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Development of Environmental Protection Wood–Based Activated Carbon Fibers Paperboard and its Application in Hygroscopic Ability

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To develop Wood–Based Activated Carbon Fibers Paperboard (WACFP) as a moisture–proof material for food use, Nadelholz/Laubholz Unbleached Kraft Pulp (NUKP/LUKP) and cardboard from recycled cartons were used as precursors for the method of physical activation with steam to prepare activated carbon fibers (ACFs). The ACFs were evaluated by a preliminary safety evaluation (Ames Test) and reverse mutation assay (antimutagenic activity). The Survival (%) for *Salmonella typhimurium* TA98 and TA100, with or without the S9 mix (an external metabolic activation system) in the test range (1.0–5.0 mg/plate of ACFs), were all higher than those of a blank (control group) by more than 80%, and the ACFs for TA98 and TA100 with or without S9 did not exceed spontaneous revertants by more than two times, indicating the ACFs had no cytotoxicity or mutagenicity. The antimutagenicity against strains for the ACFs showed an insignificant difference between his^+ revertants and the blank, suggesting the safety of the ACFs used primarily as a material for food use. WACFP was made by adding 10, 30 and 50 wt% of ACFs using the Beating CNS 12495 Method combined with preparation by the Handsheets CNS 11212 Method. The hygroscopic ability of WACFP was investigated. The water activity of all WACFP was from 0.40 to 0.45. The hygroscopicity of WACFP was 18.74–26.50% and 5.43–6.36% for 90% and 40% relative humidity (RH), and was lower than that of silica gel, 37.20 and 11.70%. While the hygroscopicity was changed from an RH 90% of absorption to an RH 40% desorption, the percent of desorption for the WACFP was reduced faster than that for silica gel. This can demonstrate that the percent weight of the tested saturated WACFP with different temperature desorptions decreased faster than that of silica gel. As a result, WACFP using ACFs prepared from NUKP, LUKP or cardboard could be a potential moisture–proof material for food use.

Key words: Activated Carbon Fibers (ACFs), Ames Test, Wood–Based Activated Carbon Fibers Paperboard (WACFP), Water Activity, Hygroscopic Ability

INTRODUCTION

Activated carbon fibers (ACFs) are generally regarded as fibrous and porous material that have a large specific surface area, a high adsorption capacity and the ability to regenerate through absorption/desorption (Asakura *et al*., 2004; Huang, *et al*., 2010; Liou, 2012). They are widely used as an adsorbent for separating and purifying gaseous or aqueous solutions (Srinivasakannan and Bakar, 2004; Zhang *et al*., 2004). Moreover, ACFs can provide better characteristics such as a low–pressure drop to mass transfer and high contact efficiency, which is different from powder or granular activated carbon (Asakura *et al*., 2004). These characteristics are due to their fibrous shape with a high aspect ratio, and ACFs prepared from softwood consisting mostly of micropores, as well as mesopores and macropores were formed in the case of the hardwood fiber (Asakura *et al*., 2004; Huang, *et al*., 2010).

Previous work established that ACFs could be prepared by physical methods with steam activation from Nadelholz/Laubholz Unbleached Kraft Pulps and cardboard from recycled cartons. SEM observation of the ACFs showed that the hollow structures and some of the pits from the fibers still rested after wood pulps or paper wastes were processed at a higher temperature. The yield of all ACFs was 6.29 to 14.35%, the range in iodine values was between 635.45 and 1077.72 mg/g and the methylene blue adsorption value was about 268.33 to 504.03 (mg/g) (Huang *et al*., 2010). It is well known that the hollow structure and good absorption of various activated carbons can be prepared from a natural material and can provide a functional adsorbent material, especially for a moisture–proof material for food use, such as: placed in salt, pepper or flavoring jars to keep them dry and prevent deliquescence and foreign flavor, or as a treatment process, e.g. purifying drinking water and conserving medical drugs (Liou, 2012; Lin, *et al*., 2014a; Lin, *et al*., 2014b). However, ACFs are a type of fibrous material with a shape between powder and granular activated carbon. This is inconvenient for placing with food con-

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sidering the dimension and shape of ACFs; even if they have excellent absorption performance.

Therefore, the study attempted to develop a type of food's moisture–proof material with a paper shape. Safety should be considered when comparing the commercial moisture–proof material, silica gel and calcium oxide (CaO) because the appearance of silica gel is a transparent particle which can be eaten as crystal sugar, possibly resulting in uncomfortable pain, and for CaO it is white or gray–white with toxicity, that, if eaten, results in an illness of the intestines and stomach (National Poison Center, 1990). Nevertheless, ACFs as a food's moisture–proof material will probably be in contact with food directly or indirectly, and could even be eaten. It is necessary to undergo a genotoxicity study (Ames Test) in accordance with Item 2, Article 3 of the Health Food Control Act and the Health Food Safety Assessment Method specified in W.S.S.Z. No. 88037803 announcement from the Ministry of Health and Welfare, 1999.

The objectives of the study were to evaluate the *Salmonella* mutagenesis assay (Ames Test) and reverse mutation assay (antimutagenic activity) of ACFs first and to develop a food moisture–proof material, Wood–Based Activated Carbon Fibers Paperboard (WACFP) with different percent weights of ACFs. WACFP was made by using the method of Beating CNS (Chinese National Standards) 12495 combined with the method of preparation of Handsheets CNS 11212. To gauge the effectiveness of moisture–proof material, the hygroscopic ability of WACFP was investigated, including water activity (Aw), hygroscopicity with either a higher or lower relative humidity at a specific temperature and the effect of absorption and desorption. This study uses wood pulp and paper wastes as the precursors to develop the products – WACFP with ACFs – that can provide a moisture–proof application for food to increase their value.

MATERIALS AND METHODS

Specimen preparation and characterization

Precursor

Nadelholz/Laubholz Unbleached Kraft Pulps (NUKP/LUKP) paperboard was provided by the Hou–li Mill, Cheng Loong Corporation in Taiwan. Cardboard from recycled cartons without a printing process was collected. The specimens, NUKP/LUKP paperboards and cardboard, with dimensions of 20 mm×20 mm were prepared and then, defibrillated by a grinder to be used as the precursor. The basic properties and morphological characteristics of the specimens refer to (Huang, *et al*., 2010; Liou, 2012).

Test strains

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 mix), as an external metabolic activation system, was prepared from Sprague–Dawley male rats treated with Aroclor 1254 (Organ Teknika Co., Switzerland).

Mutagens

The mutagens were 4–nitroquinoline–N–oxide (NQNO), a direct mutagen and the mutagenicity of aflatoxin B_1 (AFB_1), an indirect mutagen which requires metabolic activation. They were obtained from the Sigma Chemical Co. (Steinheim, Germany). All reagents used in the test were of analytical grade.

Preparation of activated carbon fibers (ACFs)

The precursor, 20 g of absolute dried specimen, 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, and the specimen was heated to the carbonization temperature of 800°C. The activation temperature was carried out at 800°C with an activation duration of 60 min. The steam was maintained at a flow rate of 1 L/h. The ACFs were then cooled by N_2 gas to a normal temperature and taken out. The preparation ACFs code was ACFs–precursor (N: NUKP; L: LUKP; R: cardboard) activation temperature, such as ACFs–N800 (Table 1). The aforesaid preparation conditions refer to (Huang *et al*., 2010). The equation for the ACFs yield (Y) is Y (%) = (absolute dry weight of ACFs/absolute dry weight of test precursor) × 100.

Iodine value

The iodine values of the ACFs were measured according to the Japanese Industrial Standard (JIS) K 1474 (1991) Test Methods for Activated Carbon. The formula for iodine adsorption capacity is: $I = [(10 - K \times f) \times 12.69 \times 5] / M$. 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: the weight of absolute dried ACFs (0.5 g).

Porosity measurements

The pore structure characteristics of the resulting ACFs were determined by N_2 gas adsorption at 77K with an automated adsorption instrument (BET–202A, Porous Materials, Inc.) in a relative pressure (*P/Po*) ranging from 10^{-2} to 1. The BET specific surface areas (S_{BET}) of the ACFs were analyzed by the standard BET equation and its 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 ($D = 4\ Vtot / S_{BET}$) was calculated (Hu and Srinivasan, 1999).

Ames Test

Cytotoxicity

1.0, 2.5 and 5.0 mg of the ACFs were examined with *S. typhimurium* TA98 and TA100 for either S9 mix (+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 greater than the bacterial count of the control group (no specimen; blank) by 80% (Survival), there was no cytotoxic-

ity. The formula used was Survival (%) = (the bacterial count of test group/the bacterial count of control group) * 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 group (blank). The detailed procedures refer to (Tseng, *et al*., 2010; Liou, 2012). If the colony count of the TA98 and TA100 test group was greater than the blank by more than two times; that is, the Mutagenicity Ratio was larger than 2, the ACFs were considered to have mutagenicity. The Mutagenicity Ratio (MR) = induced revertants per plate/spontaneous revertants per plate (blank).

Antimutagenic activity

The ACFs of the antimutagenic activity were assayed according to the Ames Method (Maron and Ames, 1983). The mutagens that were diluted with dimethyl sulfoxide (DMSO) were NQNO (1 μg/plate for TA98 or TA100) and AFB_1 (5 μg/plate for TA98 or TA100), which required an S9 mix for metabolic activation. A mutagen (0.1 mL; contained 1 μg NQNO or 5 μg AFB_1) was added to *S. typhimurium* TA 98 or TA 100, and 1.0, 2.5 and 5.0 mg of the ACFs were added to the S9 mix for NQNO or AFB_1 to the phosphate buffer (0.1 mol/L, pH 7.4). The test for antimutagenicity was carried out at 37°C for 48 h. The detailed procedures refer to (Liou, 2012; Lin, *et al*., 2014a). The mutagenicity of each mutagen in the absence of an extract is defined as 100%. The inhibition (%) of mutagenicity for ACFs is calculated as follows:

Inhibition (%) = [1 – (Number of his^+ revertants in the presence of ACFs – Number of spontaneous revertants)/(Number of his^+ revertants in the absence of ACFs – Number of spontaneous revertants)] × 100

Development of Wood–based activated carbon fibers paperboard (WACFP)

This developed method of WACFP was designed by the Laboratory of Environmental Functional Materials, Department of Wood–Based Materials, National Chiayi University in Taiwan. WACFP, based on 360 g/cm^2, with ACFs was made by using the method of Beating Chinese National Standards (CNS) 12495 combined with the method of preparation of Handsheets CNS 11212. During the method of CNS 12495, NUKP 20% and LUKP 80% were mixed with different percent weights (%) of ACFs, adding 10, 30 and 50 wt%. The aforesaid detailed steps refer to (Liou, 2012). The developing WACFP code was WACFP–precursor (N: NUKP; L: LUKP; R: Cardboard) percent weight (%) of ACFs. The abbreviation for each WACFP is shown in Table 1.

Table 1. Abbreviation of each wood–based activated carbon fiber paperboard

Specimens	ACFs (wt%)	Abbreviation of WACFP [1]
ACFs–N800 [2]	10	WACFP–N10 [3]
	30	WACFP–N30
	50	WACFP–N50
ACFs–L800	10	WACFP–L10
	30	WACFP–L30
	50	WACFP–L50
ACFs–R800	10	WACFP–R10
	30	WACFP–R30
	50	WACFP–R50

[1] WACFP: Wood–based activated carbon fiber paperboard

[2] ACFs (activated carbon fibers) – precursor (N: NUKP; L: LUKP; R: cardboard from recycled cartons) – Activated temperature

[3] WACFP – precursor (N: NUKP; L: LUKP; R: Cardboard from recycled cartons) – percent weight of ACFs

Hygroscopic Ability of WACFP

Water activity (Aw)

The Aw of the WACFP was determined using the Test for Water Activity of Food, and the procedure was determined in consultation with the CNS 5255 (1987). Six replicates of each specimen for either the WACFP or silica gel were investigated.

Hygroscopicity

Each WACFP weighing 3 g was placed in a bottle without a top. The hygroscopicity of the WACFP was examined in each measured point after a certain period of time, 2, 4, 6, 10, 16, 24, 48, 72, 96 and 114 h, with either a higher relative humidity (RH) at 90% or a lower RH at 40% at a specific temperature of 25°C (Lin *et al*., 2014a). The maximum percent weight (%) under either of the aforesaid conditions for the WACFP and silica gel was investigated.

Absorption and desorption

3 g of an absolute dried specimen of WACFP and silica gel were put in a program–adjusted conditioner (TERCHY HRM Co., Ltd.). A specific temperature of 25°C with a higher RH at 90% was set. The specimens were measured for percent weight of absorption during a period of time, 2, 4, 6, 10, 16, 24, 48, 72, 96 and 114 h, respectively. After that, the resulting specimens were continuous in the conditioner, but a lower RH 40% with a specific temperature of 25°C was altered automatically, and the percent weight of desorption was measured at the same aforementioned periods of time. The percent of absorption and desorption for the WACFP or silica gel was calculated as follows. The formula is: percent of absorption and desorption (%) = [(the weight of absorption or desorption of specimen – the absolute dried weight of specimen)/the absolute dried weight of specimen] × 100. The curve for the percent of absorption and desorption of the WACFP or silica gel was sketched.

Desorption state with different temperatures

To demonstrate that the desorption of WACFP and silica gel was influenced by the absorption state with a higher RH to the desorption state at a lower RH, 3 g of absolute dried WACFP and silica gel were adjusted to become a saturated situation with a RH 90% or 40% RH

in the conditioner. The weight of the specimens was individually measured as 100% of the percent weight, and then, was put at different temperatures (60, 80 and 100˚C) in an oven. The percent weight of desorption for the WACFP and silica gel was measured at a series of certain periods. The resulting curves of the percent weights for the WACFP or silica gel were compared.

Statistical analysis

The test results are represented by a mean (standard deviation), and the control group (blank) and test group are compared by Duncan's Analysis. If the ρ value is smaller than 0.05, meaning a significant difference between the test group and the control group, it is represented by different superscript upper case letters.

RESULTS AND DISCUSSION

Yield, iodine value and porosity of ACFs

As shown in Table 2, the yields of the activated carbon fibers (ACFs) ranged from 10.85 to 13.66% for NUKP/LUKP and cardboard prepared at carbonization temperatures of 800˚C with the carbonization duration of 60 min. The iodine values of ACFs ranged from 647.38 to 1060.97 mg/g. The highest iodine value, 1060.87 mg/g, of ACFs was obtained from NUKP. Commercial activated carbons produce iodine values normally ranging from 600 to 1000 mg/g (Hu and Srinivasan, 1999). The iodine values of the resulting ACFs are similar to the commercial values and denoted indicators of micropore amounts (Chen, 2003) because the diameter of iodine molecules was 0.56 nm (Hsieh and Teng, 1999). Micropores, with pore size diameters less than 2 nm, have been classified by the International Union of Pure and Applied Chemistry (IUPAC).

The S_{BET} and pore characteristics of ACFs from various precursors are also shown in Table 2. The S_{BET} and Vtot of ACFs–N800 and –L800, exhibiting the higher values of 779.12–877.21 m^2/g and 0.90 cm^3/g, were higher than those of ACFs–R800, 262.03 m^2/g and 0.35 cm^3/g. Commercial activated carbons exhibited S_{BET} values ranging from 500 to 2000 m^2/g (Williams and Reed, 2006). Wang (2004) reported that the greater the surface areas, the better the adsorption became, indicating that the adsorption of ACFs–N800 and –L800 is greater than that of ACFs–R800. The percentage of Vmi/Vtot showed that ACFs–N800 had many more micropores (31.5%) than ACFs–L800 and ACFs–R800, 22.5 and 15.0%. Physical activation had pore–drilling and expansion effects at 800˚C producing multiple micropores (Lua and Guo, 2000; Yun *et al*., 2001). The average pore diameters of ACFs–L800 were 4.11 nm, greater than those of ACFs–N800 at 2.14 nm. This is because softwood ACFs consist mostly of micropores and hardwood ACFs resulted in meso– and macropores as well as micropores being formed (Asakura *et al*., 2004; Huang, *et al*., 2010).

Cytotoxicity and mutagenicity of ACFs

Ames *et al*. (1975) reported that for screening of environmental mutagens and carcinogens, the Ames Test, a convenient method to evaluate mutagenic activities of these chemicals, has been developed, and a mistake in the results of mutagenicity occurred due to the decrease in the residual bacterial count with cytotoxicity. Moreover, McCann *et al*. (1975) and Shugimura *et al*. (1976) have suggested that the mutagenic activities of a number of chemicals correlate well with the carcinogenic activities. The cytotoxicity of ACFs therefore, has to be determined before testing the mutagenicity of ACFs. Cytotoxicity results for the ACFs prepared from three types of precursors and silica gel with amounts of 1.0, 2.5 and 5.0 mg/plate for *S. typhimurium* TA98 and TA100 without the S9 mix, as well as with the S9 mix are shown in Table 3.

The residual bacterial count of the control group (blank) for *S. typhimurium* TA98 and TA100 without the S9 mix was 2638 and 2630, and with the S9 mix was 2985 and 2781. For silica gel, the residual bacterial count without S9 was 2607–2714 for TA98 and 2548–2983 for TA100; for those with S9, it was 2807–3142 for TA98 and 2679–2863 for TA100. For all ACFs, the range of the residual bacterial count without S9 was 2472–2740 for TA98 and 2607–2691 for TA100, and with S9 was 2898–3132 for TA98 and 2705–2904 for TA100. Waleh *et al*. (1982) indicated that the residual bacteria rate of *S. typhimurium* must be over 80% of the control group to determine whether the test group has no cytotoxicity for *S. typhimurium*. The residual bacteria rate (Survival,

Table 2. Yield, iodine value and porosity of activated carbon fibers prepared from various precursors with 800˚C activated temperature

Specimen	Yield (%)	Iodine value (mg/g)	S_{BET} [1] (m^2/g)	*V*tot [2] (cm^3/g)	*V*mi [3]/*V*tot (%)	D [4] (nm)
ACFs–N800 [5]	11.38 (0.35)[6]	1060.97 (1.74)	779.12	0.90	31.5	2.14
ACFs–L800	10.85 (0.25)	912.03 (17.04)	877.21	0.90	22.5	4.11
ACFs–R800	13.66 (0.22)	647.38 (26.11)	262.03	0.35	15.0	5.38

[1] S_{BET}: specific surface area; [2] *V*tot: total pore volume; [3] *V*mi: micropore volume; [4] D: average diameter of pore ($4V\text{tot}/S_{BET}$); [5] Abbreviations see the Table 1; [6] Mean (standard deviation)

%) for TA98 and TA100 for all ACFs and silica gel was higher than for the control by more than 80%. This indicates that ACFs and silica gel have no cytotoxicity for the test strains in the additional range of 1.0–5.0 mg/plate, and the dose for the mutagenicity test can be selected according to this range.

Table 4 shows the mutagenicity test results for ACFs and silica gel for *S. typhimurium* TA98 and TA100. The spontaneous revertants of the blank for *S. typhimurium* TA98 and TA100 without the S9 mix were 46

Table 3. Cytotoxicity of silica gel and various ACFs toward *Salmonella typhimurium* TA98 and TA100 without the S9 (–S9) or with the S9 (+S9) mix

Specimen	Amount (mg/plate)	– S9				+ S9			
		TA98	Survival [1] (%)	TA100	Survival (%)	TA98	Survival [1] (%)	TA100	Survival (%)
blank [2]	0.0	2638 (97)^{abc} [3]	100	2630 (108)^{a}	100	2985 (103)^{abc}	100	2781 (133)^{abc}	100
Silica gel	1.0	2607 (104)^{a}	99	2722 (97)^{a}	103	2807 (95)^{a}	94	2863 (164)^{a}	103
	2.5	2714 (86)^{a}	103	2983 (105)^{b}	113	2852 (168)^{a}	96	2748 (103)^{ac}	99
	5.0	2684 (108)^{a}	102	2548 (124)^{a}	97	3142 (115)^{b}	105	2679 (99)^{a}	96
ACFs–N800 [4]	1.0	2548 (84)^{ab}	97	2643 (71)^{a}	100	2929 (81)^{abc}	98	2827 (107)^{abc}	102
	2.5	2472 (101)^{a}	94	2607 (86)^{a}	99	2931 (96)^{abc}	98	2721 (83)^{abc}	98
	5.0	2604 (98)^{abc}	99	2680 (88)a	102	2848 (129)^{a}	95	2852 (95)^{abc}	103
ACFs–L800	1.0	2486 (106)^{a}	94	2663 (66)^{a}	101	3058 (137)^{abc}	102	2904 (94)^{c}	104
	2.5	2696 (103)^{bc}	102	2674 (84)^{a}	102	2898 (94)^{ab}	97	2820 (81)^{abc}	101
	5.0	2590 (86)^{abc}	98	2657 (94)^{a}	101	3132 (112)^{c}	105	2754 (149)^{abc}	99
ACFs–R800	1.0	2712 (98)^{bc}	103	2691 (104)^{a}	102	2903 (82)^{ab}	97	2639 (122)^{a}	95
	2.5	2740 (92)^{bc}	104	2648 (100)^{a}	101	3102 (94)^{bc}	104	2705 (109)^{abc}	97
	5.0	2574 (76)^{ab}	98	2661 (67)^{a}	101	2965 (76)^{abc}	99	2843 (95)^{abc}	102

[1] Survival (%) = (the bacterial count of test group / the bacterial count of control group) * 100
[2] blank (the control group) : the specimen was without silica gel or ACFs
[3] Mean (standard deviation) within a transverse with the different superscripts are significantly different (ρ <0.05) by Duncan's multiple range tests
[4] Abbreviations see the Table 1

Table 4. Mutagenicity of silica gel and various ACFs toward *Salmonella typhimurium* TA98 and TA100 without the S9 (–S9) or with the S9 (+S9) mix

Specimen	Amount (mg/plate)	– S9				+ S9			
		TA98	MR [1]	TA100	MR	TA98	MR	TA100	MR
blank [2]	0.0	46 (2)^{abc} [3]	1.00	156 (06)^{abcd}	1.00	55 (6)^{abc}	1.00	160 (8)^{ab}	1.00
Silica gel	1.0	38 (4)^{a}	0.83	182 (20)^{b}	1.17	57 (7)^{c}	1.04	184 (12)^{b}	1.15
	2.5	43 (3)^{ab}	0.93	132 (12)^{a}	0.85	33 (4)^{a}	0.60	176 (8)^{ab}	1.10
	5.0	58 (3)^{c}	1.26	174 (18)^{b}	1.12	45 (8)^{b}	0.82	181 (17)^{ab}	1.13
ACFs–N800 [4]	1.0	44 (3)^{ab}	0.95	164 (07)^{bcde}	1.05	54 (5)^{abc}	0.99	154 (6)^{a}	0.96
	2.5	48 (3)^{abc}	1.03	168 (05)^{de}	1.07	57 (4)^{abc}	1.04	165 (4)^{abcd}	1.03
	5.0	44 (2)^{ab}	0.95	156 (08)^{abcd}	1.00	54 (3)^{abc}	0.99	161 (8)^{abc}	1.01
ACFs–L800	1.0	48 (3)^{abc}	1.05	154 (05)^{abc}	0.99	58 (2)^{a}	1.06	175 (5)^{cde}	1.09
	2.5	46 (4)^{abc}	1.01	161 (09)^{abcde}	1.03	59 (4)^{abc}	1.08	172 (4)^{bcde}	1.08
	5.0	46 (2)^{abc}	1.00	151 (05)^{ab}	0.97	52 (2)^{abc}	0.95	160 (7)^{ab}	1.00
ACFs–R800	1.0	52 (4)^{c}	1.13	152 (09)^{ab}	0.98	53 (2)^{ab}	0.97	171 (4)^{bcde}	1.07
	2.5	47 (3)^{a}	1.01	150 (06)^{a}	0.96	57 (5)^{abc}	1.03	162 (7)^{abcd}	1.01
	5.0	45 (4)^{abc}	0.97	168 (08)^{de}	1.08	55 (4)^{abc}	1.01	160 (10)^{ab}	1.00

[1] MR (Mutagenicity ratio) = induced revertants per plate/spontaneous revertants per plate (Blank)
[2] blank (the control group) : the specimen was without silica gel or ACFs
[3] Mean (standard deviation) within a transverse with the different superscripts by Duncan's multiple range tests at 5% significant level
[4] Abbreviations see the Table 1

and 156, and 55 and 160 for TA98 and TA100 with the S9 mix. For silica gel, the spontaneous revertants with amounts of 1.0, 2.5, and 5.0 mg/plate without S9 were 38–58 for TA98 and 132–182 for TA100; for those with S9, they were 33–57 for TA98 and 176–184 for TA100. For all ACFs, the range of the spontaneous revertants without S9 was 44–52 for TA98 and 150–168 for TA100, and with S9 was 52–59 for TA98 and 154–175 for TA100. The results also showed that the specimens, with or without S9, in the test range (1.0–5.0 mg/plate) did not exceed spontaneous revertants by more than two times for TA98 and TA100. In other words, the Mutagenicity Ratio (MR) was smaller than 2. According to the standards proposed by Ames *et al*. (1975), if the number of spontaneous revertants induced by the specimen is greater than the spontaneous revertants of the control group by more than two times, the specimen has mutagenicity. Therefore, the ACFs and silica gel have no mutagenicity toward *S. typhimurium* TA98 and TA100 without the S9 and with the S9.

Antimutagenicity of ACFs

ACF is one material for developing WACFP, regarding a moisture–proof material, in this study. The WACFP can easily contact the food directly or indirectly. Therefore, the antimutagenic activity of the ACFs was investigated. This is because the antimutagenic properties have an array of prospective applications in human care, such as the increasing application in drinks, food antioxidation, etc., and have not been reported for the antimutagenic activities of ACFs that have been made so far.

In the present study, NQNO and AFB1 were used as direct mutagens requiring metabolic activation and indirect acting mutagen, respectively. Doses of mutagens, 1 μg for NQNO and 5 μg for AFB_1, were selected from a dose–response curve of a preliminary experiment (Yen *et al*., 2001). The inhibitory effect of the specimen is expressed by inhibition (%), and the higher the inhibition, the more effective the antimutagenic activities are (Maron and Ames, 1983). The inhibitory effects for one of the antimutagenicity results for the ACFs and silica gel with 1.0, 2.5 and 5.0 mg/plate for NQNO and AFB_1 are summarized in Table 5.

The his^+ revertants of strain against the NQNO in the blank (without ACFs or silica gel) were 521 for TA98 and 1000 for TA100, for AFB1: they were 1514 for TA98 and 2395 for TA100. The spontaneous revertants without NQNO were 46 for TA98 and 156 for TA100, and without AFB_1 were 55 for TA98 and 160 for TA100. The his^+ revertants of strain (inhibition) against the NQNO for silica gel were 493–558 (–5.68 to –7.79%) for TA98 and 963–7062 (–7.34 to 8.65%) for TA100. For AFB_1, they were 1509–1583 (–4.72 to 3.84%) for TA98 and 2418–2584 (–8.46 to –1.03%) for TA100. The results also showed that for all ACFs, the his^+ revertants of strain (inhibition) against the NQNO were 514–546 (–5.33 to 1.47%) for TA98 and 980–1023 (–2.76 to 2.41%) for TA100. For AFB_1 with TA98, it was 1526–1573 (–4.02 to –0.80%) and with TA100, it was 2293–2455 (1.77 to

Table 5. Antimutagenicity of silica gel and various ACFs toward *Salmonella typhimurium* TA98 and TA100 without the S9 (–S9) or with the S9 (+S9) mix

Specimen	Amount (mg/plate)	NQNO (1 μg/plate)				AFB1 (5 μg/plate)			
		TA98	Inhibition [1] (%)	TA100	Inhibition (%)	TA98	Inhibition (%)	TA100	Inhibition (%)
blank [2]	0.0	521 (28)[a 3]	0.00	1000 (31)[a]	0.00	1514 (36)[a]	0.00	2395 (38)[abc]	0.00
Silica gel	1.0	548 (62)[a]	–5.68	1062 (53)[a]	–7.35	1509 (78)[a]	0.34	2584 (73)[b]	–8.46
	2.5	493 (37)[a]	5.89	963 (58)[a]	4.38	1458 (55)[a]	3.84	2418 (79)[a]	–1.03
	5.0	558 (35)[a]	–7.79	967 (39)[a]	3.91	1583 (64)[a]	–4.73	2487 (84)[ab]	–4.12
ACF–N800 [4]	1.0	546 (32)[a]	–5.33	990 (67)[a]	1.18	1561 (42)[a]	–0.14	2293 (87)[a]	4.56
	2.5	539 (27)[a]	–3.79	995 (38)[a]	0.59	1544 (44)[a]	–2.06	2365 (56)[abc]	1.34
	5.0	532 (36)[a]	–2.32	1023 (31)[a]	–2.73	1528 (38)[a]	–0.94	2379 (47)[abc]	0.72
ACF–L800	1.0	550 (25)[a]	–6.04	992 (41)[a]	0.95	1569 (29)[a]	–3.77	2345 (49)[abc]	2.24
	2.5	514 (19)[a]	1.47	1019 (64)[a]	–2.25	1526 (70)[a]	–0.82	2455 (63)[abc]	–2.68
	5.0	517 (17)[a]	0.84	949 (43)[a]	6.04	1573 (36)[a]	–4.04	2349 (53)[abc]	2.06
ACF–R800	1.0	523 (40)[a]	–0.42	983 (54)[a]	2.01	1523 (43)[a]	–0.62	2341 (44)[abc]	2.42
	2.5	541 (26)[a]	–4.14	980 (39)[a]	2.37	1563 (49)[a]	–3.36	2342 (52)[abc]	2.37
	5.0	536 (57)[a]	–3.09	1006 (49)[a]	–0.71	1572 (43)[a]	–3.98	2323 (79)[abc]	3.22
Spontaneous revertants		46		156		55		160	

[1] Inhibition (%) = [1 – (Number of his^+ revertants in the presence of the ACF of silica gel – Number of spontaneous revertants) / (Number of his^+ revertants in the absence of the ACF of silica gel – Number of spontaneous revertants)] × 100

[2] blank (the control group) : the specimen was without silica gel or ACFs

[3] Mean (standard deviation) within a transverse with the different superscripts are significantly different (ρ <0.05) by Duncan's multiple range tests

[4] Abbreviations see the Table 1

4.55%). The test groups and blank were compared by Duncan's Analysis. The results showed that the anti-mutagenicity against strains for the ACFs and silica gel displayed insignificant differences between his+ revertants and the blank. In other words, the ACFs and silica gel, a dose–dependent inhibitory from 1.0 to 5.0 mg/plate, does not affect either NQNO or AFB_1 in TA98 and TA100.

In this study, the ACFs showed no cytotoxicity (Table 3)/mutagenicity (Table 4), and were without anti-mutagenic effect (Table 5) against strains. These suggests the safety of the ACFs primarily as a material for food use.

Hygroscopic ability of WACFP

Water activity (Aw) is usually defined as the percent of relative humidity generated in equilibrium with the product in a closed system at a constant temperature, and indicates the amount of water in the total water content available to micro–organisms (Chang *et al.*, 2006); that is, each of the species of micro–organisms (bacteria, yeast and mold) has its own minimum Aw. Furthermore, the Aw control is an important factor for the chemical stability of foods. Table 6 shows the results of Aw and maximum percent weight of WACFP and silica gel in high/low relative humidity conditions at either 90 or 40% with a constant temperature of 25°C. The AW of the blank, without ACFs or silica gel, was 0.42. The Aw of all WACFPs found by adding different percent weights of ACFs was 0.41–0.45, which was higher than that for silica gel at 0.36. The Aw of WACFP was lower than that of micro–organism growth (0.60). The growth of micro–organisms is no longer possible when the Aw is below 0.65–0.95 (Chang *et al.*, 2006).

The results of hygroscopicity for WACFP by adding 10, 30, and 50% ACFs or silica gel at either RH 40 or 90% are also shown in Table 6. The maximum percent weight of WACFP at RH 90% was 18.74 to 26.50%, higher than that of silica gel at 37.20%. For RH 40%, the maximum percent weight of WACFP was 5.43–6.36%, higher than that of silica gel at 11.07%. It is indicated that no matter what the RH is, the maximum percent weight of WACFP is lower than that of silica gel, but higher than that of the blank. According to Duncan's Analysis, at either RH 40 or 90%, the maximum percent weights of WACFP and the blank were obviously different, and the increase by adding amounts of ACFs to WACFP was a higher maximum percent weight.

Table 6. Water activity and maximum percent weight of WACFP and silica gel in high/low relative humidity conditions at either 90 or 40% with a constant temperature at 25°C

Specimen	Aw [1]	Maximum percent weight (%)	
		RH [2] 90%	RH 40%
blank [3]	0.42 (0.00) [4]	17.65 (2.27)^{aA1} [5]	5.10 (0.12)^{aA1}
WACFP–N10 [6]	0.43 (0.01)	21.00 (0.33)^{b}	5.54 (0.36)^{b}
WACFP–N30	0.41 (0.00)	24.00 (0.86)^{c}	6.01 (0.16)^{c}
WACFP–N50	0.43 (0.00)	26.50 (0.58)^{d}	6.36 (0.20)^{c}
WACFP–L10	0.40 (0.01)	20.34 (0.50)^{B}	5.50 (0.29)^{B}
WACFP–L30	0.45 (0.01)	23.26 (0.56)^{C}	6.00 (0.06)^{C}
WACFP–L50	0.45 (0.00)	26.09 (0.67)^{D}	6.25 (0.07)^{C}
WACFP–R10	0.42 (0.02)	18.74 (0.53)^{1}	5.43 (0.29)^{2}
WACFP–R30	0.43 (0.01)	20.78 (1.11)^{2}	5.97 (0.06)^{3}
WACFP–R50	0.43 (0.01)	23.73 (0.72)^{3}	6.14 (0.08)^{3}
Silica gel	0.36 (0.00)	37.20 (0.10)	11.70 (0.17)

[1] Aw: Water activity; [2] RH: Relative humidity; [3] blank (without ACFs or silica gel); [4] Mean (standard deviation); [5] Mean (standard deviation) separation within column to blank with different alphabet and number are significantly different ($\rho < 0.05$) by Duncan's multiple range tests; [6] Abbreviations see the Table 1

Absorption and desorption of WACFP

To evaluate the hygroscopicity change from absorption to desorption, the percent of absorption and desorption for WACFP and silica gel were investigated. WACFP and silica gel were conditioned with high humidity at RH of 90% and temperature at 25°C and then, the percent of absorption at the period of time (114 days) was measured. As shown in Fig. 1, while the specimens reached a saturated situation, the percents of absorption for WACFP–N30, WACFP–L30, WACFP–R30 and silica gel were 18.30, 19.60, 20.71 and 22.08%, respectively. The resulting specimens were continuously conditioned with a lower 40% RH at 25°C. The percent of desorption was 5.27, 5.72 and 7.31% for WACFP–N30, –L30 and –R30, but 15.33% for silica gel. The difference in absorption/desorption percentages for WACFP with different types of ACFs was from 13.30% to 13.88%, and after Duncan's Analysis, the percents of desorption at three measured periods for WACFP were insignificant, but obviously different than silica gel at 7.19%. This indicated that the ability of absorption and desorption of WACFP is better than that of silica gel. Especially hygroscopicity was changed from absorption to desorption; the percent of desorption for WACFP reduced faster than that for silica gel.

To demonstrate the tested saturated WACFP with different temperature desorptions decreased faster than silica gel, the percent weight of WACFP with adding 30% ACFs and silica gel was investigated from saturated situ-

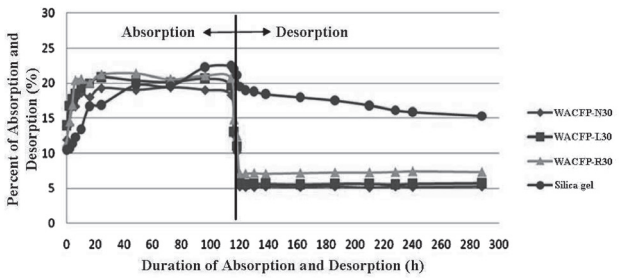

Fig. 1. Percent of Absorption and desorption of silica gel and WACFP with 30% of ACFs
Legend: Abbreviations see the Table 1.

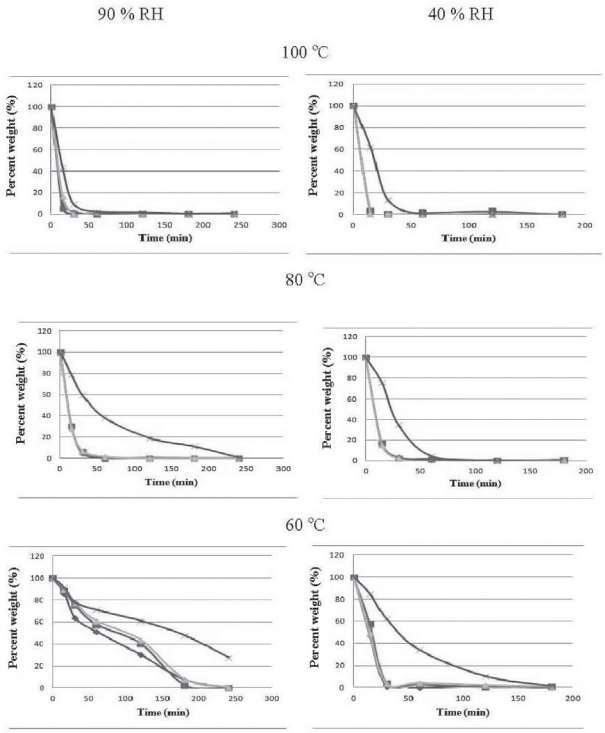

Fig. 2. Weight percent of WACFP with adding 30% of ACFs and silica gel with different temperature desorption
Legend: –◆– : WACFP–N30; –■– : WACFP–L30; –●– : WACFP–R30; –×– : Silica gel; Abbreviations see the Table 1

ation conditioning during RH 90% or 40% at a desorption state with different temperatures at 60, 80, and 100°C. The results are shown in Fig. 2. The percent weight of three types of WACFP with 100°C was no different, reaching a state of absolute dryness after about 30 min, but for silica gel this took about 60 min. At 60 and 80°C, the WACPF had about the same desorption time to reach the state of absolute dryness, and the silica gel needed a longer period by more than four times, indicating that the percent weight of the tested saturated WACFP with different temperature desorptions decreased faster than that of silica gel. In other words, because the change for the percent of absorption and desorption is able to keep or liberate the moisture for food during the different RH environments, the WACFP as a moisture–proof material is probably safe to use for food storage, and it is said that the hygroscopic ability of WACFP is better than that of silica gel from the results of this study.

CONCLUSIONS

ACFs were prepared from NUKP, LUKP and cardboard using the method of physical activation with steam. The yield of ACFs was 10.85 to 13.66%, the range of iodine number was between 647.38 and 1060.97 mg/g, the specific surface area was from 262.03 to 877.21 m^2/g and the average pore diameter was 2.14 to 5.38 (nm). ACFs showed neither cytotoxicity nor mutagenicity toward *S. typhimurium* TA98 and TA100 with or without the S9 mix, and expressed a dose–dependent inhibitory effect at 1.0–5.0 mg/plate against both NQNO and AFB_1 in *S. typhimurium* TA98 and TA100, indicating that the safety of biological action for ACFs can primarily be regarded as a material for food use. WACFP was made with 10, 30 and 50% percent weight of ACFs. The Aw of WACFP was from 0.40 to 0.45. The hygroscopicity of the WACFP was higher than that of the blank (without ACFs) in RH 90% or RH 40% at 25°C, and it was lower than that of silica gel. The percent of desorption for WACFP reduced faster than that for silica gel, demonstrating that for saturated WACFP with different temperature desorption, the change in moisture content decreased faster than that for silica gel. This indicated that WACFP with ACFs prepared from NUKP, LUKP or cardboard can be a potential moisture–proof material for food use.

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