Effectiveness of acetic, propionic and butyric anhydrides to protect rubberwood from decayed by white rot *Trametes versicolor*

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Abstract. Fresh rubber tree was harvested, cut into sized (100 cm x 14 cm x 25 mm) (l x w x t) and kiln dried (10-12 %) MC. The specimen sized of 5 mm x 20 mm x 20 mm (l x w x t) were prepared, undergone soxhlet extraction with toluene/methanol/acetone (4:1:1 by volume) for 8 hours, followed by oven dried at 103 °C for 24 hours and cooled in silica gel. The specimens were vacuum impregnated and then reacted with anhydrides for times ranging from 0.25 to 24 hours at 120 °C. The weight percent gain (WPG) was calculated and the chemical bonding was analysed with FTIR. All the specimens were leached in dionised water according to EN 84 (1997) and exposed to white rot in incubation room set at 22 °C (16 weeks). The study was found that modification only managed to reduce the rate of decay; without totally protected the rubberwood. Modified rubberwood was still classified as durability class 1 when compared to scott pine and beech woods. The final MC had a positive correlation with the weight loss following decay. The SEM showed that the hyphae were penetrated in the cells in both rubberwood.

1. Introduction
Rubberwood is the most famous wood for manufacturing both local and export oriented furniture as a result of its inexpensive, availability and adaptable processing technology. Rubberwood sawn timber is the top raw material used in the furniture industry, which make up 85% of total wood furniture exports for Malaysia in 2013 [1]. Rubberwood emerged as an alternative source of timber for the wood industry, when restriction in logging activities was implemented by the Malaysian government. Rubberwood is recognized as the most ecologically friendly lumber used in today's furniture industry. Generally, rubber trees are fell and replanted after 26-30 years for latex tapping. The wood is then harvested for use as sawn timber, moldings, furniture, medium density fibreboard, chipboard, builders, joinery and carpentry and wooden frames [2].

Despite the success story of rubberwood products, the fact that low dimensional stability and biodegradation problems are the main reasons for rubberwood to be less attractive for building materials. Products from rubberwood were generally less susceptible to bio deterioration agents than solid wood, unless they were used in situations where exposure to moisture or risk of deterioration was likely [3].
Rubberwood appeared to be the most susceptible to soft rot decay compared to other non-durable such as punggai (Coelostegia griffithii) and jelutong (Dyera costulata); which this was caused by a variety of micro fungi. However, rubberwood was found to be more susceptible than non-durable jelutong and ramin (G. bancanus) against the white rot Trametes versicolor [5-6].

Wood modification is getting attention in this new millennium which offers less or no toxic solution to extend the wood service life, as alternative for toxic copper chromium and arsenic (CCA). The CCA was reported to be able to leach out into soils from wood during its service. As a consequence, the CCA treated wood was banned for toys at playground directly contact with children skin [7]. Alternatively, chemical modification offers the best solution to improve wood dimensional stability and decay resistance, without impairing the mechanical properties especially the load bearing [8-9].

One of the most excellent chemical modification treatments is acetylation [9]. The increased resistance of acetylated wood against fungi caused by the bulking effect of the chemical in wood cell walls and the reduction of hydroxyl (OH) groups, which reduced the cell wall moisture content [7, 9]. In acetylated rattan (C. manan), the reduction of moisture content was as a result of decreasing in cell wall void volume following with modification maybe the factor which reducing the fungal attack. The moisture content of acetylated rattan was lower than the untreated rattan [8, 10].

Despite its supreme decay resistance, the acetylation process produced a byproduct of acetic acid needs further separation process to recycle the acetic anhydride [7, 9]. Further studies using a longer chain of carboxylic acid anhydrides such as propionic and butyric anhydrides reported a good decay protection. The performance varies either the source is dicotyledon or monocotyledon plants, derived by variation in the amount of structural organic chemical and microstructure.

The aim of this study was to investigate the effectiveness of acetic, propionic and butyric anhydrides to protect rubberwood against Trametes versicolor.

2. Materials and Method

2.1 Source of material
Rubberwood (Hevea brasiliensis), aged 25 to 30 years were obtained by local plantation. They were processed at Forest Research Institute Malaysia (FRIM), Kuala Lumpur; cut into sized of 25 mm x 14 cm x 100 cm (r x t x l) and kiln dried to 10-12 % moisture content. The specimens with sized of 20 mm x 20 mm x 5 mm (r x t x l) were cut using small band saw.

2.2 Preparation of the specimens
The rubberwood specimens were sanded from any loosely adhering fibers. Then it was placed in a soxhlet extractor for solvent extraction using toluene/methanol/acetone (4:1:1 by volume) for 8 hours. Specimens were then oven dried in an oven for 24 hours at 103 °C, weighed to three decimal places and its volume was measured using Mitutoyo digimatic height gage, transferred to a desiccator and allowed to cool to ambient temperature over silica gel. The rubberwood specimens were arranged in container by reaction time, weighed under metal wrapped with aluminium foil and submerged in absolute acetic, butyric and propionic anhydrides (Table 1). The containers were vacuum impregnation at 0.7 kPa and the specimens were kept submerged under the anhydride for 3 days to maintain in wet and swollen state before transferred into reaction flask.

| Table 1. The molecular weight of anhydrides used in this study. |
|-----------------------------|---------------------|-----------------|
| Anhydride                   | Molecular weight    | Molecular formula |
| Acetic anhydride            | 102.09 g/mol        | (CH₃CO)₂O       |
| Propionic anhydride         | 130.14 g/mol        | (CH₃CH₂CO)₂O    |
| Butyric anhydride           | 158.19 g/mol        | (CH₃CH₂CH₂CO)₂O |
2.3 Reaction procedures

The reaction procedures were conducted according to Hisham et al., 2014 [11]. The impregnated specimens were heated with oil in bath for the time ranging from 0.25, 4, 10, and 24 hours at 120 °C to obtain weight percent gain (WPGs) ranging from lowest (4.7 to 8.3), low (7.2 to 12.4), medium (10.2 to 14.0) to the highest (13.3 to 15.5) as shown in Figure 1. At the end of the reaction period, the reaction was quenched in ice until liquid temperature reached 20 °C and washed with cold acetone at the interval time of 1 hour for three times. The modified rubberwood was finally extracted using soxhlet consist of toluene/methanol/acetone solution mixture (4:1:1) for 8 hours and oven dried at 103 °C for 24 hours. Dry specimens were cooled, weighed and measured followed by air dried in conditioning room at 20 °C and 65 % RH to constant weight. The WPG was calculated as the following formula:

\[
\text{Weight percent gain (\%) } = \frac{[W_m - W_{um}]}{W_{um}} \times 100
\]

Where: \( W_m \) = mass of modified wood, \( W_{um} \) = mass of unmodified wood.

![Figure 1. The averages WPG of acetylated rubberwood, propionylated rubberwood and butyrylated rubberwood.](image)

2.4 FTIR analysis

The FTIR analysis was performed using Perkin Elmer Spectrum 100, FT-IR spectrometer to confirm the chemical bonding of rubberwood and anhydrides. The specimens were shaved prior to the FTIR test. The infrared spectra of each specimen which represented unmodified and modified rubberwood were obtained and evaluated with FTIR.

2.5 Decay test

All rubberwood specimens were leached in water following EN 84 (1997) [12] standard, and oven dried at 103 °C for 24 hours above. The specimens were then sterilised with propylene oxide for 5 days and exposed to decay over 4% malt extract agar in vented 500 mL squat jars following procedures in EN 113 (1996) [13]. For these purposes, 60 ml of 4% malt agar (40 g L\(^{-1}\) Oxoid powdered malt extract, 20 g L\(^{-1}\) Oxoid no. 3 agar, deionised water) was dispensed into 500 mL squat jars. These were sealed with lids vented with non-absorbent cotton wool plugs and the jars were autoclave sterilised. *Trametes versicolor* (CTB863A) was allowed to grow on the medium at 22 °C, 65% relative humidity for 2 weeks before exposure of the blocks. The blocks, one reacted and one untreated, were exposed over a sterilised polypropylene mesh in each jar. Twelve replicates were used for each reaction period and anhydride. Similarly sized of Scots pine (*Pinus sylvestris*) sapwood and European beech (*Fagus sylvatica*) blocks
were exposed, six jars each with two blocks as reference specimens. All blocks were incubated for 16 weeks. At the end of the test, excessive mycelium was removed and the moisture content and mass loss due to decay were determined. The durability classification was then determined following the guidance given in EN 350-1 (1994) where a ratio, expressed as the ‘x value’ is determined in comparison to the reference species, i.e. Scots pine or beech, i.e. x= average mass loss of test specimens/average mass loss of reference species. In this case, x value using both reference species was calculated. Durability classes were assigned according to EN 350-1 where class 1 (very durable) with an x value of x ≤ 0.15, 2 (durable) is 0.15 < x ≤ 0.30, 3 (moderately durable) is 0.30 < x ≤ 0.60, 4 (slightly durable) is 0.60 < x ≤ 0.90 and 5 (not durable) is x > 0.90.

2.6 Scanning electron microscopy (SEM)
A thin layer surface of decayed specimens was cut using a sharp razor-blade for a smooth surface. The specimen was then separated into vials and fixed in 4% glutaraldehyde for two days at 4 °C, washed with 0.1 m sodium cacodylate buffer for 30 minutes (three times), and post fixed in 1% osmium tetroxide for 2 hours at 4 °C. The specimens were washed again with 0.1 M sodium cacodylate buffer for 30 minutes for three times. They were dehydrated with acetone at concentration of 35 %, 50 %, 75 %, 95 % for 30 minutes and lastly 100 % for 1 hour (3 times). The specimens were critically dried with critical point drying for 30 minutes and coated with a gold using sputter coater. Scanning electron microscope was performed using PHILIPS XL30 ESEM.

3. Results and Discussion

3.1 Fourier Transform Infra-Red (FTIR) analysis
The FTIR spectra were differentiated by large and small peak of the line graph in the FTIR spectra. Larger peak means high intensity and smaller peak means lower intensity. The FTIR spectrum of untreated and modified rubberwood is shown in Figure 2. It showed that the spectrum of unmodified rubberwood had a lower intensity of intermolecular bonded hydroxyl groups at 3339.94 cm⁻¹. The peak intensity of bonded hydroxyl groups was found higher in acetylated rubberwood at 3338.45 cm⁻¹, for propionylated rubberwood at 3338.65 cm⁻¹ and, for butyrylated rubberwood at 3338.97 cm⁻¹. A weak C-H stretch appeared at 2900 cm⁻¹ for all spectra. A sharp intensity ester carbonyl vibration appears at 1720 cm⁻¹ to 1730 cm⁻¹ region confirmed the formation of ester bonds or esterification. The unmodified rubberwood spectrums had a lower intensity in this region of 1729.70 cm⁻¹. The increased in the intensity of C=O bonds occurred for all modified wood with 1729.46 cm⁻¹ for butyrylated rubberwood, 1728.81 cm⁻¹ for acetylated rubberwood and 1728.51 for propionylated rubberwood.
3.2 Decay resistance
The percent weight loss of untreated and modified rubberwood decayed by *Trametes versicolor* are shown in Figures 3 to 5. The weight loss of untreated rubberwood was almost identical when exposed together with each set of acetylated rubberwood (26.07 % to 36.65 %), propionylated rubberwood (23.07 % to 37.57 %) and butyrylated rubberwood (17.48 % to 28.78 %). The weight loss was gradually declined with increasing the reaction times. The weight loss was gradually decline from 16.13% to 4.31%, 18.88 to 4.41 % and 25.41 to 5.08 % for acetylated rubberwood, propionylated rubberwood and butyrylated rubberwood, respectively. The lowest percent weight losses were obtained by the highest WPGs of 14.6 (acetylated rubberwood), 15.5 (propionylated rubberwood) and 13.3 (butyrylated rubberwood) after reacted for 24 hours. In all the cases, the percent weight losses of modified rubberwood in this study were above 3 % and the test was valid according to EN113 [13]. In terms of effectiveness, these anhydrides treatment were not effective enough to protect rubberwood against *T. versicolor*. In case of chemical modifications, the decay protection of wood is referred as decay protection threshold or the percent weight gain above which no microbial degradation occurs [7]. The decay protection threshold of 10 WPG in acetylated Corsican pine (*Pinus nigra*) gave 0 % weight loss after 16 weeks exposed to *T. versicolor* [15]. In acetylated beech (*Fagus sylvatica*), threshold of 20 WPG gave 0 % weight loss. In acetylated rattan (*Calamus manan*) aged 10 and 13 years, threshold 13.4 and 9 WPGs gave 0 % weight loss after exposed for 16 weeks [8]. A less effectiveness of anhydrides to protect rubberwood was not only due to its low WPG. Another reason is that rubberwood was difficult to react with anhydrides. Acetylated rubberwood obtained 13 WPG after reacted for 27 hours and the extension of reaction time for another 94 and 144 hours only gave 15 and 16.6 WPGs, respectively [16]. This probably caused by a lower kinetic reaction in rubberwood. The kinetics reaction was explained by the access reagent to the hydroxyl groups (OH) to promote the reaction or the real chemical reaction occurred between anhydrides and the OH groups in cellulose, hemicellulose and lignin [17]. The lower WPG resulted from lower reaction rate made anhydride treatment was not favourable to totally protect rubberwood from *T. versicolor*.
Figure 3. The averages weight loss of untreated rubberwood and acetylated rubberwood decayed by *Trametes versicolor*.

Figure 4. The averages weight loss of untreated rubberwood and propionated rubberwood decayed by *Trametes versicolor*.

Figure 5. The averages weight loss of untreated rubberwood and butyrylated rubberwood decayed by *Trametes versicolor*. 
The moisture content of untreated and modified rubberwood is shown in Figures 6 to 8. The untreated rubberwood took up more than 100% moisture regardless of anhydrides, except for untreated rubberwood exposed together with butyrylated rubberwood. They were ranged from 132.8% to 159.4%, 107.6% to 161.3% and 84.9% to 143.4% when exposed together with acetylated rubberwood, propionylated rubberwood and butyrylated rubberwood, respectively. In all cases, the moisture content of modified rubberwood was greatly reduced with increasing reaction times. This was consistent with the percent weight loss after decayed by fungus. The moisture content was ranged from 17.8% to 84.3%, 15.8% to 70.9% and 23.54% to 111.5% for acetylated rubberwood, propionylated rubberwood and butyrylated rubberwood respectively.

Figure 6. The averages final moisture content of untreated rubberwood and acetylated rubberwood decayed by T. versicolor.

Figure 7. The averages final moisture content of untreated rubberwood and propionated rubberwood decayed by T. versicolor.

Figure 8. The averages final moisture content of untreated rubberwood and butyrated rubberwood decayed by T. versicolor.
3.3 The correlation between the basic properties and percent weight loss of decayed untreated and modified rubberwood.

The correlation between the basic properties and percent weight loss of modified rubberwood is shown in Table 2. The final moisture content following decay was the only property positively correlated with percent weight loss. Overall, the strongest correlation was obtained by butyrylated rubberwood (P=0.84) followed by acetylated rubberwood (P=0.69) and propionylated rubberwood (P=0.65). The WPG was transversely correlated with percent weight loss. This correlation trend was consistent with acetylated rattan (*Calamus manan*) decayed by *T. versicolor* [8]. Hill et al. (2005) [19] concluded that acetylation reduced decay by reducing cell wall moisture content. Papodopoulus & Hill (2003) [19] and Papodopoulus et al. (2010) [20] proposed that the mechanism for decay protection was due to blocking of the cell wall microcapillaries, which preventing access by the low molecular weight degradative agents produced by the fungus. Both factors are valid as the role of water is for transporting food, degradative agent and enzymes as catalyse to the breakdown process [21].

Table 2. The summary of correlation between the basic properties and percent weight loss.

| Specimen                  | Basic properties       | *Trametes versicolor* |
|---------------------------|------------------------|-----------------------|
| Acetylated rubberwood     | Percent weight gain    | -0.68**               |
|                           | Final moisture content | 0.69**                |
| Propionated rubberwood    | Percent weight gain    | -0.62**               |
|                           | Final moisture content | 0.84**                |
| Butyrylated rubberwood    | Percent weight gain    | -0.26                 |
|                           | Final moisture content | 0.65*                 |

* is significant at P<0.01, ** is significant at P < 0.001

3.4 The durability classes of untreated and modified rubberwood

When Scott pine was used as the reference specimen, almost all of the untreated rubberwood was classified as either moderately durable or slightly durable (Table 3). The acetylated rubberwood, propionylated rubberwood and butyrylated rubberwood at minimum WPG of 13.1, 14.0 and 10.2 was classified as very durable (class 1).

When beech wood was used as the reference specimen, all the untreated rubberwood was classified either as moderately durable or slightly durable. Akin to Scott pine as reference specimen, the acetylated rubberwood, propionylated rubberwood and butyrylated rubberwood at minimum WPG of 13.1, 14.0 and 10.2 was classified as very durable (class 1).

Table 3. X values and durability classes of untreated and modified rubber woods with acetic, propionic and butyric anhydrides using scots pine as reference specimen.

| Anhydrides | Reaction/h | Scott pine |          |          |          |          | Beech |          |          |          |
|------------|------------|------------|----------|----------|----------|----------|-------|----------|----------|----------|
|            |            | Untreated  | Modified | Untreated | Modified |          |       | Untreated | Modified |          |
|            |            | x         | class    | x         | class    |          |       | x         | class    |          |
| Acetic     | 0.25       | 0.51      | 3        | 0.35      | 3        | 0.62     | 4     | 0.41      | 3        |          |
|            | 4          | 0.74      | 4        | 0.21      | 2        | 0.90     | 5     | 0.26      | 2        |          |
|            | 10         | 0.60      | 3        | 0.15      | 1        | 0.73     | 4     | 0.14      | 1        |          |
|            | 24         | 0.50      | 3        | 0.11      | 1        | 0.61     | 4     | 0.13      | 1        |          |
|            | Average    | 0.59      | 3        | 0.20      | 2        | 0.72     | 4     | 0.24      | 2        |          |
| Propionic  | 0.25       | 0.48      | 3        | 0.37      | 3        | 0.58     | 3     | 0.45      | 3        |          |
|       | 4   | 0.46 | 3   | 0.13 | 1   | 0.56 | 3   | 0.17 | 2   |
|-------|-----|------|-----|------|-----|------|-----|------|-----|
| 10    | 0.77| 4    | 0.12| 1    | 0.93| 5    | 0.14| 1    |     |
| 24    | 0.73| 4    | 0.10| 1    | 0.89| 4    | 0.12| 1    |     |
| Average| 0.61| 4    | 0.18| 2    | 0.74| 4    | 0.22| 2    |     |

|       | 0.25 | 0.49 | 3   | 0.58 | 3   | 0.6  | 4   | 0.71 | 4   |
|-------|------|------|-----|------|-----|------|-----|------|-----|
| 4     | 0.53 | 3    | 0.36| 3    | 0.65| 4    | 0.44| 4    |     |
| 10    | 0.38 | 3    | 0.13| 1    | 0.57| 3    | 0.15| 1    |     |
| 24    | 0.62 | 4    | 0.12| 1    | 0.75| 4    | 0.14| 1    |     |
| Average| 0.51 | 3    | 0.32| 3    | 0.64| 4    | 0.39| 3    |     |

X values and durability classes of untreated and acetylated rubber wood using Scots pine and beech as reference specimen

3.5 Microstructure of decayed untreated rubberwood and rubberwood modified with a linear chain carboxylic acid anhydrides.

The microstructures of untreated rubberwood, acetylated rubberwood, propionylated rubberwood and butyrylated rubberwood decayed by fungi are shown in Figures 9 to 11. In almost all cases the hyphae penetration was more prominent at longitudinal direction than those from transverse direction. All the untreated and modified rubberwood were penetrated by hyphae even at the highest WPG. This indicated that the fungi hyphae can penetrated the wood cells but the access was limited due to slightly lower moisture content as shown in Figures 6 to 8. This study was consistent with others finding [8, 18].
Fig 9. The microstructure of untreated rubberwood and acetylated rubberwood decayed by *Trametes versicolor* (left side: transverse, right side: longitudinal)
Fig 10. The microstructure of untreated rubberwood and propionylated rubberwood decayed by *Trametes versicolor* (left side: transverse, right side: longitudinal)
Fig 11. The microstructure of untreated rubberwood and butyrylated rubberwood decayed by *Trametes versicolor* (left side: transverse, right side: longitudinal).

4. Conclusion

Modification of rubberwood with anhydrides at certain degree, only improve the decay resistance to *T. versicolor* but the treatment was not totally protected from deterioration. All the modified rubberwood at maximum WPG was classified as durability class 1 when compared to Scott pine and beech. The final moisture content had a positive correlation with the percent weight loss following decay. The SEM observation confirmed that the hyphae were penetrated in the cells either in untreated or modified rubberwood.

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