Chapter 4

Preliminary Safety Evaluation of Bamboo Pyrolysis Products: Charcoal and Vinegar

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Additional information is available at the end of the chapter

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Abstract

The bamboo charcoal is manufactured in earth kilns with the temperature at 700–800°C from Moso bamboo (*Phyllostachys heterocycla*). Bamboo vinegars, by-products of the charcoal, are collected from the chimney outflow of earthen kiln at six different temperatures at 80–150°C, and with categories over 80, 90–92, 99–102, 120–123, and 145–150°C during the pyrolysis of the charcoal. The preliminary safety evaluation using the Ames test for the bamboo charcoals has no cytotoxicity and mutagenicity toward *Salmonella typhimurium* TA98 and TA100 with S9 mix and without S9 mix. This suggests that the charcoal can not only be considered to be a safe pigment for food but also be used as a natural moisture-proof material. The safety of the bamboo vinegars shows that neither cytotoxicity nor mutagenicity toward *S. typhimurium* TA98 and TA100 with S9 mix at the diluting percent content of vinegars is lower than 20.00% or less and without S9 mix is at 33.33% or less, and the reverse mutation assay (antimutagenic activity) denotes that the vinegars express this dose-dependent inhibitory effect against both 4-nitroquinoline-*N*-oxide and aflatoxin B_1 in *S. typhimurium* TA98 and TA100. The main compounds in the vinegars may partially account for the safety evaluation of biological action.

Keywords: pyrolysis, bamboo charcoal, bamboo vinegar, Ames test, mutagenicity

1. Introduction

Bamboo is regarded as a natural and renewable bioresource, a green and environmentally friendly material, and has chemical and physical qualities/properties similar to wood. It quickly reaches its full potential size, which usually only takes approximately 60–70 days after the bamboo culms emerge from the ground [1]. According to the annual statistics of the agricultural reports in Taiwan (2015), the volume of diverse bamboos is about 1,581,330,000 pieces. The three main types of bamboo used include *Phyllostachys makinoi* Hayata (Makino

bamboo), *Phyllostachys heterocycla* Milf (Moso bamboo), and *Dendrocalamus latiflorus* Munro (Ma bamboo) [2]. This indicates that bamboo is one of the economic green resources in Taiwan. Among these bamboos, Moso bamboo has been mainly used as a material for the making of furniture, handicrafts, and athletic/leisure goods, because it can grow over 20 m tall and 60–150 mm in diameter in one growing season [3]. Moreover, bamboo is accumulated from organic compounds from which a bamboo converts carbon dioxide (CO_2) by photosynthesis, but it can naturally decay by organic compound oxidization or be burned, and this produces CO_2 that returns to the atmosphere. To decrease bamboo decaying or burning, the preparation of charcoal becomes one of the selected methods because carbon can extend the lifetime of bamboo [4]. In other words, the charcoals can also become earth friendly materials because they can slow down the increase of CO_2 concentration in the atmosphere.

Bamboo and its products show good prospects for commercial applications, when considering the need for the protection of our wood resources and environmental balance. It is therefore important to study the characteristics of bamboo and its by-products in order to make good use of them. Domestic and foreign manufacturers and researchers have invested a great deal of money, labor and time to discover the characteristics and functions of charcoal in recent years. Bamboo charcoals are mainly derived from 4-year-old or older bamboo as raw materials [4–7]. The utilization of bamboo charcoal, conventionally regarded as fuel, is widely applied to daily life or/and industry. The use of bamboo charcoal is more wide and diverse because it is a porous material with a high specific surface area that has lots of functions, such as indoor deodorization, humidity control, water quality improvement, air purification and so on [5, 8, 9]. Recently, there has been a tendency to maintain good health from food products. Some food producers have added charcoal materials into food products, for example, charcoal bread/cookies, charcoal peanuts, charcoal ice cream, etc. This is advertised as being able to absorb unclean substances, such as heavy metal elements, and producers have exaggerated that these materials can clean the intestines and stomach after eating. However, in May of 2006, the Department of Health's Executive Yuan, Taiwan, announces that the charcoals can only be used as colorants of food without any medical and health effects, that is, as a natural black pigment only [10]. The charcoals can be added as a pigment in food, but it is a profound question whether or not residue *in vivo* causes any harm by inducing cell lesions or carcinogens.

The application of bamboo vinegar (brown-red transparent liquids), even the compounds that are complex and different, is mainly able to be divided into three main portions: acid, phenol, and neutral compounds [11]. The vinegar consists of 80–200 compounds: 32% organic acid, 40% phenolic compound, 3% aldehyde, 5% alkone compound, 5% alcohol compound, 4% ester compound, and 5% others. When bamboo vinegar is dehydrated, there is usually 80% water [12–15]. The organic compounds in bamboo vinegars may have practical applications even when present in only trace quantities [14, 16], such as in improving soil, promoting crops and preventing worm growth, as well as reducing agricultural chemicals, compost odor and sterilization [12]. Recently, bamboo vinegars have been developed that are beneficial for promoting growth of plants to as a plant root growth promoter or a pH value adjuster of cultural media [17–19]. It is also effective when used against allergies [20], in healthy drinks [13, 21, 22], as a virus/fungi/bacterial resistant [16, 23–27] and as an agent of antioxidation, especially for a resistant lipid oxidation effect [15]. As stated in the above references, the commercial production of bamboo vinegar is being increased and highly valued for its diverse effective uses

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in Taiwan. However, bamboo vinegars collect at the exit of chimney of earthen or furnace kiln, when the carbonization temperature of bamboo is raised to over 500°C, produce some carcinogenic polycyclic aromatic hydrocarbons (PAHs), such as naphthalene and phenanthrene [28–30]. The concentrations of these toxics increase with the increase in temperature as well. Even though the council of agriculture in Taiwan has submitted certified agricultural standards of forest products (2004) to prove that it is necessary for bamboo vinegars to be collected at 80–150°C at an exit of chimney of earthen kiln and below 350°C for a furnace kiln [31], the collected bamboo vinegars from above this range of temperatures are necessary to evaluate the potential of mutagenic and carcinogenic agents, due to the fact that they are omnipresent in the human environment and seem impossible to completely eliminate.

Ames *et al*. [32] reports that for screening of environmental mutagens and carcinogens, the Ames test (safety evaluation), a convenient method to evaluate mutagenic activities of these chemicals, has been developed, and McCann *et al*. and Sugimura *et al*. have suggested that the mutagenic activities of a number of chemicals correlate well with the carcinogenic activities [33, 34]. The main goal of this chapter is to realize fundamental knowledge of the bamboo charcoal/vinegars as functional additives, while at the same time understanding the preliminary safety evaluation of both using the bacterial mutation assay on *Salmonella typhimurium* (*S. typhimurium*) TA98 and TA100 strains with and without an extrinsic metabolic activation system. Moreover, because the reverse mutation assay (antimutagenic activity) has an array of prospective applications in human care, such as the increasing application in drinks and food antioxidation, and has not been reported for the antimutagenic activities of bamboo vinegars that have been made so far, the antimutagenic activity of the vinegars is reported. Besides, the basic compounds of vinegars, collected at different temperatures from the exit of earthen kiln chimney and analyzed by using gas chromatography-mass spectroscopy, are considered, in order to realized the effect of bamboo vinegars' compounds on preliminary safety evaluation.

2. Ames test for bamboo charcoal and vinegars

The preliminary safety evaluation, including cytotoxicity and mutagenicity, is performed in accordance with the Ames test [32], a widely used convenient and short-term assay with predictable accuracy for carcinogen up to 72–91% [35]. Referring to the Ames test, as proposed by Ames *et al*. [32], Waleh *et al*. [36], which indicates that if the sample is toxic for the strain, the bacterial count decreases, and the test result is likely to be misjudged. Therefore, the specimen cytotoxicity has to be tested before the mutagenicity test, in order to evaluate whether the growth of the strain is influenced. The mutagenicity can be implemented if there is no toxicity [32, 36]. In this report, the *S. typhimurium* TA98 and TA100 are used as test strains. TA98 is the strain sensitive to frame shifting mutation, as caused by specific mutagens; TA100 is the strain for testing base substitution variation [37].

2.1. Cytotoxicity

The methods of the cytotoxicity for the bamboo charcoal and vinegars are taken 1.0, 2.5, 5.0, 7.5, and 10.0 mg of bamboo charcoal, as well as 0.1 mL of bamboo vinegars, collected at different

temperatures (80–150, over 80, 90–92, 99–102, 120–123, and 145–150°C), from the exit of chimney of earthen kiln are diluted to a percent content of 50, 33.33, 25, 20, 13.33, and 10%, respectively. Both of them are examined with *S. typhimurium* TA98 and TA100 for either S9 (+S9) or zero S9 (-S9) in accordance with the Ames test and the experimental procedure referred to [32, 37–39]. The colony count is calculated; if the bacterial count of the test group (+S9 or -S9) is larger than the bacterial count of the control group (no bamboo charcoal/vinegars) by 80% (the bacterial count rate, Survival), there is no toxicity [35]. The Survival (%) is the residual bacteria rate that is the percentage relative to the control (100%). The formula of the Survival of cytotoxicity is: Survival (%) = (the bacterial count of test group/the bacterial count of control group) × 100.

2.2. Mutagenicity

The mutagenicity is analyzed by using the method proposed by Maron and Ames [40]. The test charcoal and vinegars for this mutagenicity test, with or without S9 mix, are the same as for the cytotoxicity test, and the experimental procedure is referred by [37–39]. If the colony count of the TA98 and TA100 test group is larger than the control group by more than two times; that is, the mutagenicity ratio is larger than 2, the specimen for bamboo charcoal/vinegars is considered to have mutagenicity. The mutagenicity ratio is calculated as: mutagenicity ratio (MR) = induced revertants per plate/spontaneous revertants per plate (blank).

2.3. Antimutagenic activity

The test vinegars of the antimutagenic activity are assayed according to the Ames method [40]. The mutagens are 4-nitroquinoline-*N*-oxide (NQNO) and aflatoxin B_1 (AFB_1) that are diluted with dimethyl sulfoxide (DMSO). NQNO (1 μg/plate for TA98 and TA100, respectively), a direct-acting mutagen and AFB_1 (5 μg/plate for TA98 and TA100, respectively), an indirect mutagen which requires metabolic activation, require S9 mix for metabolic activation, respectively. A mutagen (0.1 mL; contained 1 μg NQNO or 5 μg AFB_1) is added to the mixture of a strain (TA98 or TA100), and 0.1 mL of each test vinegar is added to S9 mix for AFB_1 or to the phosphate buffer (0.1 mol/L, pH 7.4) for NQNO. The mutagenicity of each mutagen in the absence of an extract is defined as 100%. The inhibition (%) of mutagenicity of test vinegar is calculated as follows:

Inhibition (%) = [1–(number of His^+ revertants in the presence of the test vinegar—number of spontaneous revertants)/(number of His^+ revertants in the absence of the test vinegar—number of spontaneous revertants)] × 100.

3. Safety evaluation of bamboo charcoal

3.1. Basic properties of bamboo charcoal

The true density, BET specific surface area and average pore diameter of Moso bamboo charcoals are 1.68 (g/cm^3), 138.70 (m^2/g), and 2.41 (nm), respectively. Moreover, the heavy metal element of bamboo charcoal for Br, Pb, Hg, Cr and Cd is analyzed using X-ray Fluorescent Analyzer of XGT-1000WR [39]. Br and Cr are not detected because their amounts are probably

very low. Both Cd and Hg in the charcoal are closed to 0.5 ppm. The Pb in the charcoal is 2.9 ppm. That meets the Sanitation Standard for Edible Natural Colorants, Food Sanitation Standards (1989), Ministry of Health and Welfare in Taiwan at below 40 ppm [41].

3.2. Cytotoxicity of bamboo charcoal

The cytotoxicity test results with 1.0, 2.5, 5.0, 7.5 and 10.0 mg of Moso bamboo charcoal for *S. typhimurium* TA98 and TA100 are shown in **Figure 1**. The Survival (%) of the Moso bamboo charcoal with either zero S9 (-S9) or S9 (+S9) is higher than 80%. Waleh *et al*. [36] indicate that the amount of residual bacteria of S. *typhimurium* must be over 80% of the control group (Control) to determine that the test group has no cytotoxicity for S. *typhimurium* [36]. The Survival of the charcoals is higher than that of control by more than 80%, indicating that the Moso bamboo charcoal has no cytotoxicity for the test strains in the additional range of 1–10 mg/plate, and the dose for the mutagenicity test can be selected according to this range.

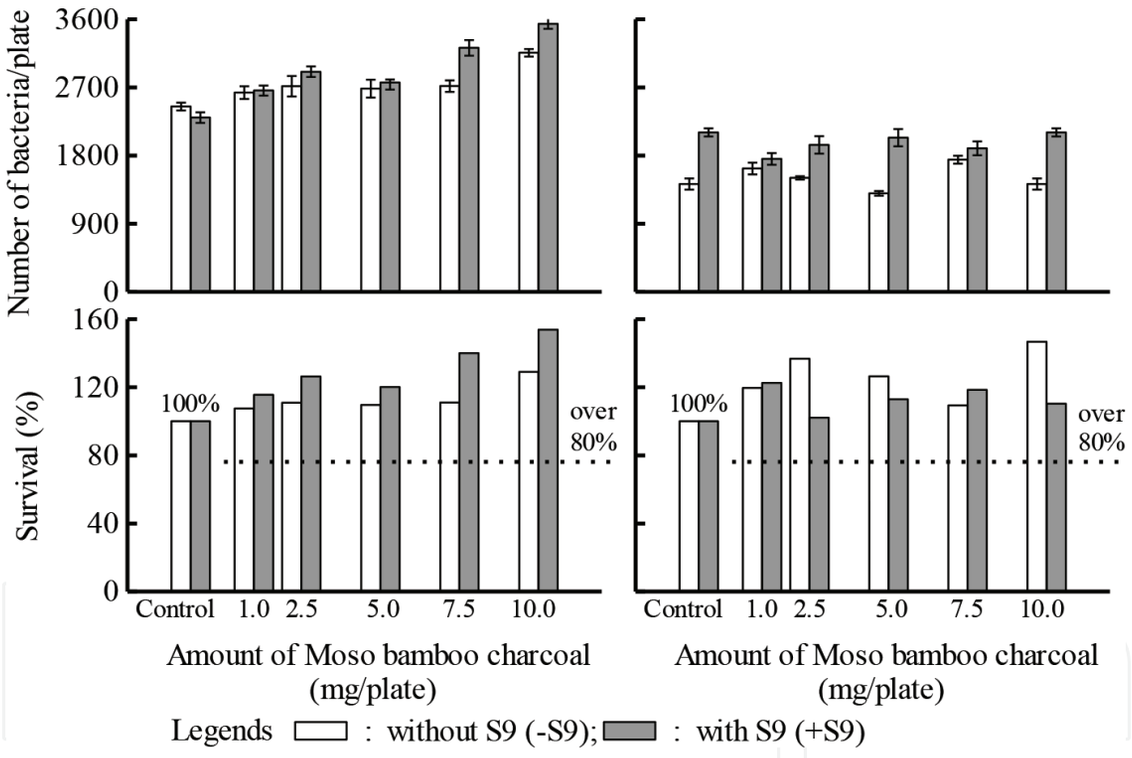

Figure 1. Cytotoxicity of Moso bamboo charcoal toward *S. tyhpimurium* TA98 and TA100 without or with S9 mix.

3.3. Mutagenicity of bamboo charcoal

Figure 2 shows the mutagenicity test results of the Moso bamboo charcoal for *S. typhimurium* TA98 and TA100. The bamboo charcoal, without or with S9, in the test range (1–10 mg/plate) does not exceed spontaneous revertants by more than two times for TA98 and TA100, that is, the mutagenicity ratio (MR) is smaller than 2. According to the standards proposed by Ames *et al*. [32], if the number of spontaneous revertants induced by the specimen is larger than the spontaneous revertants of the control group by more than two times, the specimen has

mutagenicity. Therefore, the bamboo charcoals have no mutagenicity toward *S. typhimurium* TA98 and TA100. Furthermore, Weng reports that the maximum percent weight of Moso bamboo at RH 40% is 6.7% and at RH 90% is 9.9% [39]. The water activity of the bamboo charcoal (0.57), compared to Aw for the growth of a microorganism environment, is below 6.0. The heavy metal contents (as Pb ppm base) of charcoal are below 40 ppm and meet the Sanitation Standard for Edible Natural Colorants, Food Sanitation Standards as well. For the Ames test, the bamboo charcoal has no toxicity and mutagenicity toward *S. typhimurium* TA98 and TA100. In the scope of this report, the above results indicate that the bamboo charcoal can be expected to be the same as the materials of edible natural colorants.

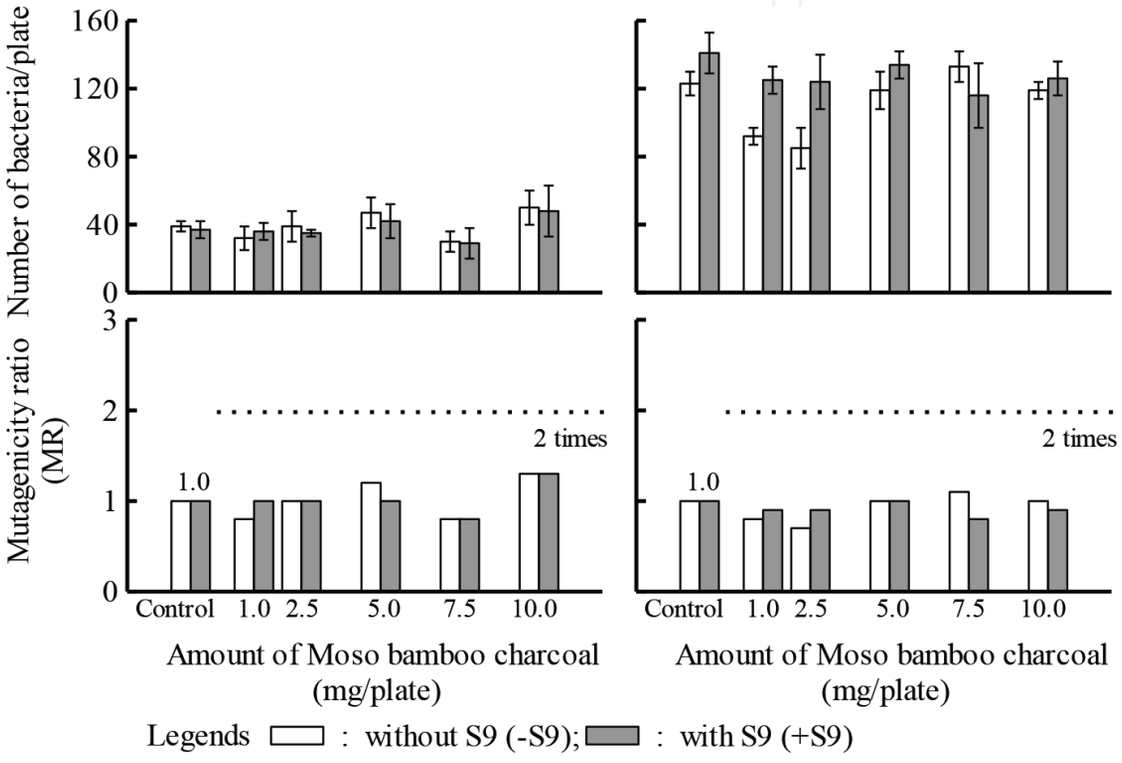

Figure 2. Mutagenicity of Moso bamboo charcoal toward *S. tyhpimurium* TA98 and TA100 without or with S9 mix.

4. Safety evaluation of bamboo vinegars

4.1. Cytotoxicity of bamboo vinegars

One of the cytotoxicity results for the bamboo vinegars collected at temperatures of 90–92°C with the original vinegar (no diluting) and a range of diluting percent contents of 50.00, 33.33, 25.00, 20.00, 13.33 and 10.00% for *S. typhimurium* TA98 and TA100 without S9 mix, as well as for with S9 mix with diluting percent contents of 33.33, 25.00, 20.00, 13.33 and 10.00% is shown in **Table 1**.

The residual bacterial count of the control group (Blank) toward *S. typhimurium* TA98 and TA100 is 1838 and 1500, respectively. For bamboo vinegars collected at temperatures of

S9 mixture	Diluting percent content (%)	Bamboo vinegar (90–92°C)			
		TA98	Survival[1] (%)	TA100	Survival (%)
	Blank[2]	**1838[3]**	**100.00**	**1500**	**100.00**
–S9	Original vinegar	0	0.00	87	5.82
	50.00	336	18.26	285	19.00
	33.33	1652	89.86	1284	85.58
	25.00	1822	99.13	1201	80.04
	20.00	1736	94.43	1264	84.27
	13.33	1829	99.53	1353	90.20
	10.00	1830	99.56	1641	109.42
S9	Blank	2165	100.00	2585	100.00
	33.33	1605	74.16	1736	67.15
	25.00	1960	90.53	2209	85.46
	20.00	1938	89.53	2243	86.75
	13.33	2046	94.53	2885	111.60
	10.00	2000	92.39	2249	87.00

[1]Survival (%) = (the bacterial count of test group/the bacterial count of control group) × 100.

[2]Blank (the control group) was added without bamboo vinegars.

[3]Mean.

Table 1. Cytotoxicity of bamboo vinegar collected at the temperature of 90–92oC toward *Salmonella typhimurium* TA98 and TA100 without or with S9 mix.

90–92°C, the residual bacterial count without S9 for diverse diluting percent contents is 0–1830 for TA98 and 87–1641 for TA100; for those with S9, it is 1605–2000 for TA98 and 1736–2249 for TA100. Waleh *et al*. indicate that the residual bacteria rate of *S. typhimurium* must be over 80% of the control group to determine that the test group has no cytotoxicity for *S. typhimurium* [36]. The residual bacteria rate (Survival, %) toward TA98 and TA100 for bamboo vinegar without S9 mix at a diluting percent content 33.3% or less, and with S9 mix at 25.00% or less is greater than 80%. In other words, the bamboo vinegars collected at temperatures of 90–92°C has cytotoxicity for *S. typhimurium* at a diluting 33.3% less. For vinegars collected at all different temperatures, the Survivals are shown in **Table 2**.

The Survival of all bamboo vinegars collected from 80 to 150, over 80, 90–92, 99–102, 120–123, and 145–150°C without S9 mix at a diluting percent content of 33.33% or less is all higher than those for Blank by more than 80%. However, the Survival of the bamboo vinegar collected at temperatures of 99–102°C at a diluting percent content of 25% shows that the cytotoxicity toward *S. typhimurium* TA98 and TA100 with S9 mix is lower than 80%, indicating "with toxicity." It is asserted that the Survival of bamboo vinegars collected at all different temperatures at a diluting percent content of 20.00% less for either with or without S9 mix is no cytotoxicity absolutely, and the dose for the mutagenicity test can be selected according to the results (**Table 2**).

Specimens	Diluting percent content (%)		Collection temperature (°C)					
			Over 80		90–92		99–102	
			TA98	TA100	TA98	TA100	TA98	TA100
Bamboo vinegars	–S9	33.33	95.16[1]	85.42	89.86	85.58	91.69	90.18
		25.00	115.54	88.91	99.13	80.04	99.13	105.82
		20.00	86.43	109.11	94.43	84.27	84.64	86.20
		13.33	95.30	88.11	99.53	90.20	99.53	90.56
		10.00	106.58	94.69	99.56	109.42	101.16	84.53
	S9	25.00	88.81	92.83	90.53	85.46	76.36	70.81
		20.00	95.40	84.84	89.53	86.75	86.53	106.65
		13.33	92.12	120.27	94.53	111.60	98.66	104.95
		10.00	84.19	99.79	92.39	87.00	94.73	83.29
Specimens	**Diluting percent content (%)**		**120–123**		**145–150**		**80–150**	
			TA98	**TA100**	**TA98**	**TA100**	**TA98**	**TA100**
Bamboo vinegars	–S9	33.33	109.12	90.76	95.92	112.16	109.79	104.98
		25.00	104.13	115.42	90.86	99.82	112.82	102.42
		20.00	111.19	115.69	107.04	123.40	124.23	108.62
		13.33	114.93	118.91	109.61	102.42	95.88	128.20
		10.00	113.75	111.47	122.63	96.44	127.98	119.71
	S9	25.00	89.79	96.39	103.87	122.07	86.94	89.12
		20.00	113.32	90.36	89.11	89.27	85.19	89.74
		13.33	126.73	115.78	143.39	140.28	84.85	97.83
		10.00	117.83	99.02	120.54	98.09	102.71	131.10

Unit: %.

[1]Survival (%) = (the bacterial count of test group/the bacterial count of control group) × 100.

Table 2. Survival of diverse bamboo vinegars collected at different temperatures toward *Salmonella typhimurium* TA98 and TA100 without or with S9 mix.

4.2. Mutagenicity of bamboo vinegars

Ames *et al*. report that the number of spontaneous revertants induced by the specimen is less than that for the control group by more than two times; the specimen has no mutagenicity [32]. **Table 3** shows the mutagenicity results for the bamboo vinegar collected at temperatures of 90–92°C with a diluting percent content of 25.00, 20.00, 13.33 and 10.00% for *S. typhimurium* TA98 and TA100 without S9 mix and with S9 for a diluting percent content of 20.00, 13.33 and 10.00%. The spontaneous revertants of Blank are 65 for TA98 and 132 for TA100 and of bamboo vinegars without S9 mix are from 41 to 53 for TA98 and from 109 to 129 for TA100. With S9 mix, the spontaneous revertants of Blank and bamboo vinegars are 69–76 for TA98

and 140–206 for TA100. The results on the same **Table 3** also show that the bamboo vinegars in the test range do not exceed spontaneous revertants by more than two times for TA98 and TA100 without/with S9 mix; that is, the MR is less than 2. The spontaneous revertants of the other bamboo vinegars collect at diverse temperatures, 80–150, over 80, 99–102, 120–123 and 145–150°C, at the ranges of the diluting percent content are smaller than those of Blank by more than two times [38].

S9 mixture	Diluting percent content (%)	Bamboo vinegar (90–92°C)			
		TA98	MR[1]	TA100	MR
	Blank[2]	**65[3]**	**1.00**	**132**	**1.00**
–S9	25.00	53	0.81	129	0.98
	20.00	42	0.64	121	0.91
	13.33	53	0.81	109	0.83
	10.00	41	0.63	115	0.87
S9	Blank	76	1.00	140	1.00
	20.00	69	0.90	195	1.39
	13.33	73	0.96	204	1.45
	10.00	74	0.97	206	1.47

[1]MR (mutagenicity ratio) = induced revertants per plate/spontaneous revertants per plate (control).

[2]Blank (the control group) was added without either bamboo or wood vinegars.

[3]Mean.

Table 3. Mutagenicity of diverse bamboo vinegar collected at the temperature of 90–92°C toward *Salmonella typhimurium* TA98 and TA100 without or with S9 mix.

The MR of the bamboo vinegars collected at all different temperatures toward *S. typhimurium* TA98 and TA100 without S9 mix for a diluting percent content of 25.00, 20.00, 13.33 and 10.00% or with S9 mix for a diluting percent content of 20.00, 13.33 and 10.00% is shown in **Table 4**. No matter what the vinegars are for all collected temperatures and *S. typhimurium* TA98 and TA100 without/with S9 mix, the MR is less than 2. Neither mutagenicity nor toxicity is observed for bamboo vinegars collected at diverse temperatures toward *S. typhimurium* TA98 or TA100 without/with S9 mix. The bamboo vinegars, therefore, have no mutagenicity toward *S. typhimurium* TA98 at 25% less and TA100 at 20% less.

4.3. Antimutagenic activity of bamboo vinegars

If mutagenicity occurred in a treated material, the results of the antimutagenic assay would be confused due to increased or decreased numbers of revertants of TA98 and TA100 [42]. Hence, without S9 mix a diluting percent content of 25.00–10.00% and with S9 mix at 20.00–10.00% a diluting percent content are selected for the antimutagenic activity. NQNO and AFB_1 are used as direct mutagens requiring metabolic activation and indirect acting mutagen, respectively

Specimens	Diluting percent content (%)		Collection temperature (°C)					
			Over 80		90–92		99–102	
			TA98	TA100	TA98	TA100	TA98	TA100
Bamboo vinegars	–S9	25.00	0.72[1]	1.08	0.81	0.98	–	–
		20.00	0.68	1.02	0.64	0.91	0.65	0.90
		13.33	0.70	0.86	0.81	0.83	0.72	0.78
		10.00	0.61	0.93	0.63	0.87	0.75	1.00
	S9	20.00	1.10	1.47	0.90	1.39	0.88	1.57
		13.33	0.91	1.51	0.96	1.45	0.89	1.62
		10.00	1.04	1.42	0.97	1.47	0.96	1.66
Specimens	**Diluting percent content (%)**		**120–123**		**145–150**		**80–150**	
			TA98	**TA100**	**TA98**	**TA100**	**TA98**	**TA100**
Bamboo vinegars	–S9	25.00	0.74	0.63	0.68	0.50	0.62	0.80
		20.00	0.81	0.62	0.59	0.54	0.67	0.76
		13.33	0.52	0.57	0.57	0.50	0.68	0.72
		10.00	0.69	0.77	0.69	0.56	0.73	0.60
	S9	20.00	1.18	1.19	1.32	1.32	1.16	0.83
		13.33	1.14	1.29	1.22	1.26	1.21	1.04
		10.00	1.00	1.28	1.20	1.40	1.35	1.05

Unit: %.

[1]MR (mutagenicity ratio) = induced revertants per plate/spontaneous revertants per plate (control).

Table 4. Mutagenicity ratio of diverse bamboo vinegars collected at different temperatures toward *Salmonella typhimurium* TA98 and TA100 without or with S9 mix.

[37]. Doses of mutagens, 1 μg for NQNO and 5 μg for AFB_1, are selected from a dose-response curve of a preliminary experiment [43]. The His^+revertants of strain are less than those of the control group (Blank), indicating that with antimutagenic activities. Meanwhile, the inhibitory effect of the specimen is expressed by inhibition (%), and the higher the inhibition, the more effective the antimutagenic activities [40]. The inhibitory effects for one of the antimutagenic activity results for the bamboo vinegar collected at temperatures of 90–92°C with a diluting percent content of 25.00, 20.00, 13.33 and 10.00% for NQNO and at 20.00, 13.33 and 10.00% for AFB_1 are summarized in **Table 5**.

The His^+ revertants of strain against the NQNO in Blank (without bamboo vinegars) are 1128 for TA98, and 1445 for TA100, for AFB_1: they are 1824 for TA98 and 2406 for TA100. The spontaneous revertants without NQNO are 65 for TA98 and 132 for TA100 and without AFB_1 are 76 for TA98 and 140 for TA100. The His^+ revertants of strain (inhibition,%) against the NQNO for bamboo vinegar at 90–92°C with different diluting percent contents are 807–985 (30.18–13.49%) for TA98 and 425–616 (77.65–63.15%) for TA100. For AFB_1, they are 1114–1319 (40.63–28.88%)

Mutagens	Diluting percent content (%)	Bamboo vinegar (90–92°C)			
		TA98	Inhibition[1)] (%)	TA100	Inhibition (%)
NQNO (1 μg/plate)	Blank[2]	1128[3]	0.00	1445	0.00
	25.00	807	30.18	425	77.65
	20.00	841	27.04	446	76.05
	13.33	891	22.33	594	64.80
	10.00	985	13.49	616	63.15
Spontaneous revertants		65		132	
AFB_1 (5 μg/plate)	Blank	1824	0.00	2406	0.00
	20.00	1114	40.63	843	68.97
	13.33	1292	30.44	1012	61.53
	10.00	1319	28.88	1202	53.14
Spontaneous revertants		76		140	

[1]Inhibition (%) = [1–(number of His^+revertants in the presence of the test vinegar—number of spontaneous revertants)/(number of His^+revertants in the absence of the test vinegar—number of spontaneous revertants)] × 100.

[2]Blank (the control group) is added without either bamboo or wood vinegars.

[3]Mean.

Table 5. Antimutagenic activity of diverse bamboo vinegar collected at the temperature of 90–92°C toward *Salmonella typhimurium* TA98 and TA100 with S9 mix.

for TA98 and 843–1202 (68.97–53.14%) for TA100 from bamboo vinegar at 90–92°C with different diluting percents. The results also show that the higher diluting percent content, the greater the inhibition, as well as, no matter what the vinegar is, the inhibition for TA100 is greater than that of TA98. Moreover, the inhibition of the bamboo vinegars collected at all different temperatures against the NQNO for diluting percent contents of 25.00, 20.00, 13.33 and 10.00% or against the AFB_1 at 20.00, 13.33 and 10.00% is shown in **Table 6**.

The inhibition of the bamboo vinegars to TA98 is 0.19–30.18% for NQNO and 0.92–40.63% for AFB_1. For TA100 against NQNO (18.18–81.77%) and AFB_1 (37.43–75.24%), they are better than those for TA98. The antimutagenic activity to NQNO is effective for bamboo vinegars collected at diverse temperatures with a diluting percent content of 25.00% or less, and for AFB_1, it is also effective at a 20.00% or less diluting percent content. Furthermore, the bamboo vinegars show that the inhibitory effect on NQNO or AFB_1 toward TA100 is greater than that toward TA98. It is also indicated that the inhibition of the vinegars against AFB_1 toward TA98 and TA100 is better than that against NQNO.

4.4. Effect of bamboo vinegars' compounds on preliminary safety evaluation

The identified compounds of bamboo vinegars collected from different temperatures in the category of 80–150°C, over 80, 90–92, 99–102, 120–123 and 145–150°C are analyzed by gas chromatography-mass spectroscopy [15, 38, 44]. The acid, phenol, ketone and other

Specimens	Diluting percent content (%)		Collection temperature (°C)					
			Over 80		90–92		99–102	
			TA98	TA100	TA98	TA100	TA98	TA100
Bamboo vinegars	NQNO	25.00	25.03[1]	70.14	30.18	77.65	-	-
		20.00	23.15	65.34	27.04	76.05	23.21	75.22
		13.33	12.80	60.64	22.33	64.80	20.58	58.91
		10.00	11.29	59.45	13.49	63.15	15.18	57.97
	AFB_1	20.00	24.38	62.94	40.63	68.97	37.80	66.35
		13.33	21.13	57.88	30.44	61.53	26.36	66.00
		10.00	18.31	37.43	28.88	53.14	24.26	54.88
Specimens	**Diluting percent content (%)**		**120–123**		**145–150**		**80–150**	
			TA98	**TA100**	**TA98**	**TA100**	**TA98**	**TA100**
Bamboo vinegars	NQNO	25.00	27.85	74.45	2.76	81.77	22.96	68.97
		20.00	11.67	64.45	1.19	60.23	4.20	61.38
		13.33	4.08	61.55	1.00	40.71	0.75	57.31
		10.00	1.07	28.52	0.63	18.18	0.19	42.74
	AFB_1	20.00	20.03	75.24	11.63	73.06	20.14	74.86
		13.33	18.73	68.97	8.54	66.41	17.09	73.27
		10.00	13.20	59.88	0.92	60.50	7.44	58.44

[1]Inhibition (%) = [1–(number of His^+revertants in the presence of the test vinegar—number of spontaneous revertants)/(number of His^+revertants in the absence of the test vinegar—number of spontaneous revertants)] × 100.

Table 6. Inhibition of diverse bamboo vinegars collected at different temperatures toward *Salmonella typhimurium* TA98 and TA100 with S9 mix.

compounds of bamboo vinegars are about 10.65–20.09%, 57.87–65.98%, 10.13–18.76% and 9.66–15.30, respectively. The acid compounds included butanoic acid, 2-methoxyethyl acetate, 4-hydroxy-butanoic acid and 4-hydroxy-3-methoxy-butanoic acid. The maximum fraction of acid compounds is the bamboo vinegar collected from 80 to 150°C. The phenol (5.93–16.60%), 2-methoxy-phenol (8.27–16.39%) and 4-ethyl-phenol (3.68–9.48%) are the main fractions of phenol compounds for bamboo vinegars. For ketone compounds, the 2-hydroxy-3-methyl-2-cyclopentenone-1-one, 2, 3-dimethyl-2-cyclopentenone-1-one and maltol can be measured for bamboo vinegars collected at all temperatures. The maximum fraction of ketone compounds is the bamboo vinegar collected from 120 to 123°C.

According to the former, results of safety evaluation (**Tables 1** and **2**; **Tables 3** and **4**) and antimutagenic activity (**Tables 5** and **6**) are present in diverse bamboo vinegars, the mutagenicity is occurred in the diluting percent content of vinegars that are higher than 20.00%, and without S9 mix is at 33.33%, but the diluting percent content of vinegars of 20.00% or less, expressed the amount-dependent inhibitory effect against both NQNO with 1 μg/plate

and AFB_1 with 5 μg/plate in *S. typhimurium* TA98 and TA100. This is because the antimicrobial activity is affected by some phenol compounds [45]. In this report, the main fractions of bamboo vinegars are phenol compounds, 57.87–65.98% [38]. It is inferred that the phenol compounds may partially account for the biological action of bamboo vinegars.

5. Conclusion

The biological action of bamboo charcoal and vinegars is evaluated by *Salmonella* mutagenesis assay, as a safety evaluation and antimutagenic activity. The charcoal can be expected to be similar to the materials of edible natural colorants and/or natural drying agents, because the water activity of charcoal is below 6.0, the Pb base of charcoal is below 40 ppm, and the bamboo charcoals have no toxicity and mutagenicity toward *Salmonella typhimurium* TA98 and TA100. For the bamboo vinegars, the main percent content of the phenol compounds for bamboo vinegars is phenol (5.93–16.60%) and 2-methoxy-phenol (8.27–16.39%). The diluting percent content of vinegars is lower than 20.00% or less with S9 mix and 33.33% or less without S9 mix in cytotoxicity and mutagenicity toward *Salmonella typhimurium* TA98 and TA100 because the rest of the bacterium (Survival) these percent contents is higher than 80% of the control group (Blank), and the mutagenicity ratio (MR) is less than for the control group by more than two times. The diluting percent content of vinegars of 20.00% or less expressed the amount-dependent inhibitory effect against both the mutagenicity of 4-nitroquinoline-N-oxide (NQNO) with 1 μg/plate and aflatoxin B1 (AFB_1) with 5 μg/plate in *Salmonella typhimurium* TA98 and TA100. It is suggested that bamboo vinegars with a diluting percent content to the least extent of 20.00% or less have no cytotoxicity and mutagenicity, and their antimutagenic activity with NQNO and AFB_1 is effective. The bamboo vinegars in the extent of this report can be as the reference of functional additives, due to their prospective applications in human care, such as in the portion of drinks or/and food.

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