EFFECTS OF ENSILAGE ON THE PRESERVATION OF BAMBOO SHOOT SHELLS AND THEIR FIBRE CHARACTERISTICS

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Received September 2010

JIA YF, SHI WY, WU LH & WANG HL. 2011. Effects of ensilage on the preservation of bamboo shoot shells and their fibre characteristics. Bamboo shoot shells (BSS) can be used as feedstock for the production of natural fibre. The purpose of this study was to preserve BSS in ensilage for further application as fibrous material. Lower silage pH and higher concentrations of dry matter and protein were found in the treatments with addition of cellulase and hemicellulase, and lactic acid bacteria (LAB) than in the control with no additives. The LAB treatment was most effective resulting in the highest concentration of total organic acids and lowest concentration of ammonia nitrogen. The structural changes in ensilaged fibres with LAB treatment were analysed and compared with untreated fresh BSS fibres using infrared spectrometer, X-ray diffraction and thermal analysis system techniques. Infrared spectrometer analysis demonstrated changes in the content of cellulosic components in the ensilaged fibres. X-ray diffraction analysis showed increase in the crystallinity of the ensilaged fibres. The thermal stability of the ensilaged fibres was improved, with slight shift of the maximum decomposition rate under higher temperature, from 320 to 327 °C. The beneficial characteristics of BSS fibres imply that ensilage can potentially be used as an effective storage method, which is essential for commercial production of high value fibrous materials.

Keywords: Fibrous material, storage, lactic acid bacteria, enzymes, structural changes

JIA YF, SHI WY, WU LH & WANG HL. 2011. Kesan ensilaj terhadap pengawetan kulit rebung dan ciri gentiannya. Kulit rebung (BSS) boleh dijadikan sumber bagi penghasilan gentian asli. Kajian ini bertujuan untuk mengawet BSS dalam ensilaj supaya ia dapat digunakan sebagai bahan gentian pada masa hadapan. Rawatan dengan selulase dan hemiselulase serta dengan bakteria asid laktik (LAB) merekodkan nilai pH silaj yang lebih rendah dan kepekatan jirim kering dan protein yang lebih tinggi berbanding kawalan yang tiada bahan tambahan. Rawatan LAB paling berkesan dan menghasilkan jumlah asid organik yang tertinggi dan kepekatan ammonia nitrogen yang terendah. Perubahan strukur gentian ensilaj yang dirawat dengan LAB dianalisis dan dibandingkan dengan gentian BSS yang tidak dirawat menggunakan teknik spektrometer inframerah, belauan sinar-X dan sistem analisis haba. Analisis spektrometer inframerah menunjukkan perubahan dalam kandungan komponen selulosa gentian ensilaj. Analisis belauan sinar-X menunjukkan pertambahan dalam penghabluran gentian. Kestabilan haba gentian ensilaj bertambah baik dan menunjukkan sedikit peningkatan dalam kadar penguraian maksimum dengan kenaikan suhu dari 320 °C hingga 327 °C. Keistimewaan gentian BSS menunjukkan yang ensilaj berpotensi digunakan sebagai kaedah penyimpanan yang berkesan. Ini penting untuk penghasilan bahan bergentian yang bermutu tinggi.

INTRODUCTION

Bamboo shoots are young, tender sprouts of bamboo and have long been used as a vegetable in both China and South-East Asia (Lu et al. 2009). In China, the current annual production of bamboo shoots is above 5 000 000 tonnes (Huang & Lu 2008), the majority of which is either canned or dried and sold all over the world. During the canning process, the shells of bamboo shoots, comprising 60 to 70% of the shoot, are usually discarded, generating a large amount of waste.

The demand for fibre products has increased dramatically in the last 50 years, especially in developing countries, as the world population grows rapidly (Ren et al. 2006). In consequence, natural fibres from agricultural residues previously left to waste are now considered as attractive commodities and have the potential to increase economic returns from existing agricultural production industries. Since over 70% of the shells of bamboo shoot consist of fibres, this has motivated several investigations to determine

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whether they can be used in textile, composite or other natural fibre applications (Jia et al. 2007, Li et al. 2007, Wu & Lv 2007). While these studies have indicated potential of bamboo shoot shells (BSS) in natural fibre materials, testing and extraction at an industrial scale have been limited. Limited industrial interest in the use of BSS fibres is related to the storing of BSS. The seasonal production of BSS coupled with their high moisture content of over 90% (Liu et al. 2001) requires effective storage for year-round manufacturing in commercial operation.

Ensilage is traditionally utilised by ruminant producers to preserve high fibre feedstuff for year-round use. It can also be used to store lignocellulosic biomass before industrial bioprocessing (Ren et al. 2007). Generally, biomass with an adequate level of fermentable substrate in the form of dry matter and water soluble carbohydrates, and with relatively low buffering capacity is easy to preserve (Liu et al. 2001). The characteristics of BSS are such that they are difficult to ensile without additives or salt (Liu et al. 2001). In studies to preserve different kinds of forage grass, various additives with antifungal properties, microbial inoculants and enzyme additions have been used to improve aerobic stability (Kleinschmit et al. 2005). For example, bacterial inoculants such as *Lactobacillus* were used for potato pulp, alfalfa and other cereal grain silages (Okine et al. 2005, Kizilsimsek et al. 2007, Murphy et al. 2007). Enzymes were also shown to improve fermentation in some high lignocellulose materials such as alfalfa, core stover and kenaf core fibre (Wang et al. 2002, Ren et al. 2006, Murphy et al. 2007). Apart from one study investigating the quality of BSS silage prepared using rice straw and wheat bran (Liu et al. 2001), no studies have been done to determine the effect of additives on BSS preservation as a fibrous material and/or the impact of preservation on fibre structure.

The purpose of this study was to determine the effects of adding either *Lactobacillus* inoculants or cellulase enzyme complex on the fermentation quality and nutrient composition of BSS during ensilage. The best ensilage method identified was also used to determine the effect of the storage on the structure of BSS fibres.

MATERIALS AND METHODS

Bamboo shoot shells

Fresh shells of bamboo shoots (*Phyllostachys pubescens*) were obtained from a bamboo shoot processing plant in City of Lishui (Zhejiang, China) and washed using fresh water. The BSS for ensilage were cut into 30–50 mm pieces using a harvester chopper and air dried until the moisture was about 80%.

Ensilage process

The BSS silages were prepared with four treatments: (1) no additives (CK); (2) enzymes (cellulase: hemicellulase activity ratio was at 1:12, cellulase added at a rate of 750 IU/g of fresh forage, E); (3) lactic acid bacteria (added at a rate of 2.5×10^5 cfu/g of fresh forage, LAB); and (4) a combined application of the two additions (E + LAB). For each treatment, five replicates of 5 kg sample were vacuum-sealed in polyethylene bags and incubated at room temperature (25 ± 5 °C). Samples for chemical analyses were obtained at 90 days, dried at 45 °C for 24 hours, homogenised and then ground to particles smaller than 0.4 mm.

Analytical procedure

The dry matter (DM) content of the BSS silage was determined by drying in forced-air oven at 60 °C for 48 hours. Crude protein (CP) content was determined using a Kjeldahl apparatus (AOAC 1990). Contents of neutral detergent fibres (NDF) and acid detergent fibres (ADF) were determined according to Van Soest et al. (1991) and water soluble carbohydrates (WSC) according to Dubois et al. (1956). The buffering capability (BC) was measured by the method of Playne and McDonald (1966).

Water extracts were prepared by shaking 50 g of silage sample with 150 ml of refrigerated water for 24 hours, followed by filtering through four layers of cloth. An aliquot of the filtrate was acidified with 25% of metaphosphoric acid and centrifuged for 10 min at 20 000 rpm. The supernatant was used for determination of pH, ammonia-N (NH_3-N) and organic acids. The pH was measured using pH meter. The NH_3-N concentration was determined by steam distillation into boric acid and titration with dilute hydrochloric acid. Organic acids were analysed using gas chromatograph. The supernatant (2 µl) was injected into a HP-INNOWAX column. Temperatures of the injector and detector were 200 and 220 °C respectively and nitrogen (N_2) was used as a carrier gas at flow rate of 63.8 ml min^{-1}. Levels of lactic acid were determined using a similar procedure, with N_2 at a flow rate of 80.7 ml min^{-1}, and the injector and detector set at 250 and 270 °C respectively.

Fourier transform infrared spectroscopy

Fourier transform infrared (FT-IR) spectrometer was used to obtain the spectra of samples. Fibres were ground and mixed with KBr, then pressed into transparent thin pellets. Spectra for FT-IR were from 4000–400 cm^{-1}. Spectral outputs were recorded in transmittance mode as a function of wave number.

X-ray diffraction and crystallinity measurements

The crystallinity of samples were examined by an ARL X'TRA equipped with a Cu Kα (λ = 0.154 nm) target. The X-ray tube was set at 40 kV and 30 mA. The X-ray scattering angle was recorded from 3 to 70 ° 2θ, set at scanning speed of 0.04° s^{-1}.

Thermal characterisation

Thermal stability of samples was determined using thermal analysis system with a heating rate of 10 °C min^{-1} in nitrogen.

Statistical analysis

Statistical analysis was carried out using SYSTAT (Version 9 for Windows, 1999). One-way analysis of variance (ANOVA) was performed to evaluate whether the means were significantly different ($p < 0.05$).

RESULTS AND DISCUSSION

Chemical composition and subjective evaluation of BSS

Fresh BSS was low in dry matter (DM) and water soluble carbohydrat with very high buffering capacity (Table 1). After 90 days of fermentation, BSS in CK showed brown colour and smelled of ammonia. Seepage was evident. Colour was similar for the BSS in the E treatment. However, seepage was reduced and the odour was milder. In contrast, BSS in the LAB and E + LAB treatments were yellow with sweet and acidic smell.

Change in nutrient composition of BSS after ensilage

Contents of DM and CP in all additive treatments were significantly ($p < 0.05$) higher than that of the control, with the highest in LAB, followed by the E + LAB and E (Table 2). Some of the protein might have decomposed in the control, resulting in a significantly higher value of NH_3-N (Table 3). Contrary to DM and CP losses, the control had the highest concentrations of NDF and ADF (Table 2). A similar result was reported by Ren et al. (2006) who suggested that the decrease in biodegradable constituents and thus total mass might have caused the increase in cellulose content. Addition of the cellulase enzyme complex in the E and E + LAB significantly lowered the concentrations of NDF and ADF (Table 2) compared with the control and LAB. This could be because the enzymes improved silage fermentation through hydration

Table 1 Chemical composition, buffering capacity and pH of fresh bamboo shoot shells

Variable	Value
Dry matter (DM) (g kg^{-1})	207.5 ± 2.4
Crude protein (g kg^{-1} DM)	132.7 ± 3.3
Water soluble carbohydrate (WSC) (g kg^{-1} DM)	28.3 ± 0.6
Neutral detergent fibre (g kg^{-1} DM)	708.4 ± 1.6
Acid detergent fibre (g kg^{-1} DM)	311.4 ± 1.6
Buffering capacity (mEinstein kg^{-1} DM)	579 ± 3.9
pH	5.69 ± 0.1

Values are means of three determinations ± standard deviations.

of structural carbohydrates (hemicellulose and cellulose) into additional fermentable sugars (Murphy et al. 2007).

There was greater concentration of acetic acid than lactic acid in the control (Table 3). This was the only treatment where butyric acid was found in considerable amount. This indicated that secondary fermentation had occurred in the control, converting fermentable sugars and lactic acid into other organic acids. In all the additive treatments (E, LAB and E + LAB), lactic acid was the dominant acid produced, causing a significant reduction in pH to less than 4.1 after 90 days (Table 3).

Addition of enzymes only (E) had beneficial effects on the fermentation of BSS compared with the control. However, when combined with lactic acid bacteria (E + LAB), there was no improvement in fermentation over the LAB treatment as indicated by the NH_3-N and organic acid concentration (Table 3). It was possible that conditions were not optimum for enzyme hydrolysis to occur, resulting in little fermentable carbohydrate released from the BSS for further fermentation by LAB during ensilage. The addition of the enzyme complex and lactic acid had no significant effect on the fermentation quality of BSS as compared with addition of LAB alone, which was consistent with other studies (Kung et al. 1990, Nia & Wittenberg 1999). The most encouraging result was ensiling BSS with LAB alone and it was used to determine the effect of the storage on the structure of BSS fibres.

Spectroscopic analysis

In general, the FT-IR spectra of untreated and ensilaged BSS were similar, indicating that the fibres had similar structure (Figure 1). The dominant peaks at 3423 and 2894 cm^{-1} were due to the O–H and C–H bands respectively, and corresponded to the aliphatic moieties in lignin, cellulose and hemicellulose (Sain & Panthapulakkal 2006). A vibration peak at 1732 cm^{-1} in the untreated BSS fibre was attributed to either the acetyl and uronic

Table 2 Chemical compositions of the bamboo shoot shells after 90 days of ensiling without additive (CK), with enzyme (E), with lactic acid bacteria (LAB) and with a combination of the two (E + LAB)

Variable	CK	E	LAB	E + LAB	SEM
Dry matter (DM) (g kg^{-1})	142.2 d	166.1 c	188.4 a	178.1 b	3.06
Crude protein (CP) (g kg^{-1} DM)	74.4 c	103.3 b	110.2 a	110.1 a	3.04
Neutral detergent fibre (NDF) (g kg^{-1} DM)	734.5 a	638.2 c	663.4 b	635.1 c	7.51
Acid detergent fibre (ADF) (g kg^{-1} DM)	438.7 a	355.7 c	379.1 b	342.7 d	8.27

Values are means of four samples; means in a row with different letters differ significantly ($p < 0.05$); SEM = standard error of the mean

Table 3 pH, ammonia nitrogen (NH_3-N) and organic acid contents of bamboo shoot shells after 90 days of ensiling without additive (CK), with enzyme (E), with lactic acid bacteria (LAB) and with a combination of the two (E + LAB)

Variable	CK	E	LAB	E + LAB	SEM
pH	5.27 a	4.04 b	3.74 c	3.73 c	0.02
NH_3-N (TN) (g kg^{-1})	111.76 a	82.6 b	53.0 d	64.3 c	4.95
Organic acids (g kg^{-1} DM)					
Lactic acid	4.4 d	31.1 c	64.2 a	53.7 b	2.6
Acetic acid	31.5 a	29 b	16.5 d	24.5 c	0.9
Propionic acid	2.4 a	–	–	–	0.3
Butyric acid	22.5 a	5.4 b	0.2 c	0.4 c	0.5

– = not detected; values are means of four samples; means in a row with different letters differ significantly ($p < 0.05$); SEM = standard error of the mean; TN = total ammonia-N.

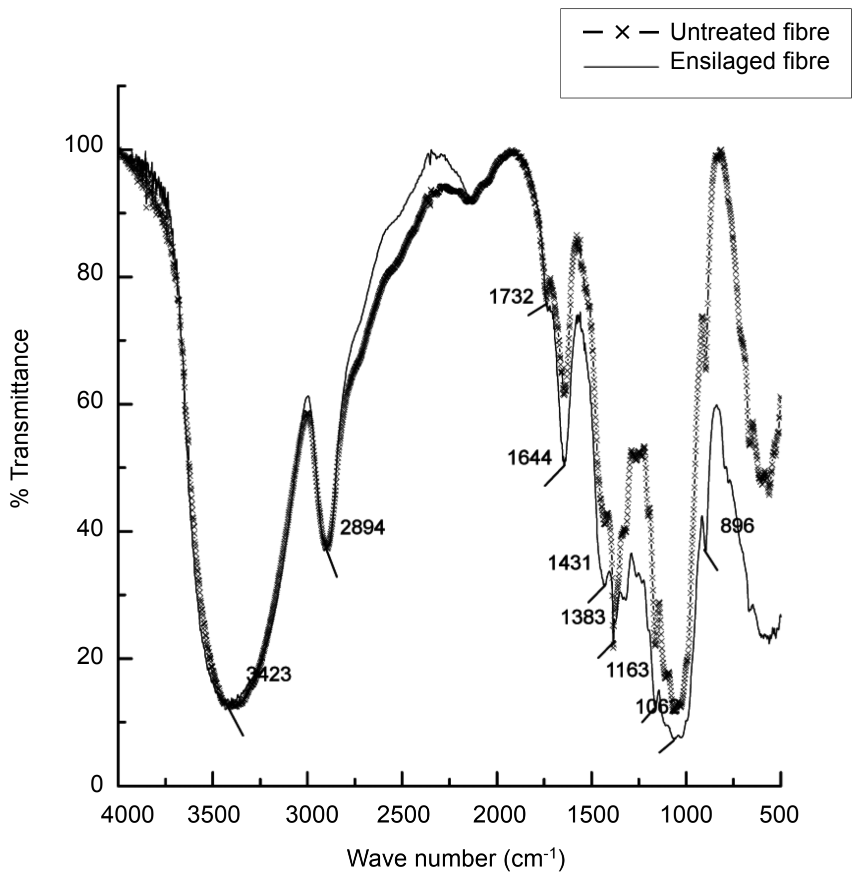

Figure 1 Fourier transform infrared spectra of bamboo shoot shells fibers before and after ensilage

ester groups of the hemicelluloses or the ester linkage of the carboxylic group of the ferulic and p-coumaric acids of lignin and/or hemicelluloses. The intensity of this peak decreased in the ensilaged BSS fibres possibly reflecting degradation of the hemicelluloses in the acidic silage environment. The peak at 1644 cm^{-1} was associated with adsorbed water and the decrease of the intensity of the peak in the ensilaged BSS fibres was possibly due to the loss of hemicelluloses. The aromatic C=C stretch from aromatic ring of lignin made the peak at 1431 cm^{-1} (Xiao et al. 2001). The sharp peak at 1381 cm^{-1} reflected C–H asymmetric deformations (Sun et al. 2005). The region of 1200–1000 cm^{-1} represented C–O stretching (Xiao et al. 2001). The dominant band at 896 cm^{-1} in BSS fibres indicated the typical structure of cellulose (θ-anomer in pyranose ring) (Pradhan et al. 2009). The lateral crystallinity index of the fibres was evaluated as the intensity ratio between IR absorptions at 1431 and 897 cm^{-1} assigned to the CH_2 symmetric bending mode and C_1 group frequency respectively. After ensilage treatment, the crystallinity index of BSS fibers was increased from 0.63 to 0.86.

Crystallinity of untreated and treated fibers

Cellulose crystallinity is a key factor that determines the mechanical properties of fibres. The change in physical structure of the BSS fibres during ensilage is shown in Figure 2. Before ensilage, the BSS fibres showed typical cellulose I pattern, with no crystalline transformation of the crystalline structure following silage. Using Jade 5 (XRD Pattern Processing Materials Data Inc 1999), the degree of cellulose crystallinity was calculated as a ratio of the diffraction portion from the crystalline part and the total diffraction from the sample. The crystallinity of untreated BSS fibres was estimated as 43.23%, which was lower than other commonly used natural fibres such as cotton, hemp and kenaf (Reddy & Yang 2008). The lower percentage crystallinity of BSS fibres was mainly due to the lower cellulose and higher lignin and hemicellulose contents

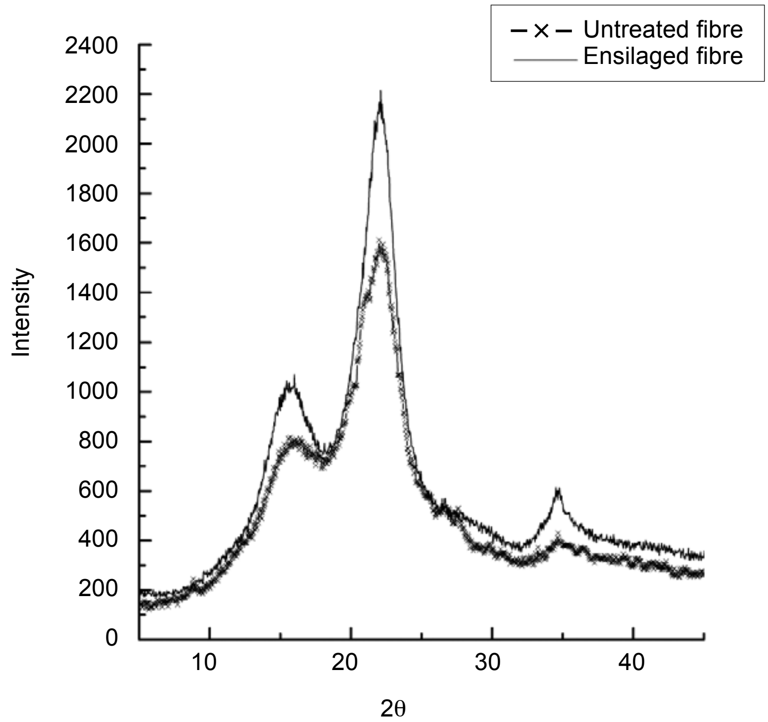

Figure 2 Diffractogram of fresh bamboo shoot shells fibres before and after ensilage

as compared with the other natural fibres. However, the crystallinity of the BSS fibres was increased to 53.06% after ensilage. During the ensiling process, the amorphous cellulose and hemicellulose were hydrolysed by water and fermentation microorganisms, resulting in little damage to the crystal region of the fibres. The increase in the number of crystallinity regions may increase the rigidity of cellulose and lead to higher tensile strength. The ensiled treatment also had other positive impacts on mechanical properties such as internal bond strength and modulus of elasticity (Ren et al. 2006), which could widen the application of ensilaged BSS fibres.

Thermal degradation of fibres

The thermal properties of natural fibres are important for their commercial application. The themogravimetric analysis thermogram and differential thermogravimetry curves (Figures 3 and 4) showed that the thermal stability of the BSS fibres changed after ensilage. There was slight shift in the maximum decomposition rate to higher temperature, from 320 to 327 °C after ensilage. The implied improvement in thermal stability of ensilaged fibres may be attributed to changes in the cellulosic component content and the higher crystallinity of the cellulose. These were consistent with the results from the FT-IR and crystallinity tests.

CONCLUSIONS

The characterisation of fibres from BSS confirms their feasibility as an alternative source for natural cellulose fibres. Adding lactic bacteria alone to the BSS was the most effective ensilage treatment, resulting in the highest concentration of total organic acid and lowest concentration of ammonia-N. Hence, the physical and thermal properties of the ensilaged fibre with LAB alone were characterised. The results revealed that the crystallinity and thermal stability of ensilaged BSS fibres were improved and the structure of the cellulose was preserved, as there were changes in the cellulosic component content in the acidic silage environment. The enhanced properties of BSS fibres imply that ensilage can potentially be used as an effective storage method for BSS, which is essential for commercial production of high value fibrous materials.

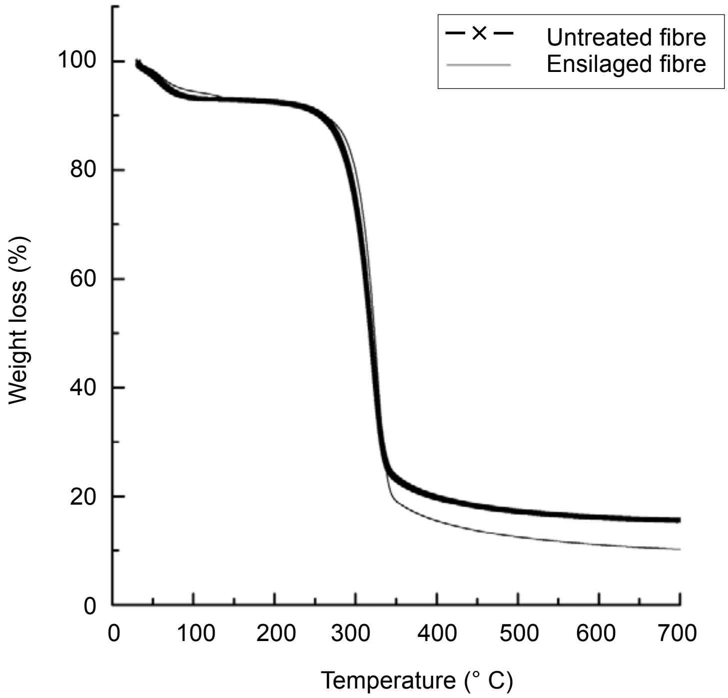

Figure 3 Themogravimetric analysis curves of fresh bamboo shoot shell fibres before and after ensilage

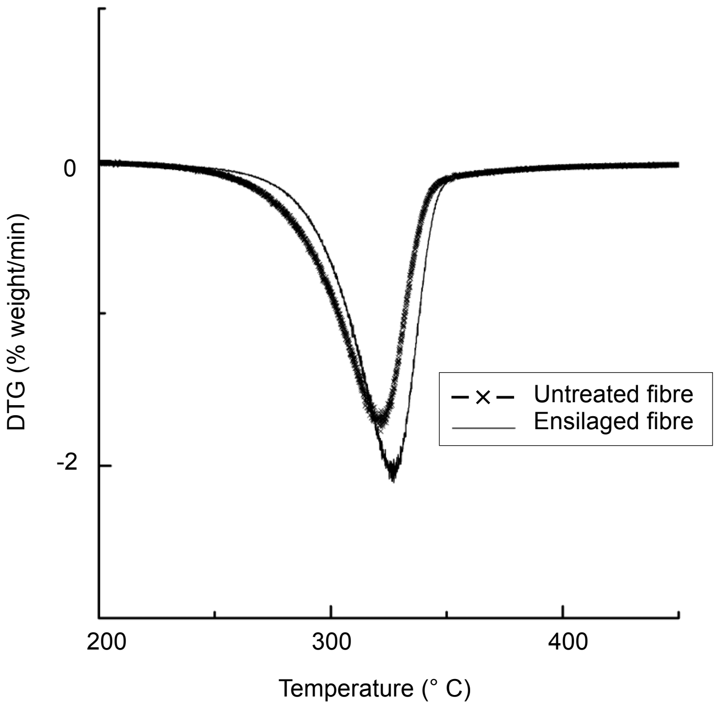

Figure 4 Differential thermogravimetry (DTG) curves of fresh bamboo shoot shell fibres before and after ensilage

ACKNOWLEDGEMENTS

The authors would like to thank GL Lv for assistance in the XRD study and C Rolando for constructive comments on the manuscript.

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