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Study on wood vinegars for use as coagulating and antifungal agents on the production of natural rubber sheets

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ABSTRACT

Coagulating and antifungal properties of wood vinegars in the preparation process of *Hevea brasiliensis* natural rubber (NR) sheets were investigated and compared with those of formic and acetic acids. The wood vinegars produced from biomasses such as inner coconut shell, bamboo and *Eucalyptus* woods were evaluated. It was found that plasticity retention index, Mooney viscosity and mechanical properties of NR coagulated by wood vinegars were similar to those using acetic acid and better than using formic acid. The antifungal efficiency of coagulants determined from a fungi growth area on NR sheet surfaces was found in the following order: coconut shell wood vinegar > bamboo wood vinegar ≈ *Eucalyptus* wood vinegar > acetic acid ≈ formic acid. The antifungal efficiency of the wood vinegars was strongly depended upon their phenolic compound contents and confirmed through the inhibitory growth of the main fungi, *Penicillium griseofulvum*, on potato dextrose agar.

1. Introduction

Natural rubber (NR) latex from *Hevea brasiliensis* trees is usually coagulated by acid to prepare the sheets for the production process. In this acid coagulation process, the negative charge of the phospholipid–protein complex on the surface of colloidal NR particles [1] was neutralized by the positive charge of the acid hydrogen ions. The commercial acids used for this purpose are formic and acetic acids. Since these acids are petrochemicals, an alternative, more sustainable materials are on high demand with rising petrol prices. In this study, wood vinegar, an acid by-product of wood charcoal production, was investigated as a coagulant in the production of NR sheets and compared with formic and acetic acids.

Raw wood vinegar is a condensed liquid, which is collected during the pyrolysis or carbonization of wood in airless conditions at a temperature range of 400–500 °C. The wood vinegar composes of many chemical components with acetic acid as the main substance [2]. For agricultural use, tar residue in the raw wood vinegar is usually extracted by coagulation, since the tar residue can stick as a coat on plant leaves. The remaining wood vinegar is called tar-extracted wood vinegar. The use of wood vinegar as a coagulant in NR sheets' preparation has been investigated and reported [3]. However, they did not compare the properties of NR coagulated by different wood vinegar types. In this study, we added more results on NR analysis data such as dirt and volatile contents, plasticity retention index and vulcanized NR modulus.

High moisture content of the NR sheets encourages fungi growth. This is a serious problem in NR production, since it may affect the production conditions as well as the quality of the final products. Therefore, antifungal agents need to be added during the NR production. Most commercial antifungal agents are highly toxic and not environmental friendly. An environmentally

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friendly antifungal agent with low toxicity is desired. Acetic acid and phenolic compounds contained in wood vinegar have been reported as anti-germination agent [2] and termiticides [4]. Due to their acetic acid and phenolic compound contents, *Guayle* wood vinegar and biomass slurry exhibit fungicidal and termiticidal properties and can be used as wood preservatives [5,6].

Therefore, the coagulating efficiency of different wood vinegars on the production process of NR was determined to compare with those of commercial formic and acetic acids in this work. Physical, chemical and mechanical properties of the NR sheets were measured by standard methods. The antifungal efficiency of wood vinegars was also investigated from fungi growth areas on the NR sheet surfaces and inhibitory growth of the main fungi on potato dextrose agar

2. Materials and methods

2.1. Materials

Tar-extracted wood vinegars produced from inner coconut shell, bamboo and *Eucalyptus* woods were kindly supplied by the Appropriate Technology Association (Thailand) and used without further purification. The all wood vinegars were reddish brown in color with pH $\approx$ 3.0. Formic acid (94%) and acetic acid (99.7%) were purchased from BSH and Labscan, respectively.

Fresh natural rubber (NR) latex was tapped and collected from RRIM 600 clone of *H. brasiliensis* trees in Mahasarakham, Thailand. Total solid content (TSC) of fresh NR latex was determined by using the moisture evaporation method No. D1076:1988 of the American Society for Testing and Materials (ASTM). To determine the amount of Dry Rubber Content (DRC), 5% (v/v) acetic acid was used to coagulate fresh NR latex. Following these procedures, %TSC and %DRC of fresh latex were $31.2 \pm 0.5\%$ and $27.5 \pm 0.7\%$, respectively.

2.2. Preparation of NR sheets

Each NR sheet was prepared in a pan by adding coagulant into diluted NR latex (2,000 mL filtered fresh NR latex + 3000 mL water). For formic and acetic acids, 300 mL 2% (v/v) were used. In the case of wood vinegars, 300 mL 50% (v/v) was chosen for preparing the NR sheets. The mixture of diluted latex and coagulant was left for 2 h for complete coagulation. The obtained slabs were compressed between two steel rolls and then washed with water before drying at room temperature with relative humidity of 50–60% for a week. The product obtained from this process is called pre-drying NR sheet and its further processed by drying at 50–60 °C for 2 days to be the dry NR sheets. The NR sheets coagulated by using formic acid and wood vinegar mixtures (3 mL formic acid + 75 mL wood vinegar + 222 mL water for each pan) were also prepared for antifungal efficiency comparison. The pH of all coagulant aqueous solutions was in the range of 2.6–3.1.

2.3. Characterization of NR sheets

FTIR spectra were obtained from NR films by using a Perkin-Elmer Spectrum GX FTIR spectrometer. The NR film was prepared by evaporation casting method of 2% (w/v) NR solution in toluene. The solvent was evaporated at room temperature for a week with the remaining solvent being dried in a vacuum oven at room temperature for another week before the NR film was analyzed with 32 scans and resolution of 4 cm^{-1}. Dirt content [7], volatile content [8], plasticity retention index (PRI) [9] and Mooney viscosity [10] of pre-drying and dry NR sheets were determined by the Standard Malaysian Rubber (SMR) methods. Vulcanization or curing time (t_{90}) [ACS#1 formulation 1, (ODR type, TECH PRO) Arc 1.5° at 150 °C] of the dry NR sheets was obtained using the method of the International Standardization Organization (ISO) [11]. Mechanical properties of vulcanized NR were determined by tensile testing (ISO 37 type 1) [12].

2.4. Antifungal efficiency of coagulants

The antifungal efficiency of the coagulants was determined from a fungi growth area on the pre-drying NR sheet surfaces after leaving them for a week. Percentage of the fungi growth area was calculated by the following equation.

$$\%\text{fungi growth area} = \frac{A_{\text{fungi}}}{A_{\text{NR}}} \times 100 \quad (1)$$

where A_{fungi} = Fungi growth area on NR sheet surface (cm^2)
A_{NR} = Total area of NR sheet surface (cm^2)

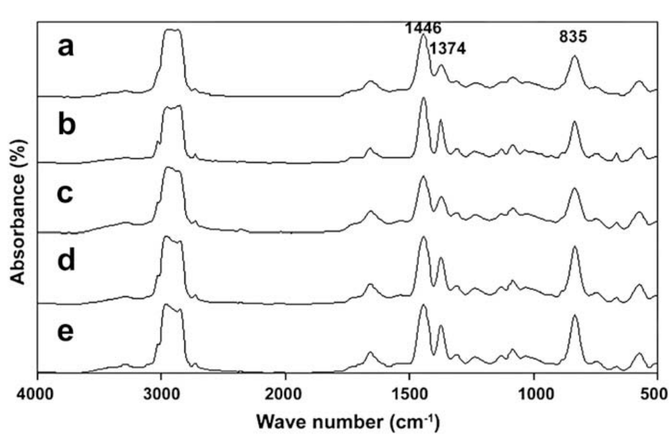

Fig. 1 – FTIR spectra of NR films coagulated by (a) formic acid, (b) acetic acid, (c) coconut shell wood vinegar, (d) bamboo wood vinegar and (e) *Eucalyptus* wood vinegar.

Table 1 – Physical–chemical properties and fungi growth area of pre-drying and dry NR sheets.

Properties	Coagulants				
	Formic acid	Acetic acid	Coconut shell wood vinegar	Bamboo wood vinegar	*Eucalyptus* wood vinegar
Pre-drying sheets					
Dirt content (%)	0.043 ± 0.006	0.050 ± 0.004	0.044 ± 0.004	0.043 ± 0.007	0.049 ± 0.003
Volatile content (%)	0.77 ± 0.04	0.80 ± 0.04	0.77 ± 0.04	0.79 ± 0.03	0.82 ± 0.04
PRI	95.4 ± 3.0	110.8 ± 1.7	111.0 ± 1.4	111.8 ± 1.2	110.0 ± 3.0
Mooney viscosity	50.3 ± 0.3	51.8 ± 0.4	52.3 ± 0.5	52.2 ± 0.6	52.9 ± 0.4
Dry sheets					
Dirt content (%)	0.031 ± 0.006	0.038 ± 0.004	0.036 ± 0.004	0.035 ± 0.004	0.035 ± 0.004
Volatile content (%)	0.43 ± 0.06	0.48 ± 0.05	0.42 ± 0.03	0.42 ± 0.04	0.47 ± 0.04
PRI	90.3 ± 4.2	104.6 ± 2.5	106.3 ± 1.3	106.3 ± 0.7	104.7 ± 2.5
Mooney viscosity	55.8 ± 1.0	59.2 ± 0.3	59.0 ± 0.3	59.6 ± 0.4	59.0 ± 0.2
Curing time (min.)	19.1 ± 2.0	14.8 ± 1.9	15.0 ± 0.6	14.8 ± 0.3	16.5 ± 1.8

Phenolic compound content in the wood vinegar was determined by using a Perkin-Elmer Lambda 25 UV–vis spectrophotometer at an adsorption band in the range of 268–273 nm [4].

The main fungus on the NR sheet surfaces was purified three times on Potato Dextrose Agar (PDA) before measurement. The antifungal efficiency of the coagulants against this fungus was measured by mixing the coagulant into the PDA. For this experiment, 2 mL of 2% (v/v) formic and acetic acids and 50% (v/v) wood vinegars were used. Fungus growth on the PDA was observed after incubating at 25 °C for a week.

2.5. *Statistical analysis*

The data were expressed as means and standard deviations (S.D.). Statistical analysis was performed using a one-way analysis of variance (one-way ANOVA). Comparisons of means between groups were performed by using the Duncan's test, and a p value of less than 0.05 was considered significant.

3. Results and discussion

3.1. *Physical and chemical properties of NR sheets*

FTIR spectra of the NR films prepared from different coagulants are shown in Fig. 1. The absorption bands at 835, 1374 and 1446 cm^{-1} can be assigned to $=CH$, CH_3 and CH_2 deformations for *cis*-1,4-polyisoprene, respectively according to the literature [13]. The NR, mainly consisting of *cis*-1,4-polyisoprene isolated from *H. brasiliensis* , shows these absorption bands [14]. The FTIR results suggest that the various NR coagulants prepare a NR very similar in structure and conformation.

Weights of the NR sheets obtained with each wood vinegar are not different with formic and acetic acids. The weights of pre-drying and dry NR sheets are in the ranges of 550–580 and 510–525 g, respectively.

The physical and chemical properties of various pre-drying and dry NR sheets are summarized in Table 1. All coagulants prepare NR sheets with similar dirt and volatile contents. Many ingredients in wood vinegars did not affect dirt nor volatile contents in the NR sheets.

The PRI and Mooney viscosity values of the NR sheets coagulated by all wood vinegars are similar to acetic acid. This is probably due to the fact that acetic acid is the major component in wood vinegar. However, these values of the NR sheets coagulated by wood vinegars were higher than those of sheets coagulated by formic acid. This may be explained that the NR latex usually experiences bacteria contamination [15] during the tapping and collecting processes, which damages the antioxidants through a protein degradation process. The NR molecules are therefore easily oxidized which results in PRI and Mooney viscosity values to be decreased. The results suggest that both acetic acid and wood vinegars may act as an antibacterial agent.

Before NR can be used as a product, the NR molecules have to be cross-linked together for increasing elasticity. Usually, NR products with 90% cross-linking are prepared. The time to prepare 90% cross-linking is called vulcanization or curing time (t_{90}). The t_{90} of various dry NR is not different, as shown in Table 1.

Table 2 – Mechanical characteristics of vulcanised NR sheets.

Properties	Coagulants				
	Formic acid	Acetic acid	Coconut shell wood vinegar	Bamboo wood vinegar	*Eucalyptus* wood vinegar
Tensile strength at break (MPa)	4.5 ± 0.7	6.0 ± 0.4	6.2 ± 0.3	6.3 ± 0.3	6.0 ± 0.4
Elongation at break (%)	625 ± 26	655 ± 30	682 ± 11	685 ± 9	686 ± 22
300% Modulus (MPa)	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1

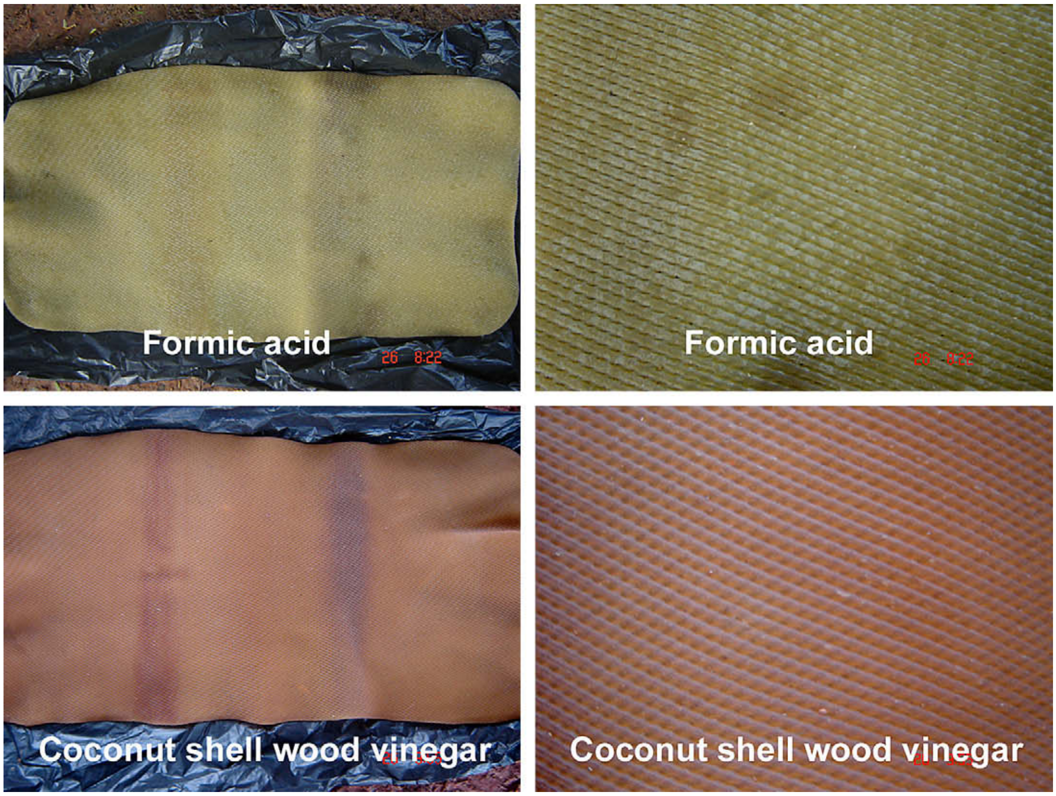

Fig. 2 – Pre-drying NR sheets (left) and its surfaces (right) coagulated by formic acid and coconut shell wood vinegar.

3.2. *Mechanical properties of vulcanized NR sheets*

Table 2 reports the mechanical properties of the vulcanized NR sheets. Tensile strength and percentage of elongation at break of the vulcanized NR sheets coagulated by acetic acid and wood vinegars show higher than formic acid, according to their PRI and Mooney viscosity of the dry NR sheets. However, the %elongation at break and the 300% modulus of all vulcanized NR sheets were not significantly different.

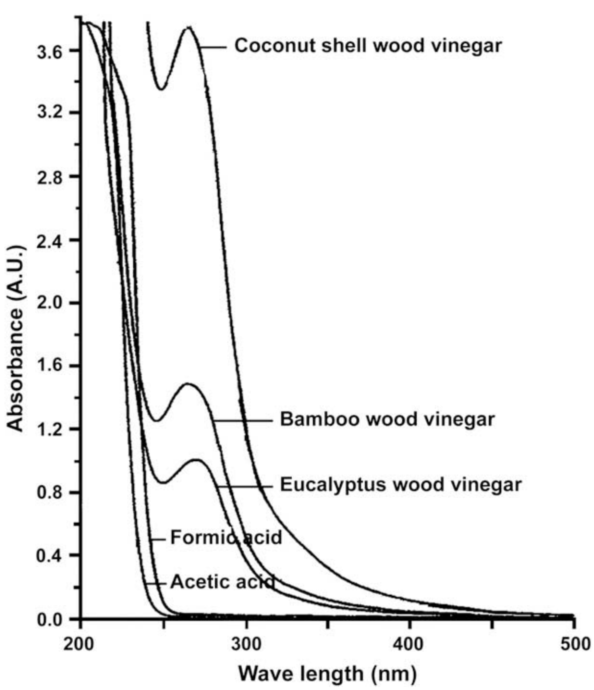

Fig. 3 – UV–vis spectra of formic acid, acetic acid and wood vinegars.

3.3. *Antifungal efficiency of coagulants*

The antifungal efficiency of coagulants was compared from a fungi growth area on the pre-drying NR sheet surface. The %fungi growth areas were calculated by Equation (1). It was found that the %fungi growth areas of pre-drying NR sheets prepared with formic and acetic acids were 100%, as example of which is shown in Fig. 2 (above). Whereas the wood vinegars can completely inhibit the fungi growth on the NR sheet surfaces [Fig. 2 (below)]. The results suggested that all wood vinegars had higher antifungal efficiency than both formic and acetic acids. However, when the formic acid and wood vinegar mixture was used to prepare pre-drying NR sheets, the %fungi growth areas were 4 ± 2, 16 ± 4 and $22 \pm 2\%$ for coconut shell, bamboo and *Eucalyptus* wood vinegars, respectively. Then it can be concluded that the antifungal efficiency of the wood vinegars is in the following order: coconut shell wood vinegar > bamboo wood vinegar ≈ *Eucalyptus* wood vinegar. The phenolic compound contained in the wood vinegar might exhibit some antifungal property [5,6]. The UV–vis spectra of the coagulants are shown in Fig. 3. The all wood vinegars contained phenolic compounds whereas formic and acetic acids did not. The phenolic compounds in wood vinegars are in order of coconut shell wood vinegar > bamboo wood vinegar > *Eucalyptus* wood vinegar correspond to their fungi growth

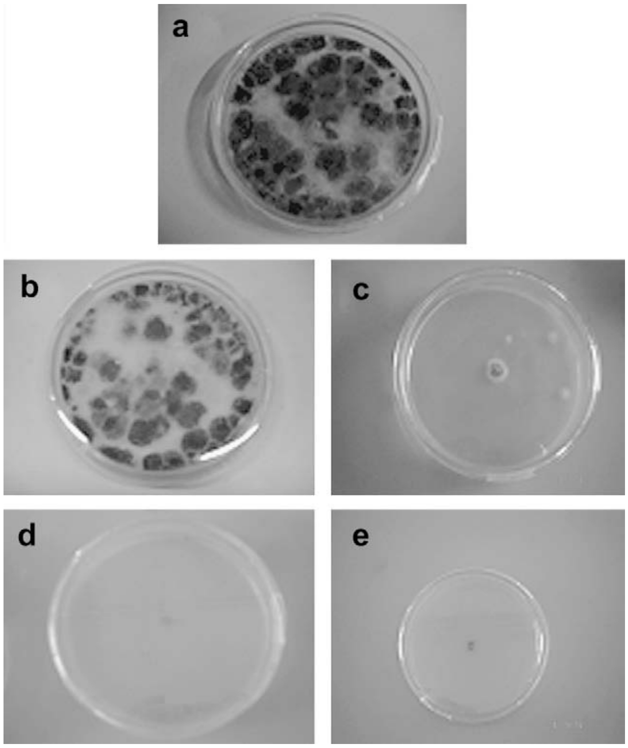

Fig. 4 – Growth of *Penicillium griseofuvum* fungus after incubation for 7 days on PDA mixed with (a) formic acid, (b) acetic acid, (c) *Eucalyptus* wood vinegar, (d) bamboo wood vinegar and (e) coconut shell wood vinegar.

inhibition property on the NR sheets. This indicates that the phenolic compounds in wood vinegar are antifungal agents.

Finally, the antifungal efficiency of the coagulants can be confirmed by inhibition of the main fungus growth on PDA substrate. The purified fungus grown on the pre-drying NR sheets has been determined as *Penicillium griseofulvum* [16]. Fig. 4 shows the fungus grown on PDA mixed with different coagulants after incubation at 25 °C for a week. All wood vinegars show higher antifungal efficiency than formic and acetic acids.

4. Conclusions

The results of this research show that all wood vinegars prepared from different resources can be used as coagulating and antifungal agents to replace formic and acetic acids. The coagulating efficiency of all wood vinegars was similar to acetic acid and better than formic acid. The wood vinegars showed higher antifungal efficiency than both formic and acetic acid. The antifungal property of wood vinegars are in order of coconut shell wood vinegar > bamboo wood vinegar > *Eucalyptus* wood vinegar according to their phenolic compound contents.

Since NR farmers can produce wood vinegar by themselves through charcoal production, it could also reduce the cost of NR sheet's production. Therefore, wood vinegars could become important factors in NR industry with ever rising costs of petrochemical products. This study also provided the first evidence that wood vinegars can inhibit the fungi growth on NR sheets which makes them an environmental friendly substitute for highly toxic antifungal agents.

From our experience with the wood vinegars, our recommendation to NR production is: substitute formic and acetic acids with wood vinegar.

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