Study on the Mould-Resistant Properties of Moso Bamboo Treated with High Pressure and Amylase

Xiao-Dong Huang,[a,*] Chung-Yun Hse,[b] and Todd F. Shupe [c]

Starch of moso bamboo mainly exists in the elongated parenchyma cells, and it is difficult for amylase to enter moso bamboo and dissolve the starch. Therefore, the mould resistance capability of moso bamboo's products cannot meet the need for bamboo to resist fungal decay. In this experiment, moso bamboo blocks were first treated at six levels of pressure and for six different treatment durations. The results showed that reducing sugar content was decreased dramatically from 0.92 mg/L to 0.19 mg/L and the starch content decreased from 1.18% to 0.96% when the pressure was increased from 0 psi to 100 psi. Regression analysis showed that the effects of an individual amylase reaction and individual pressure treatment on the starch or reducing sugar content were significant with a high correlation coefficient. Three traditional types of moso bamboo moulds (*Aspergillus niger*, *Penicillium citrinum*, and *Trichoderma viride*) were then used for mould resistance testing. The results revealed that the mould resistance capability of moso bamboo blocks could be greatly improved by the combined effect of enzyme activity and pressure treatment. Mould resistance was enhanced by increasing the pressure or prolonging the treatment time. This research could provide a new method for the protection of bamboo from mould attack.

Keywords: Moso bamboo; Starch; Pressure; Amylase treatment; Mould resistance capability

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INTRODUCTION

Moso bamboo (*Phyllostachys pubescens* Mazei ex H. de Lebaie) accounts for about 70% of the total bamboo forest in the world (Sun *et al.* 2011). The species has been developed rapidly in China since the 1990s as an important forest resource alternative to wood due to its fast growing rate, high strength and stiffness, easy workability, and local availability (Jiang 2002; Zhang 1995). The selection of moso bamboo for industrial use and structural purposes is closely related not only to the physical and mechanical properties, but also to the chemical composition (particularly starch and sugar) of this material. This is important, as these properties could be associated with age and culm heights which thus affect the final use of moso bamboo (Abd. Latif 1987; Abd. Latif *et al.* 1990, 1991). In contrast to timber, bamboo attracts fungi much more easily due to the high content of starch and sugar, which act as food for fungi. This results in degraded performance, shortened service life, and reduced value (Jiang 2002; Okahisa *et al.* 2005; Wu 1992). Therefore, it is necessary to protect moso bamboo from mould or fungi (Liese

and Hamburg 1987; Liese 2003; Zhang 1995, 2003). Though treatments such as heat treatment (Cheng *et al.* 2013), solvent, or chemical reagents treatment (Sun *et al.* 2012; Tang *et al.* 2012; Lee *et al.* 2001) have been applied to protect bamboo from mould, a simpler and faster process for a more environmental, industrial, an economical available technique for the protection of bamboo from mould has so far not been found.

In the traditional moso bamboo block antifungal and anti-corrosion process, it is also difficult to determine how the chemical reagents can be introduced internally into the moso bamboo blocks (Huang 2008). A primary reason for this problem is that the distribution of moso bamboo's main vascular tissue system is longitudinal, with no horizontal transmission system (Jiang 2002). Moreover, since moso bamboo's starch mainly exists in the elongated parenchyma cells, it is difficult for amylase to enter the moso bamboo and react under normal conditions. The starch and reducing sugar content of moso bamboo samples treated with optimal amylase treatment method were the lowest (1.18% and 0.92 mg/L) compared to the other amylase treatment method. Thus, merely treating the system externally with alpha-amylase was found not to be effective.

High-pressure treatment is a safe and effective method for improving the permeability of the wood to chemical reagents. In short, a high pressure difference was applied in the container with the moso bamboo samples during the amylase treatment process. The effects of high pressure amylase treatment on the starch and reducing sugar content were first investigated, and then the growth of fungi on moso bamboo surfaces with respect to the starch and reducing sugar content was observed. The object of this study was to evaluate the high pressure amylase treatment on the mould resistance of moso bamboo.

EXPERIMENTAL

Materials

Mature moso bamboo was collected from Franklinton, Louisiana, USA. Bamboo blocks with dimensions 50 mm (length) × 20 mm (width) × 5 mm (thickness) were chosen for laboratory tests, with the green and yellow faces planed off. Twelve specimens per concentration of amylase treatment solvent were chosen for laboratory tests, of which half were with knots and half were without knots.

Three kinds of mould fungi were purchased from the Chinese Academy of Forestry, Beijing, China, including *Aspergillus niger*, *Penicillium citrinum*, and *Trichoderma viride.* These were used for the laboratory mould resistance tests. The amylase product was purchased from Sigma-Aldrich Co. Ltd. Its enzyme activity was 40000 u/g. Commercially available products including amylaceum, 3.5-dinitrosalicylic acid, NaOH, potassium sodium tartrate tetrahydrate, phenol, KI, NaHSO3, HCl, corn starch, and phenolphthalein indicator were purchased from VWR, USA and used without further purification.

Methods

High pressure amylase treatment

In previous studies it was found that with this bamboo sample, the optimal amount of enzyme activity was 120 u/mL and the optimal amylase treatment temperature was 95 °C. In a typical treatment, the bamboo samples and 120 u/mL amylase solvents

were combined in the high pressure treatment vessel and kept tightly sealed until the completion of the experiment. For laboratory tests, moso bamboo samples were treated at six levels of pressure (0 psi, 20 psi, 40 psi, 60 psi, 80 psi, and 100 psi) and six lengths of time (6 h, 12 h, 18 h, 24 h, 30 h, and 36 h) according to the experimental plan. Both the treated and untreated moso bamboo blocks were placed into the fume hood for about two weeks before the mould resistance test.

Starch and reducing sugar content analysis

The moso bamboo samples were treated with amylase and high pressure, and then the bamboo samples were immersed in cold distilled water for 10 min with magnetic stirring. This was followed by 24 h of drying at 105 °C in an oven. The oven-dried samples, including treated and untreated moso bamboo blocks, were milled, and the particles sized 40 to 60 mesh were collected in a sealed bag. The starch and reducing sugar content analyses were carried out according to TAPPI T 419 and the DNS (3.5-dinitrosalicylic acid method for the determination of reducing sugar), respectively.

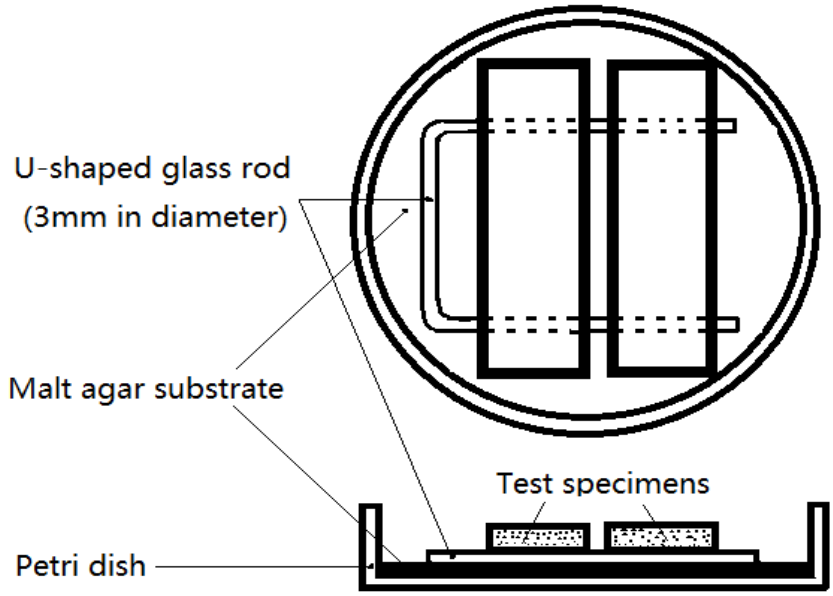

Fig. 1. The mould resistance laboratory test

Mould resistance test

For laboratory tests, moso bamboo samples were treated at six levels of pressure (0 psi, 20 psi, 40 psi, 60 psi, 80 psi, and 100 psi) and six lengths of time (6 h, 12 h, 18 h, 24 h, 30 h, and 36 h) according to the experimental plan. The treated moso bamboo blocks were placed on a 3-day culture of the test fungus malt agar (2% malt extract and 2% agar) in petri dishes (Fig. 1) and incubated for 30 days at 25 °C ± 2 under controlled humidity between 85 and 90% (ASTM: D4445-03).

Evaluation of the test

The growth of fungi in the laboratory test was visually analyzed and scored twice daily. When the samples began to mildew, a vernier caliper was applied to measure the

length and width of the mouldy part. Mould resistance of samples in the laboratory test was evaluated by the infection area (IA), which was calculated as follows,

$$IA = \frac{\sum_{i=1}^{12} IAi}{2700 \times 12} \times 100\% \quad (1)$$

where IA is the percentage of infection area, IAi is the infection area of sample i (mm^2), $2700 = 50 \times 20 \times 2 + 50 \times 5 \times 2 + 20 \times 5 \times 2$ is the constant for the area of each sample (mm^2), and 12 is the number of replicate samples.

RESULTS AND DICUSSION

Amylase Treatment

The effect of amylase and pressure treatment on the starch and reducing sugar content of bamboo samples was investigated, and the results are shown in Fig. 2. The sugar content decreased dramatically from 0.92 mg/L to 0.25 mg/L when the pressure was increased from 0 to 60 psi, whereas the reducing sugar content remained stable when the pressure was further increased from 60 to 100 psi. Amylase and pressure treatment were apparently able to convert the starch into reducing sugar monomers, which are soluble in water. Therefore, the solutions resulting from pressure treatment can have a relatively high concentration of reducing sugar. Part of the reducing sugar was absorbed in the moso bamboo sample's surface, but final reducing sugar content decreased dramatically from 0.92 mg/L to 0.19 mg/L because the moso bamboo samples must be cleaned in distilled water for 10 min before the reducing sugar content analysis. The reducing sugar was easily soluble in water, but the product of starch enzymatic hydrolysis was dextrin, which blocked up the vascular longitudinal tissue of moso bamboo. The dextrin prevented the amylase from entering the moso bamboo and reacting with the starch. As shown in Fig. 2, the starch content decreased smoothly from 1.18% to 0.96% when the pressure was increased from 0 to 100 psi. The most interesting finding was that a curvilinear equation was found between the pressure and starch content (R^2=0.9852), and another curvilinear equation was found between the pressure and reducing sugar content (R^2=0.9985). These results revealed that the combined effect of amylase and pressure treatment on the bamboo blocks could reduce the starch and reducing sugar contents.

The influence of pressure treatment duration on the content of starch and reducing sugar are shown in Fig. 3 and 4. The starch and reducing sugar content were decreased dramatically when the treatment duration increased from 6 h to 24 h. After that, the starch and reducing sugar content were stable when the treatment duration was increased from 24 h to 36 h. The starch and reducing sugar content of the bamboo samples without enzyme activity treatment decreased with water and 100 psi pressure as the treatment duration increased. However, under the same conditions, declining starch and reducing sugar content of bamboo samples with only pressure treatment was limited compared with the bamboo samples with the enzyme and pressure treatment. An interesting finding from the amylase and pressure reaction curvilinear equation was the high coefficient of determination of the regression for the starch content and different

treatment duration (R^2=0.9984), as shown in Fig. 3, and the coefficient of determination

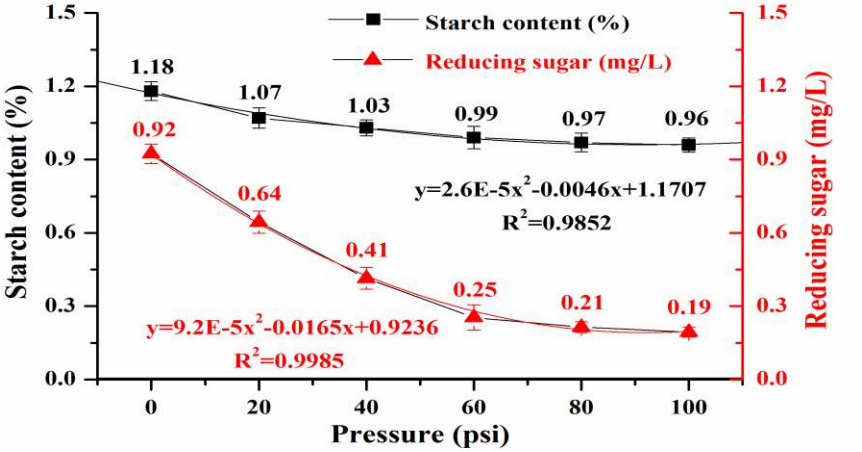

Fig. 2. Starch and reducing sugar content of moso bamboo samples was treated with six levels of pressure

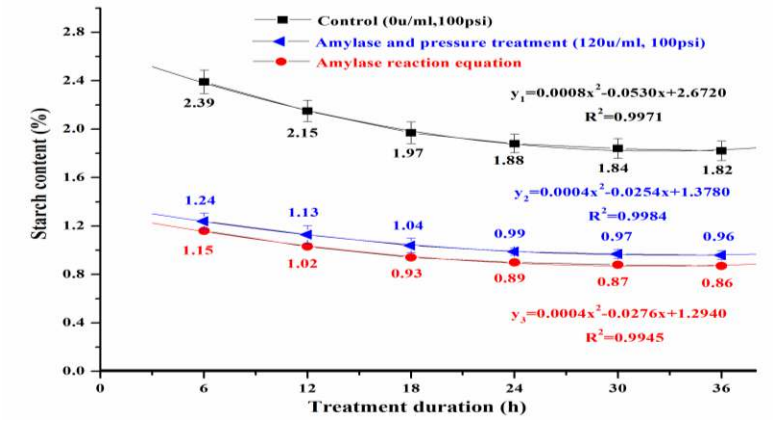

Fig. 3. The moso bamboo sample's curvilinear equation of the starch content and different treatment durations

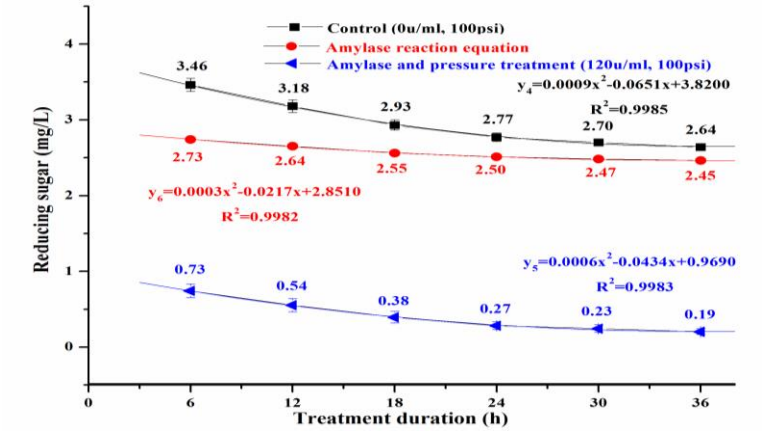

Fig. 4. The moso bamboo sample's curvilinear equation of the reducing sugar content and different treatment durations

for the regression between the reducing sugar content and different treatment duration (R^2=0.9983) as shown in Fig.4. An interesting aspect of the hot water and pressure reaction curvilinear equation was the high coefficient of determination for a regression of the starch content and different treatment duration (R^2=0.9971) as shown in Fig. 3, and the regression of reducing sugar content and different treatment duration (R^2=0.9985) as shown in Fig. 4. These results revealed that amylase and pressure both can play an important role in decreasing the starch and the reducing sugar content of moso bamboo blocks, and the proper prolongation of amylase and pressure treatment time on the bamboo blocks could effectively reduce starch and reducing sugar content.

Another important finding is that the starch and reducing sugar content of moso bamboo blocks treated for 12 h with amylase and pressure were 1.13% and 0.54 mg/L. The combination of amylase and pressure treatment could reduce the starch and reducing sugar contents of moso bamboo blocks to far below the 36 h of only amylase treatment levels (1.18% and 0.92 mg/L). This pressurized method can save a lot of amylase treatment time and become an economical and efficient new technique for the protection of bamboo from mould.

Decreasing the starch and reducing sugar content of bamboo samples was attributed to the combined effect of amylase, pressure, and hot water. The starch content of moso bamboo sample's fitting equation (moso bamboo sample treated with hot water and 100 psi pressure) is,

$$y_1 = 0.0008x^2-0.0530x+2.6720 \quad (2)$$

where y is the starch content of the moso bamboo sample, and x is the treatment duration for the moso bamboo sample.

Because the equation of the starch content involves the combined effect of amylase and pressure, one can obtain,

$$y_2 = 0.0004x^2-0.0254x+1.3780 \quad (3)$$

where y_2 is the moso bamboo sample's curvilinear equation terms for the starch content as a function of different treatment durations with 120 u/mL enzyme activity and 100 psi pressure. An individual amylase reaction equation aimed at the starch content of bamboo samples is:

$$y_3 = y_1-y_2= 0.0004x^2-0.0276x+1.2940 \quad (4)$$

The reducing sugar content of moso bamboo sample's fitting equation (moso bamboo sample treated with hot water and 100 psi pressure) is:

$$y_4=0.0009x^2-0.0651x+3.8200 \quad (5)$$

Because of the equation of the reducing sugar content is the combined effect of amylase and pressure, the result can be expressed as,

$$y_5 = 0.0006x^2-0.0434x+0.9690 \quad (6)$$

where y_5 is the moso bamboo sample's curvilinear equation terms for the reducing sugar content as a function of different treatment durations with 120 u/mL enzyme activity and 100 psi pressure. An individual amylase reaction equation aimed at the reducing sugar content of bamboo samples is:

$$y_6 = y_4 - y_5 = 0.0003x^2 - 0.0217x + 2.851 \quad (7)$$

Theoretically, the solid amylase cannot enter the moso bamboo's elongated parenchyma cells and react with the starch. It must use water as a carrier to enter the interior and complete the decomposition. Previous experiments have shown that amylase plays an important role in decreasing the starch and the reducing sugar contents of moso bamboo. We determined an individual amylase reaction curvilinear equation using a mathematical method, aimed at the starch and reducing sugar contents of moso bamboo samples. This equation demonstrated a high coefficient of determination for amylase treatment duration and starch content (R^2=0.9945), as well as another coefficient of determination for amylase treatment duration and reducing sugar content (R^2=0.9982).

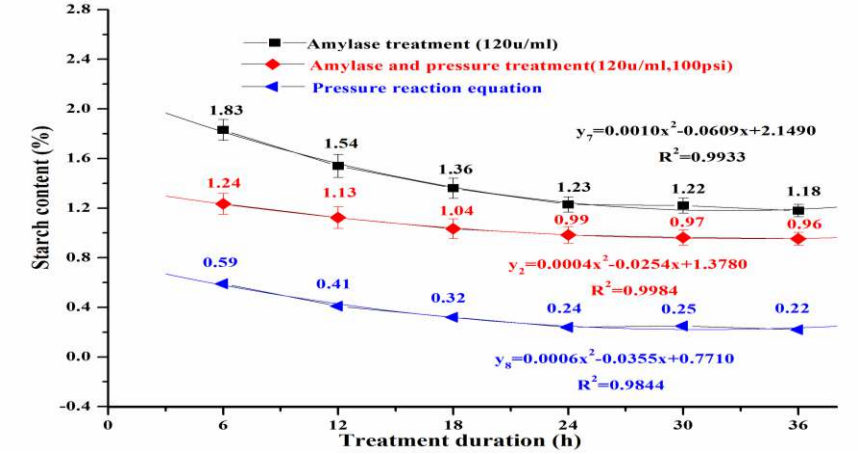

Fig. 5. Starch content of moso bamboo samples treated with pressure and amylase for different treatment durations

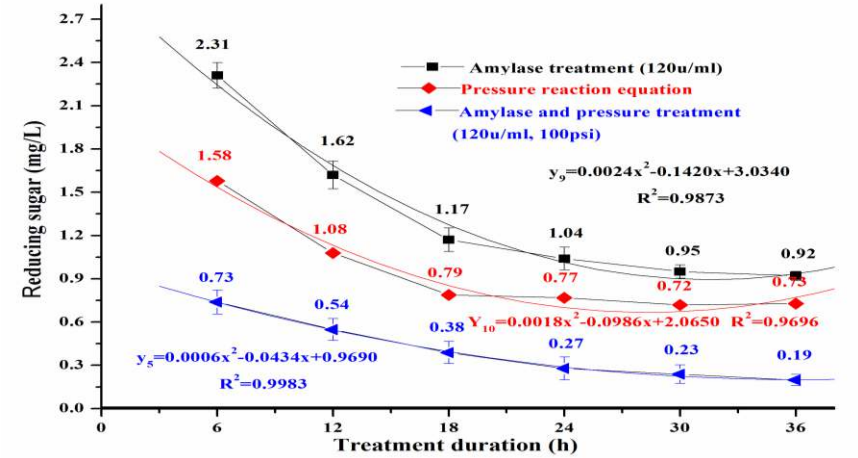

Fig. 6. Reducing sugar content of moso bamboo samples treated with pressure and amylase for different treatment durations

The starch and reducing sugar contents of bamboo samples treated with either only amylase or amylase and pressure are shown in Figs. 5 and 6. In Equation 8, y_7 is the starch content of moso bamboo samples treated with 120 u/mL enzyme activity during the different treatment durations, and y_2 is the starch content of moso bamboo samples treated with the combined effect of 120 u/mL enzyme activity and 100 psi pressure treatments. Because the pressure is an important influencing factor, we derived an individual pressure reaction equation aimed at the starch content of moso bamboo samples as follows:

$$y_8 = y_7 - y_2 = 0.0006x^2 - 0.0355x + 0.7710 \quad (8)$$

The equation for y_9 is the reducing sugar content of moso bamboo samples treated with 120 u/mL enzyme activity during the different treatment durations, and the y_5 equation is the reducing sugar content of moso bamboo samples treated with the combined effect of 120 u/mL enzyme activity and 100 psi pressure treatments. Therefore, an individual pressure reaction equation aimed at the reducing sugar content of bamboo samples is:

$$y_{10} = y_9 - y_5 = 0.0018x^2 - 0.0986x + 3.0340 \quad (9)$$

Then, to the surprise of the researchers, an individual pressure reaction curvilinear equation aimed at the starch content of bamboo samples was found between the pressure treatment duration and starch content (R^2=0.9844), and another individual pressure reaction curvilinear equation aimed at the reducing sugar content of bamboo samples was found between the pressure treatment duration and reducing sugar content (R^2=0.9696).

Mould Resistance in the Laboratory Test

Resistance against *Aspergillus niger*, *Penicillium citrinum*, and *Trichoderma viride* are shown in Figs. 7 and 8. Three types of moso bamboo samples included untreated moso bamboo blocks, treated with 120 u/mL amylase solvents, or the combined effect of 120 u/mL enzyme activity and 100 psi pressure. Both the treated and untreated moso bamboo blocks were placed into the fume hood for about two weeks before mould resistance testing. The results revealed that the untreated moso bamboo blocks (0 u/mL, 36 h) had no resistance against the three test fungi; the mould's mycelia spread rapidly once it climbed onto the moso bamboo blocks (day 2), and all surfaces were covered with mycelia by day 9.

When the moso bamboo blocks were treated with 120 u/mL amylase, the mould resistance capability of the moso bamboo blocks increased significantly. The moso bamboo blocks had similar resistances against the three test fungi under the same treatment conditions, and the mycelia spreading speeds decreased to varying degrees in every infection value. Mycelia spread slowly once they climbed onto the moso bamboo blocks (days 5 to 6), and all surfaces became covered with mycelia after at least 21 days.

To study the most efficient method of mould resistance of moso bamboo blocks, the moso bamboo blocks were treated with 120 u/mL amylase solution at 100 psi. The results showed that the mould resistance capability of the moso bamboo blocks greatly increased with only the 120 u/mL amylase solvent treatment. Mycelia spread slowly once it reached the moso bamboo blocks (day 10), and all surfaces were covered with mycelia

by day 25.

The reason for the improved mould resistance of moso bamboo blocks may involve synergistic results of multiple factors. Amylase treatment not only could dissolve the starch and sugar nutrients of the moso bamboo blocks required for mould fungi, but could also change the starch into reducing sugar and dissolve the reducing sugar in water. The distribution of moso bamboo's main vascular tissue system is longitudinal, with no horizontal transmission system. With this in mind, traditional moso bamboo blocks' antifungal and anti-corrosion processes and the internal immersion of chemical reagents in the moso bamboo blocks is always a difficult problem. Moreover, moso bamboo's starch mainly is present in elongated parenchyma cells, making it difficult for amylase to enter the moso bamboo and react under normal conditions. High-pressure conditions can improve the permeability of moso bamboo blocks, allowing the amylase to enter the blocks and dissolve the starch and sugar nutrients. However, the results of the mould resistance tests showed that mould species have little effect on moso bamboo blocks.

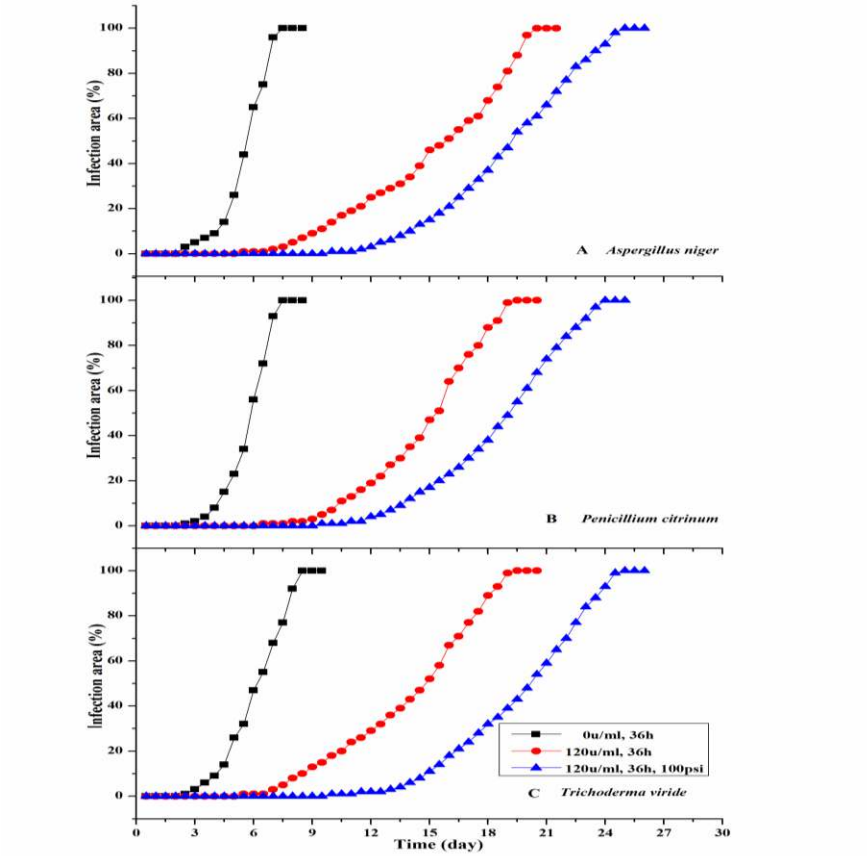

Fig. 7. Mould resistance of moso bamboo blocks against *Aspergillus niger*, *Penicillium citrinum* and *Trichoderma viride* during one month of cultivation

After determining the most efficient method of mould resistance of moso bamboo blocks, the moso bamboo samples were treated at 100 psi for various treatment durations (12, 24, and 36 h). The results showed that for higher treatment time, the capability of blocks of the three species to resist mould was also higher. This finding shows that the combined effect of amylase and high pressure plays an important role in improving the mould resistance capability of moso bamboo blocks.

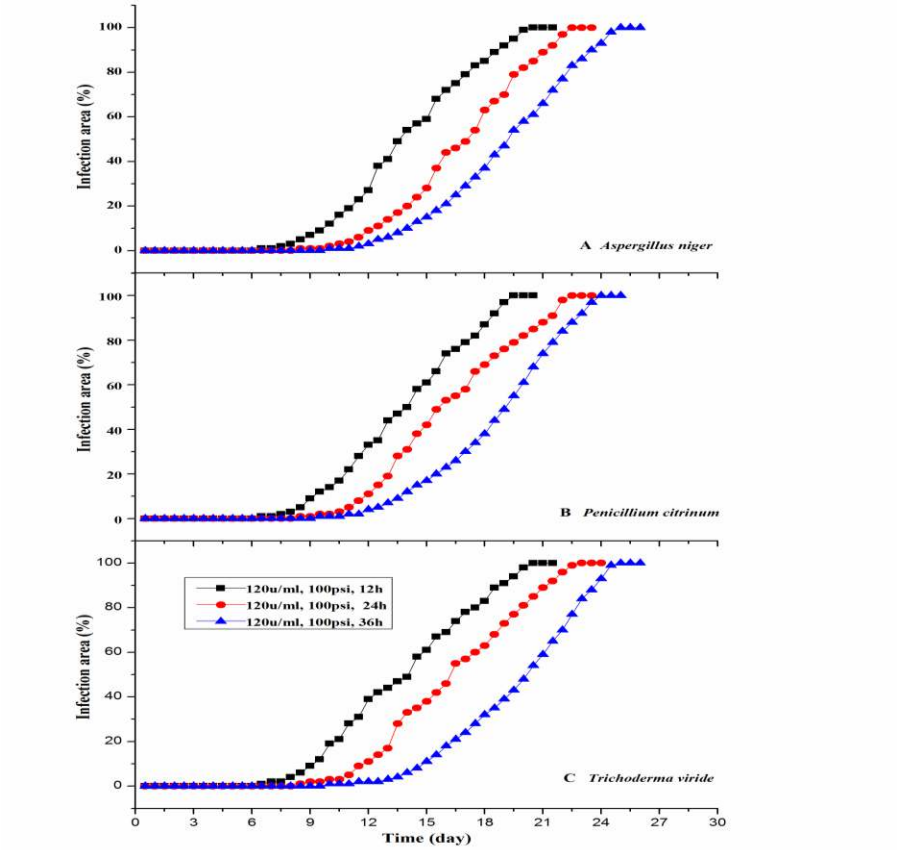

Fig. 8. Mould resistance of different treatment durations of moso bamboo blocks against *Aspergillus niger*, *Penicillium citrinum*, and *Trichoderma viride* during one month of cultivation

Application of Linear Regression to the Analysis of Mould Resistance Properties of Moso Bamboo

All of the untreated and treated bamboo, as well as the combined effect of amylase and pressure treatment conditions, reveal different degrees of inhibition of growth of the three types of mould fungus on the bamboo samples (Figs. 7 and 8, and Table 1). One month was required for the three types of mould fungus growth to cover all surfaces of bamboo block samples with mycelia. A characteristic of mould growth phenomena is that an increase in growth at any moment is proportional to the size already attained. This is sometimes called the law of compound interest. During each phase of culture bacterial growth, the numbers of organisms follow such a law. The relation is nicely illustrated by the mould's growth from 1 to 30 days. The graph of the infection value ascends more rapidly as age increases, and Eq. 10 shows the regression equation that is commonly associated with such growth,

$$W = (A)(B^{X}) \quad (10)$$

where A and B are constants to be evaluated. Applying the natural logarithm to this equation, the following equation results,

$$\ln(W) = \ln(A) + (\ln(B))X, \text{ or } Y = a + b\mathrm{X} \quad (11)$$

where $Y = \ln(W)$, $a = \ln(A)$, and $b = \ln(B)$. This means that if $\ln(W)$ instead of W is plotted against X, the graph will be linear. When using the natural logarithm instead of the quantity itself, the data are said to be rectified.

The values of $Y = \ln(W)$ are set out in the last column of the table and are plotted opposite X in the figure. The regression equation, computed in the familiar manner from columns X and Y, is shown in Table 1. For example, the regression equation for the moso bamboo samples with the best resistance against *Aspergillus niger* treated with 120 u/mL enzyme activity at 100 psi for 36 h amylase treatment duration is

$$y = 0.1412x+1.1285 \quad (12)$$

The regression line fit the data points well, with the correlation between Y and X being 0.8937. As measured by the infection value, the mycelia grew in accordance with the exponential law. The logarithm of the infection value increased at the estimated uniform rate of 0.1412 per day. This is the lowest infection value data of all three types of mould resistance tests.

Table 1. Summary of Linear Regression for the Mould Resistance Properties of Moso Bamboo

Amylase treatment conditions		Linear Equation	R^2	IA_{50}(I)(day)	IA_{50}(II)(day)
I:	0 u/mL, 36h	y=0.7558x-0.6702	0.9741	6.1	5.7
	0psi, 120 u/mL, 36h	y=0.2657x-0.4037	0.8857	16.2	16
	100psi, 120 u/mL, 12h	y=0.1461x+1.2506	0.8468	18.2	13.6
	100psi, 120 u/mL, 24h	y=0.1559x+1.0641	0.8853	18.3	17.1
	100psi, 120 u/mL, 36h	y=0.1412x+1.1285	0.8937	19.7	19.3
II:	0 u/mL, 36h	y=0.9412x-1.8906	0.9596	6.2	5.8
	0psi, 120 u/mL, 36h	y=0.3592x-1.7764	0.9207	15.8	15.4
	100psi, 120 u/mL, 12h	y=0.1552x+1.2328	0.8462	17.3	14
	100psi, 120 u/mL, 24h	y=0.1521x+1.1070	0.8325	18.4	15.6
	100psi, 120 u/mL, 36h	y=0.1533x+0.9133	0.9123	19.6	19.1
III:	0 u/mL, 36h	y=0.7071x-0.8125	0.9109	6.9	6.2
	0psi, 120 u/mL, 36h	y=0.2701x-0.1839	0.8692	15.2	14.8
	100psi, 120 u/mL, 12h	y=0.1403x+1.2649	0.8189	18.9	14.1
	100psi, 120 u/mL, 24h	y=0.1463x+0.9851	0.8589	20	16.2
	100psi, 120 u/mL, 36h	y=0.1583x+0.6507	0.9242	20.6	20.2

I, *Aspergillus niger* ; II, *Penicillium citrinum* ; III, *Trichoderma viride* ; $IA_{50,}$ up to half of the infection area of time; IA_{50}(I), predicted time; IA_{50}(II), measured time

IA_{50}(I) is the predicted time required for mycelia to spread to 50% of the surfaces of all moso bamboo samples, and IA_{50}(II) is the corresponding measured time. Table 1 shows that the predicted time for *Aspergillus niger* (100 psi, 120 u/mL, 36 h) was 19.7 days, that for *Penicillium citrinum* (100 psi, 120 u/mL, 36 h) was 19.6 days, and that for *Trichoderma viride* (100 psi, 120 u/mL, 36 h) was 20.6 days. Meanwhile, the measured time for *Aspergillus niger* under the same conditions was 19.3 days, that for *Penicillium citrinum* was 19.1 days, and that for *Trichoderma viride* was 20.2 days. IA_{50}(II) was

greatly prolonged for 36 h of pressure treatment over the 36-h treatment with only amylase. Both the laboratory test and the mathematical natural logarithm regression equation demonstrate that 120 u/mL enzyme activity, 100 psi pressure, and 36 h treatment duration are the optimal amylase treatment conditions for all the moso bamboo mould resistance tests.

CONCLUSIONS

1. The starch and reducing sugar content of moso bamboo samples decreased with pressure and amylase treatment as time increased.
2. The starch and reducing sugar contents of moso bamboo blocks treated for only 12 h with amylase and pressure were 1.135 and 0.54 mg/L, respectively; these contents were far less than those of the amylase-only treatment for 36 h (1.18% and 0.92 mg/L, respectively). Thus the pressurized method can save significant amylase treatment time.
3. Regression analysis showed that the effects of an individual amylase reaction and individual pressure treatment on the starch or reducing sugar content were significant with a high correlation coefficient.
4. The mould resistance test results showed that high pressure can improve the permeability of moso bamboo blocks, allowing the amylase to enter the blocks and dissolve the starch into its sugar monomers. The mould resistance capability of moso bamboo blocks increased significantly, and the combined effect of amylase and pressure played an important role.

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