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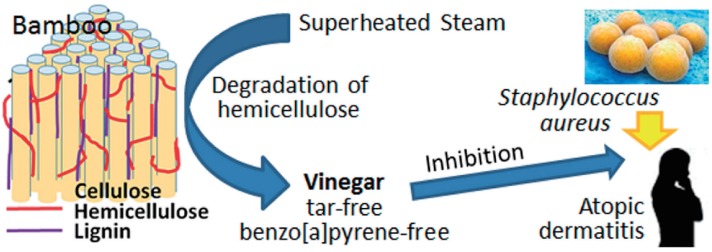

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Wood and bamboo vinegars are liquors with strong smoke flavors that have been obtained as a by-product during the traditional carbonization process of wood and bamboo. These vinegars are composed of water and organic compounds such as acetic acid, propionic acid, furfural, 2-cyclopentenone, and 2-hydroxy-3-methyl-2-cyclopentenone.[1] So far, the vinegars have been conventionally used for treatment of atopic dermatitis without any scientific basis.

Very recently, Kobayashi et al.[2] reported that Adam17fl/flSox9-Cre mice developed eczematous dermatitis with naturally occurring dysbiosis, similar to that observed in atopic dermatitis. *Staphylococcus aureus* was found to prominently drive eczema formation, and Langerhans cells were required for eliciting immune responses against *S. aureus* inoculation. With this finding, the wood and bamboo vinegars may get a potential scientific basis to be used for eczema treatment.

Moreover, the vinegars have been used as substances to stimulate plant growth, accelerate the speed of plant seed germination, and serve as herbicides in agriculture.[1] Nowadays, these favorable medical and environmental effects of the vinegars have attracted much attention.

On the other hand, the traditional vinegars include unfavorable components; for example, tar as a main product of carbonization which primarily composes phenolics[3] that have attracted attention due to the harmful influence on soil and crops when sprayed onto plants. The tar has been reported to include benzo[*a*]pyrene, a well-known carcinogen. In order to avoid the carcinogenic effect, the vinegars are used after removal of tar components through some refining processes such as stationing, filtration, and evaporation. However, the stationing requires a long time for refining.

Steam explosion, a hydrothermal method using pressurized steam has been employed for controlled conversion of biomass to useful compounds.[4–6] In this study, hydrothermal treatment using superheated steam (SHS) controlled at low temperature range (210–230 °C) was employed to obtain tar-free and benzo[*a*]pyrene-free vinegar from Moso bamboo (Figure 1). Moreover, its selective antibacterial activity against *S. aureus* concerned with the atopic dermatitis and *Bacillus cereus* well known to cause food poisoning in humans was confirmed.

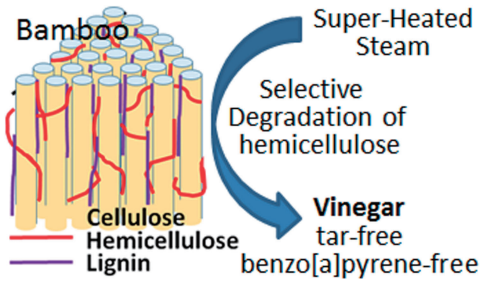

Figure 1. Bamboo vinegar preparation using superheated steam.

Phyllostachys heterocycla f. pubescens (Moso bamboo) (diameter: 10–20 cm, height: 10–20 m) was collected from Yame city in Japan. The bamboo was cut to 40 cm length and treated with superheated steam (SHS) at 210, 220, and 230 °C and a constant flow rate of 6 kg h^{-1} for 5 h in a SHS oven model NHL-1 (Naomoto Corp., Japan; inner dimensions: W 590 × D 385 × H 555 mm^3) with an internal fan for agitation of atmosphere. Discharged steam, which included selectively hydrolyzed products, was cold-condensed and collected as a bamboo hydrolyzed and steam distilled solution (BHS). The BHS solution was collected at hourly intervals.

Main components in the obtained BHS were analyzed with a Shimadzu high-performance liquid chromatograph (HPLC) LC-10A equipped with a UV detector SPD-10A VP. The chromatography was conducted using a hybrid silica-based ODS column YMC-Triart C18 (particle size 3 µm, pore size 12 nm) and a phosphate buffer (20 mM) solution eluent at a flow rate of 0.425 mL min^{-1} at 37 °C. A BHS sample was diluted 10 times with distilled water and a 10 µL portion of the diluted sample was injected. Calibration curves for main components of hydrolyzed products were prepared by using standard chemicals obtained from Wako Pure Chemical Industries, Ltd. and Tokyo Chemical Industry Co., Ltd.

Polycyclic aromatic hydrocarbons (PAHs): benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, and chrysene were quantitatively analyzed with the same HPLC. The chromatography was conducted using a general-purpose silica-based column Shim-pack VP-ODS column (particle size 5 µm, 150 mm × 4.6 mm i. d.) and a acetonitrile/phosphate buffer (10 mM) 8:2 (v/v) solution eluent in a flow rate of 0.8 mL min^{-1} at 37 °C. A 25 µL portion of BHS sample was injected. Calibration curves for PAHs were also prepared by using standard chemicals obtained from Wako Pure Chemical Industries, Ltd. and Tokyo Chemical Industry Co., Ltd. PHAs contents were also redetermined with a high-resolution gas chromatograph/high-resolution mass spectrometer at Shimadzu Techno-Research, Inc.

A typical BHS sample, which was collected by SHS treatment of Moso bamboo at 210 °C for 3 h, was used for examining its antibacterial properties. Four kinds of bacteria: *S. aureus*, *Escherichia coli*, *B. subtilis*, and *B. cereus*, which are well known as indigenous bacteria in natural environments and the human body, were employed for inhibitory tests of the BHS sample. These bacteria were obtained from the Department of Microbiology, Universiti Putra Malaysia.

All bacteria were cultured in nutrient broth (NB) and incubated in an incubator shaker (150 rpm) for 18 h at 30 °C for *B. cereus* and *B. subtilis* and 37 °C for *E. coli* and *S. aureus*. For inhibitory test of BHS sample against these bacteria, a 150 µL of the bacterial culture broth ($OD_{600} \approx 0.5$) was transferred to a nutrient agar (NA) plate. The inoculant was then spread on the NA by using hockey stick. A sterile 5 mm in diameter filter paper was used in order to determine the inhibition zone around the BHS sample on the NA plate. Sterile filter paper was immersed in the BHS sample for about 30 s before being placed at the center of the NA plate. Another sterile filter paper without prior immersion in the BHS sample was used as control. The test plate was then incubated in an incubator at 30 °C for *B. cereus* and *B. subtilis* and 37 °C for *E. coli* and *S. aureus*, respectively. Inhibitory zone formation around the filter paper was examined after 24 h incubation time.

Moso bamboo was hydrolyzed by SHS in a low temperature range of 210–230 °C to preferentially degrade hemicellulose component. The main reason for selection of the temperature range was because the molecular weight of cellulose crystalline parts remained constant within the temperature range.[7] Recovered BHSs were transparent and yellowish solutions without any tar droplets. Component composition of BHS samples, in particular, PAHs contents were quantitatively analyzed with a HPLC equipped the appropriated columns for the components.

A typical HPLC profile measured with YMC-Triart C18 column of BHS sample, which was recovered at 210 °C during 1–2 h of treatment time, was depicted in Figure 2. Many peaks were detected in the profile. Main peaks were characterized as organic acids and furan derivatives.

Quantitative analysis of the HPLC peaks was achieved by preparing calibration curves for the main components and comparing the peak areas, resulting in time courses of degradation products at each temperature as shown in Figure 3. The main components based on the quantitative evaluation were

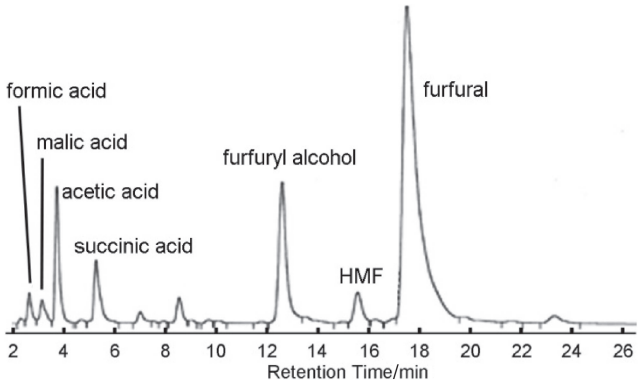

Figure 2. HPLC profile of BHS sample recovered at 210 °C during 1–2 h of steaming time.

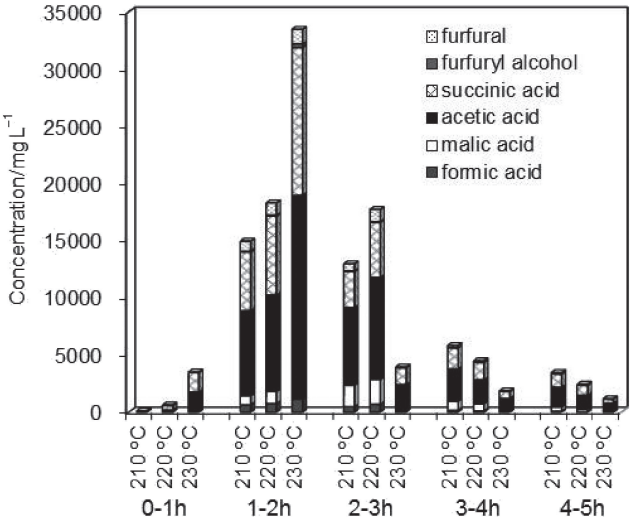

Figure 3. Time courses of main components composition in BHS recovered at 210, 220, and 230 °C.

Table 1. HPLC analysis of PAHs in BHS sample recovered at 210 °C for 3 h

PAHs	Detection limit /ppb	Peak intensity
Benzo[*a*]pyrene	50	N. D.
Benzo[*a*]anthracene	50	N. D.
Benzo[*b*]fluoranthene	50	N. D.
Chrysene	50	N. D.

acetic acid, succinic acid, furfural, malic acid, and formic acid. Some amounts of phenolics such as phenol and guaiacol were detected as noticeable small peaks, however, no tar formation was observed.

At all the temperatures, the maximum recovery of BHS was achieved during 1–2 h of the SHS treatment. Acetic acid was detected as the major component at all temperature ranges for treatment time of 1–5 h. Interestingly, production of succinic acid and malic acid as main components was found. It is a specific feature of SHS-treated products, because to the best of our knowledge only a few papers have been reported for the production of both acids from oxidative hydrolysis/pyrolysis of biomass.[8,9] Therefore, it is suggested that there is some contribution of oxygen during the SHS treatment under normal pressure.

PAHs: benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, and chrysene in a BHS sample recovered at 210 °C during the treatment of 0–3 h were analyzed with the Shim-pack VP-ODS column. Results are listed in Table 1. All the PAHs were undetected in the BHS sample, meaning the values were under detection limit (50 ppb) or less. This is due to the hydrolysis of biomass at lower temperatures in comparison with general pyrolysis conditions at higher temperatures.[10] The transparency of obtained BHS reflects the absence of tar and PAHs, meaning the relative safety of BHS towards the human body and environment compared to the pyrolysis vinegars at higher temperatures including PAHs.

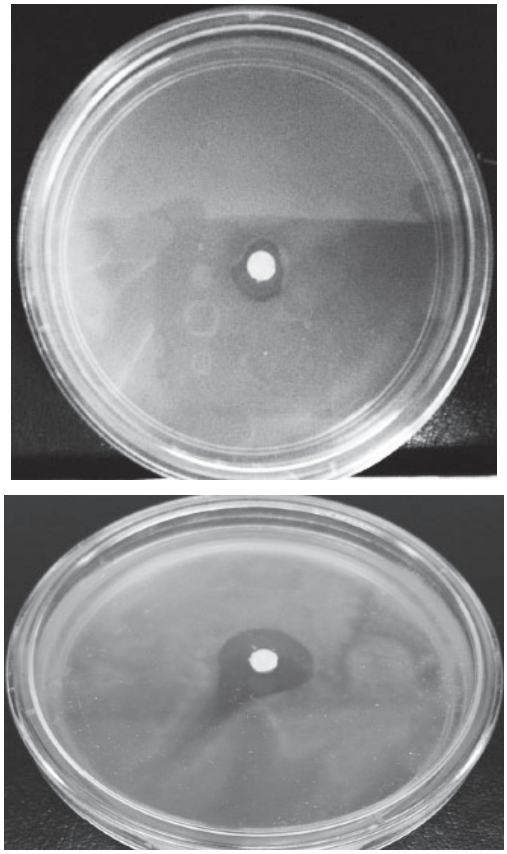

Figure 4. Growth inhibitory tests of *S. aureus* and *B. cereus* around BHS impregnated paper on NA plates.

Antibacterial activity of BHS was examined by an inhibitory test of bacteria on NA plates. Filter papers impregnated with the BHS, which was collected by SHS treatment of Moso bamboo at 210 °C for 3 h, were placed at the center of NA plates inoculated with the four kinds of bacteria. Results are shown in Figure 4. Clear inhibitory zones were created around the filter papers on NA plates inoculated with *S. aureus* and *B. cereus*. It was suggested that the inhibition effect depended on the concentration of BHS, because the inhibitory zone became clearer when the filter paper was impregnated with a BHS concentrated to double. Both *S. aureus* and *B. cereus* are well known to cause food poisoning in humans. Furthermore, *S. aureus* has been reported to be strongly involved in atopic dermatitis.[2] Tanaka et al.[11] reported that Moso bamboo shoot (sprout) skin and its dichloromethane extract had antibacterial activity against *S. aureus*. However, no information about the antibacterial components in the extract has been reported.

On the other hand, no inhibitory zone was found on the plates inoculated with *E. coli* and *B. subtilis*, meaning no antibacterial effect of BHS against the bacteria, which are indigenous bacteria in natural environments. This suggests the safety of BHS when it is used in the environment.

Yamamoto et al.[12] suggested the inhibitory activity of organic acids on food spoilage bacteria in connection with the rate of undissociated molecules of the organic acids, which are related to cell membrane permeability. In the report, they found the effective antibacterial activity of some organic acids: acetic acid, propionic acid, succinic acid, formic acid, and lactic acid, which are main components of BHS. However, these organic acids showed activity not only against Gram positive bacteria: *B. subtilis*, *B. cereus*, and *S. aureus*, but also against Gram negative bacteria: *E. coli*, *Pseudomonas fluorescens*, and *Salmonella typhimurium*. Therefore, the selective inhibitory activity of BHS may depend on a complex action of BHS components including organic acids.

In conclusions, Moso bamboo was treated by the hydrothermal method with SHS at controlled temperatures to obtain the tar-free and benzo[*a*]pyrene-free transparent BHS. Selective antibacterial activities against *S. aureus* and *B. cereus* were confirmed, suggesting an effect on atopic dermatitis and the food poisoning in humans, while no effect was found against *E. coli* and *B. subtilis* as being indigenous bacteria in natural environments. These features of BHS: safety and selective antibacterial property may make it possible to be utilized more effectively. Just now, the study of inhibitory effect against some pathogenic microorganisms and viruses is ongoing. Results will be reported elsewhere.

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References and Notes

1 J. Mu, T. Uehara, T. Furuno, *J. Wood Sci.* **2004**, *50*, 470.
2 T. Kobayashi, M. Glatz, K. Horiuchi, H. Kawasaki, H. Akiyama, D. H. Kaplan, H. H. Kong, M. Amagai, K. Nagao, *Immunity* **2015**, *42*, 756.
3 S. P. Mun, C. S. Ku, *J. Wood Sci.* **2010**, *56*, 47.
4 C. Asada, Y. Nakamura, F. Kobayashi, *Biochem. Eng. J.* **2005**, *23*, 131.
5 S. Shao, G. Wen, Z. Jin, *Wood Sci. Technol.* **2008**, *42*, 439.
6 H. de Lasa, E. Salaices, J. Mazumder, R. Lucky, *Chem. Rev.* **2011**, *111*, 5404.
7 K. Yamashiro, H. Nishida, *Int. J. For. Res.*, submitted.
8 B. D. Schutt, B. Serrano, R. L. Cerro, M. A. Abraham, *Biomass Bioenergy* **2002**, *22*, 365.
9 I. Hasegawa, Y. Inoue, Y. Muranaka, T. Yasukawa, K. Mae, *Energy Fuels* **2011**, *25*, 791.
10 D. C. Elliott, in *Pyrolysis Oils from Biomass: Producing, Analyzing, and Upgrading* in *Acs Symposium Series*, **1988**, Vol. 376, Chap. 6, pp. 55–65. doi:10.1021/bk-1988-0376.ch006.
11 A. Tanaka, H. J. Kim, S. Oda, K. Shimizu, R. Kondo, *J. Wood Sci.* **2011**, *57*, 542.
12 Y. Yamamoto, K. Higashi, H. Yoshii, *Nippon Shokuhin Kogyo Gakkaishi* **1984**, *31*, 525.