvesting such as the method of harvesting, moisture content, storage conditions and crop maturity (Savoie et al., 1992, 1994; Chalaux et al., 1995; Flegg, 1997). However, little research has been done on the consequences of variability in straw quality on mushroom cultivation, and correlation between measurements estimating the straw quality and its ability to support mushroom production have not been determined (Savoie et al., 1994).

Straw consists of the aboveground fractions of cereal plants after removal of the grains. Wheat straw fractions are characterised by the predominance of lignocellulose cell wall material (fibre) with cellulose, hemicellulose (structural polysaccharides) and lignin as the main components (Theander and Åman, 1984).

A great deal of plant breeding research is underway in the different Agricultural Research Institutes of South Africa to develop high-yielding wheat cultivars, though straw quality is not included as a selection parameter. Habib et al. (1995) found no relationship between grain yield and in vitro dry matter digestibility (IVDMD) of the straw but rather a genotypic dependency, which suggested that it should be possible to select wheat varieties that produce high quality straw without sacrificing grain yield. These observations demonstrate that wheat varieties can be identified which combine the desirable characteristics of high grain yield and superior straw quality.

It was evident that: (1) straw as a by-product received little interest as far as its quality is concerned; (2) mushroom cultivation utilises straw, therefore it is important to be able to determine straw quality and (3) among the factors responsible for variation in straw quality the differences between cultivars seem to be an important factor. As a consequence of these observations the aim of this study was to investigate whether

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biochemical measurements on straw could be used to determine the correlation between straw quality and its use as a substrate for *P. ostreatus* cultivation.

2. Methods

2.1. Wheat straw

The straw used was from selected spring wheat cultivars; Inia, Kariga, Marico, Palmiet, SST 38, SST 55, SST 57, SST 65, SST 822, SST 825, SST 875, SST 876, T 4, 95-46 and 96 PT/9. The straw was gathered at harvest during the November 1997 season from experimental plots of the Small Grain Institute, under irrigation and situated in the warmer northern summer rainfall region of South Africa. All cultivars were grown in the same field. For each cultivar four replicates of six rows (5.1 m²) each were planted in a randomised block design and received identical treatments of nitrogen (120 kg ha⁻¹) and phosphate (200 kg ha⁻¹) fertilisation, and irrigation. Each replicate from the different cultivars was harvested individually when the genotypes were considered physiologically mature judged from their colour and brittleness. At harvesting the four replicates per cultivar were harvested manually using sickles. The grain and straw from each replicate were separated with a threshing machine. Air dried straw samples of each replicate were individually chopped into smaller pieces with a garden compost shredder, and subsamples of each replicate were ground with a mini-hammermill, and stored separately in sample bottles at room temperature.

2.2. Chemical analysis

The wheat straw from each replicate was ground to a powder, sieved through a 0.5 mm sieve, and oven-dried for 18 h at 40°C. The ash content was determined by placing a known mass of ground straw in a muffle furnace at 550°C for 3 h and weighing the residue. An analysis laboratory (Sylvan Africa) determined the total nitrogen content of straw by the Kjeldahl method.

2.3. Enzymatic hydrolysis/saccharification of wheat straw

All chemicals were of the highest grade and were obtained from Sigma-Aldrich SA, South Africa. Estimation of water-soluble dry matter (WS) was done according to a modified method of Chalaux et al. (1995). The sieved straw (0.5 g) was placed in a 50 ml centrifuge tube with 40 ml of water containing 100 mg Thimerosal l⁻¹ as a preservative. The tube was rotated end-over-end at 100 rpm on an orbital shaker for 2 h at room temperature. After centrifugation at 12 000 g for 15 min, the supernatant was removed and another 2 h water extraction was carried out. The straw residue was collected

on a pre-weighed borosilicate glass microfibre filter and rinsed with 150 ml distilled water. The glass microfibre filter and WS residue was dried at 80°C for 24 h and the weight loss was calculated to determine the WS component.

The dried water-extracted straw was used to estimate the in vitro degradability of cell-wall polysaccharides by enzymatic hydrolysis as described by Chalaux et al. (1995). Xylanase, 184 units mg⁻¹ solid (EC.3.2.1.8) and cellulase, 1.1 units mg⁻¹ solid (EC.3.2.1.4) used in this study were commercially derived from *Trichoderma viride* and supplied as a lyophilised powder. Incubation of 40 mg of WS residue with 10 ml of enzyme solution was performed at 38°C for 24 h. As a control, the 10 ml enzyme solution was substituted with 10 ml acetate buffer solution. The solutions were centrifuged within an hour after incubation.

2.4. Sugar analyses

After centrifugation at 19 500 g and 4°C for 15 min, the above supernatants were assayed for soluble carbohydrates resulting from polysaccharide hydrolysis by using two methods (Chalaux et al., 1995). In the first method, all hexoses, but no pentoses, reacted with anthrone and the coloured product was measured at 620 nm (Dische, 1962). In the second method, all hexoses and pentose reducing sugars reacted to give coloured products measured at 530 nm (Miller, 1959).

2.5. Fungal strain

A commercial strain of *Pleurotus ostreatus* (Jacq. ex Fr.) Kummer was used and maintained at 4°C on malt extract agar (MEA) slants with periodic transfers.

2.6. Liquid inoculum medium preparation

A liquid medium was prepared according to the method in Stamets (1993) and inoculated with *P. ostreatus* culture. The liquid inoculum medium was incubated on an orbital shaker at 150 rpm at 25°C for a week until mycelium pellets formed (myceliated fluid).

2.7. Spawn preparation

Grain sorghum (1.6 l) and water (400 ml) were pre-cooked in an autoclave in a 60 µm plastic bag for 30 min at 121°C. When slightly cooled, 50 ml lime (CaCO₃): gypsum (CaSO₄) mixture (1:1) was mixed with the moist sorghum grains. After thorough mixing, an aluminium ring fixed at the bag opening was plugged with non-absorbent cottonwool, sealed with aluminium foil, and the whole autoclaved for 15 min at 121°C. When cool, the sterile sorghum grain mixture was aseptically inoculated with 50 ml liquid inoculum medium. The inocu-

lated sorghum grain bags were incubated at 25°C for 2–3 weeks to complete colonisation of the sorghum grains by the mushroom mycelium (spawn).

2.8. Wheat straw substrate preparation for oyster mushroom cultivation

Wheat straw from the different cultivars (four replicates per cultivar) was chopped into small pieces with a garden compost shredder and pre-wetted in plastic bags. The moist straw (ca 70% of water) was placed in a wire basket in a drum and pasteurised with steam for 2 h at 65–70°C. Grain spawn was mixed (4% rate) into the cooled pasteurised wheat straw substrate and plastic tubes (500 mm diameter) were each filled with 10 kg of spawned substrate. Holes were punched in the bottom and sides of the substrate bags for drainage and aeration. The substrate bags were incubated in the dark in a phytotron at 22–24°C and 90% relative humidity (RH). When the substrate was colonised by the mushroom mycelium (ca 21 days), the bags were exposed to an 8 h photoperiod of 400 lux per day. The air temperature was decreased to 20°C for basidiome initiation. Oyster mushroom pins developed at the holes in the plastic tubes. Mature mushrooms were harvested by twisting off the whole cluster and cutting into individual mushrooms. Oyster mushroom yield (means of four replicates per cultivar) was determined over three flushes and expressed as kg t⁻¹.

2.9. Statistical design and analyses

The Genstat 5 statistical program (Genstat 5 Committee, 1997) was used for all statistical analyses. Analysis

of variance (ANOVA) was performed on each variable separately, as well as canonical variate analysis (CVA).

3. Results and discussion

The cultivars were coded with a number (Table 1). The univariate ANOVAs of the six straw quality measurements (ash, nitrogen, reducing sugars, anthrone reactive-carbohydrates, WS and mushroom yield (Table 1)) indicated highly significant differences ($P < 0.001$, except WS $P < 0.07$) between cultivars (genotypes). However, the objective of this study was to determine which of these quality measurements were the most important in determining differences in straw quality. Thus, CVA or linear discriminate analysis was performed on these six measurements to determine if it was possible to discriminate between the 15 cultivars. The first two components or axes, known as canonical variates CV1 and CV2 accounted for 80.3% of the total variation in the data, of which CV1 alone accounted for 65.1%. A graphical representation of the first two CVs (Fig. 1) indicated the groups or contrasts between the cultivars more clearly. In such multivariate graphs, points close together indicate similarity in response while those further apart are dissimilar. The main contrast in CV1 (x -axis) is between cultivar 3 and the other cultivars. The measurements mainly discriminating between these were reducing sugars, anthrone reactive-carbohydrate and percentage nitrogen (Table 2) as they all had correlation coefficients $r > 0.65$ with the CV1 scores. As these were all positive, cultivar 3 was below average for reducing sugars, anthrone reactive-carbo-

Table 1
Definition of straw samples from fifteen wheat genotypes and means ($n = 4$) of six quality variates

Cultivar no.	Genotype	¹ DNS	¹ ANT	Ash(%)	N(%)	Yield	WS
1	95-46	17.9	10.2	10.1	0.6	246	76.5
2	Palmiet	16.9	6.0	10.5	0.6	177	78.8
3	96 PT/9	13.1	7.4	8.2	0.5	158	81.8
4	SST 65	21.8	13.2	10.0	0.8	147	82.0
5	SST 55	19.3	17.3	10.2	0.7	193	84.3
6	SST 38	40.7	20.3	10.8	1.0	169	89.8
7	T 4	38.6	19.6	10.5	0.9	123	90.8
8	Marico	30.4	11.0	11.1	0.7	167	90.8
9	Kariga	21.9	9.8	11.3	0.9	181	88.5
10	SST 822	23.0	14.1	10.8	1.0	262	96.0
11	SST 825	18.3	9.5	11.7	0.7	217	81.5
12	Inia	23.5	13.16	10.5	1.0	172	88.5
13	SST 876	17.6	10.8	11.0	0.7	200	84.8
14	SST 875	18.8	11.0	10.0	0.8	202	88.5
15	SST 57	18.4	12.2	10.5	0.7	163	94.5

DNS – reducing sugars.

ANT – anthrone-reactive-carbohydrates.

¹DNS & ¹ANT (mg equivalent gluc g⁻¹ initial straw).

N – nitrogen.

Yield – mushroom yield (kg t⁻¹).

WS – water soluble dry matter (mg g⁻¹).

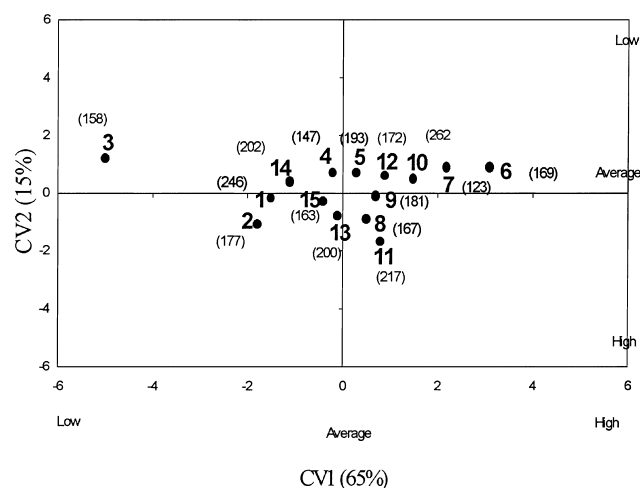


Fig. 1. Discrimination of 15 straw cultivars by the first two components (CV1 and CV2) of a canonical variate analysis (CVA) obtained with reducing sugars, anthrone-reactive-carbohydrates released by enzyme hydrolysis, nitrogen and ash content estimating straw biodegradability for oyster mushroom production. Mushroom yields (kg t^{-1}) in brackets.

Table 2

Correlation coefficients of the most important measurements of straw quality for 60 different straw samples, and their correlations with the first two canonical variates

	¹ DNS	¹ ANT	Ash(%)	N(%)	WS
DNS	1.000				
ANT	0.705 **	1.000			
Ash	0.111	-0.088	1.000		
N	0.510 **	0.506 **	0.300 *	1.000	
WS	0.290 *	0.339 **	0.160	0.581 **	1.000
CV1	0.658	0.656	0.648	0.775	
CV2	0.318	0.651	-0.75	0.317	

Correlation coefficients (r) * = 5% significance, ** = 1% significance.

¹DNS & ¹ANT (mg equivalent gluc g^{-1} initial straw).

DNS – reducing sugars.

ANT – anthrone-reactive-carbohydrates.

N – nitrogen.

WS – water soluble dry matter (mg g^{-1}).

hydrate and percentage nitrogen, while cultivar 6 and 7 were above average and all cultivars close to the zero line were average. Superimposed on Fig. 1 are the mushroom yields in brackets. Thus, it is clear that mushroom yield cannot be predicted from the measurements made in this study as for example, cultivar 10 yielded highest and cultivar 7 which yielded lowest are grouped closely together. CV2, which accounted for only another 15% of the variation, mainly discriminated between cultivars by percentage ash ($r = -0.752$) of which cultivar 11 had the maximum and cultivar 3 the minimum values. Thus this CVA indicated that yield was not predictable from the differences between straw quality of cultivars, also it did not correlate with either of the quality or yield measurements.

According to Koekemoer, ARC-Small Grain Institute (personal communication) differences in biochemical measurements could be due to genetic variation. Green wheat plants store nutrients produced during photosynthesis in the above-ground plant structures. Cultivars with a longer growing period theoretically can store more nutrients than those with a shorter growing period. Nutrients are usually translocated from the plant structures to the wheatears after the pollen has been shed (post anthesis). Environmental (excessive heat or drought) and genetic factors (such as solid stem) could influence the translocation of nutrients. In the cultivars with a solid stem some of the nutrients may be converted into the development of pith tissue. Although cultivars 6 and 7 had higher nutrient values than the other cultivars (Table 1), other factors could have inhibited the availability of the nutrients to the mushroom mycelium. Cultivars 1, 3, 11 and 13 share the same genetic background except that cultivar 11 lacks the solid stem gene. It seems that the solid stem gene in cultivars 1, 3 and 13 had an influence on the nutrient content of these straws. Of all the cultivars, cultivar 3 had the lowest nutrient content, which could have been due to some of the nutrients in the plant being used by the sponge tissue in the development of the solid stem. However, mushroom yield of cultivar 1 was significantly higher than that of cultivar 3 probably due to cultivar 1 having a lower ash content than cultivar 3. The measurement values of the cultivars (2, 4 and 15) usually grown in the winter rainfall region could have been influenced by environmental conditions in the summer rainfall experimental plot. Cultivar 12 has a long straw and could have stored more nutrients before anthesis. The most popular cultivars planted in the summer rainfall irrigation regions are 5, 8, 9, 10 and 11 while cultivars 2, 4 and 15 are winter rainfall cultivars.

When treated with commercial cellulases, release of glucose from straw could be an indication of the availability of the cellulose in the straw for the utilisation by fungal mycelium. During the first phases of growth, the easily digestible soluble carbohydrates are utilised for mycelial growth. According to Platt et al. (1984) it is possible to distinguish two separate phases in the degradation of straw by *P. ostreatus* 'Florida'. Phase one involves the degradation of small water-soluble materials and high activity of cellulase, phenol-oxidases and peroxidase. Phase two is mainly lignin degradation. One can conclude that perhaps in vitro hydrolysis is only indicative of the first phase of straw colonisation.

A good initial colonisation is necessary but not sufficient to ensure a good mushroom yield, which probably could be influenced by other factors in the utilisation of nutrients by mushroom mycelium.

It is possible that geographical, cultural and environmental factors might affect the degradability of wheat straw. Based on the study reported here, one may conclude that there is a need for further research on several

aspects of wheat straw quality such as: development of specific measurements for wheat straw quality, development of standardised methods for in vitro tests, verification of results in laboratory and pilot-scale trials.

It is also unlikely that any one single test could reveal the standard of quality of a batch of raw material, therefore, in vitro experiments on a larger number of samples over a longer period of time would be necessary before any conclusion could be reached on the influence of cultivar on wheat straw quality. It would help mushroom growers considerably if some reliable and readily definable measurements could be available by which suppliers to the industry could operate to evaluate straw quality.

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