Journal of Environmental Science and Technology

ISSN 1994-7887

science alert

ANSInet
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Journal of Environmental Science and Technology 1 (4): 157-163, 2008
ISSN 1994-7887

Utilization of Wood Vinegars as Sustainable Coagulating and Antifungal Agents in the Production of Natural Rubber Sheets

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Abstract: The coagulating and antifungal properties of *Eucalyptus globulus* wood vinegars (raw and tar-extracted types) in the production process of *Hevea brasiliensis* Natural Rubber (NR) sheets were investigated and compared with those of formic and acetic acids. It was found that plasticity retention index, Mooney viscosity, curing times (t_{90}) 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 wood vinegars, acetic acid and formic acid was determined from a fungi growth area on NR sheet surfaces. The antifungal efficiency of the coagulants was found in the following order: raw wood vinegar > tar-extracted wood vinegar > acetic acid > formic acid. The antifungal efficiency of the wood vinegars was confirmed through the inhibitory growth of the main fungus, *Penicillium griseofulvum*, on potato dextrose agar.

Key words: Wood vinegar, natural rubber, *Hevea brasiliensis*, coagulant, antifungal agent

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 surfaces of colloidal NR particles (Paiphansiri and Tangboriboonrat, 2005) is 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 material is 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. Wood vinegar composes of many chemical components with acetic acid as the main substance (Yatagai *et al*., 2002). 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 raw wood vinegar as a coagulant in NR sheets' preparation has been investigated and reported by Ferreira *et al*. (2005). However, they did not compare the properties of NR coagulated by raw and tar-extracted wood vinegars. In this study, we add 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 product. Therefore, antifungal agents need to be added during the NR production. Most commercial antifungal agents are highly toxic and not environmental friendly. An environmentally friendly antifungal agent

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with low toxicity is desired. Acetic acid and phenolic compounds in wood vinegar have been reported by Yatagai *et al.* (2002) and Mu *et al.* (2004). 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 (Nakayama *et al.*, 2001; Kartal *et al.*, 2004).

Therefore, the aim of this study is to examine the coagulating and antifungal properties of raw and tar-extracted *Eucalyptus globulus* wood vinegars on the production process of NR and to compare them with those of commercial formic and acetic acids.

MATERIALS AND METHODS

Materials

Raw and tar-extracted wood vinegars of *Eucalyptus globulus* were kindly provided by Kiengmool Group Ltd. (Thailand) and used without further purification. The pH values of raw and tar-extracted wood vinegars are 3.2 and 3.4, respectively. Both wood vinegars are reddish brown in color. 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 *Hevea 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 NR latex were 30.7±0.7% and 28.4±0.2% (w/w), respectively.

Methods

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 + 3,000 mL water). For formic and acetic acids, 300 mL 2% (v/v) were used. In the case of raw and tar-extracted wood vinegars, 300 mL 2% (v/v), 150 mL undiluted and 300 mL 50% (v/v) were tested for preparing the NR sheets. The mixture 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 for a week. The product obtained from this process is called pre-drying NR sheet and is further processed by drying at 50-60°C for 2 days to be the dry NR sheets.

Characterization of NR Sheets

FT-IR spectra were obtained from NR films by using a Perkin-Elmer Spectrum GX FT-IR 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 (32 scans and resolution of 4 cm^{-1}). Dirt content (Bulletin No. 7-1992 Part B.4), volatile content (Bulletin No. 7-1992 Part B.5), plasticity retention index (PRI, Bulletin No. 7-1992 Part B.8) and Mooney viscosity (Bulletin No. 7-1992 Part B.9) 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 No. 3417 of the International Standardization Organization (ISO). Mechanical properties of vulcanized NR were determined by tensile testing (ISO 37 type1).

Antifungal Efficiency of Coagulants

The antifungal efficiency of the coagulants was studied 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.

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

Where:

A_{NR} = Total area of NR sheet surface (cm^2)

A_{fungi} = Fungi growth area (cm^2)

The phenolic compound content in the wood vinegar was determined by UV-Vis spectrophotoscopy at maximum absorption peak (λ_{max}) in the range of 268-273 nm as described by Mu *et al.* (2004).

The main fungus on the NR sheet surface was purified three times on Potato Dextrose Agar (PDA) before determination. The antifungal efficiency of the coagulants against the main 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) raw and tar-extracted wood vinegars were chosen. As a control, 2 mL distilled water were used. Fungus growth on the PDA was observed after incubating at 25°C for a week.

RESULTS AND DISCUSSION

Volume and Concentration Testing

During the NR latex coagulation process, latex completely coagulates within 2 h when using formic or acetic acids [300 mL 2% (v/v)]. When using raw or tar-extracted wood vinegars in the same volume and concentration, however, the diluted latex does not completely coagulate within 2 h, which could be due to wood vinegar weaker acidity compared to formic and acetic acids. This coagulation process also produces a strong odour, which might be connected to bacteria activity in the NR latex (John, 1974). When using 150 mL undiluted wood vinegar, on the other hand, complete coagulation within 2 h was obtained, due to its high content of acetic acid (Yatagai *et al*., 2002). However, the color of the obtained pre-drying NR sheet is inconsistent as shown in Fig. 1a. This indicates that the wood vinegar is not well distributed in the diluted NR latex during the coagulation process. A possible cause

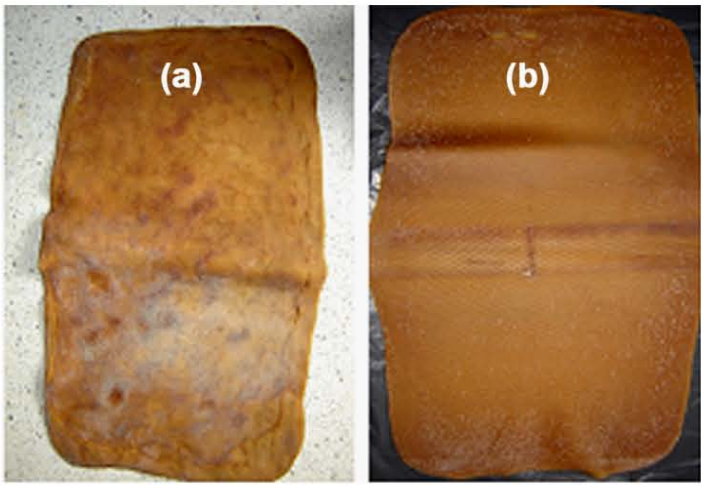

Fig. 1: Pre-drying NR sheets coagulated using (a) 150 mL undiluted and (b) 300 mL 50% (v/v) raw wood vinegars

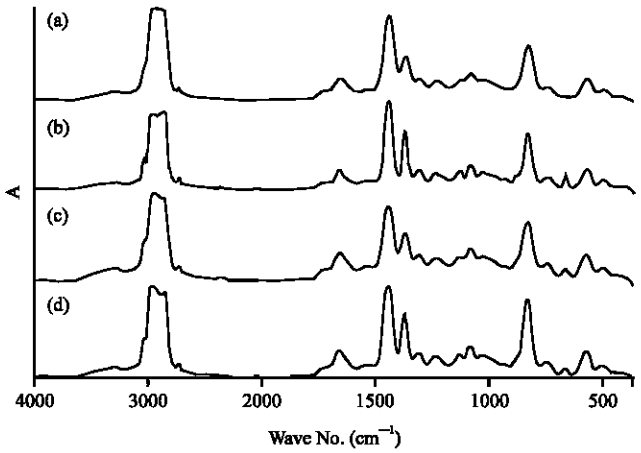

Fig. 2: FT-IR spectra of NR films coagulated using (a) formic acid, (b) acetic acid, (c) raw wood vinegar and (d) tar-extracted wood vinegar

could be traces of organic compounds such as n-butanoic acid and phenolic compounds in the wood vinegar (Mu *et al*., 2004). The inconsistent color of the NR sheet probably shows inhomogeneous physical and chemical properties.

When using 300 mL 50% (v/v) wood vinegar as a coagulant instead of 150 mL undiluted wood vinegar, the obtained pre-drying NR sheet shows more consistent color (Fig. 1b). Therefore, the 300 mL 50% (v/v) wood vinegar was used in further work for preparing the NR sheets.

Physical and Chemical Properties of NR Sheets

All spectra are similar, exhibiting the absorption bands at 837 cm^{-1}. This band can be assigned to C = CH out-of-plane deformation for *cis*-1,4-polyisoprene. The absorption bands at 1376 and 1480 cm^{-1} can be assigned to CH_3 and CH_2 deformations, respectively (Fig. 2). According to Santos *et al*. (2005), the absorption bands of CH_2, CH_3 and = CH stretching can be observed at 2928, 2962 and 3036 cm^{-1}, respectively. Natural rubber, mainly consisting of *cis*-1,4-polyisoprene isolated from *Hevea brasiliensis*, shows these absorption bands (Marinho and Monterio, 2000). The FT-IR results suggest that the various NR coagulants prepare a NR very similar in structure and conformation.

Weights of NR sheets obtained with wood vinegars are not different with formic and acetic acids. Weights of pre-drying and dry NR sheets are in the ranges of 560-580 and 510-520 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 raw and tar-extracted 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 both 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 both wood vinegars were slightly higher than those of sheets coagulated by formic acid. During the tapping and collecting processes, NR latex usually experiences bacteria contamination (John, 1974), 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 the wood vinegar may act as an antibacterial agent.

Table 1: Physical-chemical characteristics of pre-drying and dry NR sheets (analysis of 3 NR sheet samples)

	Coagulants			
NR sheets	Formic acid	Acetic acid	Raw wood vinegar	Tar-extracted wood vinegar
Pre-drying				
Dirt content (% w/w)	0.043 (0.007)	0.050 (0.006)	0.047 (0.002)	0.065 (0.016)
Volatile content (% w/w)	0.77 (0.03)	0.80 (0.02)	0.82 (0.01)	0.82 (0.01)
PRI	95.40 (3.50)	110.80 (1.00)	108.90 (4.40)	110.00 (1.00)
Mooney viscosity	50.30 (0.10)	51.80 (0.20)	52.00 (0.50)	52.90 (0.40)
Fungi growth area (%)[a]	100.00 (5.00)	64.00 (14.0)	10.00 (4.00)	17.00 (5.00)
Dry				
Dirt content (% w/w)	0.031 (0.005)	0.038 (0.007)	0.020 (0.005)	0.050 (0.004)
Volatile content (% w/w)	0.43 (0.02)	0.48 (0.05)	0.46 (0.07)	0.47 (0.06)
PRI	90.30 (2.00)	104.60 (1.50)	95.40 (3.20)	104.70 (2.50)
Mooney viscosity	55.80 (0.50)	58.30 (0.80)	57.10 (0.70)	57.30 (0.50)
Curing time (min)	19.08 (1.66)	14.83 (2.05)	15.46 (2.10)	16.50 (1.53)

Values in parentheses are standard deviations, [a]Calculated from Eq. 1

Table 2: Mechanical characteristics of vulcanized NR (analysis of 3 NR sheet samples)

Coagulants	Tensile strength (MPa)	Elongation at break (%)	300% modulus (MPa)
Formic acid	4.5 (0.5)	625 (23)	1.0 (0.1)
Acetic acid	6.0 (0.3)	655 (32)	1.0 (0.1)
Raw wood vinegar	7.2 (0.5)	781 (10)	1.0 (0.1)
Tar-extracted wood vinegar	6.1 (0.2)	686 (12)	1.0 (0.1)

Values in parentheses are standard deviations

Before NR can be used as a product, the NR molecules have to be cross-linked together for increased 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}). Table 1 shows that the t_{90} of various dry NR are in the following order: acetic acid < raw wood vinegar < tar-extracted wood vinegar < formic acid. This suggests that both wood vinegars are better coagulant than formic acid because of their lower curing time.

Mechanical Properties of Vulcanized NR Sheets

According to their PRI and the Mooney viscosity of the dry NR sheets, tensile strength and percent elongation at break of the vulcanized NR sheets show the following order: raw wood vinegar > tar-extracted wood vinegar > acetic acid > formic acid. However, the 300% modulus of all vulcanized NR sheets was not significantly different (Table 2).

Antifungal Efficiency of Coagulants

The antifungal efficiency of coagulants was compared from a fungi growth area on the pre-drying NR sheet surface. The percent fungi growth areas were calculated by Eq. 1 and the calculated data are also presented in Table 1. The antifungal efficiency of the coagulants is in the following order: raw wood vinegar > tar-extracted wood vinegar > acetic acid > formic acid. The acetic acid and phenolic compound contained in the wood vinegar might exhibit some antifungal property (Nakayama *et al*., 2001; Kartal *et al*., 2004). The UV-Vis spectra of the coagulants are shown in Fig. 3. Both raw and tar-extracted wood vinegars contain phenolic compounds whereas formic and acetic acids do not. The phenolic compounds in raw wood vinegar are more than in the tar-extracted type. It is possible that some phenolic compounds have been removed during the tar-extraction process. The content of phenolic compounds in the coagulants shown in the UV-Vis spectra in Fig. 3 corresponds to their fungi growth inhibition property (Table 1). This indicates that the synergy of acetic acid and phenolic compounds in wood vinegar improves fungi growth inhibition.

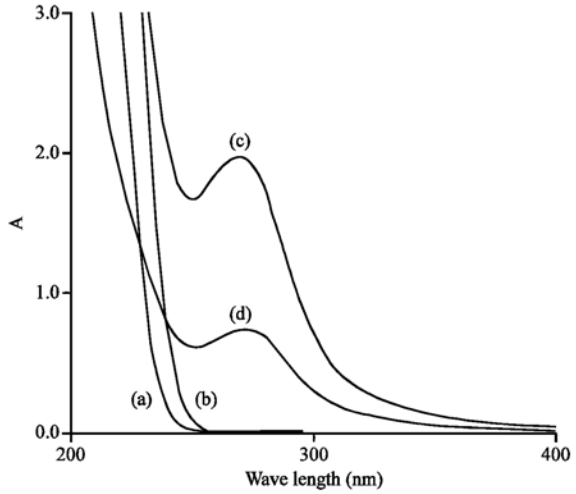

Fig. 3: UV-Vis spectra of (a) formic acid, (b) acetic acid, (c) raw wood vinegar and (d) tar-extracted wood vinegar [0.5% (v/v) aqueous solution]

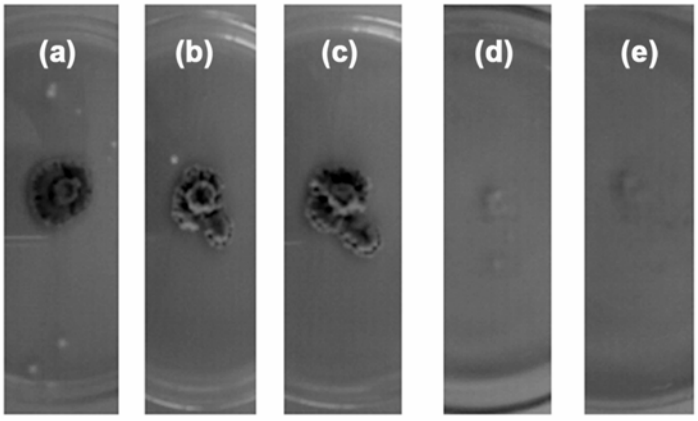

Fig. 4: Growth of *Penicillium griseofuvum* on PDA mixed with (a) distilled water, (b) formic acid, (c) acetic acid, (d) raw wood vinegar and (e) tar-extracted wood vinegar (incubation for a week at 25°C)

Finally, the antifungal efficiency of the coagulants can be confirmed by inhibition of main fungus growth on PDA substrate. The purified main fungus grown on the pre-drying NR sheets has been determined as *Penicillium griseofulvum* (Barnett and Hunter, 1987). Figure 4 shows this fungus grown on PDA mixed with different coagulants after incubation at 25°C for a week. Both wood vinegars show higher antifungal efficiency than formic and acetic acids.

CONCLUSIONS

The results of this study show that both raw and tar-extracted wood vinegars prepared from *Eucalyptus globulus* can be used as coagulating and antifungal agents to replace formic and acetic acids with raw wood vinegar being more efficient than tar-extracted wood vinegar.

Since NR farmers can produce wood vinegar by themselves through charcoal production, it could also reduce the cost of NR sheets' 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 both wood vinegars, our recommendation to NR production is: substitute formic and acetic acids with raw wood vinegar.

ACKNOWLEDGMENTS

This research was funded by the Small Project on Rubber (SPR), Nation Research Project on Rubber, the Thailand Research Fund (grant No. RDG4850058) and the Center for Innovation in Chemistry: Postgraduate Education and Research Program in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education, Thailand. The authors would like to thank Dr. Rungruedee Thiwthong, Department of Biology, Faculty of Science, Mahasarakham University for her suggestions on fungus culture.

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