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Adsorption and Preliminary Safety Evaluation of Activated Carbons Refined from Charcoals

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The objectives of this study were to investigate the absorption and preliminary safety of activated carbon (AC) refined from Makino bamboo, Moso bamboo and Japanese cedar charcoals with carbon dioxide (CO_2) at an 800 and 900°C activation temperature with 90, 120 and 150 min of activation time. The yield of AC was from 47.5 to 90.4%. The benzene absorption (10.8–37.4%) of AC was also higher than that (1.3–9.4%) of charcoals. The iodine value (562–1088 mg/g) of AC was higher than that (141–628 mg/g) of charcoals. The true density (2.11–2.17 g/cm^3) and BET surface area (791.22–829.78 m^2/g) of AC were obviously higher than those (1.68–1.86 g/cm^3 and 58.95–356.62 m^2/g) of charcoals. The AC belongs to a mesopore structure because the results of average pore diameters were from 2.22 to 2.69 nm. No matter what the relative humidity (RH) was in high/low RH at either 90 or 40% with a constant temperature of 25°C, the hygroscopic ability of AC was better than that for charcoals, and the water activity of AC was from 0.45 to 0.46. The heavy metal content as the Pb (Plumbum) ppm base of AC was below 40 ppm and met the Sanitation Standard of Edible Natural Colorants from the announcement by the Department of Health's Executive Yuan, in Taiwan. The preliminary safety evaluation using the Ames test for the charcoals and AC had no cytotoxicity and mutagenicity toward *Salmonella typhimurium* TA98 and TA100. It is suggested that the AC, refined from the charcoals, can be expected to be a natural moisture–proof material.

Key words: activated carbon, adsorption, water activity, Ames test, mutagenicity

INTRODUCTION

Either wood or bamboo is accumulated from organic compounds from which a tree/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 wood/bamboo decaying or burning, the preparation of charcoal/carbon becomes one of the selected methods because carbon can extend the lifetime of wood/bamboo (Lin and Hwang, 2006). In other words, the charcoal/carbon can become earth friendly materials because they can slow down the increase of CO_2 concentration in the atmosphere.

Domestic and foreign manufacturers and researchers have invested a great deal of money, labor and time to discover the characteristics and functions of charcoal/carbon in recent years. The utilization of charcoal, conventionally regarded as fuel, is widely applied to daily life, industry, etc. The use of charcoal is more wide and diverse because it can be used for carbon textiles, cleaning products, etc. Charcoal, prepared under high temperatures in the absence of oxygen, is made using wood and bamboo as raw materials in Taiwan. The raw materials of wood charcoals are made from broadleaf trees, such as *Acacia confuse, Euphoria longana, Lagerstroemia sucostata* and *Litchi chinensis*. Bamboo charcoals are mainly derived from 4–year–old or older bamboo as raw materials, such as *Phyllostachys makinoi, Phyllostachys heterocycla* and *Dendrocalamus latiflorus* (Hung, 2004; Wang *et al*., 2004; Wang, 2004; Lin and Hwang, 2006).

Charcoal 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 (Lin *et al*., 2003; Hung, 2004). 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, charcoal peanuts, charcoal ice cream, charcoal moon cakes, charcoal cookies, 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, announced 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 (Lee, 2007). The charcoals can be added as a pigment in food. It is a profound question whether or not residue *in vivo* causes any

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harm by inducing cell lesions or carcinogens.

Charcoal, after being refined by activation, becomes activated carbon (AC) that still retains the charcoal's characteristics (porous structure, large specific surface area, etc.). The specific surface area and total pore volume of AC are obviously higher than those of charcoal (Chang *et al*., 2000). AC is also a good adsorbent for gaseous and liquid adsorption and is widely applied in the purification, de–colorization, removal of toxic substances and treatment of waste water (Liu, 1998). A trend toward the demand of AC has increased year by year, according to the import and export data from the Directorate General of Customs, Taiwan. In 2010, 12 564 tons of AC was imported, and it increased to 15 283 tons (about NT$ 72 million) in 2011. Therefore, charcoal is used to prepare AC that can expand the applications of charcoal, increase the economic effect and decrease the domestic demand of AC in Taiwan.

The objectives of this study were to investigate the absorption of charcoal and AC, refined from three charcoals: Makino bamboo, Moso bamboo and Japanese cedar charcoals. The refined methods were carried out by physical activations with CO_2 at various activation temperatures and times. Charcoal can be used as a colorant in food, but the safety of the residue in a human body is still uncertain. The preliminary safety evaluation, including cytotoxicity and mutagenicity, was performed in accordance with the Ames test (Ames *et al*., 1975), a widely used convenient and short–term assay with predictable accuracy for carcinogen up to 72–91% (Ames *et al*., 1976). If the preliminary safety evaluation shows no toxicity and mutagenicity, charcoal and AC can not only be considered to be safe pigment materials for food, but also used as a natural moisture–proof material due to their good absorption.

MATERIALS AND METHODS

Specimen preparations and characteristics

Precursor

The specimens, activated carbon (AC), were prepared from Makino bamboo, Moso bamboo and Japanese cedar charcoals as the precursors. The charcoals were manufactured in earth kilns with the temperature at 700–800°C (Hung, 2004; Lin and Hwang, 2006). The precursors prepared were ground and sieved to the size of 10–40 mesh, boiled in water for 30 min, and then, dried in a vacuum oven at 105°C overnight.

Salmonella typhimurium

Test strains of *Salmonella typhimurium* (*S. typhimurium*) TA98 and TA100 were bought from the Bioresource Collection and Research Center, Food Industry Research and Development Institute.

Rat liver mixture

One of the enzymes in a rat liver mixture (S9) is the rat liver cell extract. This enzyme is added to simulate the intravital metabolism of organisms and used in the preliminary safety (Ames tests) of the charcoals and AC.

Ash

According to the Chinese National Standards (CNS) 5581 (1980) Method of Test for Total Ash Content of Activated Carbon, the ash content of charcoals is measured. The formula used is as follows: Ash (%) = [(the weight of crucible with precursor ash – the weight of crucible) / (the weight of crucible with test precursor – the weight of crucible)] * 100

Preparation of AC

The precursor, 20 g of oven dried charcoal, was refined in a closed container of super–high temperature vacuum carbonization activation equipment (Chi–How Heating Co., Ltd.). Nitrogen (N_2 gas) was added to make the container oxygen free. The heating rate was set at 10°C/min to the carbonization temperature in the range of 700 to 850°C. The activation temperature was carried out at 700, 750, 800, 850, 900 and 950°C with the activation time for 90, 120 and 150 min, respectively. The imported gas was CO_2 (purity of 99.99%), and the flow rate was maintained at 100 cm^3/min. The specimens were then cooled by N_2 gas to a normal temperature and taken out. The aforesaid preparation conditions refer to (Chang *et al*., 1998; Juang *et al*., 2000; Tseng *et al*., 2007). The equation for the AC yield (Y) is Y (%) = (bone dry weight of AC / absolute dry weight of test precursor) × 100.

Benzene adsorption

The benzene adsorption equipment was designed by the Laboratory of Environmental Functional Materials, Department of Wood Based Materials, National Chiayi University (NCYU) in Taiwan. 200 mg of oven dried specimens was put into an U type glass tube. The benzene adsorption used the vacuum percentage for air and benzene solution at 1:10, and the flow rate was maintained at 167 mL/min. The water temperature in a water–bath was kept at 20°C during absorption. The charcoal/AC was first weighed after being in contact for 60 min with the benzene adsorption, and then, measured at 30 min intervals, respectively. The equation for benzene adsorption (%) = [(specimen weight after absorption balance – the weight of test specimen) / the weight of test specimen] × 100.

Iodine value

According to the Japanese Industrial Standard (JIS) K 1474 (1991) Test Methods for Activated Carbon, the iodine value of charcoal/AC were individually measured. The formula for iodine adsorption capacity is: (I) = [(10 – K × f) × 12.69 × 5] / M. The abbreviations for the formula are I: iodine adsorption capacity (mg/g); K: the volume of titrated sodium thiosulfate (mL); f: the ratio of 0.1 N sodium thiosulfate to 0.1 N iodine solution and M: weight of oven dried specimens (0.5 g).

True density

The oven dried charcoal or AC was measured using a true density analyzer (Ultrapycnometer 1000, Quanta chrome). The measuring equipment uses helium to displace the air of a specimen pore to obtain the true density (g/cm^3).

Characterization measurements

The BET specific surface areas (S_{BET}) of the chars and the resulting AC were determined by N_2 gas adsorption at 77 K with an automated adsorption instrument (BET–202A, Porous Materials, Inc.) in a relative pressure (*P/Po*)

ranging from 10^{-2} to 1. The S_{BET} of the chars and AC were analyzed by the standard BET equation and procedure developed by Barrett *et al*. (1951). The total pore volume (Vtot) was obtained by converting the amount of N_2 gas adsorbed (expressed in cm^3/g STP) at a relative pressure of 0.99 to the volume of liquid adsorbate. The average pore diameter (4 Vtot / S_{BET}) can be calculated (Hu and Srinivasan, 1999). The micropore surface area (S_{micro}) was determined by the *t*–plot method (Gregg and Sing, 1982).

Hygroscopicity

This test method of hygroscopic ability was also designed by the Laboratory of Environment Functional Materials, Department of Wood Based Materials, NCYU in Taiwan. The hygroscopic ability of the specimen was examined in each measured point after a certain period of time with either a higher relative humidity (RH) at 90% or a lower RH at 40% with a specific temperature of 25°C. The maximum percent weight (%) under either of the aforesaid conditions for the chars and the resulting AC was investigated.

Water activity

Water activity (Aw) is usually defined as the percent of relative humidity generated in equilibrium with the product specimen in a closed system at a constant temperature. The Aw of the charcoal and the AC were determined by the Chinese National Standards (CNS): Method of Test for Water Activity of Food, and the procedure developed was determined in consultation with the CNS 5255 (1987). Six replicates of each specimen for either the charcoal or the AC were investigated.

Heavy metal element

Analysis of the heavy metal elements for the chars and the AC were determined by using an X–ray Fluorescent Analyzer (XRF) of XGT–1000WR (Horiba). The resulting heavy metal elements included bromine (Br), chromium (Cr), cadmium (Cd), plumbum (Pb), mercury (Hg) and so on.

Ames test

Cytotoxicity

1.0, 2.5, 5.0, 7.5 and 10.0 mg of various charcoals and AC (activation temperature 900°C with 120 min of activated time) were 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 by Ames *et al*. (1975). The colony count was calculated; if the bacterial count of the test group (+S9 or –S9) was larger than the bacterial count of the control group (no specimen) by 80% (survival), there was no toxicity. The survival (%) is the residual bacteria rate that is the percentage relative to the control (100%).

Mutagenicity

The mutagenicity was analyzed by using the method proposed by Maron and Ames (1983). The test dose selected for this mutagenicity test with +S9 or –S9 was the same as the aforesaid cytotoxicity test. The test for mutagenicity was carried out at 37°C for 48 h, and the phosphate buffer saline was used as the control. If the colony count of the TA98 and TA100 test group was larger than the control group by more than two times; that is, the Mutagenicity ratio was larger than 2, the specimen was considered to have mutagenicity. The Mutagenicity ratio (MR) = induced revertants per plate/ spontaneous revertants per plate (Control).

Statistical analysis

The test results are represented by the mean (standard deviation). The control group and test groups were compared by Duncan's analysis. If the ρ value was smaller than 0.05, this meant that there was a significant difference between the test group and control group; it is represented by different letters.

RESULTS AND DISCUSSION

Basic characteristics of charcoal and AC

The ash of the precursors, yield of refined activated carbons, iodine value and benzene absorption of charcoals and AC are shown in Table 1. The charcoal ash was between 1.3 and 3.1 %. The ash of Moso bamboo charcoal was the same as the results (1.2–3.7%) of the study conducted by Chen (2003). The yields from Makino bamboo, Moso bamboo and Japanese cedar AC were 54.6–90.4%, 55.4–87.9% and 47.5–82.7%, respectively. The highest (90.4%) was from Makino bamboo AC with CO_2 at 800°C activation temperature for 90 min; the lowest (47.5%) was Japanese cedar AC with CO_2 at 900°C for 150 min. No matter what kind of precursor (charcoal) was used, results were dependent on the activation temperature and time, and the influence of the activation temperature was greater than that of the activation time (Chang *et al*., 1998 and 2003; Tseng *et al*., 2007; Wu *et al*., 2010; Huang *et al*., 2010; Peng *et al*., 2010).

The benzene absorption of AC was 10.8–37.4%, higher than that (1.3–9.4%) of charcoal (Table 1) and higher than that of the precursors by more than 1.5–28.8 times. The increase of benzene absorption for Makino bamboo AC was higher than that for the other two. These results are similar to Wang's study of Taiwan's giant bamboo charcoals and AC (2004). No matter what kind of AC is used, the results indicate that there is a significant difference for the benzene value of AC prepared at different activation times in accordance with Duncan's analysis.

The iodine value of AC was 562–1088 mg/g (Table 1), and higher than that of charcoals (141–628 mg/g). The iodine value of Makino bamboo AC, prepared with CO_2 at 900°C for 150 min was the highest, 1088 mg/g, and was higher than that of the Makino bamboo charcoal by more than 7.6 times. Moreover, the iodine value of AC increased obviously as the activation temperature increased. According to Duncan's analysis, there is no obvious difference in the iodine value of AC prepared at 800°C compared to the activation time for Japanese cedar AC at 900°C, but the iodine value of Makino and Moso bamboo AC increases apparently at the activation temperature of 900°C. This is because the iodine values are indicators of micropore amounts of AC (Chen, 2003), and

Table 1. Ash of charcoals and yield, benzene absorption and iodine value of activated carbons refined from charcoals

Precursor	Activation		Ash (%)	Yield (%)	Benzene absorption (%)	Iodine value (mg/g)
	Temperature (°C)	Time (min)				
Makino bamboo charcoal	Control	–	3.1 (0.02)	– [1)]	1.3 (0.19) [aA 2)]	153 (5.7) [aA]
	800	90	–	90.4 (0.43) [a]	15.2 (0.16) [b]	664 (11.4) [b]
		120	–	87.6 (0.30) [b]	14.3 (0.16) [c]	683 (20.7) [b]
		150	–	86.4 (0.94) [c]	15.5 (0.52) [c]	682 (2.6) [b]
	900	90	–	70.3 (1.26) [A]	25.2 (0.22) [B]	920 (21.1) [B]
		120	–	64.0 (1.04) [B]	27.8 (0.40) [C]	1009 (6.5) [C]
		150	–	54.6 (2.93) [C]	37.4 (0.64) [D]	1088 (8.6) [D]
Moso bamboo charcoal	Control	–	2.4 (0.01)	–	1.9 (0.29) [aA]	141 (12.1) [aA]
	800	90	–	87.9 (0.13) [a]	10.8 (0.67) [b]	562 (12.1) [b]
		120	–	85.6 (0.33) [b]	14.5 (0.14) [c]	602 (21.7) [c]
		150	–	85.8 (1.51) [b]	14.7 (0.26) [c]	623 (4.7) [c]
	900	90	–	69.6 (0.90) [A]	24.2 (0.15) [B]	866 (18.3) [B]
		120	–	64.6 (0.50) [B]	29.0 (0.32) [C]	992 (16.9) [C]
		150	–	55.4 (2.01) [C]	33.8 (0.66) [D]	1068 (21.0) [D]
Japanese cedar charcoal	Control	–	1.3 (0.03)	–	9.4 (0.34) [aA]	628 (68.1) [aA]
	800	90	–	80.1 (0.63) [a]	13.8 (0.09) [b]	756 (0.9) [b]
		120	–	82.7 (0.24) [b]	15.5 (0.34) [c]	737 (6.7) [b]
		150	–	79.3 (0.56) [a]	16.0 (0.13) [d]	722 (1.7) [b]
	900	90	–	60.7 (1.25) [A]	25.6 (0.45) [B]	984 (2.0) [B]
		120	–	53.0 (0.94) [B]	28.9 (0.29) [C]	1029 (7.8) [B]
		150	–	47.5 (0.40) [C]	28.3 (0.26) [C]	1043 (19.6) [B]

[1)] – : Non detected

[2)] Mean (standard deviation) separation within columns by Duncan's multiple range tests at 5% significant level. Alphabets express the different activation time at the same activation temperature

the pore sizes of AC have a selection of adsorbate (Hsu, 2008). Micropores, with the diameter of pore sizes less than 2 nm, have been classified by the International Union of Pure and Applied Chemistry (IUPAC), and the diameter of iodine molecules is 0.56 nm (Hsieh and Teng, 1999). In other words, the iodine value of AC is related to the amount of micropores. The iodine value increases as the amount of micropores of AC increases and the absorption of AC becomes better. The iodine value standard of commercial AC is 600–1000 mg/g (Wu and Tseng, 1999). The results in this study show that, except for the AC prepared at 800°C for 90 min, the value of refined AC is from 602 to 1088 mg/g, determined to meet commercial values.

For the following experiments, AC was selected with an activation temperature of 900°C with an activation time of 120 min because AC has the best yield results, benzene absorption and iodine value.

True density and characteristics of charcoal and AC

Table 2 shows that the true densities of charcoals are between 1.68 to 1.86 g/cm^3, and these are the same as the results of other studies (Lo and Wang, 2007; Lan *et al*., 2008; Lin *et al*., 2010), 1.347–1.957; 1.42–1.88; 1.68–1.82 g/cm^3. The true density of refined AC prepared from Makino bamboo, Moso bamboo and Japanese cedar charcoals was 2.14, 2.11 and 2.17 g/cm^3, respectively. The true density of AC is higher than that of charcoals, indicating that there are more pores in AC than in charcoal.

The S_{BET} of AC prepared from Makino bamboo, Moso bamboo and Japanese cedar AC was from 791.22 to 829.78 m^2/g, as shown in Table 2, meeting the commercial standard of 500–1500 m^2/g (Huang, 2000). The results for the S_{BET} of AC were greater than for charcoal. Wang (2004) reported that the surface areas increased, and the adsorption became better, so that the surface area could be an adsorption indicator of charcoal. It is said that the adsorption of AC is greater than that of charcoal.

The percentage of S_{micro}/S_{BET} using the t–plot method (Gregg and Sing, 1982) shows that Moso bamboo AC has many more micropores (93.68%) than Moso bamboo charcoal, 17.08%. Physical activation has pore–drilling and expansion effects at 800°C producing multiple micropores (Lua and Guo, 2000; Yun *et al*., 2001). The average pore diameters of Moso bamboo charcoal and AC were 2.41 and 2.22 nm, respectively. The S_{micro} of Moso bamboo AC is 741.21 m^2/g, which is greater than that of Moso bamboo charcoal, 23.69 m^2/g, by more than 30 times. The pore characteristics of Makino bamboo charcoal/AC have the same tendency as the above results for Moso bamboo. For Japanese cedar AC, the Smicro/S_{BET}

Table 2. True density, BET specific surface area and average pore diameter of Makino bamboo, Moso bamboo, Japanese cedar charcoals and activated carbons refined from charcoals

Precursor / Specimen		True density (g/cm^3)	SBET[1] (m^2/g)	*t*–plot		Average pore diameter (nm)
				S_{micro}[2] (m^2/g)	S_{micro}/S_{BET} (%)	
Makino bamboo	Charcoal	1.83	058.95	127.05	2.16	5.92
	Activated carbon [1]	2.14	829.78	772.04	93.04	2.27
Moso bamboo	Charcoal	1.68	138.70	23.69	17.08	2.41
	Activated carbon	2.11	791.22	741.21	93.68	2.22
Japanese cedar	Charcoal	1.86	356.62	324.09	90.88	2.30
	Activated carbon	2.17	815.94	667.62	81.82	2.69

[1] S_{BET}: BET specific surface area; [2] S_{micro}: micropore surface area; [3] Average pore diameter: 4 Vtot / S_{BET}; [4] Preparation conditions were activation temperature at 900°C with 120 min of activation time

showed that AC had fewer micropores (81.82%) than Moso bamboo charcoal, 90.88%, but the SBET was 667.62 m^2/g, greater than that of charcoal, 324.09 m^2/g. When the activation temperature rises, as the expansion effect is greater than the pore–drilling effect, multiple mesopores or macropores are produced (Walker and Almagro, 1995), so that the average pore diameter for AC is 2.69 nm, larger than that for charcoal, 2.30 nm.

Hygroscopicity, Aw and heavy metal element analysis

The results of hygroscopicity for AC and charcoal at either RH 40 or 90% is shown in Table 3, no matter what the RH is, the maximum percent weight of the AC is bet-

Table 3. Maximum percent weight of charcoals, activated carbons and silica gel in high/low relative humidity conditions at either 90 or 40% with a constant temperature at 25°C

Precursor	Activation		Maximum percent weight (%)	
	Temperature (°C)	Time (min)	40% RH	90% RH
Makino bamboo charcoal	Control	–[1]	9.2 (0.48) aA 2)	11.3 (0.63) aA
	800	90	12.1 (0.24) b	15.2 (0.93) b
		120	13.0 (0.24) c	17.1 (0.78) c
		150	13.8 (0.51) d	18.2 (0.75) c
	900	90	21.3 (0.78) B	28.5 (0.65) B
		120	24.3 (0.94) C	34.6 (1.06) C
			30.6 (2.96) D	45.1 (0.97) D
Moso bamboo charcoal	Control	–	6.7 (031) aA	9.9 (0.26) aA
	800	90	11.6 (0.28) b	15.6 (0.49) b
		120	12.5 (0.25) c	17.3 (0.94) c
		150	13.1 (0.29) d	17.6 (0.41) c
	900	90	20.0 (1.25) B	29.1 (0.71) B
		120	22.1 (1.34) C	29.9 (1.10) B
		150	23.8 (1.09) D	40.3 (0.75) C
Japanese cedar charcoal	Control	–	9.5 (0.52) aA	13.3 (0.45) aA
	800	90	10.1 (0.64) b	17.4 (1.03) b
		120	10.5 (1.61) c	17.9 (2.28) b
		150	10.7 (0.57) d	18.7 (1.02) b
	900	90	15.1 (1.08) B	32.7 (1.82) B
		120	16.1 (1.02) C	35.7 (3.37) B
		150	16.8 (1.40) D	33.4 (2.10) B
Silica gel	–	–	11.7 (0.10)	37.2 (0.17)

[1] – : Non condition

[2] Mean (standard deviation) separation within columns by Duncan's multiple range tests at 5% significant level. Alphabets express the different activation time at the same activation temperature to control

ter than that of charcoal. According to Duncan's analysis, at either RH 40 or 90% the maximum percent weight of AC and charcoal is obviously different. The maximum percent weight of Makino bamboo, Moso bamboo and Japanese cedar AC at RH 40% was 12.1–30.6%, 11.6–23.8% and 10.1–16.8%, higher than that of silica gel, 11.07%.

For the RH 90%, the maximum percent weight of AC was 15.2–45.1%, higher than that (9.9–13.3%) of charcoals by more than 1.5–3.4 times. The highest (45.1%) was Makino bamboo AC with CO_2 at 900°C activation temperature for 150 min higher than silica gel (37.2%); the lowest (15.2%) was also Makino bamboo AC with CO_2 at 800°C for 90 min. This indicates that the increase of the adsorption is based on the increase of both activation temperature and activation time. Moreover, no matter what kind of AC is used, higher hygroscopic ability is concerned with the activation temperature and time, and the influence of the activation temperature is greater than that of the activation time (Wu *et al*., 2010; Huang *et al*., 2010).

Aw indicates the amount of water in the total water content available to micro–organisms. Each of the species of micro–organisms (bacteria, yeast and mould) has its own minimum Aw. The growth of micro–organisms is no longer possible when the Aw is below 0.65–0.95 (Chang *et al*., 2006). The Aw control is also an important factor for the chemical stability of foods. Most foodstuffs contain carbohydrates and proteins, as well as being subject to non–enzymatic browning reactions (Maillard reaction). The Maillard reaction gets stronger with increasing Aw and reaches its peak at Aw 0.6 to 0.7. With any further increase in Aw, this reaction gets weaker rapidly (Qyaizu, 1986). Table 4 shows the results of Aw and heavy metal element analysis of charcoals and AC. The Aw of charcoals and AC was 0.56–0.58 and 0.45–0.46, respectively. After charcoals were refined by using activation, the Aw of AC decreased and was lower than the Aw of micro–organism growth, as well as possibly being able to help the Maillard reaction.

Table 4. Water activity and heavy metal element analysis of charcoals and activated carbon

Precursor / Specimen		Water activity	Heavy metal element		
			Cd (ppm)	Pb (ppm)	Hg (ppm)
Makino bamboo	Charcoal	0.57 (0.01) [1)]	1.6 (2.10)	0.7 (0.63)	1.2 (0.62)
	Activated carbon [2)]	0.45 (0.00)	1.4 (0.87)	1.4 (1.08)	0.7 (0.30)
Moso bamboo	Charcoal	0.56 (0.00)	0.5 (0.64)	2.9 (0.70)	0.5 (0.51)
	Activated carbon	0.46 (0.00)	0.6 (0.44)	0.3 (0.02)	1.1 (0.43)
Japanese cedar	Charcoal	0.58 (0.00)	0.5 (0.27)	0.7 (0.69)	0.8 (0.39)
	Activated carbon	0.45 (0.00)	1.5 (0.37)	0.3 (0.10)	0.8 (0.53)
Silica gel		0.36 (0.00)	– [3)]	–	–

[1)] Mean (standard deviation)
[2)] Preparation conditions of the AC were activation temperature at 900°C with 120 min of activation time
[3)] – : Non detected

Table 5. Cytotoxicity of Moso bamboo charcoal and activated carbon toward *S. tyhpimurium* TA 98 and TA 100 with or without S9 mixture

Precursor/ Specimen	Amount (mg/plate)	Number of bacteria / plate							
		TA98				TA100			
		– S9	Survival (%)[1)]	+ S9	Survival (%)	– S9	Survival (%)	+ S9	Survival (%)
Moso bamboo charcoal	Control [2)]	2449 (51) [3)]	100.0	2303 (70)	100.0	1425 (74)	100.0	2107 (54)	100.0
	1.0	2633 (084)	107.5	2660 (67)	115.5	1630 (77)	119.6	1757 (77)	122.5
	2.5	2717 (135)	110.9	2909 (70)	126.3	1506 (22)	136.8	1942 (116)	102.1
	5.0	2686 (119)	109.6	2766 (94)	120.1	1302 (31)	126.4	2037 (114)	112.9
	7.5	2719 (96)	111.0	3224 (104)	140.0	1747 (53)	109.3	1897 (91)	118.4
	10.0	3159 (50)	129.0	3542 (66)	153.8	1425 (74)	146.7	2107 (54)	110.3
Moso bamboo activated carbon [4)]	1.0	2515 (79)	102.7	2749 (44)	119.4	1685 (2)	141.5	1714 (74)	99.7
	2.5	2304 (50)	94.1	2753 (61)	119.5	2143 (57)	179.9	1873 (107)	108.9
	5.0	2150 (88)	87.8	2680 (88)	116.4	1606 (90)	134.9	1792 (53)	104.2
	7.5	2411 (93)	98.4	2547 (80)	110.6	2397 (81)	201.3	1858 (67)	108.0
	10.0	3051 (122)	124.6	2791 (39)	121.2	2493 (109)	209.3	1597 (97)	92.8

[1)] Survival (residual bacteria rates, %) is the percentage relative to control (as 100%); [2)] Control: no specimen; [3)] Mean (standard deviation)

Table 6. Mutagenicity of Moso bamboo charcoal and activated carbon toward *S. tyhpimurium* TA 98 and TA 100 with or without S9 mixture

Precursor/ Specimen	Amount (mg/plate)	Number of bacteria / plate							
		TA98				TA100			
		– S9	MR [1]	+ S9	MR [1]	– S9	MR	+ S9	MR
	Control [2]	39 (3) [3]	1.0	37 (5)	1.0	123 (7)	1.0	141 (12)	1.0
Moso bamboo charcoal	1.0	32 (7)	0.8	36 (5)	1.0	92 (5)	0.8	125 (8)	0.9
	2.5	39 (9)	1.0	35 (2)	1.0	85 (12)	0.7	124 (16)	0.9
	5.0	47 (9)	1.2	42 (10)	1.0	119 (11)	1.0	134 (8)	1.0
	7.5	30 (6)	0.8	29 (9)	0.8	133 (9)	1.1	116 (19)	0.8
	10.0	50 (10)	1.3	48 (15)	1.3	119 (5)	1.0	126 (10)	0.9
Moso bamboo activated carbon [4]	1.0	42 (6)	1.1	42 (7)	1.1	135 (20)	1.1	125 (8)	0.9
	2.5	26 (6)	0.7	40 (8)	1.1	136 (18)	1.1	127 (12)	0.9
	5.0	43 (5)	1.1	37 (10)	1.0	146 (29)	1.2	129 (14)	0.9
	7.5	46 (11)	1.2	37 (9)	1.0	185 (13)	1.5	134 (13)	1.0
	10.0	39 (12)	1.0	39 (2)	1.0	156 (7)	1.3	125 (23)	0.9

[1] MR (Mutagenicity ratio) = induced revertants per plate/spontaneous revertants per plate (Control); [2] Control: no specimen; [3] Mean (standard deviation); [4] Preparation conditions of the AC were activation temperature at 900˚C with 120 min of activation time

For heavy metal element analysis of charcoals and AC, Br, Pb, Hg, Cr and Cd were analyzed using XRF, as shown in Table 4. Br and Cr were not detected because their amounts were probably very low (the results are not shown in Table 4). Both Cd and Hg in the charcoal and AC were 0.5–1.6 and 0.5–1.2 ppm. The Pb in charcoal and AC prepared with CO_2 at 900˚C as the activation temperature for 150 min, was 0.3–2.9 ppm. That met the Sanitation Standard for Edible Natural Colorants, Food Sanitation Standards (1989), Ministry of Health and Welfare in Taiwan at below 40 ppm. The Pb results for activated carbons from agricultural waste corn cobs, bagasse piths and mushroom stalks at different activation temperatures (Tseng, 2007) was also below 40 ppm. This indicates that the charcoal and AC refined from the charcoals can be expected to be the same as the materials of edible natural colorants.

Cytotoxicity and mutagenicity of charcoals and AC

The cytotoxicity test results of 1.0, 2.5, 5.0, 7.5 and 10.0 mg of Moso bamboo charcoal and Moso bamboo AC (activation temperature 900˚C with 120 min of activated time) for *S. typhimurium* TA98 and TA100 are shown in Table 5. The survival (residual bacteria rates, %) of the Moso bamboo charcoal and Moso bamboo AC with either S9 (+S9) or zero S9 (–S9) is higher than 80 %. Waleh *et al.* (1982) indicated that the amount of residual bacteria of *S. typhimurium* must be over 80% of the control group to determine that the test group has no cytotoxicity for *S. typhimurium*. The results show that the survival was higher than the control by more than 80%. Therefore, the Moso bamboo charcoal and Moso bamboo AC have 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. Moreover, the survival of the other specimens (Makiko bamboo charcoal, Makiko bamboo AC, Japanese cedar charcoal, and Japanese cedar AC) with +S9 or –S9 in the test range (1–10 mg/plate) were all higher than those of the control by more than 80% (results not shown in this paper).

Table 6 shows the mutagenicity test results of the Moso bamboo charcoal and Moso bamboo AC for *S. typhimurium* TA98 and TA100. The specimens, with or without S9, in the test range (1–10 mg/plate) did not exceed spontaneous revertants by more than two times for TA98 and TA100; that is, the mutagenicity ratio (MR) was smaller than 2. The MR of the other specimens was also smaller than 2 (results not shown in this paper). According to the standards proposed by Ames *et al.* (1975), 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 charcoals and the AC, refined from the charcoals, have no mutagenicity toward *S. typhimurium* TA98 and TA100.

CONCULSION

The objectives of this study were to investigate the absorption and the preliminary safety evaluations of charcoals and AC refined from charcoal. The ash of each charcoal was between 1.3 and 3.1%. The yield of refined AC was between 47.5 and 90.4%. The benzene absorption and the iodine value of AC were all higher than those of charcoal. The true density of the charcoal and AC was 1.68 to 1.86 and 2.11 to 2.17 g/cm^3, and the BET surface area of the AC was obviously higher than that of charcoal. The average pore diameter was from 2.22 to 2.69 nm. For the hygroscopicity, no matter what the RH was, the hygroscopic ability of AC was better than that of charcoal. The Aw of charcoal and AC, compared to Aw for the growth of a microorganism environment, was below 6.0. The heavy metal content (as Pb ppm base) of

charcoal and AC were below 40 ppm and met the Sanitation Standard for Edible Natural Colorants, Food Sanitation Standards. For the Ames test, all the charcoals and AC had no toxicity and mutagenicity toward *S. typhimurium* TA98 and TA100. It is suggested that charcoal and AC, refined from the charcoals, can be expected to be similar to the materials of edible natural colorants and/or natural drying agents.

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