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Storage temperature and time and its influence on feed quality of fungal treated wheat straw

Lei Mao [a], Anton S.M. Sonnenberg [b], Jeroen van Arkel [c], John W. Cone [a,*], Ric C. H. de Vos [c], J. Leon M. Marchal [a,d], Wouter H. Hendriks [a]

[a] Animal Nutrition Group, Wageningen University & Research, Wageningen, the Netherlands
[b] Plant Breeding, Wageningen University & Research, Wageningen, the Netherlands
[c] Bioscience, Wageningen University & Research, Wageningen, the Netherlands
[d] DuPont, Leiden, the Netherlands

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ABSTRACT

Degradation of lignocellulose by selective white-rot fungi can significantly improve the nutritional value of high lignocellulose containing biomass by affecting the access of rumen microbes to structural carbohydrates. To determine if such treated biomass is stable in time to allow it to be conserved for subsequent feeding to ruminant animals, wheat straw (WS) pre-treated for 7 weeks with either *Ceriporiopsis subvermispora* or *Lentinula edodes* was stored anaerobically up to 10 weeks at different temperatures (24.7–52.4 °C). Substrates were subsequently analysed for changes in pH, titratable acidity, fibre composition, *in vitro* gas production (IVGP) and colour, as well as polar metabolites by GC–MS and ceriporic acids by LC–MS. The increased titratable acidity of fungal treated WS during storage indicated acidification of the straw. A significant decrease in hemicellulose and an increase in acid detergent lignin content was observed at 52.4 °C. No negative effect of the storage condition on the degradability of both fungal treated WS in rumen fluid was observed. A darker colour was observed for substrates incubated at higher temperatures, coinciding with a strong accumulation of several organic acids and sugars. A decrease in ceriporic acid A, B, C and G produced by *C. subvermispora* was observed when stored at 52.4 °C from week 2–10. The results show that, although the chemical composition changes, anaerobic storage of fungal treated WS at different temperatures does not affect its fermentation potential for ruminants.

1. Introduction

The aerobic treatment of lignocellulosic biomass, such as cereal straws, with white-rot fungi has great potential to increase its feed value for ruminants, as well as the utilization in the production of chemicals and biofuels (Van Kuijk et al., 2015a). White-rot fungi degrade the lignin of the biomass and, thereby, increase the accessibility of the remaining polysaccharides for rumen microbiota. Especially selective white-rot fungi, such as *Ceriporiopsis subvermispora* and *Lentinula edodes*, show a great delignification ability on wheat straw (WS) with a low simultaneous utilization of cellulose (Tuyen et al., 2012). These fungi can cleave the lignocellulose linkages and degrade lignin in the biomass by secreting oxidative enzymes and diverse low molecular mass compounds (Ohashi et al.,

* Corresponding author at: PO Box 338, 6700 AH, Wageningen, the Netherlands.
E-mail address: john.cone@wur.nl (J.W. Cone).

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2011; Pollegioni et al., 2015). During colonization, fungal hyphae mainly accumulate in the lumen of plant cells where enzymes (*e.g.* manganese peroxidases and laccases) are secreted to degrade the cell wall. However, the plant cell walls are too dense to allow the penetration of the relative large enzymes. Low molecular-weight metabolites (*e.g.* alkoxy- and carbon-centered radicals) formed in the process of degradation by *Ceriporiopsis subvermispora* and *Lentinula edodes* can diffuse into the plant cell walls and help breaking down the lignin (Blanchette et al., 1997; Ohashi et al., 2011).

While the enzymes and metabolites produced by the white-rot fungi decompose lignin, released carbohydrates from the biomass are utilised as a carbon and energy source to meet the requirements for growth and metabolic activity; hence a longer fungal incubation shows higher losses of dry matter and carbohydrates (Van Kuijk et al., 2015b; Nayan et al., 2017). As such, the timing of terminating the fungal activity is essential to optimise the nutritional value of the biomass. Several studies have shown that substrates are acidified during the aerobic culture with *C. subvermispora* and *L. edodes* (Mäkelä et al., 2002; Kwak et al., 2016; Mao et al., 2018). Wheat straw treated with *C. subvermispora* and *L. edodes* can thus be stored under anaerobic conditions at 20 °C for up to 64 days, without the use of additives (Mao et al., 2018). The aerobic fermentation by fungi can be performed year-round. As large amounts of high lignocellulosic biomass are often generated at specific harvest times (*e.g.* rice straw), the subsequently produced fungal treated biomass must be able to be stored and retain its nutritional quality under variable climatic conditions. Although fungi can at best only slowly degrade cell wall components during anaerobic storage, some fermentation activity still occurs for yet unknown reasons (Yang et al., 2001; Mao et al., 2018). This fermentation activity in turn might also be affected by storage temperature, and can affect the acceptance of the treated WS by ruminant animals. It is therefore important to investigate the effect of storage temperature on the nutritional quality of fungal treated biomass if this biotechnology is to be used in practice.

The present study investigated storage stability of fungal treated WS in the range of 25−50 °C. Changes over time were measured in pH, titratable acidity, fibre composition and fermentability by estimating *in vitro* gas production (IVGP), as well as in straw metabolite composition analysed by gas chromatography-mass spectrometry (GC–MS) and liquid chromatography-mass spectrometry (LC–MS).

2. Materials and methods

2.1. Fungal strains and spawn preparation

The fungi *C. subvermispora* (CBS 347.63; USA) and *L. edodes* (CCBAS389; Czech Republic) were recovered from the liquid nitrogen (N) collection of Plant Breeding (Wageningen University & Research, The Netherlands), and mycelium was grown on malt agar plates containing 10 g L^{-1} malt extract (Oxoid Ltd, Hampshire, UK) and 17.5 g L^{-1} micro agar (Duchefa Biochemie B.V, Haarlem, The Netherlands) at 25 °C for one to two weeks (depending on the fungus) until the agar plate was fully covered. Two agar pieces (~ 1.5 cm^2) were then transferred to plastic boxes (OS60 + OD60; Combiness, Nevele, Belgium) containing approximately 75 g of sterilized sorghum grain and incubated in a climate controlled room at 25 °C. The fully colonized grains were stored at 4 °C for further use.

2.2. Aerobic culture of fungi on wheat straw

Organic wheat (*Triticum aestivum* L.) straw was obtained locally and mechanically (Pierret Industries, Corbion, Belgium) chopped into pieces of 0.5–1.5 cm. The WS was filled in monofilament net bags (50 × 80 cm, pore size: 0.15 × 0.15 cm) and immersed in tap water for three days and then drained for 5 h. The fungal inoculation and incubation with *C. subvermispora* and *L. edodes* was carried out in 4 l plastic containers (TP4000 + TPD4000; Combiness, Nevele, Belgium) containing a filter in the lid allowing gas exchange. Before fungal inoculation, all containers filled with mixed wet WS (dry weight of WS in each container was approximate 160 g) were autoclaved at 121 °C for 1 h and then left overnight in a flow cabinet. Sixteen g of spawn of each fungus was aseptically inoculated on the autoclaved wheat straw (AWS), gently mixed by hand in a flow cabinet and then incubated at 25 °C for 7 weeks in a climate controlled room. The incubated substrate comprised of untreated autoclaved WS (AWS), *C. subvermispora* (CS) treated AWS (CS-AWS) and *L. edodes* (LE) treated AWS (LE-AWS).

2.3. Anaerobic storage

After 7 weeks of aerobic incubation, each box of AWS, CS-AWS and LE-AWS was mixed and transferred into a 0.5 l glass jar, sealed by a locking lid with rubber seal. The substrates in the glass jars were manually compacted as much as possible to press out air. The jars were stored in a climate controlled room with an average temperature of 24.7 °C, and in ovens with an average temperature measured over the incubation period of 35.0, 45.9 and 52.4 °C. Data loggers (LIBERO CB; ELPRO, Buchs, Switzerland) were used to monitor the temperature at 1 h intervals during the storage period. Subsequently, three glass jars for each temperature were opened after 2, 4, 6, 8 and 10 weeks of storage. In case of week 0, substrate was collected directly after the aerobic incubations, without filling glass jars. Approximately 20 % of the content of the glass jars was stored at -20 °C for further analysis of pH and assessing titratable acidity. The remainder of the samples was freeze dried prior to the analysis of the fibre composition, the IVGP and colour measurements.

2.4. Analytical methods

2.4.1. Fibre composition

Freeze dried samples were ground in a hammer mill over a 1 mm sieve (100 AN; Peppink, Olst, The Netherlands). Residual dry matter (DM) content of the freeze dried material was determined after drying in an oven at 103 °C for 4 h (ISO 6496, 1999) and

subsequently ash content was determined after incinerating in a muffle furnace at 550 °C for 3 h (ISO 5984, 2002). The N content was analysed by the Kjeldahl method (ISO 5983, 2005). Fibre analysis was conducted according to the methods described by Van Soest et al. (1991), using an Ankom fiber analyzer (A2000I; ANKOM Technology, Macedon, NY, USA). Neutral detergent fiber (aNDFom) was determined using a heat-stable amylase (thermamyl) and alcalase. The cellulose content was calculated as the difference between acid detergent fiber (ADFom) and acid detergent lignin (Lignin (sa), ADL), and hemicellulose as the difference between aNDFom and ADFom.

For titratable acidity, 30 g of fresh sample was mixed with 270 mL of demineralised water and homogenised using a stomacher (400 Circulator; Seward, Worthing, UK) at 230 rpm for 5 min. The initial pH of the stomacher extract was determined and subsequently the extract was titrated with 0.1 mol L^{-1} NaOH (Titrisol sodium hydroxide solution; Merck, Darmstadt, Germany) to pH 7.0 using a titrando machine (Metrohm, Herisau, Swizerland). Titratable acidity was calculated as mmol NaOH per kg DM substrate required to achieve pH 7.0.

2.4.2. *In vitro gas production*

In vitro gas production was performed according to Cone et al. (1996). In brief, rumen fluid was collected from three lactating rumen fistulated cows, fed *ad libitum* corn silage and grass silage. The strained rumen fluid was filtered through two layers of cheese cloth and mixed with a mineral buffer solution. All procedures were conducted under continuous flushing with CO_2. Approximately 0.5 g of sample was incubated with 60 mL of buffered rumen fluid for 72 h and gas production was recorded automatically. Gas production was corrected for blank gas production (*i.e.* gas production in buffered rumen fluid without sample).

2.4.3. *Colour measurement*

The colour of the freeze dried samples was measured by a colorimeter (CR-200; Minolta, Japan). The colour parameters *L*, *a* and *b* were used to show the colour change in brightness, redness and yellowness with *L* representing the lightness of the sample from 0 (black) to 100 (white), *a* has a negative value with green and a positive value with red, and *b* has a negative value with blue and a positive value with yellow. The instrument was calibrated with a white calibration plate with parameter $L = +97.79$; $a = -0.44$; $b = +2.04$. Overall change in colour (ΔE) of samples was calculated from parameters *L*, *a* and *b* according to the equation:

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \quad (1)$$

where L_0, a_0 and b_0 represent the control values of samples of AWS, CS-AWS and LE-AWS after 0 weeks of storage. The *L*, *a* and *b* parameters in the equation represent the values of the corresponding stored samples.

2.4.4. *Metabolite analysis in fungal treated wheat straw*

The relative contents of polar metabolites in CS-AWS and LE-AWS samples collected at week 0, 2 and 10 at temperatures of 24.7 and 52.4 °C were determined by GC–MS according to the method described by Lisec et al. (2006). Modifications were performed aimed for a better extraction of components from the specific freeze dried materials. In brief, approximately 50 mg (48−52 mg) of ground sample was weighed in a 2 mL Eppendorf tube, to which 1.4 mL of 80 % pre-cooled methanol and 60 μl of ribitol (0.2 mg ml^{-1}, internal standard) was added. The extracts were vortexed for 10 s, and subsequently incubated in a thermomixer (Vortemp 56; Labnet International Inc., Edison, USA) at 950 rpm at 70 °C for 10 min, followed by centrifugation for 10 min at max speed (21,000 × g) in an Eppendorf centrifuge. Afterwards, 500 μl of supernatant was transferred in a new Eppendorf tube and mixed with 375 μl of chloroform and 750 μl of H_2O for 10 s, and the phases were separated by centrifugation at max speed (21,000 × g) in an Eppendorf centrifuge. Subsequently 100 μl of the methanol-water phase was dried in a 180 μL glass insert in a 2 mL vial by vacuum centrifugation. The dried samples were derivatized online using a Combi PAL autosampler (CTC Analytics AG; http://www.ctc.ch) and metabolite analyses were performed as described and adapted by Carreno-Quintero et al. (2012). Two μl of the derivatized samples was analysed by a GC–MS system consisting of an Optic 3 injector (ATAS GL Int., Eindhoven, the Netherlands) and an Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA) coupled to a Pegasus III time-of-flight mass spectrometer (Leco Instruments, Inc., St. Joseph, MI).

Ceriporic acids A, B, C and G were analysed by LC coupled to an LTQ-Orbitrap FTMS hybrid system (in short LC–MS) in CS-AWS samples collected at week 0, 2 and 10 at temperatures of 24.7 and 52.4 °C. The extraction of fungal treated WS was performed using the protocol described by De Vos et al. (2007). In short, approximate 100 mg (97−103 mg) of ground sample was weighed in a 2 mL Eppendorf tube, and extracted with 1.5 mL of 75 % methanol containing 0.1 % formic acid. The extract was vortexed for 10 s and sonicated for 10 min. The mixture was then centrifuged for 10 min in an Eppendorf centrifuge (16,000 × g), an aliquot of the supernatant (180 μl) was transferred to a glass vial with insert for LC–MS analysis. Chromatographic separation, mass detection methods and the LC–MS equipment were as described by Mokochinski et al. (2018). Ceriporic acid A, B and C were identified by matching their observed specific accurate masses and mass spectra with those provided by Amirta et al. (2003) and Van Kuijk et al. (2017), while the mass spectrum of ceriporic acid G was according to Nishimura et al. (2012b).

2.5. *Data processing and statistical analysis*

All measured parameters were evaluated for their main effects of storage temperature and storage time and the interactions of the two factors were analysed by the GLM procedure in SAS 9.3 version. Changes in pH, titratable acidity, IVGP and colour were determined after 0, 2, 4, 6, 8 and 10 weeks of storage. Moreover, the fibre composition was determined in samples stored for 0, 2 and 10 weeks. All biological samples (fungal treatment of WS) were performed in triplicate and significance was declared at $P < 0.05$.

The GC–MS data were processed in an untargeted manner according to the steps described by Mokochinski et al. (2018). In short, Metalign software was used for unbiased mass peak picking and alignment, after which MSClust software was used to assemble all mass signals originating from the same metabolite. The relative intensity of the resulting clusters (putative metabolites) were used in subsequent data analyses. Principal component analysis (PCA) was performed using the software Simca (version 14) after log-transformation and Pareto-scaling of the relative abundance values of the metabolites. Mass spectra of clusters, together with their observed retention index, were used to annotate selected compounds by comparing data obtained with authentic standards and the dedicated Golm EI-spectral database (http://gmd.mpimp-golm.mpg.de).

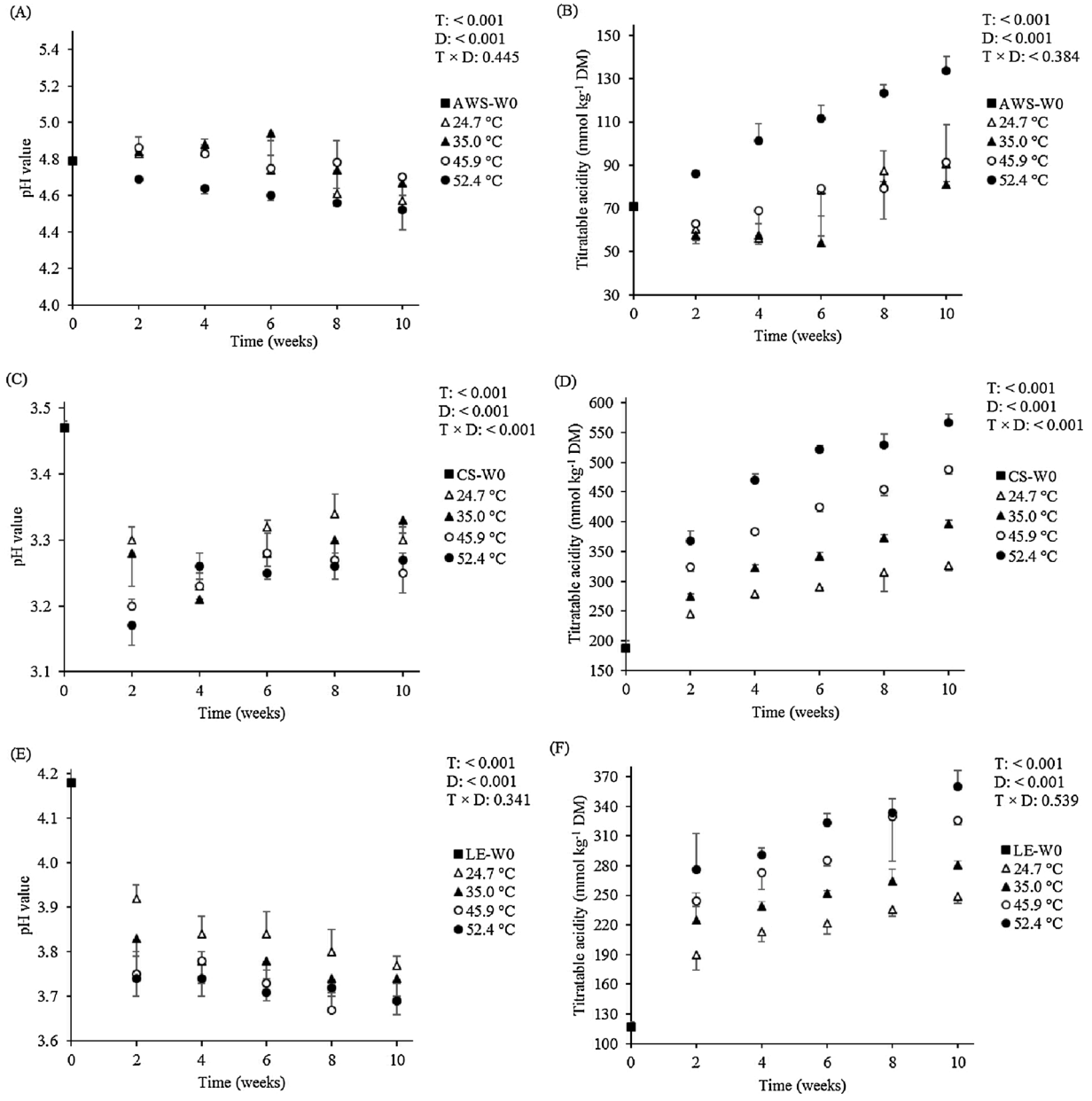

Fig. 1. Changes in pH and titratable acidity of autoclaved wheat straw (AWS) (A and B), *Ceriporiopsis subvermispora* (CS) treated AWS (C and D) or *Lentinula edodes* (LE) treated AWS (E and F) stored anaerobically at different temperatures (T: 24.7, 35.0, 45.9 and 52.4 °C) and duration (D: 2, 4, 6, 8 and 10 weeks). W0 represent the start of anaerobic storage (AWS was aerobically pre-treated without or with CS or LE at 25 °C for 7 weeks). Error bars indicate standard deviations (n = 3).

3. Results

3.1. Change in pH and titratable acidity during anaerobic storage

Changes in pH and titratable acidity (mmol NaOH per kg DM required to reach pH 7.0) of AWS, CS-AWS and LE-AWS stored anaerobically at 24.7, 35.0, 45.9 and 52.4 °C are illustrated in Fig. 1 panels A–F. Minor changes in pH were observed with the storage of AWS (Fig. 1 A). An increasing and higher titratable acidity of AWS was seen when stored at 52.4 °C (Fig. 1 B). The initial pH of the AWS was 3.47 and 4.18 for *C. subvermispora* or *L. edodes*, respectively (Fig. 1 C and E). At all storage temperatures, a reduction in pH value during the first 2 weeks of storage of CS-AWS (3.17–3.30) and LE-AWS (3.74–3.92) was observed. Temperature ($P < 0.001$) and time of incubation ($P < 0.001$) significantly affected the pH of both CS-AWS and LE-AWS. No interaction was found between temperature and time for LE-AWS ($P = 0.341$; Fig. 1E). No obvious decline in pH was observed for CS-AWS from 2 to 10 weeks, while a clear (but small) trend for a decrease in pH was observed for LE-AWS.

CS-AWS showed a higher initial titratable acidity than LE-AWS (187.5 *vs* 116.6 mmol kg^{-1} DM) at week 0 (Fig. 1 panel D and F). A sharp increase in titratable acidity during the first 2 weeks of storage of CS-AWS and LE-AWS was observed at all temperatures and for both there was an effect of temperature and time. An interaction was observed between storage temperature and duration on titratable acidity for CS-AWS but not for LE-AWS ($P = 0.539$).

3.2. Change in fibre composition of fungal treated wheat straw

No interaction between storage temperature and duration was found with ash, N, cellulose and hemicellulose content in AWS (Table 1). For ADL, three of the four temperatures showed an increase. No significant change was found for the ash content in CS-AWS and LE-AWS during the storage at different temperatures (Table 2). The content of cellulose was significantly affected by temperature (CS: $P = 0.015$; LE: $P < 0.001$), while hemicellulose and ADL in both CS-AWS and LE-AWS were affected ($P < 0.05$) by both storage temperature and time. A reduction in hemicellulose content at all temperatures was observed from 2 to 10 weeks of storage, which was significant ($P < 0.05$) at 52.7 °C in CS-AWS, and at 45.9 and 52.4 °C in LE-AWS. The ADL content was affected by temperature and duration of storage, but no interaction was observed in LE-AWS.

3.3. Changes in IVGP with storage

Aerobic incubation for 7 weeks with *C. subvermispora* and *L. edodes* increased the IVGP from 246.2–288.4 mL g^{-1} OM and 221.5 to 244.7 mL g^{-1} OM, respectively (Fig. 2). The IVGP of untreated and fungal treated AWS during 10 weeks of anaerobic storage showed no significant changes.

3.4. Colour change during anaerobic storage at different temperatures

Overall change in colour of AWS and fungal treated AWS stored at different temperatures and for different durations is shown in Fig. 3. ΔE of untreated and treated WS was significantly affected by temperature ($P < 0.001$) and time ($P < 0.05$). A clear increase in ΔE of AWS was seen when stored at 52.4 °C (Fig. 3A). A rapid increase in ΔE value was observed especially in the first 2 weeks and increasing more gradually thereafter to remain relatively constant for CS-AWS and LE-AWS (Fig. 3B and C). There was an interaction between temperature and time for CS- and LE-AWS unlike ($P = 0.279$) AWS.

Table 1
Composition (g kg-1 dry matter) of autoclaved wheat straw stored anaerobically at different temperatures and duration.

Temperature (°C)	Duration (Week)	Ash	Nitrogen	Cellulose	Hemicellulose	ADL
Control	0	38.6	2.51	468.0	288.8	68.2
24.7	2	38.6	2.49	474.2	275.9	66.6^{bc}
	10	38.9	2.43	473.6	272.5	68.8^{abc}
35.0	2	39.0	2.15	470.8	280.6	67.7^{bc}
	10	39.3	1.86	468.1	276.9	65.7^{c}
45.9	2	37.6	1.91	475.3	273.0	68.8^{abc}
	10	37.6	2.00	470.7	273.8	70.9^{ab}
52.4	2	37.1	1.96	474.1	280.8	68.9^{abc}
	10	38.5	1.99	461.6	268.0	73.2^{a}
RMSE		1.22	0.123	5.41	6.37	1.70
P value						
Temperature		0.133	<0.001	0.212	0.483	0.002
Duration		0.339	0.305	0.034	0.084	0.028
Temperature × Duration		0.764	0.108	0.285	0.335	0.037

ADL, acid detergent lignin; RMSE, root mean square error.
[a–e] Values (n=3) with different superscripts within a column are significantly ($P < 0.05$) different.

Table 2
Composition (g kg^{-1} dry matter) of *Ceriporiopsis subvermispora* and *Lentinula edodes* treated autoclaved wheat straw stored anaerobically at different temperatures and duration.

Temperature (°C)	Duration (Week)	*C. subvermispora*					*L. edodes*				
		Ash	Nitrogen	Cellulose	Hemicellulose	ADL	Ash	Nitrogen	Cellulose	Hemicellulose	ADL
Control	0	43.3	3.58	497.6	141.1	26.9	42.3	3.72	507.5	164.9	45.3
24.7	2	42.4	3.50	495.7	135.7^{a}	27.7^{bc}	41.9	3.89^{a}	503.9^{de}	158.2^{ab}	42.7
	10	42.8	3.71	494.2	124.2^{a}	26.7^{c}	39.9	2.51^{b}	504.0^{de}	158.1^{ab}	47.1
35.0	2	43.0	3.56	498.1	135.6^{a}	28.5^{bc}	41.0	2.48^{b}	505.1^{cde}	158.8^{ab}	43.2
	10	43.7	3.66	495.5	133.3^{a}	29.7^{bc}	41.5	2.55^{b}	498.6^{e}	158.4^{ab}	43.5
45.9	2	42.5	3.66	502.3	134.5^{a}	26.4^{c}	41.2	2.47^{b}	508.2^{bcd}	167.6^{a}	46.1
	10	42.6	3.68	501.0	115.3^{a}	31.9^{b}	41.4	2.44^{b}	512.1^{abc}	144.1^{bc}	47.8
52.4	2	42.9	3.64	503.2	128.0^{a}	30.2^{bc}	41.1	3.33^{a}	513.7^{ab}	155.6^{ab}	48.3
	10	43.7	3.56	513.4	80.7^{b}	40.4^{a}	41.2	3.85^{a}	516.5^{a}	135.5^{c}	55.4
RMSE		0.896	0.171	6.69	7.70	1.65	1.25	0.223	2.87	6.36	3.21
P value											
Temperature		0.296	0.885	0.015	<0.001	<0.001	0.939	<0.001	<0.001	0.008	0.002
Duration		0.152	0.384	0.675	<0.001	<0.001	0.555	0.037	0.950	<0.001	0.020
Temperature × Duration		0.880	0.521	0.332	<0.001	<0.001	0.329	<0.001	0.027	0.007	0.300

ADL, acid detergent lignin; RMSE, root mean square error.
$^{a-e}$ Values with different superscripts within a column are significantly ($P < 0.05$) different. n=3.

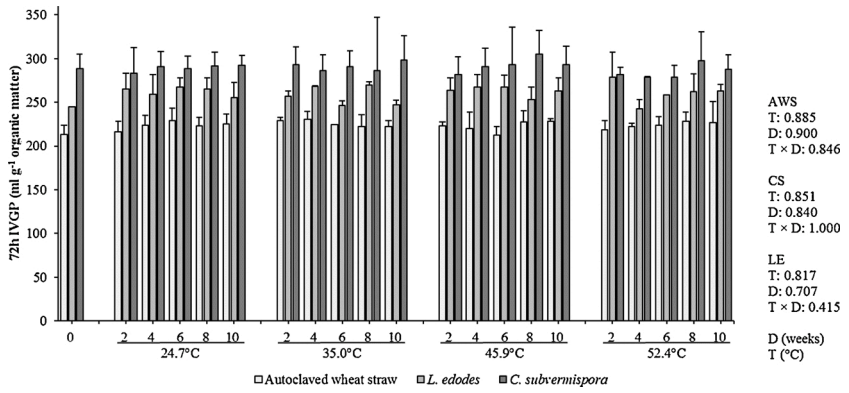

Fig. 2. Cumulative (72 h) *in vitro* gas production (IVGP) of autoclaved wheat straw (AWS), *Ceriporiopsis subvermispora* (CS) or *Lentinula edodes* (LE) treated AWS stored anaerobically at different temperatures (T: 24.7, 35.0, 45.9 and 52.4 °C) and duration (D: 2, 4, 6, 8 and 10 weeks). Week 0 represent the start of anaerobic storage (AWS was aerobically pre-treated without or with CS or LE at 25 °C for 7 weeks). Error bars indicate standard deviations (n = 3).

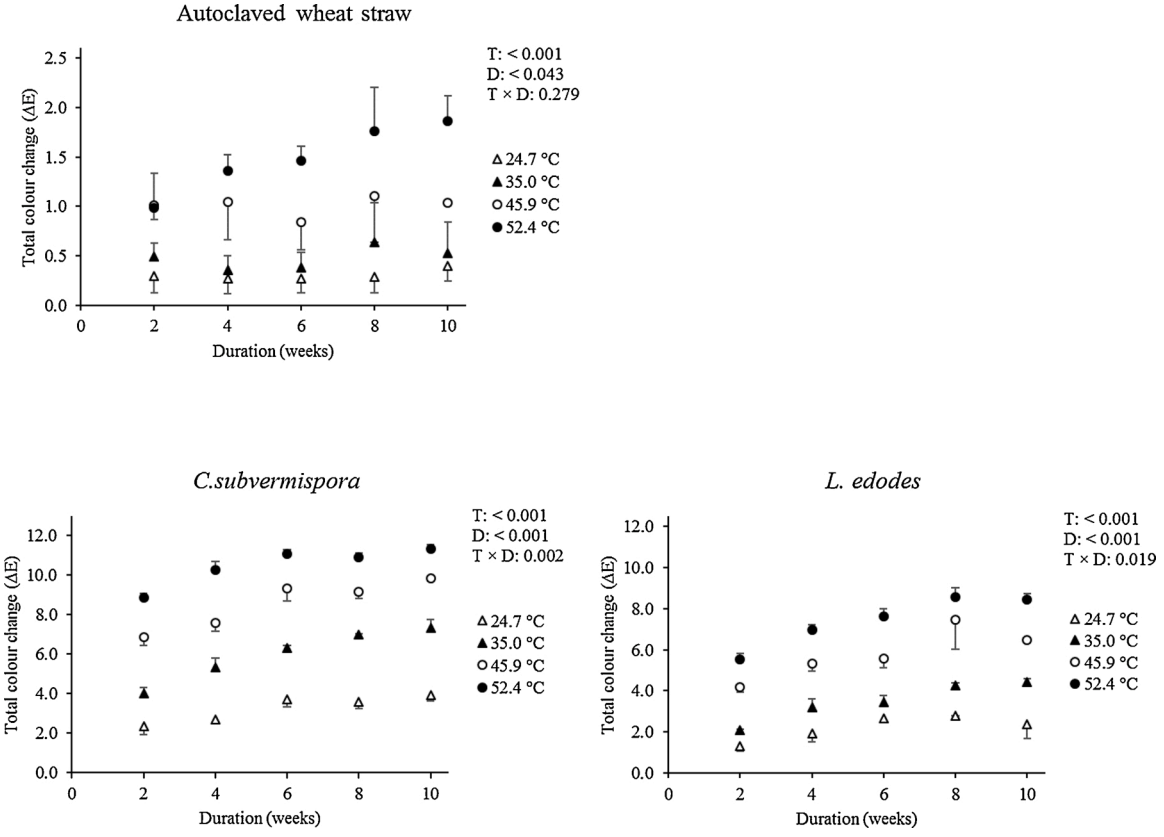

Fig. 3. Changes in colour (ΔE) of autoclaved wheat straw (AWS), *Ceriporiopsis subvermispora* treated or *Lentinula edodes* treated AWS stored anaerobically at different temperatures (T: 24.7, 35.0, 45.9 and 52.4 °C) and duration (D: 2, 4, 6, 8 and 10 weeks). ΔE is the colour difference between stored fungal treated AWS and control, which represent the start of anaerobic storage (AWS was aerobically pre-treated without or with *C. subvermispora* or L. *edodes* at 25 °C for 7 weeks). Error bars indicate standard deviations (n = 3).

3.5. Metabolite analysis of stored fungal-treated wheat straw

The data generated by the GC- and LC—MS analytical techniques represented relative abundancy values. The close grouping of biological replicates in the PCA plot (Fig. 4) indicates a relative high reproducibility of both the experimental and the analytical methods used. The first two principle components (PC's), indicated by (R2x[1] and R2x[2], respectively, together explain 53.2 % of

the total GCM—S metabolite variation (Fig. 4). The largest effect (PC1) coincides with the time of incubation, while PC2 corresponds to the incubation temperature. For both fungal species the largest relative distance between sample groups was between week 0 and week 2, at both 24.7 °C and 52.4 °C.

The metabolites observed and annotated using GC—MS were broadly classified into three groups: organic acids, carbohydrates and others (Table 3). Among the organic acids analysed, an accumulation was seen of glycolic, succinic, glyceric, malic, pipecolic, 2,3,4-trihydroxybutyric and vanillic acids with storage and temperature of CS-AWS and LE-AWS, while storage of LE-AWS resulted in an accumulation of formic acid. Except for some variation, lactic, malonic, tartaric, ribonic and protocatechoic acid also showed an accumulation with storage. Oxalic acid was present in both fungal treatments and remained relatively constant during the entire anaerobic storage period depending on temperature. Galacturonic acid was not detectable in CS-AWS, while it was clearly detected in LE-AWS (Table 3).

A series of sugars increased during anaerobic storage, including monosaccharides and disaccharides (Table 3). In contrast with non-stored fungal treated AWS (week 0), pentose (xylose, arabinose), and hexoses (mannose, glucose and fructose) increased in both fungal treated straws during storage.

The relative levels of the ceriporic acids A, B, C and G in CS-AWS were determined (Fig. 5). All ceriporic acids were detected in relatively high levels at the start of storage (W0) and remained relatively constant or showed an increase at 24.7 °C up to 10 week of storage. A decrease was observed when storage occurred at 52.4 °C from week 2 and 10. The accumulation of glycerol was observed in both CS-AWS and LE-AWS during storage.

4. Discussion

A low pH seems to be an important factor for fungi for a quicker growth and more efficient lignin degradation (Magnuson and Lasure, 2004). Results of the current study are in line with those of Mäkelä et al. (2002) who found in other white-rot fungi that the decrease in pH was caused by secretion of acids. Results show that a larger amount and different types of acids were produced by *C. subvermispora* than by *L. edodes*.

The acidity of the fungal treated AWS changed after storage under anaerobic conditions. Whereas pH is an indication of active acidity, titratable acidity is a better indication of the total acid concentration (Sadler and Murphy, 2010). The increased acidity might indicate an accelerated acid formation either by the still active excreted fungal enzymes or occurrence of other chemical reactions. The former might be explained by the continuing metabolic activity of the fungi under decreasing oxygen concentrations. Growth of basidiomycete fungi can go on for a short time under low oxygen conditions and change the chemistry of decomposition of lignocellulose (Nsolomo et al., 2000; Pavarina and Durrant, 2002), which might also explain to some extent the decrease in pH during the first 2 weeks of anaerobic incubation. It is, however, also possible that chemical reactions increased the amount of acids in fungal treated AWS. Part of the increase in titratable acidity with temperature and time was also seen in AWS but the major changes were due to the fungal treatment. The increased acidity of AWS could be caused by some enzymatic activity, but more likely by chemical

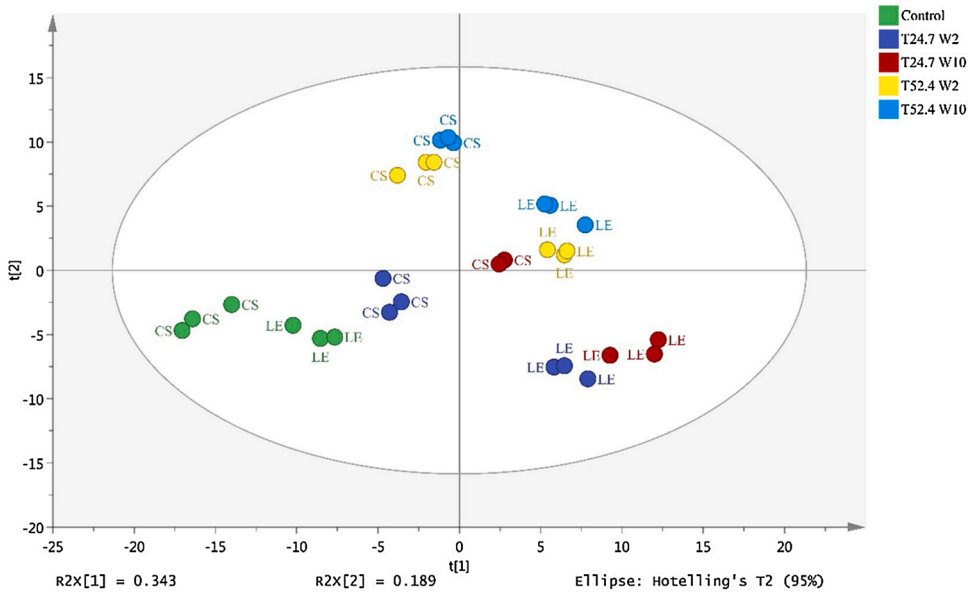

Fig. 4. Principal components analysis of fungal treated wheat straw samples based on their composition of polar metabolites. Autoclaved wheat straw (AWS), aerobically pre-incubated with either *Ceriporiopsis subvermispora* (CS) or *Lentinula edodes* (LE) at 25 °C for 7 weeks, was anaerobically stored at either 24 °C (T24.7) or 52.4 °C (T52.4) for 2 (W2) or 10 (W10) weeks. Polar metabolites were analysed by GC–MS. The controls (green) represent the start of anaerobic storage (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 3
Metabolites in *Ceriporiopsis subvermispora* or *Lentinula edodes* treated autoclaved wheat straw stored at either 24.7°C (T24.7) or 52.4°C (T52.4) for 2 (W2) or 10 (W10) weeks.

					C. subvermispora					*L. edodes*				
Compound type	Centrotype	RIexp	Name	Annotation level*	W0	T24.7 W2	T24.7 W10	T52.4 W2	T52.4 W10	W0	T24.7 W2	T24.7 W10	T52.4 W2	T52.4 W10
Organic acid	303	1074	Lactic Acid, 2TMS	1	0	382	1320	2278	1760	55	45	207	114	466
	514	1088	Glycolic acid, 2TMS	1	1740	8096	17166	34542	55540	1478	7218	14669	34540	50374
	1076	1141	Oxalic acid, 2TMS	1	8806	8076	9784	7240	7057	7590	9015	8445	8922	7713
	2828	1301	Succinic acid, 2TMS	1	516	7329	7942	8516	11078	1078	5550	6066	7269	9746
	2965	1312	Glyceric acid, 3TMS	2	4867	12448	26531	40623	57660	8161	14171	29485	41924	65710
	3675	1384	Formic acid, 1TMS	2	2321	2236	2384	2149	2629	132	737	1299	1018	1734
	3756	1392	Malonic acid, 2TMS	2	81	244	728	321	682	125	1284	3178	2918	3308
	4535	1473	Malic acid, 3TMS	1	206	448	831	2148	2794	1577	4131	7250	20917	20668
	5045	1515	Pipecolic acid, 2TMS	2	191	573	1541	2111	3015	868	1159	2279	3470	5546
	5362	1543	2,3,4-Trihydroxybutyric acid, 4TMS	2	2194	6158	11334	9181	12280	1424	8775	18015	14914	19845
	6103	1623	Tartaric acid, 4TMS	1	2137	2919	3823	4628	6517	668	481	875	1642	2296
	8528	1749	Ribonic acid, 4TMS	1	3615	11802	24256	9118	15048	3223	15029	35210	26615	37517
	8758	1765	Vanillic acid, 2TMS	1	4921	5970	6659	10205	14831	3549	4247	6325	7926	9809
	9263	1816	Protocatechuic acid, 3TMS	1	314	340	683	1344	1809	130	93	179	526	974
	11840	1924	Galacturonic acid, methyloxime 1, 5TMS	1	12	0	0	0	0	2982	2464	2482	1912	1555
Carbohydrate	3340	1345	Dihydroxyacetone, 2TMS	2	1323	2036	3229	3271	3364	1485	2455	2102	2851	2293
	4761	1489	Threitol, 4TMS	1	423	5975	6810	5080	7617	921	3523	4572	4181	6138
	6170	1627	Arabinose, 4TMS, methyloxime	1	2452	3488	4147	5065	5893	2441	2144	3086	3396	5044
	6502	1636	Arabinose, 4TMS, methyloxime, metyloxime (isomer 2)	1	2632	21060	35224	35203	72447	8262	71697	166775	147619	200522
	7129	1647	Xylose, 4TMS, methyloxime (anti)	1	10486	181217	550238	801498	1856391	47640	455837	1055308	603618	1470409
	7529	1662	Xylose, 4TMS, methyloxime (syn)	1	426	1073	1648	2899	6255	1142	4533	9560	6145	10581
	7959	1704	Arabitol, 5TMS	1	107622	44076	69699	68527	59056	183294	211731	204031	195803	168479
	9832	1857	Fructose, 5TMS, methyloxime (anti)	1	1569	20069	50164	69412	61680	2105	12829	33919	106493	102331
	10004	1867	Fructose, 5TMS, methyloxime (syn)	1	871	14570	37003	53086	48990	1201	5610	18909	82844	80112
	10263	1877	Mannose, 5TMS, methyloxime (1Z)	1	665	14488	39414	7768	17461	2340	48802	102114	16858	23620
	10566	1883	d-Glucose, 5TMS, o-methyloxyme, (1E)	1	7989	541724	1195726	272283	292221	14657	1225119	1564505	616553	552862
	11099	1903	d-Glucose, 5TMS, o-methyloxyme, (1Z)	1	1625	112224	263684	54267	58290	3343	263592	502472	122071	116492
	11450	1914	Mannitol, 6TMS	1	48626	62949	48047	56719	57849	381193	649740	447671	523277	549200
	12090	1941	Myo-Inositol, 6TMS	1	4988	4071	2234	5628	5686	5837	5921	2893	5118	5169
	13205	2074	Myo-Inositol, 6TMS	2	1955	7101	9363	6265	7300	5795	11348	13183	9840	10553
	14498	2356	Xylobiose, 6TMS, methyloxime 1	1	3658	325162	722229	165269	249002	41679	182714	154089	16835	32379
	14812	2376	Xylobiose, 6TMS, methyloxime 2	1	908	93431	186725	47070	65997	12454	54766	43402	10110	10788
	16191	2545	Sucrose, 8TMS	1	11903	10151	6971	2501	5068	21030	21408	20050	5669	3927
	16763	2625	Maltose, 8TMS, methyloxime (isomer 1)	1	66	4426	5374	651	1608	70	86	308	992	1457
	16978	2631	Trehalose, 8TMS	1	5582	44	70	3639	4407	25502	197	934	22234	20517
	17820	2779	Uridine, 3TMS	1	245	174	364	9112	1398	1446	532	506	15999	9806
Others	2308	1257	Glycerol, 3TMS	1	15775	108825	108793	60130	70920	15287	42116	55027	39826	46789

TMS, Trimethylsilyl; RIexp, experimentally determined retention index.
* The metabolite annotation level is according to the rules described by Sumner et al. (2007).

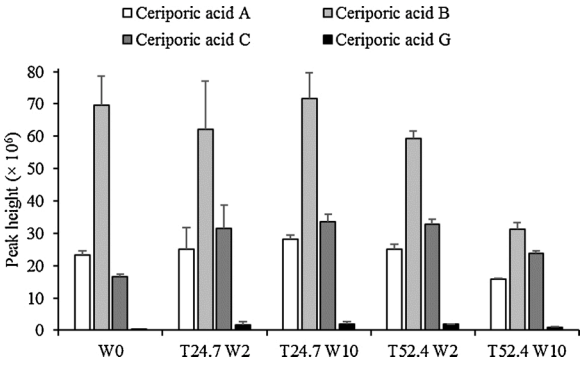

Fig. 5. Relative levels of ceriporic acids A, B, C and G in *Ceriporiopsis subvermispora* treated wheat straw stored anaerobically at 24.7 °C (T24.7) and 52.4 °C (T52.4) for 2 (W2) or 10 (W10) weeks. W0 represent the start of anaerobic storage. Error bars indicate standard deviations (n = 3).

reactions in the organic matter.

The total organic matter remained stable during the anaerobic storage. Although fungal growth and excretion of enzymes will decrease at some point after the onset of the anaerobic storage, a continuing activity of hydrolytic enzymes might lead to some degradation of hemicellulose. No significant changes of ADL was expected since oxygen is required for degradation of lignin. It has been reported that the disappearance of carbohydrates, mainly hemicellulose, can be the result from an acidic environment during ensiling (Morrison, 1979; Singh et al., 1996). Hence, acidic conditions generated by fungi might also be a reason for hemicellulose hydrolysis or solubilisation during the anaerobic storage, especially at an elevated storage temperature and for a prolonged period. Yang et al. (2001) conserved *Penicilium decumbens* treated corn straw (with wheat bran) anaerobically at 25, 30, 35 and 40 °C with addition of lactic acid bacteria and molasses for pH reduction. These authors showed that reducing sugars are increased with a longer storage period and at higher temperatures after an initial decrease at the beginning of the storage period.

The IVGP is an effective indicator for the degradability of organic matter in buffered rumen fluid (Cone et al., 1996). Although a significant decrease in hemicellulose content occurred in CS-AWS and LE-AWS ($P < 0.05$) (Table 2) stored anaerobically at 52.4 °C, this did not affect the IVGP. The method used to assess the amount of cellulose and hemicellulose is based on gravimetric measurements of residues after extraction (Van Soest et al., 1991). This apparent discrepancy between the fibre content (Table 2) and IVGP results (Fig. 2) can be explained by an increase in solubility and conversion into free sugars of the hemicellulose. The latter will not significantly affect the IVGP at 72 h but does affect the measurement of hemicellulose.

The colour of WS became lighter, redder and yellower with incubation of *C. subvermispora* and *L. edodes*. This is in line with Arora et al. (2011), who indicated that redness and yellowness are higher in *C. subvermispora* treated WS than control WS. However, they reported a slightly lower *L* value in *C. subvermispora* treated WS, which is opposite to the results in the current study. Fungal degradation of biomass results in a variety of changes in physical, biological and chemical properties (Blanchette et al., 1997; Tuyen et al., 2012; Van Kuijk et al., 2015b). White-rot fungi generally have a bleaching effect on lignocellulose since lignin and hemicellulose are removed in the early stage of colonization, leading to an enrichment in "whitish" cellulose (Hatakka and Hammel, 2011; Martínez et al., 2011). The ΔE is mainly influenced by a decrease in the *L* value. The increase in brownness of stored fungal treated WS at different temperatures might result from some chemical reactions. It is possible that browning by the Maillard reactions occurs as a result of the accumulation of reducing sugars in the fungal treated substrates (Yang et al., 2001), which are able to react with amino groups. Caramelisation can also contribute to the colour change during the first few days when there is still oxygen present. The changes in colour might also be caused, in part, by changes in polyphenols during storage. Since the major change in colour occurs at the beginning of the storage (between 0 and 2 weeks), it is possible that polymerization of mono-phenols occurs by laccase activity using the remaining oxygen present. Polymerized phenols are dark brown, dark orange, and dark wine red (Sun et al., 2013) and the increase in the ADL content with temperature and storage would indicate that polymerization of mono-phenols may have occurred.

In this research we applied, for the first time, a large-scale and essentially untargeted metabolomics approach for identifying the main changes in the composition of polar compounds in wheat straw treated with two contrasting white-rot fungi, in response to time and temperature of subsequent anaerobic incubation. The PCA analysis shows that the largest metabolite changes occurred at the beginning of anaerobic incubation. This correlates with the changes in pH, titratable acids and colour, which also show the largest changes within the first 2 weeks of storage. The relative levels of organic acids were already different between CS-AWS and LE-AWS at the start of the anaerobic storage (week 0), indicating that the two fungi accumulated different organic acids during the aerobic pre-incubation period of 7 weeks. Before the fungal treatment, wheat straw was submerged in water for 3 days to obtain an optimal moisture content. It is very likely that during this period anaerobic conditions were generated and available sugars were fermented by microorganisms into lactic acid. Lactic acid disappeared almost completely after 8 weeks of fungal treatment indicating degradation by the inoculated fungi (data not showed). The accumulation of lactic acid during anaerobic storage of fungal treated wheat straw might be due to unknown chemical reactions or by fermentation of sugars (generated by the fungi) by anaerobic microorganisms, such as lactic acid bacteria, that either persisted in treated wheat straw or where reintroduced when treated straw was mixed and transferred into the jars. It is unlikely that glyceric and glycolic acids are only produced by fungal metabolism: while these compounds are linked to the pentose phosphate pathway that takes place in the fungal cytosol (Magnuson and Lasure, 2004), it is very unlikely that the

enzymes of this aerobic pathway are still active under the anaerobic conditions of fungal treated AWS storage, in contrast to excreted enzymes that are still active even after the fungi are not metabolically active anymore. The accumulation of glycolic and glyceric acids might be due to unknown chemical reactions proceeding more efficiently at higher temperatures. We cannot exclude that yeasts also may have played a role in the production these compounds. Other acids that accumulated over time and correlating with temperature might also be formed by yet unknown chemical reactions. The difference in the level of measured acids present in LE-AWS compared to CS-AWS stored samples may be related to the observed differences in fibre composition of the pre-treated substrate at the end of their 7 week aerobic incubation (Table 2, CS and LE control samples). Vanillic acid and protocatechuic acids are products of lignin degradation (Mäkelä et al., 2015) and the higher level of vanillic acid and protocatechuic acids detected in CS-AWS than LE-AWS is expected since it is known that CS degrades lignin more effectively than LE (Nayan et al., 2018). The accumulation of both phenolic acids during anaerobic storage indicates that lignin degradation continued to some extent although Table 2 would indicate that lignin appears to increase with storage. The latter may be caused by reaction of previously formed lignin fragments yielding larger lignin molecules which are then measured in the gravimetric assay used to determine lignin. Oxalic acid is a known organic acid produced by white-rot fungi (Mäkelä et al., 2002; Aguiar et al., 2006; Kwak et al., 2016). Acids that are specifically produced by *C. subvermispora* are ceriporic acids, which are alk(en)yl-itaconic acids (Enoki et al., 2002; Amirta et al., 2003; Nishimura et al., 2008, 2012a, 2012b). The observed decrease in ceriporic acids in CS-AWS upon anaerobic storage at the high temperature might be due to degradation of ceriporic acids *via* yet unknown reactions. Galacturonic acid is a major component of pectin (Mäkelä et al., 2015). The difference between *C. subvermispora* and *L. edodes* treated straw samples at the end of the storage period might be due to the continuing pectin degradation during the first weeks of anaerobic incubation by *L. edodes*, either by its higher activity of pectin degrading enzymes or merely due to the higher amount of fungal biomass of *L. edodes* compared to *C. subvermispora*.

Since the main organic acids were not quantified, it is difficult to explain which acids are the main cause of the changes in pH and titratable acidity and which explain the differences in pH between the two fungi. Minor levels of malic, tartaric and malonic acid were found in aqueous extracts from *C. subvermispora* treated *Pinus taeda* wood chips, while tartaric, citric, succinic and malonic acids were detected in liquid cultures of *C. subvermispora* with glucose as the single carbon source (Aguiar et al., 2006). Malic, succinic and tartaric acid were also reported in the fruit body of *L. edodes* (Chen et al., 2015).

The accumulation of detected sugars might be partly caused by a continuous activity of plant cell wall degrading enzymes during anaerobic storage. Cellulases and hemicellulases from *C. subvermispora* have optimal activity at a low pH between 3.5 and 5.0 and high temperatures of 60–70 °C (Heidorne et al., 2006). The detected xylose (and xylobiose) and arabinose most likely originate from hemicellulose and pectin, while glucose originates from cellulose (Hatakka and Hammel, 2011; Mäkelä et al., 2015). Mannitol and trehalose are fungal storage molecules and may play a role in water stress (Rast and Pfyffer, 1989; Fillinger et al., 2001). The differences between the two fungi in mannitol and trehalose levels at the beginning of the storage (week 0) is likely due to the differences in the amount of mycelium (Nayan et al., 2018). Glycerol is a major by-product during anaerobic production of ethanol (Medina et al., 2010).

5. Conclusions

The anaerobic storage of wheat straw treated with the white-rot fungi *C. subvermispora* and *L. edodes* to increase their nutritional quality, results in clear changes in the fibre composition and colour of the straw, likely due to remaining enzyme activity and various chemical reactions. Although organic acids and sugar monomers accumulated in time, the nutritional quality of the treated straw remained stable.

Author statement

The concept of the research was done by Lei Mao, Wouter Hendriks, Anton Sonnenberg, John Cone and Leon Marchal.

The work was carried out by Lei Moa, who was supervised by Wouter Hendriks, John Cone, Anton Sonnenberg and Leon Marchal.

The biochemical analysis were performed at the laboratory of Bioscience of WUR in Wageningen. That research was also performed by Lei Mao and guided by Jeroen van Arkel and Ric de Vos.

The manuscript was written by Lei Mao and checked by all co-authors.

Declaration of Competing Interest

The authors report no declarations of interest.

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References

Aguiar, A., De Souza-Cruz, P.B., Ferraz, A., 2006. Oxalic acid, Fe^{3+}-reduction activity and oxidative enzymes detected in culture extracts recovered from *Pinus taeda* wood chips biotreated by *Ceriporiopsis subvermispora*. Enzyme Microb. Technol. 38, 873–878.

Amirta, R., Fujimori, K., Shirai, N., Honda, Y., Watanabe, T., 2003. Ceriporic acid C, a hexadecenylitaconate produced by a lignin-degrading fungus, *Ceriporiopsis subvermispora*. Chem. Phys. Lipids 126, 121–131.

Arora, D.S., Sharma, R.K., Chandra, P., 2011. Biodelignification of wheat straw and its effect on *in vitro* digestibility and antioxidant properties. Int. Biodeterior. Biodegradation 65, 352–358.

Blanchette, R.A., Krueger, E.W., Haight, J.E., Akhtar, M., Akin, D.E., 1997. Cell wall alterations in loblolly pine wood decayed by the white-rot fungus, *Ceriporiopsis subvermispora*. J. Biotechnol. 53, 203–213.

Carreno-Quintero, N., Acharjee, A., Maliepaard, C., Bachem, C.W.B., Mumm, R., Bouwmeester, H., Visser, R.G.F., Keurentjes, J.J.B., 2012. Untargeted metabolic quantitative trait loci (mQTL) analyses reveal a relationship between primary metabolism and potato tuber quality. Plant Physiol. 158, 1306–1318.

Chen, W., Li, W., Yang, Y., Yu, H., Zhou, S., Feng, J., Li, X., Liu, Y., 2015. Analysis and evaluation of tasty components in the pileus and stipe of *Lentinula edodes* at different growth stages. J. Agric. Food Chem. 63, 795–801.

Cone, J.W., Van Gelder, A.H., Visscher, G.J.W., Oudshoorn, L., 1996. Influence of rumen fluid and substrate concentration on fermentation kinetics measured with a fully automated time related gas production apparatus. Anim. Feed Sci. Technol. 61, 113–128.

De Vos, R.C.H., Moco, S., Lommen, A., Keurentjes, J.J.B., Bino, R.J., Hall, R.D., 2007. Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. Nat. Protoc. 2, 778–791.

Enoki, M., Honda, Y., Kuwahara, M., Watanabe, T., 2002. Chemical synthesis, iron redox interactions and charge transfer complex formation of alkylitaconic acids from *Ceriporiopsis subvermispora*. Chem. Phys. Lipids 120, 9–20.

Fillinger, S., Chaveroche, M.-K., Van Dijck, P., De Vries, R., Ruijter, G., Thevelein, J., d'Enfert, C., 2001. Trehalose is required for the acquisition of tolerance to a variety of stresses in the filamentous fungus *Aspergillus nidulans*. Microbiol. 147, 1851–1862.

Hatakka, A., Hammel, K.E., 2011. Fungal biodegradation of lignocelluloses. In: Hofrichter, M. (Ed.), Industrial Applications. Springer, Berlin, pp. 319–340.

Heidorne, F.O., Magalhães, P.O., Ferraz, A.L., Milagres, A.M.F., 2006. Characterization of hemicellulases and cellulases produced by *Ceriporiopsis subvermispora* grown on wood under biopulping conditions. Enzyme Microb. Technol. 38, 436–442.

ISO, 6496, 1999. Determination of Moisture and Other Volatile Matter Content. Animal feedingstuffs, Geneva, Switzerland.

ISO, 5984, 2002. Determination of Crude Ash. Animal feeding stuffs, Geneva, Switzerland.

ISO, 5983, 2005. Determination of Nitrogen Content and Calculation of Crude Protein Content–part 1: Kjeldahl Method. Animal feeding stuffs, Geneva, Switzerland.

Kwak, A.-M., Lee, I.-K., Lee, S.-Y., Yun, B.-S., Kang, H.-W., 2016. Oxalic acid from *Lentinula edodes* culture filtrate: antimicrobial activity on phytopathogenic bacteria and qualitative and quantitative analyses. Mycobiol. 44, 338–342.

Lisec, J., Schauer, N., Kopka, J., Willmitzer, L., Fernie, A.R., 2006. Gas chromatography mass spectrometry–based metabolite profiling in plants. Nat. Protoc. 1, 387–396.

Magnuson, J.K., Lasure, L.L., 2004. Organic acid production by filamentous fungi. In: Tkacz, J.S., Lange, L. (Eds.), Eds.), Advances in Fungal Biotechnology for Industry, Agriculture, and Medicine. Springer, Boston, pp. 307–340.

Mäkelä, M., Galkin, S., Hatakka, A., Lundell, T., 2002. Production of organic acids and oxalate decarboxylase in lignin-degrading white rot fungi. Enzyme Microb. Technol. 30, 542–549.

Mäkelä, M.R., Marinović, M., Nousiainen, P., Liwanag, A.J.M., Benoit, I., Sipilä, J., Hatakka, A., De Vries, R.P., Hildén, K.S., 2015. Chapter two - aromatic metabolism of filamentous fungi in relation to the presence of aromatic compounds in plant biomass. Adv. Appl. Microbiol. 91, 63–137.

Mao, L., Sonnenberg, A.S.M., Hendriks, W.H., Cone, J.W., 2018. Preservation of *Ceriporiopsis subvermispora* and *Lentinula edodes* treated wheat straw under anaerobic conditions. J. Sci. Food Agric. 98, 1232–1239.

Martínez, A.T., Rencoret, J., Nieto, L., Jiménez-Barbero, J., Gutiérrez, A., del Río, J.C., 2011. Selective lignin and polysaccharide removal in natural fungal decay of wood as evidenced by *in situ* structural analyses. Environ. Microbiol. 13, 96–107.

Medina, V.G., Almering, M.J.H., van Maris, A.J.A., Pronk, J.T., 2010. Elimination of glycerol production in anaerobic cultures of a *Saccharomyces cerevisiae* strain engineered to use acetic acid as an Electron acceptor. Appl Environ Microb 76, 190–195.

Mokochinski, J.B., Mazzafera, P., Sawaya, A.C.H.F., Mumm, R., De Vos, R.C.H., Hall, R.D., 2018. Metabolic responses of *Eucalyptus* species to different temperature regimes. J. Integr. Plant Biol. 60, 397–411.

Morrison, I.M., 1979. Changes in the cell wall components of laboratory silages and the effect of various additives on these changes. J. Agric. Sci. 93, 581–586.

Nayan, N., Sonnenberg, A.S.M., Hendriks, W.H., Cone, J.W., 2017. Differences between two strains of *Ceriporiopsis subvermispora* on improving the nutritive value of wheat straw for ruminants. J. Appl. Microbiol. 123, 352–361.

Nayan, N., Sonnenberg, A.S.M., Hendriks, W.H., Cone, J.W., 2018. Screening of white-rot fungi for bioprocessing of wheat straw into ruminant feed. J. Appl. Microbiol. 125, 468–479.

Nishimura, H., Tsuda, S., Shimizu, H., Ohashi, Y., Watanabe, T., Honda, Y., Watanabe, T., 2008. *De novo* synthesis of (Z)- and (E)-7-hexadecenylitaconic acids by a selective lignin-degrading fungus, *Ceriporiopsis subvermispora*. Phytochem. 69, 2593–2602.

Nishimura, H., Murayama, K., Watanabe, T., Honda, Y., Watanabe, T., 2012a. Diverse rare lipid-related metabolites including ω-7 and ω-9 alkenylitaconic acids (ceriporic acids) secreted by a selective white rot fungus, *Ceriporiopsis subvermispora*. Chem. Phys. Lipids 165, 97–104.

Nishimura, H., Sasaki, M., Seike, H., Nakamura, M., Watanabe, T., 2012b. Alkadienyl and alkenyl itaconic acids (ceriporic acids G and H) from the selective white-rot fungus *Ceriporiopsis subvermispora*: a new class of metabolites initiating ligninolytic lipid peroxidation. Org. Biomol. Chem. 10, 6432–6442.

Nsolomo, V.R., Solheim, H., Venn, K., 2000. *In vitro* effects of oxygen stress on fungi growing in wood of *Ocotea usambarensis*. Mycol. Res. 104, 1480–1484.

Ohashi, Y., Uno, Y., Amirta, R., Watanabe, T., Honda, Y., Watanabe, T., 2011. Alkoxyl-and carbon-centered radicals as primary agents for degrading non-phenolic lignin-substructure model compounds. Org. Biomol. Chem. 9, 2481–2491.

Pavarina, E.C., Durrant, L.R., 2002. Growth of lignocellulosic-fermenting fungi on different substrates under low oxygenation conditions. Appl. Biochem. Biotechnol. 98, 663–667.

Pollegioni, L., Tonin, F., Rosini, E., 2015. Lignin-degrading enzymes. FEBS J. 282, 1190–1213.

Rast, D.M., Pfyffer, G.E., 1989. Acyclic polyols and higher taxa of fungi. Bot. J. Linn. Soc. 99, 39–57.

Sadler, G.D., Murphy, P.A., 2010. pH and titratable acidity. In: Nielsen, S.S. (Ed.), Food Analysis, Food Science Texts Series. Springer Science+Business Media, LLC, Boston, MA. https://doi.org/10.1007/978-1-4419-1478-1_13, 219.

Singh, K., Honig, H., Wermke, M., Zimmer, E., 1996. Fermentation pattern and changes in cell wall constituents of straw-forage silages, straws and partners during storage. Anim. Feed Sci. Technol. 61, 137–153.

Sumner, L.W., Amberg, A., Barrett, D., Beale, M.H., Beger, R., Daykin, C.A., Fan, T.W.-M., Fiehn, O., Goodacre, R., Griffin, J.L., Hankemeier, T., Hardy, N., Harnly, J., Higashi, R., Kopka, J., Lane, A.N., Lindon, J.C., Marriott, P., Nicholls, A.W., Reily, M.D., Thaden, J.J., Viant, M.R., 2007. Proposed minimum reporting standards for chemical analysis. Metabolomics 3, 211–221.

Sun, X., Bai, R., Zhang, Y., Wang, Q., Fan, X., Yuan, J., Cui, L., Wang, P., 2013. Laccase-catalyzed oxidative polymerization of phenolic compounds. Appl. Biochem. Biotechnol. 171, 1673–1680.

Tuyen, V.D., Cone, J.W., Baars, J.J.P., Sonnenberg, A.S.M., Hendriks, W.H., 2012. Fungal strain and incubation period affect chemical composition and nutrient availability of wheat straw for rumen fermentation. Bioresour. Technol. 111, 336–342.

Van Kuijk, S.J.A., Sonnenberg, A.S.M., Baars, J.J.P., Hendriks, W.H., Cone, J.W., 2015a. Fungal treated lignocellulosic biomass as ruminant feed ingredient: a review. Biotechnol. Adv. 33, 191–202.

Van Kuijk, S.J.A., Sonnenberg, A.S.M., Baars, J.J.P., Hendriks, W.H., Cone, J.W., 2015b. Fungal treatment of lignocellulosic biomass: importance of fungal species, colonization and time on chemical composition and *in vitro* rumen degradability. Anim. Feed Sci. Technol. 209, 40–50.

Van Kuijk, S.J.A., Sonnenberg, A.S.M., Baars, J.J.P., Hendriks, W.H., del José, J.C., Rencoret, J., Gutiérrez, A., de Ruijter, N.C.A., Cone, J.W., 2017. Chemical changes and increased degradability of wheat straw and oak wood chips treated with the white rot fungi *Ceriporiopsis subvermispora* and *Lentinula edodes*. Biomass Bioenergy 105, 381–391.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.

Yang, X., Chen, H., Gao, H., Li, Z., 2001. Bioconversion of corn straw by coupling ensiling and solid-state fermentation. Bioresour. Technol. 78, 277–280.