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Journal of the Taiwan Institute of Chemical Engineers 117 (2020) 198?208

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2**Saponin-intercalated organoclays for adsorptive removal of b-carotene: Equilibrium, reusability, and phytotoxicity assessment**

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## ARTICLE INFO

**9Article History: Received 9 August 2020 Revised 23 November 2020 Accepted 30 November 2020 Available online 6 December 2020**

**Keywords:** Rarasaponin Microwave irradiation b-carotene Adsorption isotherm Reusability Phytotoxicity

**ABSTRACT** Acid-activated clays play an essential role in the edible oil refining industry to remove colored pigments and impurities to meet consumer demands and specific purposes. Despite its high bleaching activity, the use of highly corrosive acids in significant quantities for producing the activated clay raises safety and environmental concerns. Herein, we demonstrate an environmentally friendly and low-cost preparation of organoclay-type bleaching earth via aqueous phase intercalation of bentonite with natural surfactant (rarasaponin) under microwave irradiation. The influence of the rarasaponin concentrations on the textural and chemical characteristics of the resultant organoclays was investigated with relevant techniques, including SEM, XRD, FTIR, and N<sub>2</sub> sorption. The results revealed that the intercalation of rarasaponin causes a basal spacing increase to 1.50 nm, surface area to 99.5 m<sup>2</sup>/g, and pore volume to 0.85 cm<sup>3</sup>/g, while the cation exchange capacity (CEC) value decreased to 19.1 meq/100 g; where the untreated bentonite has a basal spacing of 1.39 nm, a surface area of 86.8 m<sup>2</sup>/g, a pore volume of 0.69 cm<sup>3</sup>/g, and a CEC value of 30.1 meq/100 g. The RSB-2 organoclay exhibits the best bleaching activity, with ~91% b-carotene removal efficiency achieved in degummed palm oil compared to the untreated bentonite (72% efficiency). The equilibrium behavior of b-carotene adsorption onto RSB-2 organoclay was best fit with the Redlich-Peterson isotherm model, giving the theoretical maximum sorption capacity (q<sub>max</sub>) 78.09 mg/g, which represents the best-reported values among the investigated samples. A reusability study of the spent bleaching clay demonstrates that the adsorptive removal of b-carotene remained greater than 90% after five consecutive cycles. The spent RSB-2 organoclays also showed no appreciable phytotoxicity toward Arabidopsis seed germination but a slight inhibitory activity on the root development, suggesting environmentally friendly behavior and allowing for the landfill disposal. © 2020 Taiwan Institute of Chemical Engineers. Published by Elsevier B.V. All rights reserved.

**1. Introduction** Smectite represents one of the most important swelling clay families for various applications, including engineered barriers for nuclear waste repositories, catalyst support, and the oil recovery process [1-3]. Among the industrial applications of

clay material, the use of clays in the refinery process has attracted widespread attention. Bleaching is a crucial treatment process in refining crude vegetable oils aside from the degumming and deodorization steps. All of the three steps above are known as the physical refinery technique, in \* Corresponding author. E-mail address: shella\_p5@yahoo.com (S.P. Santoso). which the removal of undesired constituents in the crude palm oil (CPO) takes place [4,5]. The degumming step using phosphoric acid is used to remove the phospholipids, where high amounts of phospho- lipids can interfere with the oxidative stability of oils and bleaching power of the bleaching earths [6]. The bleaching and deodorization steps are mainly used to remove the unwanted pigments from oil by means of adsorption and thermal degradation, respectively. [7]. Bleaching is considered the more crucial step than the deodorizing step since most pigment removal occurs in this step. Meanwhile, the deodorizing step is used to degrade the remaining pigments ther- mally [8,9]. <https://doi.org/10.1016/j.jtice.2020.11.036> 1876-1070/© 2020 Taiwan Institute of Chemical Engineers. Published by Elsevier B.V. All rights reserved. Carotenes, especially b-carotene, are the primary compounds that are removed during the bleaching operation. Despite its benefits as the anti-oxidant properties, the high amount of b-carotene can greatly decrease the oxidation stability of oils since this compound is resistant to oxidative reaction [10,11]. The removal of b-carotene occurs by means of adsorption using bleaching earths. Clay materials, such as bentonite, kaolin, and sepiolite, are the common bleaching agents (also called bleaching earth) for CPO [12?17]. In the prepara- tion of bleaching earths, an activation step was employed to increase their specific surface area and porosity [18?20]. Chemical activation using concentrated mineral acids is the most popular method used for activating the clay minerals due to its simplicity. However, the acid residue from the activation process can cause severe corrosion to the process equipment and also harmful effects on the environ- ment [21]. Several studies have proposed an alternate activation technique by surfactant intercalation [20,22,23]. For example, Guna- wan et al. demonstrated that the cetyltrimethylammonium bromide (CTAB) intercalated bentonite could achieve 65.44% color removal of crude palm oil (CPO). In comparison, the raw bentonite merely removes 49.35% of the color [24]. However, synthetic surfactant use may raise another health issue; e.g., CTAB is known to be potentially toxic to the cells of living organisms with a concentration as low as 10 mM [25,26]. Saponins represent an important class of natural surfactants derived from the fruit pericarps of tropical/subtropical tree species belonging to the Sapindaceae (or soapberry) family. In comparison to the synthetic surfactants, saponins exhibit many desirable attributes, such as biodegradability, renewability, and unique bioactivity, as well as exert relatively low (or nil) toxicity since it can hardly absorb by the cells of living organisms [25,26]. More recently, plant-based saponins have also been employed as intracellular delivery systems of protein drugs for improved therapeutic and bioimaging applications [26], which further highlighting its biocompatible nature. In the previous study by Kurniawan et al. [20], rarasaponin has been utilized as an intercalating agent to prepare organobentonites with enhanced adsorption capacity toward organic dyes. However, to the best of our knowledge, there have been no reports investigating the perfor- mance of rarasaponin-intercalated bentonite (RSB) for b-carotene bleaching in vegetable oils. The use of RSB organoclay as bleach- ing earth can provide a low-cost and greener alternative to com- mercial acid-activated bentonite clays. The aims of the present study are to investigate the influence of rarasaponin concentra- tion during the microwave-assisted intercalation process on the physical textural and chemical properties of the resultant organo- bentonites and to optimize the concentration with respect to the bleaching performance. The physical characteristics evaluation includes the

20cation exchange capacity (CEC), specific surface area, and

pore structures. The bleaching performance was assessed based on the b-carotene removal efficiency in oil as the model oil, which is regarded as the most important parameter in assess- ing the bleaching efficiency. In addition, we also evaluated the reusability and the environmental-safety aspects of the spent bleaching earth through in vivo plant assay based on the inhibi- tory activity of seed germination and the root growth of Arabi- dopsis thaliana. 2. Experimental section 2.1. Materials Hydrogen peroxide solution (H<sub>2</sub>O<sub>2</sub>, 30 wt%), HPLC grade n-hexane (C<sub>6</sub>H<sub>14</sub>), and phosphoric acid (H<sub>3</sub>PO<sub>4</sub>, 85 wt%) were

**8**obtained from Sigma-Aldrich, Singapore. Sodium hydroxide (NaOH, 96

%), ethanol (C<sub>2</sub>H<sub>5</sub>OH, 96%), and methylene blue (C<sub>16</sub>H<sub>18</sub>ClN<sub>3</sub>S, 85% dye content, C. I. 52,015)

**8**were obtained from Merck, Germany. Unless otherwise specified, **all chemicals were used as received without further purification. The water used to prepare aqueous solutions** was purified with a Millipore Milli-Q system

to a resistivity of 18.2 MV cm. Natural calcium bentonite was collected from Pacitan city, East Java, Indonesia. The bentonite chunks were pulverized into fine powders using a laboratory hammer mill (Janke & Kunkel, IKA Labortechnik) and sieved pass through an 80/100 mesh screen, followed by purification using 10% (v/v) H<sub>2</sub>O<sub>2</sub> according to the procedure described elsewhere [27]. The CEC of purified bentonite powders was determined to be 30.1 ± 3.7 mequiv per

**16**100 g clay based on the standard ASTM C837-99

method. Elemental chemical analysis of bentonite powders by X-ray fluorescence spectroscopy (XRF) shows the following metal (as oxide) contents: Al<sub>2</sub>O<sub>3</sub> 30.20%, SiO<sub>2</sub> 49.31%, Fe<sub>2</sub>O<sub>3</sub> 2.98%, CaO 3.91%, MgO 0.71%, K<sub>2</sub>O 0.21%, MnO 0.72%, and Na<sub>2</sub>O 1.63%. Plant-derived rarasaponin was extracted from the dried pericarps of soapberry fruits (*Sapindus rarak*) using a 50% (v/v) ethanol-water mixture at 30 °C for 24 h. Quantification of total saponin content of the crude extract (19.83 mg g<sup>-1</sup>) was carried out following the spectrophotometric method described by Medina-Meza et al. [28]. CPO was supplied by a local company (PT Best Palm Product International, Surabaya) and was degummed using 0.1 wt% H<sub>3</sub>PO<sub>4</sub> at 90 °C for 20 min. The fatty acid composition of degummed oil (DPO) was analyzed by a gas chromatography/flame ionization detection (GC/FID) method [29] and the results are as follow:

**4**lauric acid (C<sub>12:0</sub>) = 2.07%, **myristic acid (C<sub>14:0</sub>) = 2.01%, palmitic acid (C<sub>16:0</sub>**

) = 41.28%,

**4**stearic acid (C<sub>18:0</sub>) = 4.48%, **oleic acid (C<sub>18:1</sub>) = 39.01%, linoleic acid (C<sub>18:2</sub>) = 3.90%, linolenic acid (C<sub>18:3</sub>) = 6.88%, and arachidic acid (C<sub>20:0</sub>**

) = 0.35%. 2.2. Preparation of rarasaponin-intercalated bentonite (RSB) The aqueous phase intercalation method described previously by Kurniawan et al. (2011) was adopted to prepare RSB with minor modifications [20]. Briefly, a clay suspension (2 wt%) was prepared by dispersing 1 g of bentonite powder in 50 mL of DI water and ultrasonicated for 30 min. Subsequently, aqueous rarasaponin solution (50 mL) with different concentrations (i.e., 0.2, 1, 2, and 10 wt%)

**14**was added to the bentonite suspension and stirred for another 10 min. The mixture was then

transferred into a porcelain pan and homogenously heated on a rotating turntable of a domestic microwave oven (Panasonic NN ?CD671, 2.45 GHz) at 700 W for 90 s. Afterward, the mixture was centrifuged (5000 rpm, 15 min) to collect the solids, washed three times with DI water, and oven-dried at 105 °C overnight. The resulting orga- noclays were designated as RSB-x, where x denotes the rarasapo- nin solution concentration. The untreated bentonite (UB) was used as a control bleaching clay. 2.3. Materials characterization Scanning electron microscopy (SEM) images of the UB and RSB samples were taken using a JEOL JSM-6500F field emission scanning electron microscope operating at 10 kV.

14**Prior to the SEM imaging, the samples were sputter-coated with platinum**

to minimize charg- ing effects. High-resolution transmission electron microscopy (HRTEM) characterization was conducted on a Philips Tecnai F30

10**field emission gun electron microscope at 200 kV**

. The textural properties of the bleaching clays were characterized using nitrogen physisorp- tion

10**isotherms at 77 K in a Micromeritics ASAP 2010 gas sorption analyzer**

. All samples were vacuum degassed

10**at 150 °C for 12 h prior to measurement. The specific surface area was**

analyzed

13**using the standard Brunauer-Emmett-Teller (BET) method, and the total pore volume (VT) was estimated from the N2 adsorption amount at a rela- tive pressure (P/P0) of 0**

.95.

11**Fourier transform infrared (FTIR) spectra were recorded on a Shimadzu FTIR**

8400S spectrometer in the 4000?500 cm<sup>-1</sup> range with 4 cm<sup>-1</sup> spectral resolution using the KBr pellet technique. Powder X-ray diffraction (XRD) patterns were collected using a Phillips X'Pert X-ray diffractometer equipped with a

10**Cu Ka X-ray source**

(

$23\lambda = 0.15418 \text{ nm}$ ) operating at 40 kV and 30 mA

. 2.

**14. Bleaching of DPO** The bleaching process of DPO was conducted by adding 3 wt% of

clays (UB or RSB-x) into 100 mL of DPO at 90 °C for 30 min, which mimics the actual bleaching conditions applied in the oil refinery process [4]. The characteristics of the bleached oil were examined based on the relevant standard quality parameters, such as the total carotenoid content (expressed as

**1 b-carotene), free fatty acid** content (FFA as palmitic acid), **and the peroxide value (PV)**. The removal rate of

b-carotene (or the extent of bleaching) in the unit of mg per kg of a dry oil was calculated according to Eq. (1). Removal rate  $\delta\% = \frac{w - w_p}{w} \times \frac{C_i}{C_r} \times 100$   $\delta\% = \frac{C_i - C_r}{C_i} \times 100$

**1 where  $C_i$  and  $C_r$  denote the initial and residual concentrations of b-carotene in**

DPO, which are measured spectrophotometrically at 446 nm [30]. The FFA content and PV were determined

**4 according to the American Oil Chemists' Society (AOCS) Official Method Ca 5a-40**

and Cd 8753, respectively. 2.5. Adsorption isotherm study Static adsorption equilibrium study of b-carotene was performed by adding various amounts of bleaching clays (i.e., 1–10 wt%)

**19 into a series of Erlenmeyer flasks containing 100 mL of**

DPO (preheated to 90 °C). All the adsorptive bleaching experiments were performed for 30 min in a thermostatic silicone oil bath with a temperature-controlled unit. The adsorbed amount of b-carotene at equilibrium ( $Q_e$ ) was calculated with Eq. (2):  $Q_e (\text{mg/g}) = \frac{C_0 - C_e}{m} \times V$  where  $C_0$

**1 and  $C_e$  are the initial and equilibrium concentrations of b-carotene (mg/L), respectively,  $m$  is the mass of adsorbent (g), and  $V$  is the volume of oil (L). The**

regeneration experiments of spent bleaching clays were carried out by immersing the solids in n-hexane under constant

**19 stirring for 30 min at either room temperature**



(referred to as chemical regeneration) or 60 °C (referred to as hybrid thermal-chemical regeneration), followed by drying

21 **in an oven at 105 °C** overnight. In **the** latter regeneration **process**

, a reflux condenser was fitted to prevent solvent loss. The regenerated clays were then reused in the next bleaching test under the same conditions as for the first run. 2.6.

2 **Arabidopsis thaliana growth media supplementation** The environmental feasibility of

spent RSB-x bleaching clays was evaluated as a soil supplement for promoting the growth of plants. *Arabidopsis thaliana* ecotype Columbia (Col-0) was selected as a model plant due to its fast-growing and relatively simple root systems, as well as well-known genetics and physiological characteristics [31?33]. In a typical experiment, the surface-sterilized *A. thaliana*

22 **Col-0 seeds were germinated and grown on**

2 **half-strength Murashige and Skoog (MS) basal medium supplemented with 1% (w/v) sucrose, 0.8% (w/v) agar**

[31,32,34] under continuous light condition (day/night) at 22 °C. For the RSB treatment groups, a known amount (0.1%) of spent RSB-x organoclays was added into the basal medium. After 10 days, the seedlings were observed and then transferred to a fresh medium. After

22 **10 days, the seedlings were** observed and then **transferred to** a fresh **medium**

. Three independent biological experiments were performed for both control and RSB-treated samples, and the results are expressed as average standard deviation.

2 **Statistical analysis was conducted using GraphPad Prism 8.0 software, and significant differences between**

the treatment groups

2 **were examined by one-way analysis of variance (ANOVA) with Tukey's posthoc test. 3. Results and discussion**

3.1. Characterization and formation mechanism of RSB organoclays The CEC of clay materials represents the quantity of the interlayer cations (e.g., Na<sup>+</sup>, Mg<sup>2+</sup>, and Ca<sup>2+</sup>) that neutralizes permanent and pH-dependent charges at the edge and edge sites [35], which are replaceable by other positively charged species through an ion-exchange mechanism. The average CEC values of as-prepared UB and RSB-x

samples ( $x = 0.2, 1, 2$ , and  $10$  wt% of rarasaponin) samples are shown in Table 1. The results demonstrate that the CEC of the clays decreased after the intercalation of rarasaponin molecules, which is proportional to the increase in rarasaponin loading. The decrease in CEC may be ascribed to the interaction between the intercalated rarasaponin and the interlayer cation species through the electrostatic attractions. The interaction involving the carboxyl groups of rarasaponin is also evidenced from the blue shift of the absorption band at  $1246\text{--}1261\text{ cm}^{-1}$  corresponding to the C=O stretching vibration after rarasaponin intercalation (see Fig. 1c). Although the CEC values of RSB organoclays are lower than that of UB, some of them show no statistically significant difference (Student's  $t$ -test,  $P > 0.05$ ). It can also be observed that the measurement of the CEC value of the RSB-10 sample gave a relatively high standard deviation, which may suggest that the decrease in CEC of the clay may involve a rather complex mechanism. And, that of several factors cause the high standard deviation, such as the unequal distribution of cations, pores, and surface area of starting material where some bentonite particles may have either higher interlayer cations or lower surface areas [36]. The  $N_2$  adsorption-desorption isotherms of the UB and RSB samples at  $77\text{ K}$  are presented in Fig. 1a,

**1 while the specific BET surface area and the total pore volume**

deduced from these isotherms are given

**23 in Table 1. The adsorption and desorption behavior of all the clay samples**

reflect type II isotherm in the IUPAC classification [37], which is typical of the micro-mesoporous clay materials. The volume Table 1 The average CEC values and textural properties of RSB organoclays. Clay CECy (mequiv/100 g) Textural Parameters  $d_{001}$  (nm) Interlayer expansionz (nm) SBET ( $\text{m}^2/\text{g}$ ) VT ( $\text{cm}^3/\text{g}$ ) UB 30.1  $\pm$  3.6a RSB-0.2 27.1  $\pm$  3.2a RSB-1 22.7  $\pm$  2.4b RSB-2 19.1  $\pm$  4.4b RSB-10 19.6  $\pm$  8.0ab 86.8 0.69 99.1 0.71 99.4 0.85 99.5 0.85 99.0 0.86 1.39 0.47 1.43 0.51 1.46 0.54 1.50 0.58 1.52 0.60 y Different letters among groups indicate a statistically significant difference (Student's  $t$ -test,  $P < 0.05$ ). z Calculated as the difference between the basal distance ( $d_{001}$ ) and the thickness of dry montmorillonite 2:1 (tetrahedral-octahedral-tetrahedral) layer ( $\sim 9.5\text{ \AA}$ ). Fig. 1. Characteristic of the as-prepared UB and RSB bleaching clays. (

**25a)  $N_2$  adsorption (solid circles) and desorption (empty circles) isotherms measured at  $77\text{ K}$ . (b) XRD patterns of**

Cu K radiation, and (c) transmission FTIR spectra recorded in the  $4000\text{--}500\text{ cm}^{-1}$  range. The pink, blue, and green highlighted wavenumber regions are assigned to the stretching vibrations of C-H, C=O, and C-O-C groups in rarasaponin, respectively. The peak labeled with an asterisk at  $\sim 2350\text{ cm}^{-1}$  for rarasaponin is due to absorption by atmospheric  $\text{CO}_2$ . (

**12 For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)** filling of micropores occurs in the

low  $P/P_0$  region ( $P/P_0 < 0.05$ ) and is followed by a gradual uptake of  $N_2$  at higher relative pressures due to multilayer adsorption on the mesopores and the external surface of the samples. The large hysteresis loop characteristic of capillary condensation in the mesopores was observed in the  $P/P_0$  range of  $0.45\text{--}0.85$  for

all clay samples. A closer inspection of the shape of the hysteresis loop for RSB samples reveals that the pore structure of the resultant organoclays depends on the amount of intercalated rarasaponin. The UB sample possesses a type H4 hysteresis loop with a characteristic steep in the desorption isotherm at  $P/P_0$  range of 0.45–0.5, agreeing well with the typical features of lamellar materials with interconnected micro- and mesopores [37,38]. Upon incorporating rarasaponin molecules, both RSB-0.2 and RSB-1 organoclays exhibit a type H3 hysteresis loop, indicating porous network structure with slit-shaped mesopores. The transformation of the H4-type in UB to H3-type hysteresis loop in RSB-0.2 and RSB-1 may imply a smaller proportion of microporosity in the latter samples, which could be ascribed to the partial blocking in narrow slit-shaped micropores by the intercalating rarasaponin molecules. With increasing concentration of rarasaponin, the hysteresis loops of RSB-2 and RSB-10 samples resemble a combination between types H2 and H3 with a broader asymmetrical loop and a steeper desorption branch at high  $P/P_0$ . This result may suggest the presence of a complex pore network structure consisting of narrow-wide and slit-like channels [39]. Further details about the intercalated structure of rarasaponin molecules in the clay layers will be discussed in the next section. The textural parameter analysis (Table 1) demonstrates that the RSB organoclays exhibit higher specific

21 **BET surface area** (SBET) and total **pore volume** than **the** UB sample. **The**

SBET values are 86.8, 99.1, 99.4, 99.5, and 99.0  $\text{m}^2 \text{g}^{-1}$  for UB, RSB-0.2, RSB-1, RSB-2, and RSB-10 samples, respectively. The higher surface area of RSB organoclays can be attributed to the expanded interlayer distance that increases the accessibility of  $\text{N}_2$  molecules to the micropores. Further discussion on the correlation between the surface area and the interlayer expansion in RSB organoclays is provided in the XRD analysis below. The above results imply that the enhancement in the surface areas of RSB clays is relatively independent of the rarasaponin concentration, suggesting that the interlayer expansion appears smaller than the limiting molecular dimension of  $\text{N}_2$  (0.354 nm [40]). The slight decrease of the surface area of RSB-10 sample prepared with 10 wt% rarasaponin concentration relative to RSB-2 is likely due to the excess amounts of rarasaponin molecules occupying the interlayer spacing, which may reduce and thus restrict the access to the micropores. The intercalation of rarasaponin molecules inside the clay galleries is further confirmed by XRD patterns, as depicted in Fig. 1b. The UB clay shows the Bragg reflections of montmorillonite at  $2\theta$  values of  $6.34^\circ$  (001),  $19.94^\circ$  (011),  $20.95^\circ$  (101), and  $39.49^\circ$  (210), along with impurity phases attributed to quartz ( $\text{SiO}_2$ ,  $2\theta = 26.69^\circ$ ,  $28.50^\circ$ , and  $36.11^\circ$ ) and K-feldspar ( $\text{KAlSi}_3\text{O}_8$ ,  $2\theta = 21.80^\circ$  and  $24.53^\circ$ ) minerals [20]. The basal distance ( $d_{001}$ ) of UB clay was determined to be 1.39 nm from a Bragg peak at  $2\theta = 6.34^\circ$  by the Scherrer equation. After the insertion of rarasaponin, the change in the Bragg peak position is evident for the basal 001 reflection, which shows dependence on the rarasaponin concentration. In the case of RSB-0.2 and RSB-1 samples, the basal 001 reflection shifts to lower  $2\theta$  angles of  $6.19^\circ$  and  $5.97^\circ$ , respectively, which is indicative of the intercalated clay gallery with enlarged basal spacings of 1.43 and 1.46 nm. With greater rarasaponin loadings of 2 and 10 wt% in RSB-2 and RSB-10 samples, the basal spacing of the organoclays becomes further and vanished, suggesting that the montmorillonite layer structure becomes delaminated or distorted as a result of induced by excess rarasaponin molecules. The successful modification of the clay interlayer with rarasaponin is further verified by FTIR spectroscopy. As presented in Fig. 1c, the transmission IR spectrum of rarasaponin exhibits the characteristic vibrations of functional groups corresponding to the aglycone and sugar moieties, such as O–H stretching ( $3300 \text{ cm}^{-1}$ ), asymmetric and symmetric C–H stretching of the  $\text{CH}_2$ , and groups ( $2800$ – $2950 \text{ cm}^{-1}$ ), C=O stretching ( $1718 \text{ cm}^{-1}$ ), C–O–C stretching of the acetyl group ( $1215 \text{ cm}^{-1}$ ), and O–H bending modes ( $923 \text{ cm}^{-1}$ ). Meanwhile, the UB clay shows the stretching vibration of the OH groups at  $3700$  and  $3358 \text{ cm}^{-1}$ , which corresponds to the siloxane-bound water and the interlayer water molecules, respectively. The former

17 **band at  $3700 \text{ cm}^{-1}$  can** also **be** ascribed **to the** stretching mode **of H**

-bonded of silanol groups. The IR bands at 1644  $\text{cm}^{-1}$  and 1525  $\text{cm}^{-1}$  are associated with the  $\text{H}_2\text{O}$  bending mode of water molecules bound to the interlayer  $\text{Ca}^{2+}$  cations and  $\text{N-H}$  stretching modes. Additionally, the stretching vibrations of the siloxane bond ( $\text{Si-O-Si}$ ) and  $\text{Si-O}$  of the surface silanol ( $\text{SiOH}$ ) groups appear at 1050 and 958  $\text{cm}^{-1}$ , respectively. The low-frequency bands in the 800–1000

17 $\text{cm}^{-1}$  region can be assigned to the  $\text{O-H}$  deformation bands

from the octahedral sheet of montmorillonite clay, such as  $\text{Al-Mg-OH}$  at 844  $\text{cm}^{-1}$ ,  $\text{Al-Fe-OH}$  at 863  $\text{cm}^{-1}$ , and  $\text{Al-Al-OH}$  at 917  $\text{cm}^{-1}$ . Upon modification of bentonite with rarasaponin, additional IR bands at 2800–2900 ( $\text{C-H}$  stretch) and 1292  $\text{cm}^{-1}$  ( $\text{C=O}$  stretch) are observed, implying the presence of intercalating rarasaponin compound. Moreover, the  $\text{dH}_2\text{O}$  bending vibration band at 1620  $\text{cm}^{-1}$  also slightly shifted to lower wavenumbers in RSB samples. The intercalation of rarasaponin was also indicated by the appearance of a new absorption band corresponding to the  $\text{C=O}$  stretch of the rarasaponin carbonyl group at  $\sim 1250 \text{ cm}^{-1}$ . The typical surface morphologies of as-prepared bleaching clays are shown in Fig. 2a–e. From these SEM images, it can be observed that all the clay samples show irregularly shaped particles about a few tens of microns with rough and complex surface topography. The basal surface of both UB and RSB clays is characterized by multiple terraces and edge sites, alongside with randomly distributed nanopits ( $<100 \text{ nm}$ ), crumpled micro-islands or flakes ( $100\text{--}300 \text{ nm}$ ), and nanocrystallites ( $<50 \text{ nm}$ ). Such a complex surface structure may imply the poorly disordered state of natural bentonite clays as compared to the synthetically made clays. As expected, the intercalation of rarasaponin inside the clay gallery does not induce any appreciable change to the morphology of the materials, regardless of the rarasaponin concentration. Moreover, HRTEM lattice analysis of UB and RSB-1 clays (inset images in Fig. 2a and d) reveals the expansion of the

16 basal spacing from 1.32 nm to 1.44 nm due to the rarasaponin intercalation

, which coincides well with the XRD analysis. According to the  $\text{N}_2$  sorption, XRD, and FTIR characterizations, the formation mechanism of RSB organoclays can be proposed by considering, as schematically illustrated in Fig. 3. The formation mechanism of RSB organoclays is as follows: In a polar solvent (e.g., water), deacylation of the carbonyl groups at the hydrophobic head of rarasaponin occurs. At the same time, protonation occurs at the silanol groups of bentonite. The deacylation of the rarasaponin results in the negative charge of the molecules; meanwhile, the protonation results in a positive charge of bentonite. Consequently, the electrostatic interaction between rarasaponin and bentonite occurs due to different surface charges [20,41]. Furthermore, the negative-charged rarasaponin might bind to the cations in the bentonite interlayer, and thus, the irreversible interaction occurs. This irreversible reaction can be attributed to the reduction of the CEC value. The elution technique was used to determine whether the decrease in the CEC value of RSB organoclays was caused by the leaching of the  $\text{Ca}^{2+}$  cations from the bentonite interlayer. However, there was very little or no difference in the number of cations in the eluate of UB and RSB samples, suggesting that the decrease in CEC was not due to the leaching of interlayer cations. More in-depth research would be needed to determine the mechanisms causing the reduction of CEC in intercalated organoclays.

### 3.2. Adsorptive bleaching of RSB organoclays

The potential utility of as-prepared RSB organoclays was evaluated with respect to the adsorptive removal of  $\beta$ -carotene in DPO

Fig. 2. Plan-view SEM images of (a) UB and (b–e) RSB organoclays prepared with varying concentrations of rarasaponin. The insets in (a) and (d) are the HRTEM images showing the lattice fringe spacings (marked by yellow lines and arrows) corresponding to the (001) basal reflections in the nonintercalated UB and intercalated RSB-2 clays prepared with 2 wt% rarasaponin solution, respectively (scale bar: nm). The lattice fringe spacings of the UB and RSB clays are measured to be 1.32 and 1.44 nm, respectively, using the ImageJ software (NIH, version 1.35) and shows good agreement with the  $d_{001}$  values determined from the experimental XRD patterns (1.39 for UB and 1.47 nm for RSB-1).

**11**For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article

.) under conditions relevant to the industrial dry bleaching process (i.e., atmospheric pressure and 90 °C). As displayed in Fig. 4a (red bars), the UB clay has moderate bleaching activity with a b-carotene removal efficiency of ~72%, showing a performance comparable or superior to those of non-activated bleaching clays previously reported in the literature [16,42,43]. The modification of the inter-layer structure of nonintercalated bentonite with rarasaponin affords an enhanced bleaching power. More specifically, the b-carotene removal efficiency of DPO gradually increases from ~77% for RSB-0.2 to ~91% for RSB-2 with increasing rarasaponin concentration during the solution intercalation process. Interestingly, the bleaching activity was observed to plateau at ~91% for the RSB-10 organoclay modified with a high concentration of rarasaponin (10 wt%). This result may suggest that there is an optimum loading of rarasaponin intercalated into the bentonite interlayer spaces that play an active role for the enhanced b-carotene removal, which will be discussed in more detail later. The above results reveal that the RSB-2 organoclay prepared with 2 wt% rarasaponin solution concentration is the best-performing sorbent among all of the samples for adsorptive bleaching of DPO. In addition to high b-carotene removal efficiency, the use of a lower dosage of rarasaponin in the interlayer surface modification of Fig. 3. Schematic representation of the intercalation mechanism of rarasaponin molecules into the clay galleries to form RSB organoclays. The intercalation of rarasaponin causes the expansion of the basal spacing, as confirmed by the XRD and TEM characterizations. Fig. 4. (a) Performance of the RSB organoclays in the adsorptive bleaching of b-carotene. (b) Comparison of FTIR spectra of RSB-2 organoclay before and after the bleaching process. The pink and yellow highlighted wavenumber regions denote the vibrational bands of CH<sub>2</sub> scissoring and C=O stretching modes, respectively. (c) Schematic illustration of the oxidation of b-carotene by the oxygen radicals and its resulting oxidized species. (d) Adsorption of b-carotene on the rarasaponin-intercalated bentonite clay (RSB-2). (For interpretation

3of the references to color in this figure legend, the reader is referred to the web version of this article

.) bentonite may help minimize adverse environmental impacts and health safety to workers. Despite several studies showing the rarasaponin has relatively low toxicity and is considered to be a safe substance, the excess presence of rarasaponin in the aqueous may trigger its accumulation in the environment during the disposal of bleaching clays. The infiltration of rarasaponin into the water bodies may also induce deterioration of oxygen levels in the aquatic ecosystems. The bleaching performance of the RSB-2 organoclay under standard test conditions (90.6% at 90 °C for 30 min) also rivals that of commercial acid-activated bentonite clays (Tonsil variants). As reported in the Silva et al. study [9], the Tonsil Optimum 210 FF bleaching clay displays an 89% removal of b-carotene (355 mg/kg) under the same test conditions applied in this work, while the enhanced reduction in b-carotene content are obtained at 105 °C (96%, 383 mg/kg) and 115 °C (97%, 389 mg/kg), respectively. In another report by Ribeiro et al. [15], the same variant of Tonsil bleaching earth and non-activated bentonite (Pure-Flo B80) both demonstrate lower b-carotene uptake capacity of 578 and 533 mg/kg (58% and 54% of the total carotene content) from native Brazilian palm oil, respectively. The Tonsil Supreme 110 FF with a high surface area of 240 m<sup>2</sup> g<sup>-1</sup> also displays a lower removal capacity of b-carotene in rice bran oil at 100 °C [44]. Those results clearly suggest that the as-prepared RSB-0.2 organoclays have a prospective application to the bleaching process of vegetable oils with high adsorption capacity and environmentally friendly processability. As mentioned previously, the intercalation of rarasaponin into the clay interlayer results in enhanced adsorptive removal of b-carotene. This can be explained by the RSB organoclays could be

1 attributed to the increase in hydrophobicity of the bentonite surface due to the presence of the hydrophobic tail of the intercalated

rarasaponin. The adsorptive removal

1 mechanism of b-carotene onto the RSB clay can be

proposed as follows: (i) During the bleaching process at 90 °C, b-carotene is oxidized into some oxidized-species such as b-cyclocitral, crocetindial, and b-apo-80-carotenal (Fig. 4c) [10,11]. The oxidation of b-carotene occurs as a result of attack by peroxy radicals (OO) contained in DPO. Furthermore, the high bleaching temperature can promote a radical attack on b-carotene [45]. The oxidation phenomena of b-carotene can be evidenced by the appearance of the FTIR peak (Fig. 4b) at 1462 cm<sup>-1</sup>, which corresponding to CH<sub>2</sub> scissoring at the hydrocarbon chain [46]; this peak might occur due to the deformation of b-carotene. (ii) The adsorption of b-carotene and its oxidized species on RSB Table 2 occurs through the interaction between the radical-damaged The fitted equilibrium isotherm parameters of sites of b-carotene and the carbonyl group of rarasaponin Freundlich, Langmuir, and Redlich-Peterson models for b-carotene adsorption onto RSB-2 (Fig. 4d). This interaction is evidenced by the alteration of the organoclay. FTIR band at 1153 cm<sup>-1</sup> (Fig. 4b), which corresponds to the C=O-C out-of-plane stretch of rarasaponin. Furthermore, the Model Parameter Error analysisy adsorption of b-carotene on RSB promotes by the physical inter-Symbol Value Symbol Value action with the adsorptive sites of bentonite [47,48]. Langmuir Q<sub>max</sub> (mg/g) 97.58 R<sup>2</sup> 0.968 KL (L/mg) 0.010 SS 178.3 The changes in the FFA content and PV of oil after bleaching treat- RL 0.117 ment were also examined for quality evaluation associated with the Freundlich oxidative stability and shelf-life of the product. As can be seen from KF (mg/g) a 3.888 R<sup>2</sup> 0.927 Fig. 4a (blue and green bars), the FFA level in DPO decreased signifi- 0.954 SS 403.6 Redlich-Peterson cantly from 4.9 wt% to 0.8?1.5 wt% upon adsorptive bleaching of Q<sub>max</sub> (mg/g) 78.09 R<sup>2</sup> 0.987 b-carotene. The RSB organoclays show superior performance than bR-P (L/g) 1.423 SS 65.28 the UB in terms of the FFA reduction ability, where the bleached oil a 1.098 had a final FFA content of about 1 wt%. This FFA level is considered to be acceptable for the base-catalyzed transesterification of vegetable oils to fatty acid methyl esters (biodiesel) without inducing undesir- A closer look at the model fitting shows the apparent discrepancies able saponification reaction or significant deactivation of the solid between experimental data and model fit, particularly at both low- catalysts [49]. The PV, which is an indicator of the oxidation degree of and high-concentration regimes. This phenomenon can be ascribed oils and fats, exhibits a generally decreasing trend following bleach- to the intrinsic characteristic of the empirical Freundlich model, ing treatment with RSB organoclays. Unlike the reported earlier stud- which is less thermodynamically and lacks both Henry's law and sat- ies, the use of acid-activated bleaching clays may increase the PV, urated adsorption behaviors in the low- and high-concentration thus rendering the bleached oils easier to become rancid and reduc- regimes, respectively [53]. Thus, a deeper investigation of the most ing its life. These results indicate that FFA and peroxide compounds appropriate isotherm models between Langmuir and Red- may also be simultaneously removed with b-carotene during the lich-Peterson isotherms is focused on interpreting the physical bleaching operation. Such the adsorption of FFA (as oleic acid) and meaning of each fit parameter, albeit the latter model producing a peroxide-based compounds onto the RSB surface is facilitated by the slightly better fit to the data for the low-end regions. edge aluminol (Al-OH) and silanol (Si-OH) functional groups The equilibrium isotherm curve of b-carotene adsorption on RSB- through either electrostatic or attraction forces. 2 organoclay (Fig. 5a) shows typical L-shaped isotherm, more specifi- cally the L3-type according to the Giles et al. classification [54,55]. A 3.3. Equilibrium adsorption isotherms of b-carotene onto RSB-2 gradual increase in the adsorbed amount occurs at low concentra- organoclay tions, followed by a slower uptake before reaching saturation at high concentrations. Such the L3-type isotherm





prepared RSB-2 organoclay and commercial acidic bleaching earths obtained from the Langmuir isotherm fit toward b-carotene removal. \*Sample abbreviations: AAS = acid-activated sepiolite, AAB = acid-activated bentonite, MCCM = mesoporous carbon coated monolith. (

**5For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.) a**

dimensionless Langmuir equilibrium parameter (RL), showing a value between 0 and 1 [56]. Based on the above interpretation of the model and fit parameters, it can be concluded that the Redlich-Peterson isotherm appears to be the best-fitted model for describing the equilibrium adsorption behavior of b-carotene onto RSB-2 clay. 3.4. Evaluation of the potential utility of spent RSB-2 bleaching clay 3.4.1. Reusability evaluation The reuse potential of the spent RSB-2 bleaching clay was evaluated for five successive adsorptive bleaching/regeneration cycles, and the results

**18are presented in Fig. 6. As can be seen from the figure, the**

effectiveness of the solvent regeneration toward b-carotene loaded organoclay shows a dependence on temperature, which has never been studied or previously. The chemical regeneration of the spent bleaching clay proceeds relatively inefficient at room temperature, leading to the reduced adsorption activity of the reused Fig. 6. The removal performance of RSB-2 organoclay toward b-carotene over five consecutive adsorptive bleaching/regeneration cycles. Error bars represent the standard deviation of triplicate experiments. The spent bleaching clay obtained at the end of each adsorption cycle was regenerated using n-hexane without (chemical) and with mild heating at 60 °C (hybrid thermal-chemical). The

**1removal rate of b-carotene from 2nd to**

4th cycles was expressed as the relative value toward the 1st run as 100%. All adsorptive bleaching experiments were carried out under standard test conditions (90 °C for 30 min) with 3 wt% clay loading with respect to the treated oil. adsorbent. This explanation is readily confirmed by a sharp decline in b-carotene removal efficiency from 87.4 ± 1.6% (54.24 mg g<sup>-1</sup>) in the fresh sample to 43.0 ± 0.6% (31.19 mg g<sup>-1</sup>) after four cycles. The loss of the removal capacity was attributed to the incomplete removal of the b-carotene molecules from the interior of clay structure during regeneration, which hinders the availability of active sites in the regenerated adsorbent for the next adsorptive bleaching cycle. On the other hand, the combination of chemical and mild heating (60 °C) can enhance the regeneration efficiency of the bleaching clay, where the removal percentage of b-carotene remains as high as 91.1 ± 1.1% after the fourth cycle. This result may suggest the endothermic desorption behavior of the b-carotene molecules from the organoclay surface, which shows an agreement with the observations by Muhammad et al. [57] and Wu and Li [58] for desorption of b-carotene from mesoporous carbon coated monolith and acid-activated bentonite, respectively. Thus, the hybrid thermal-chemical method proved to be an efficient strategy to regenerate spent bleaching earths with robust stability. 3.4.2. Toxicity assessment and feasibility as a supplementation toward plant growth The disposal of spent bleaching earths into the landfill or water may exert an ecological risk toward the soils. Thus, it is also important to know the fate and potential risk. The assessment of the phytotoxic activity of as-prepared, spent, and regenerated RSB-2 organoclays were carried out based on their influences on the root system of *A. thaliana* Col-0 seedlings. As shown in Fig. 7a, the supplementation of fresh RSB-2 organoclay has no appreciable inhibitory effect on the elongation of primary roots and lateral root development in 10-day-old *Arabidopsis* Col-0 seedlings compared to the control group, suggesting minimal phytotoxicity of the clay material. The primary root length and lateral root density in RSB-2 treated seedlings were measured to be 5.65 ± 0.68 cm and 0.30 ± 0.29 roots per cm, respectively (Fig. 7b). Next,



we evaluated the inhibitory potential of the spent and regenerated RSB-2 bleaching clays against *Arabidopsis* root development. The results demonstrate that the length of primary roots is ~26% shorter (4.83  $\pm$  0.55 cm) in *Arabidopsis* Col-0 seedlings treated with b-carotene loaded RSB-2 (or spent bleaching clay) than those in the control (6.49  $\pm$  0.41 cm) and RSB-2-treated (5.65  $\pm$  0.68 cm) Fig. 7. Root development in the 10-day-old seedlings of *A. thaliana* Col-0 treated with as-prepared, spent, and regenerated RSB-2 organoclays (a) digital photos and (b) bar graph showing the primary root length (red) and lateral root density (blue; indicated by orange arrows in panel (a)) from three biological replicates; error bars indicate the standard deviation of the mean. Primary root lengths were measured from the dashed yellow line marking to the root tips with the ImageJ software. Different

2 letters above the error bars represent statistically significant differences among

treatments based on the one-way

2 ANOVA with Tukey's posthoc test

(

3  $P < 0.05$ ). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article

.) groups. No statistically significant difference was observed in the *Arabidopsis* lateral root formation for the as-prepared and spent RSB-2 treatment groups. The observed inhibitory effect of spent bleaching clay, albeit at a moderate level, may be attributed to the surface-adsorbed oil molecules that cover the seedling roots and thus impede the oxygen and water uptake. Moreover, the penetration of vegetable oil into the plant roots may induce local oxidative stress conditions mediated by reactive oxygen species (e.g., OH, HO<sub>2</sub>, and O<sub>2</sub><sup>•</sup>) that cause damage to the surrounding vital tissues and disrupt the photosynthesis process [60]. Prior studies have demonstrated the impaired germination and growth of roots, stems, and leaves in the oil-treated seedlings [59,60]. Prior studies have demonstrated the impaired germination and growth of roots, stems, and leaves in the oil-treated seedlings [59,61], likely due to the germination or reduced water uptake mechanisms. The *Arabidopsis* Col-0 seedlings treated with reused (hexane-washed) RSB-2 organoclay also showed a marked inhibition of root growth compared to roots from control plants, with an average primary root length of 4.52  $\pm$  0.20 cm and the lateral root density of 0.29  $\pm$  0.32 roots cm<sup>-1</sup>. This result appears quite surprising, given that the regenerated bleaching clay contains minimal or no oil and solvent residues, and is therefore expected to display limited phytotoxicity similar to that of the as-prepared organoclay. We attributed the growth-inhibiting activity of the reused organoclay to the competitive uptake of micro- and macronutrients by the edge aluminol and silanol groups of clay. The presence of trace hexane residue that is trapped within may also although it remains further clarification. The above results imply that the minimal toxic effect of the spent and regenerated RSB-2 bleaching clays toward germination of *Arabidopsis* seedlings, although slight to moderate inhibition effects on the elongation of both primary and lateral roots. However, it should be taken into consideration that the direct disposal of spent RSB bleaching clays still has hazardous potential to the environment with respect to accumulated release and persistence of rarasaponin into the aquatic ecosystem. Detailed studies are currently undergoing in our group to elucidate the potential toxicity of rarasaponin-loaded adsorbents toward aquatic biota.

#### 4. Conclusions

The study demonstrates an environmentally benign and low-cost production of efficiency bleaching earth via microwave-assisted aqueous phase intercalation of natural bentonite with plant-derived rarasaponin surfactant. The incorporation of rarasaponin molecules into the interlayer space results in the

**20**increase in the basal spacing, surface area, and pore volume of

bentonite clay, all benefited features toward the enhanced adsorptive removal of b-carotene. The RSB-2 organoclay prepared with an optimum rarasaponin concentration of 2 wt% exhibits the best bleaching performance with a b-carotene removal efficiency of 91.2%, representing a competitive performance to that of commercial acid-activated bleaching earths. The equilibrium adsorption data of b-carotene onto RSB-2 organoclay shows the best fit to the Redlich-Peterson isotherm model, thus suggesting the monolayer and saturation behavior of the sorption mechanisms. Hybrid thermal-chemical treatment has proven effective for the regeneration of spent bleaching clay with retained adsorption capacity (more than 90% of its original activity) over 5 cycles. Furthermore, the relatively low phytotoxicity of the spent RSB-2 organoclay toward Arabidopsis seedlings may enable direct disposal into the soil without significant environmental damage. In summary, efficient and reusable RSB-2 organoclay, in addition to its low-cost production, sustainable material resources, and environmentally friendly preparation, may manifest its promising potential as an alternative to commercial acid-activated bleaching earth in the industrial refining of vegetable oils.

**7****Declaration of Competing Interest** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Acknowledgments Financial support from the Ministry of Research, Technology and Higher Education through World Class Research

Grant No. 130D/WM01.5/N/2020 is highly appreciated.

**9****Supplementary materials** Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jtice.2020.11.036.  
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