

PREPARATION OF HIGH PERFORMANCE ACTIVATED CHARCOAL FROM RICE STRAW FOR COSMETIC AND PHARMACEUTICAL APPLICATIONS

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ABSTRACT

Objective: Rice straw is an agricultural waste material which has a negative impact on the environmental. This study's aim was to prepare high-performance rice straw activated charcoal (RSAC) for cosmetic and pharmaceutical applications using chemical activation technique.

Methods: Three cultivars of rice straw namely Dok Mali 105 (DM105), RD6 and Niaw San-Pah-Tawng were selected to determine the lignin content. The rice straw with the highest lignin content was selected to produce rice straw activated charcoal. The particle of size 20, 40 and 60 mesh was carbonized at 200, 300, 400, 500 and 600 °C for 1, 2, and 3 h. The obtained charcoal with the highest iodine number was then refluxed using potassium hydroxide and phosphoric acid as an activating agent in the ratios of 1:10 w/v at 70, 80, 90, and 100 °C for 2 (KOH) and 3 (H₃PO₄) h. The adsorption ability on iodine, methylene blue, acetaminophen, and aflatoxin B₁ was investigated. Additionally, pore structure, pore size, and surface area were also determined relative to medical-grade activated charcoal.

Results: DM105 rice straw contains the highest amount of lignin with 24.3 %w/w, followed by RD6 and Niaw San-Pah-Tawng, respectively. DM105 rice straw with particle size 60 mesh carbonized at 400 °C for 2 h (DM60T400H2) demonstrated the highest iodine number. The activation step of DM60T400H2 using 1:10 w/v potassium hydroxide at 70 and 80 °C for 2 h exhibited the highest iodine, methylene blue, acetaminophen, and aflatoxin B₁ adsorption capacity. The scanning electron microscope (SEM) image and Brunauer-Emmett-Teller (BET) showed the microporous structure of obtained RSAC.

Conclusion: RSAC prepared from rice straw of DM105 has potential in cosmetic or pharmaceutical applications.

Keywords: Rice straw, Activated charcoal, Phosphoric acid, Potassium hydroxide, Cosmetic and pharmaceutical applications

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INTRODUCTION

Activated charcoal has become one of the most technically important and widely used adsorbents because of its high adsorptive capacity [1-3]. It can be prepared by two methods: chemical and physical activation [4]. In 2005-2011, Thailand imported approximately 29-83 million tons of activated charcoal per year [5]. Activated charcoal was used in industrial processes, drinking water filtration, gas purification, liquid chemicals, catalyst supports, wastewater treatment, sugar syrup purification, cosmetics, medicinal and pharmaceutical products [6, 7]. Activated charcoal has been reported to reduce low-density cholesterol (LDL), which can cause plaque buildup in the arteries while increasing high-density cholesterol (HDL) [8, 9]. The combination of oral activated charcoal and a low protein diet effectively control the uremic symptoms in old end-stage renal disease patients [10]. Moreover, nanoporous activated charcoal has demonstrated an ability to remove pro-inflammatory cytokines from the blood [11]. Activated charcoal has been used for teeth whitening and clear skin treatment in the cosmetics industry. High porous activated charcoal helps draw toxins from pollution up and out of the skin [7]. Activated charcoal has efficacy in prevention of drug absorption when activated charcoal is administered soon after drug ingestion [13, 14]. It was the best agent to reduce absorption of acetaminophen from a single trial [15].

In Thailand, half of the population is engaged in the agriculture sector, mostly cultivation of rice, which is a staple food of most Thai people [16]. The production of rice reaches around 30 million tons per year. A significant amount of rice straw approximately 25.65 million tons remains in the fields, and this quantity is increasing every year [17]. The open burning of rice straw after harvesting is a common practice for rice straw disposal and the preparation of land

in cultivation areas. This activity has serious negative effects on the air quality, public health, and traffic [2, 18]. Rice straw is one of the most abundant waste materials in the agricultural sector. Its chemical structure consists of cellulose (32-47%), hemicellulose (19-27%) and lignin (5-24%) [19]. In the pyrolysis step, cellulose and hemicellulose make up the volatile fraction, whereas carbonized residue from lignin forms charcoal [6]. Consequently, the composition of rice straw is important in char creation and a higher amount of lignin will affect a higher yield of charcoal. Therefore, lignin is the main source for preparation of activated charcoal [20].

The demand for activated charcoal has grown in the various industries, especially in the medicinal, cosmetic, and pharmaceutical industries. This demand forces us to consider a large number of raw material obtainable for the production of activated charcoal. Thus, the aim of this research was to successfully produce high-quality rice straw activated charcoal for cosmetic and pharmaceutical applications.

MATERIALS AND METHODS

Materials

Dok Mali 105 (DM105), RD6, and Niaw San-Pah-Tawng rice straw samples were collected from the Chiang Mai Rice Research Center, Chiang Mai, Thailand on November 2015. The rice straw was cut into small pieces, washed and dried at 110 °C for 24 h. Rice straw material with a moisture content lower than 5% were selected for the further experiment. The rice straw material was ground and sieved to collect particles of size 20, 40, and 60 mesh. All solvents and chemicals used were either high-performance liquid chromatography (HPLC) grade or analytical grade and were purchased commercially from Sigma Chemical Company (St. Louis, MO), Fluka Chemical Company (Switzerland), and Merck (Darmstadt, Germany).

Methods

Determination of lignin content

Lignin content was determined using the Klason method and the AOAC official method 973.18 with slight modifications by Averous and Digabel (2006) [10]. Briefly, rice straw material was hydrolyzed by 72% H₂SO₄ and refluxed for 3 h. Then, the sample was filtrated and washed until it becomes neutral. The residue was dried at 100 °C for 20 h, cooled in a desiccator and weighed (P1). The residue was then transferred into a crucible and heated at 500 °C for 3.5 h, cooled in a desiccator and weighted (P2). The lignin content was calculated using the following formula.

$$\text{Lignin content} = \frac{P1 - P2}{M}$$

Where P1 is the weight of the sample after drying at 100 °C, P2 is the weight of the sample after heated at 500 °C, and M is the weight of the dried sample.

Carbonization step

The highest lignin content was selected to go on to the carbonization step. The rice straw material was carbonized at 200, 300, 400, 500, and 600 °C in a furnace for 1, 2, and 3 h. After carbonization, the obtained rice straw char (RSC) was cooled down to room temperature in a desiccator before being investigated for iodine and methylene blue adsorption, as well as being measured for pore structure by SEM.

Activation step

The highest iodine and methylene blue adsorption of obtained RSC was selected for the chemical activation step. The activation step was performed by reflux using 85% H₃PO₄ and 85% KOH as an activating agent in the refluxing ratios of 1:8, 1:10, 1:15, and 1:20 w/v at 70, 80, 90 and 100 °C for 1, 2, and 3 h. Then, the obtained rice straw activated charcoal (RSAC) samples were washed with hot water to remove residual acid and base. The neutral RSAC samples were then dried at 110 °C for 24 h and cooled in a desiccator. The RSAC samples were measured for iodine, methylene blue, iron, zinc, acetaminophen, and aflatoxin adsorption. Additionally, pore structure was captured using SEM.

Determination for iodine adsorption

The iodine adsorption efficiency of RSC and RSAC samples were determined relative to a positive control, a commercial medical-grade activated charcoal. The iodine number was determined according to the certified method of American Society for Testing and Materials (ASTM D4607-94) [14].

Determination for methylene blue adsorption

The methylene blue adsorption efficiency of RSC and RSAC samples were examined relative to a positive control; a commercial medical-grade activated charcoal. The methylene blue number was determined according to the method of Japanese Industrial Standards Committee standard (JIS) [12].

Determination for aflatoxin B₁ adsorption

The aflatoxin B₁ adsorption of RSAC samples was carried out as per the method described by Stroka *et al.* (2000) [14] with some modifications. Briefly, 10 µg/ml of standard aflatoxin B₁ solutions was added to 20 mg of the RSAC samples. Then, the mixture was incubated in shacking incubator at 37 °C 300 rpm for 30 min. The RSAC samples were separated from the solution using a syringe filter membrane. The residue of aflatoxin B₁ was analyzed by Agilent 1200 HPLC couple with a fluorescence detector. The mobile phase consists of acetonitrile, methanol, and water in the ratio of 20:20:60 by volume with flow rate of 0.8 ml/min. The RP-C18 Symmetry shield column (4.6 x 250 mm Waters Co., Ltd.) was used, excitation and emission wavelength were set at 365 nm and 450 nm, respectively. Each sample was analyzed in triplicate.

Determination for acetaminophen adsorption

The adsorption efficiency of RSAC samples on acetaminophen was measured by slightly modifying the method of Rey-Mafull *et al.* (2014) [21] in simulated gastric fluid (SGF) at pH 1.2 and simulated intestinal fluid (SIF) pH 6.8. Briefly, acetaminophen was added to the SGF and SIF solutions reaching a concentration 200 µg/ml. Then, 25 mg of RSAC samples were mixed with SGF and SIF solutions and incubated at 37 °C 100 rpm for 30 min in a shacking incubator. The RSAC samples were removed using a syringe filter membrane. The residue of acetaminophen was measured by Agilent 1200 HPLC coupled with UV detector [15]. The mobile phase consists of acetonitrile and 0.1 M KH₂PO₄ in the ratio of 35:65 v/v with a flow rate of 1.0 ml/min. The separation was performed using RP-C18 Symmetry shield column, and the wavelength of the detector was set at 210 nm. Each sample was analyzed in triplicate.

Determination of pore structure

The pore structure of selected RSAC samples was recorded by SEM (JEOL JSM-5910LV) at Science and Technology Service Center, Faculty of Science, Chiang Mai University.

Determination of pore size and surface area

Both of pore size and the surface of selected RSAC samples (0.1000 g) were taken and analyzed by a Surface Area Analyzer (Autosorb 1 Quantachrome, USA) using N₂ as purge Gas.

Statistical analysis

All the results were expressed as a mean of three replicate ± standard deviations (SD). All statistical analysis was conducted using SPSS (version 16). P values < 0.05 were significant.

RESULTS AND DISCUSSION

Three cultivars of rice straw namely DM105, RD6, and Niaw San-Pah-Tawng were selected to determine the lignin content, and rice straw material with highest lignin content was selected for the material to be used in charcoal preparation. The lignin content of the three cultivars of rice straw is presented in table 1.

Table 1: Lignin contents of rice straw samples

Rice straw cultivars sample	Lignin content
DM105	24.28±0.17 ^a
RD 6	20.16±0.04 ^b
Niaw San-Pah-Tawng	18.87±0.04 ^c

*all values were expressed as mean ± SD, n=3, superscript letters show the significantly (p < 0.05) different from each other.

The results indicated that DM105 rice straw has a higher lignin content than RD6 and Niaw San-Pah-Tawng. It has been reported that the raw material with high contents of lignin produces a higher yield of charcoal and a higher amount of activated charcoal. Interestingly, lignin carbon has more pore volume than cellulose-delivered carbon [4]. According to the determined lignin content, DM105 rice straw was then selected to prepare the RSC and RSAC.

Iodine and methylene adsorption values of RSC samples are presented in table 2. The results demonstrated that charcoal obtained from particle size 60 mesh, carbonized at 400 °C for 2 h (DM60T400H2) exhibited the highest iodine and methylene blue adsorption by a significant margin. The carbonization process at 500 and 600 °C was over the carbonization temperature for rice straw, while the carbonization process at 200 °C was under the carbonization temperature for rice straw. The results revealed that

iodine adsorption depends on carbonization temperature, carbonization time and particle size. The RSC samples obtained from 60 mesh (180-250 micron) particle size exhibited higher iodine adsorption values than those obtained from 40 mesh (250-425 micron) and 20 mesh (425-850 micron) particle size. Generally, the rice straw consists of 32-47% cellulose, 19-27% hemicellulose and 5-24% lignin. In this study, DM105 rice straw contains 24.3% lignin. Mohamed et al. (2013) [7] reported that a higher amount of lignin is

necessary to develop more abundant pore structure. Our results demonstrated that the carbonization temperature at 300 and 400 °C is optimum for cellulose and hemicellulose decomposition in rice straw, leading to an increase of porosity and surface area. The DM60T400H2 was then activated with KOH and H₃PO₄. The iodine and methylene adsorption of obtained RSAC samples activated by 1:10 w/v of H₃PO₄ and KOH at 70, 80, 90, and 100 °C are showed in table 3.

Table 2: % yield and an iodine number of RSC samples

Sample code	Particle size (µm)	Carbonization		Iodine number (mg/g)	%Yield
		Temp (°C)	Time (h)		
DM60T300H2	180-250	300	2	28±0.35 ⁱ	47.83
DM60T300H3		3	3	31±0.32 ⁱ	52.04
DM60T400H1		400	1	1	88±0.18 ^c
DM60T400H2	250-425	400	2	158±0.47 ^a	40.78
DM60T400H3		3	3	77±0.27 ^d	36.44
DM40T300H2		300	2	2	13±0.37 ^m
DM40T300H3	425-850	300	3	17±0.23 ^k	45.75
DM40T400H1		400	1	79±0.33 ^d	42.13
DM40T400H2		2	2	48±0.27 ^f	38.67
DM40T400H3	425-850	300	3	90±0.32 ^b	29.27
DM20T300H2		300	2	11±0.35 ⁿ	25.31
DM20T300H3		3	3	16±0.40 ^l	20.11
DM20T400H1	425-850	400	1	38±0.43 ^h	17.48
DM20T400H2		2	2	45±0.21 ^g	15.90
DM20T400H3		3	3	37±0.37 ^h	14.37

*all values were expressed as mean±SD, n=3, superscript letters show the significantly (p < 0.05) different from each other.

Table 3: The iodine and methylene blue number of RSAC samples

Activation Temperatures (°C)	Iodine number (mg/g)		Methylene blue number (mg/g)	
	H ₃ PO ₄	KOH	H ₃ PO ₄	KOH
70	600±2.39 ^g	950±4.41 ^b	60±1.43 ^{de}	63±1.08 ^d
80	646±3.62 ^f	933±3.04 ^c	57±1.12 ^e	109±2.14 ^b
90	532±3.77 ^h	855±4.07 ^e	59±2.10 ^{de}	46±1.26 ^f
100	374±4.22 ⁱ	924±3.53 ^d	65±1.14 ^c	62±1.39 ^d
DM60T400H2	158±0.07 ^k		36±1.08 ^f	
Medical-grade activated charcoal	1009±0.39 ^a		359±0.98 ^a	

*all values were expressed as mean±SD, n=3, superscript letters show the significantly (p < 0.05) different from each other.

Table 3 shows that RSAC samples activated by reflux using KOH in the ratio of 1:10 w/v as an activating agent at 70 °C for 2 h exhibited highest iodine adsorption values, while the RSAC sample activated at 80 °C demonstrated the highest methylene blue adsorption value. The activation of RSC samples using KOH and H₃PO₄ increased the surface area of RSC due to the increase of iodine and methylene blue numbers. The iodine number is directly correlated with the microporous structure (values > 0 to 20 Å, or up to 2 nm) by adsorption of iodine from the solution and it is used as an indication of the total surface area [22, 23]. Sorbents with higher iodine number perform better in removing small sized contaminants. It is the most fundamental parameter used to characterize the performance of activated charcoal [24]. Methylene blue contributes to the mesoporous prediction [25]. The RSAC samples activated by KOH and H₃PO₄ that exhibited a high iodine number might be

composed of microporous structure more than of mesoporous structure.

Aflatoxins are derivative of difurocoumarin, synthesized by various toxigenic species of *Aspergillus*. Chemically, aflatoxins are difurocoumarin and classified into two main groups. The first group includes difurocoumarolactone series of aflatoxin G₁ and G₂, while the other is the difurocoumarocyclopentenone series containing aflatoxin B₁, B₂, B₂A, M₁, M₂, M₂A and aflatoxicol [26]. Aflatoxin B₁ (C₁₇H₁₂O₆) is a common contaminant in a variety of foods including peanuts, dried chili, corn, and other grains as well as animal feeds. Aflatoxin B₁ is considered the most toxic aflatoxin and it is commonly implicated as inducing hepatocellular carcinoma in humans [27]. The adsorption ability of RSAC samples on aflatoxin B₁ are shown in table 4.

Table 4: The adsorption ability of RSAC samples on aflatoxin B₁

Activation temperatures (°C)	% Adsorption		Adsorption capacity (µg aflatoxin B ₁ /g samples)	
	H ₃ PO ₄	KOH	H ₃ PO ₄	KOH
70	66.89±0.42 ^e	71.35±0.48 ^c	0.6675±0.0090 ^E	0.7217±0.0123 ^C
80	64.79±0.32 ^f	80.67±0.54 ^b	0.6465±0.0095 ^F	0.8136±0.0137 ^B
90	65.47±0.37 ^f	67.66±0.37 ^{de}	0.6533±0.0074 ^F	0.6752±0.0095 ^{DE}
100	71.01±0.39 ^c	68.30±0.43 ^d	0.7086±0.0088 ^C	0.6825±0.0087 ^D
DM60T400H2	33.58±0.37 ^g		0.3562±0.0089 ^G	
Medical-grade activated charcoal	82.54±0.22 ^a		0.8603±0.0064 ^A	

*all values were expressed as mean±SD, n=3, superscript letters show the significantly (p < 0.05) different from each other.

All of the RSAC samples activated by H_3PO_4 and KOH exhibited higher aflatoxin B₁ adsorption capacity than those of RSC. RSAC samples activated by KOH revealed higher aflatoxin B₁ adsorption capacity than those activated by H_3PO_4 . The RSAC sample activated by 1:10 w/v KOH at 80 °C for 2 h showed the highest aflatoxin B₁ adsorption capacity but lower than those of medical-grade activated charcoal. The results corresponded to the report of Galvano *et al.* (1996) [28], which reported that 13 experimental activated charcoal samples prepared from natural waste biomass and 4 commercial activated charcoal samples exhibited *in vitro* aflatoxin B₁ adsorption abilities ranging from 44.47% to 99.82%. In addition, mesopores (i.d. from 20 to 500 Å or 2.0 to 50.0 nm) must be directly involved in aflatoxin B₁ adsorption and are able to increase adsorption ability because of a better fit to the aflatoxin B₁ molecule. On the contrary, a microporous structure (i.d.<20 Å or 2.0 nm) could hinder the

diffusion of the aflatoxin B₁ molecules inside the activated charcoal and decrease the adsorption ability.

The acetaminophen (N-acetyl-p-aminophenol, $C_8H_9NO_2$) is a drug with analgesic properties. An overdose of acetaminophen induces hepatic injury and subsequent hepatic failure [29]. The adsorption capacity of RSAC samples on acetaminophen adsorption was evaluated in SGF (pH 1.2) and SIF (pH 6.8). The results demonstrated that most of RSAC samples exhibited a higher acetaminophen adsorption capacity than those of RSC. The RSAC samples activated by KOH showed a potential for acetaminophen adsorption capacity in both of SGF and SIF while the RSAC samples activated by H_3PO_4 exhibited good acetaminophen adsorption in only SIF. The acetaminophen adsorption capacity of RSAC samples are shown in table 5.

Table 5: The adsorption capacity of RSAC samples on acetaminophen

Activation Temperatures (°C)	Adsorption capacity in SGF (g acetaminophen/g samples)		Adsorption capacity in SIF (g acetaminophen/g samples)	
	H_3PO_4	KOH	H_3PO_4	KOH
70	0.0298±0.0015 ^f	0.2153±0.0045 ^b	0.2065±0.0062 ^B	0.1971±0.0047 ^{BC}
80	0.0338±0.0019 ^e	0.1830±0.0056 ^c	0.2066±0.0055 ^B	0.1503±0.0052 ^E
90	0.0113±0.0022 ^{gh}	0.1608±0.0064 ^d	0.1769±0.0063 ^D	0.1271±0.0068 ^G
100	0.0101±0.0018 ^h	0.1739±0.0059 ^{cd}	0.1308±0.0058 ^F	0.1448±0.0057 ^E
DM60T400H2	0.0156±0.0021 ^g		0.0748±0.0045 ^H	
Medical-grade activated charcoal	0.2753±0.0039 ^a		0.4033±0.0044 ^A	

*all values were expressed as mean±SD, n=3, superscript letters show the significantly (p <0.05) different from each other.

Fig. 1 explains the morphology of obtained RSAC from reflux using H_3PO_4 and KOH as an activating agent at 70 and 80 °C. It was found

that the outer part of rice straw activated charcoal obtained from all of activating conditions created the new pore structure.

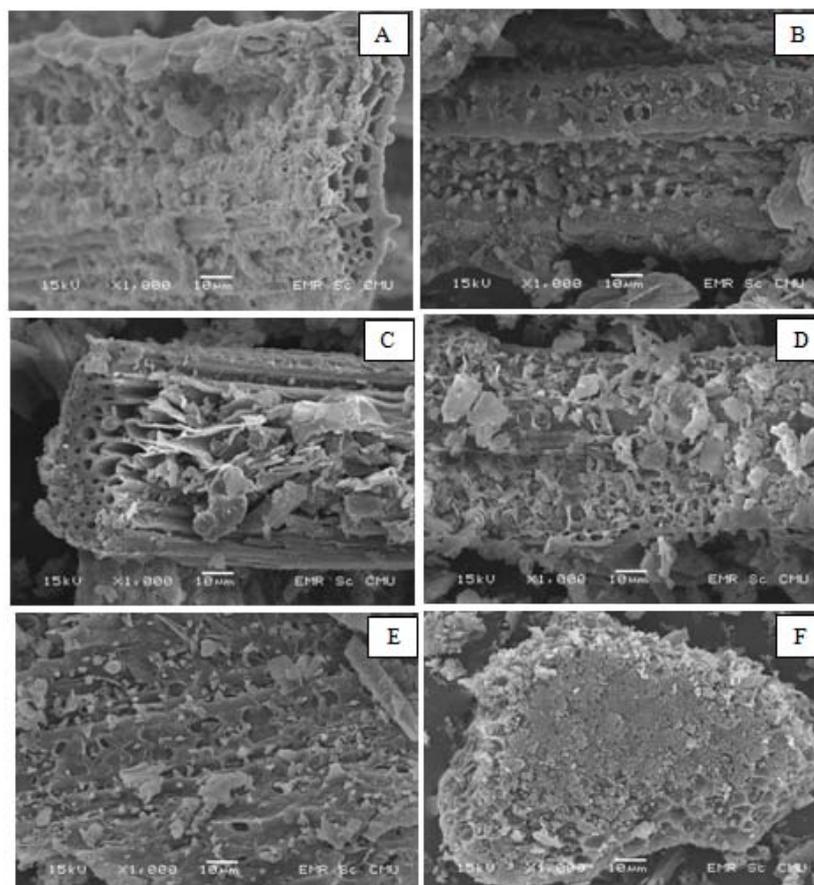


Fig. 1: SEM micrograph of the RSC and RSAC which A: DM60T400H2, B: RSAC activated by H_3PO_4 at 70 °C, C: RSAC activated by H_3PO_4 at 80 °C, D: RSAC activated by KOH at 70 °C, E: RSAC activated by KOH at 80 °C, and F: Medical-grade activated charcoal

The SEM micrograph of the RSC is shown in fig. 1A. It was found that the morphology of obtained charcoal looked like a honeycomb. This can be explained by the fact that the components and inorganic matters of rice straw decomposed and the porous structure was formed during the carbonization process whereas the lignin walls did not modify the pores. Fig. 1B and 1C showed the morphology of obtained RSAC from reflux using H_3PO_4 as an activating agent. It was found that at 80 °C, the inner part of RSAC created a new pore structure. Fig. 1D and 1E show the morphology of obtained RSAC from reflux using KOH as an activating agent. The results indicated that the lignin wall of obtained RSAC formed the new pore structure at 70 and 80 °C. In addition, the average pore size of obtained RSAC

in both KOH and H_3PO_4 activation demonstrated that it is composed of micropore structure (table 6) which correspond to the results of iodine adsorption. The results indicated that KOH treatment leads to a higher surface area than H_3PO_4 treatment. The reaction mechanism of KOH and carbon is a well-established activation method for producing porous activated charcoal materials [30]. A pore structure with the resultant high surface area is formed during KOH activation. It is suggested here that during KOH activation, the chemical reaction between KOH and carbon proceeds as $6KOH+2C=2K+3H_2+2K_2CO_3$; following either decomposition of K_2CO_3 or reaction of $K/K_2CO_3/CO_2$ with carbon, the production of H_2 will potentially open up the pores [31].

Table 6: Pore size and surface area of RSAC samples

Activation temperatures (°C)	Pore size (Å)		Surface area (m ² /g)	
	H ₃ PO ₄	KOH	H ₃ PO ₄	KOH
70	11.65	9.07	642.86	952.72
80	10.54	8.99	715.32	983.08
90	13.32	11.29	487.64	759.28
100	14.27	9.23	550.49	875.10
DM60T400H2	8.39		272.49	
Medical-grade activated charcoal	103.52		1219.52	

CONCLUSION

DM105 rice straw of particle size 60 mesh carbonized at 400 °C for 2 h could affect the physicochemical properties of obtained RSC. The chemical activation by reflux using KOH as an activating agent in ratio 1:10 at 70 and 80 °C was more efficient in producing RSAC which exerted the potential to be used for cosmetic or pharmaceutical applications.

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AUTHORS CONTRIBUTIONS

All the author contributed equally

CONFLICT OF INTERESTS

Declared none

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