Contents lists available at ScienceDirect



Journal of the Taiwan Institute of Chemical Engineers

journal homepage: www.elsevier.com/locate/jtice



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



Shella Permatasari Santoso^{a,b,*}, Artik Elisa Angkawijaya^c, Maria Yuliana^a, Vania Bundjaja^b, Felycia Edi Soetaredjo^{a,b}, Suryadi Ismadji^{a,b}, Alchris Woo Go^c, Phuong Lan Tran-Nguyen^d, Alfin Kurniawan^e, Yi-Hsu Ju^{b,c,f}

^a Department of Chemical Engineering, Widya Mandala Surabaya Catholic University, Kalijudan 37, Surabaya 60114, East Java, Indonesia

^b Department of Chemical Engineering, National Taiwan University of Science and Technology, No. 43, Sec. 4, Keelung Rd., Taipei 10607, Taiwan

^c Graduate Institute of Applied Science and Technology, National Taiwan University of Science and Technology, No. 43, Sec. 4, Keelung Rd., Taipei 10607, Taiwan

^d Department of Mechanical Engineering, Can Tho University, 3-2 Street, Can Tho City, Vietnam

^e Department of Chemistry, National Sun Yat-Sen University, No. 70, Lianhai Rd., Kaohsiung 80424, Taiwan

^f Taiwan Building Technology Center, National Taiwan University of Science and Technology, No. 43, Sec. 4, Keelung Rd., Taipei 10607, Taiwan

ARTICLE INFO

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

Keywords: Rarasaponin Microwave irradiation β -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 organoclaytype 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_2 sorption. The results revealed that the intercalation of rarasaponin causes a basal spacing increase to 1.50 nm, surface area to 99.5 m²/g, and pore volume to 0.85 cm³/g, while the cation exchange capacity (CEC) value decreased to 19.1 ± 4.4 meq/100 g; where the untreated bentonite has a basal spacing of 1.39 nm, a surface area of 86.8 m²/g, a pore volume of 0.69 cm³/g, and a CEC value of 30.1 \pm 3.6 meq/ 100 g. The RSB-2 organoclay exhibits the best bleaching activity, with ~91% β -carotene removal efficiency achieved in degummed palm oil compared to the untreated bentonite (72% efficiency). The equilibrium behavior of β -carotene adsorption onto RSB-2 organoclay) was best fit with the Redlich–Peterson isotherm model, giving the theoretical maximum sorption capacity (q_{max}) 78.09 mg g⁻¹, which represents the bestreported values among the investigated samples. A reusability study of the spent bleaching clay demonstrates that the adsorptive removal of β -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 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 phospholipids 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 thermally [8,9].

* Corresponding author.

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.

E-mail address: shella_p5@yahoo.com (S.P. Santoso).

Carotenes, especially β -carotene, are the primary compounds that are removed during the bleaching operation. Despite its benefits as the anti-oxidant properties, the high amount of β -carotene can greatly decrease the oxidation stability of oils since this compound is resistant to oxidative reaction [10,11]. The removal of β -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 preparation 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 environment [21]. Several studies have proposed an alternate activation technique by surfactant intercalation [20,22,23]. For example, Gunawan 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 µM [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 performance of rarasaponin-intercalated bentonite (RSB) for β -carotene bleaching in vegetable oils. The use of RSB organoclay as bleaching earth can provide a low-cost and greener alternative to commercial acid-activated bentonite clays. The aims of the present study are to investigate the influence of rarasaponin concentration during the microwave-assisted intercalation process on the physical textural and chemical properties of the resultant organobentonites and to optimize the concentration with respect to the bleaching performance. The physical characteristics evaluation includes the cation exchange capacity (CEC), specific surface area, and pore structures. The bleaching performance was assessed based on the β -carotene removal efficiency in oil as the model oil, which is regarded as the most important parameter in assessing 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 inhibitory activity of seed germination and the root growth of Arabidopsis thaliana.

2. Experimental section

2.1. Materials

Hydrogen peroxide solution (H_2O_2 , 30 wt%), HPLC grade *n*-hexane (C_6H_{14}), and phosphoric acid (H_3PO_4 , 85 wt%) were obtained from Sigma-Aldrich, Singapore. Sodium hydroxide (NaOH, 96%), ethanol (C_2H_5OH , 96%), and methylene blue ($C_{16}H_{18}ClN_3S$, 85% dye content, C. I. 52,015) 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 M Ω 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, JKA Labortechnik) and sieved pass through an 80/100 mesh screen, followed by purification using 10% (v/v) H₂O₂ according to the procedure described elsewhere [27]. The CEC of purified bentonite powders was determined to be 30.1 ± 3.7 meguiv per 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: Al₂O₃ 30.20%, SiO₂ 49.31%, Fe₂O₃ 2.98%, CaO 3.91%, MgO 0.71%, K2O 0.21%, MnO 0.72%, and Na2O 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^{-1}) 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% H₃PO₄ 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: lauric acid (C12:0) = 2.07%, myristic acid (C14:0) = 2.01%, palmitic acid (C16:0) = 41.28%, stearic acid (C18:0) = 4.48%, oleic acid (C18:1) = 39.01%, linoleic acid (C18:2) = 3.90%, linolenic acid (C18:3) = 6.88%, and arachidic acid (C20:0) = 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%) 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 organoclays were designated as RSB-x, where x denotes the rarasaponin 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. Prior to the SEM imaging, the samples were sputter-coated with platinum to minimize charging effects. High-resolution transmission electron microscopy (HRTEM) characterization was conducted on a Philips Tecnai F30 field emission gun electron microscope at 200 kV. The textural properties of the bleaching clays were characterized using nitrogen physisorption isotherms at 77 K in a Micromeritics ASAP 2010 gas sorption analyzer. All samples were vacuum degassed at 150 °C for 12 h prior to measurement. The specific surface area was analyzed using the standard Brunauer-Emmett-Teller (BET) method, and the total pore volume (V_T) was estimated from the N₂ adsorption amount at a relative pressure (P/P_0) of 0.95. Fourier transform infrared (FTIR) spectra were recorded on a Shimadzu FTIR 8400S spectrometer in the 4000–500 cm⁻¹ range with 4 cm⁻¹ 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 Cu K α X-ray source (λ = 0.15418 nm) operating at 40 kV and 30 mA.

2.4. 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 β -carotene), free fatty acid content (FFA as palmitic acid), and the peroxide value (PV). The removal rate of β -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 (% w/w) =
$$\left(\frac{C_i - C_r}{C_i}\right) \times 100$$
 (1)

where C_i and C_r denote the initial and residual concentrations of β -carotene in DPO, which are measured spectrophotometrically at 446 nm [30]. The FFA content and PV were determined according to the American Oil Chemists' Society (AOCS) Official Method Ca 5a-40 and Cd 8–53, respectively.

2.5. Adsorption isotherm study

Static adsorption equilibrium study of β -carotene was performed by adding various amounts of bleaching clays (i.e., 1–10 wt%) 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 β -carotene at equilibrium (Q_e) was calculated with Eq. (2):

$$Q_{\rm e}({\rm mg/g}) = \left(\frac{C_0 - C_e}{m}\right) \times V \tag{2}$$

where C_0 and C_e are the initial and equilibrium concentrations of β -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 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 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. 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 Col-0 seeds were germinated and grown on 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 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 \pm standard deviation. Statistical analysis was conducted using GraphPad Prism 8.0 software, and significant differences between the treatment groups 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⁺, Mg²⁺, and Ca²⁺) that neutralizes permanent and pH-dependent charges at the edge and edge sites [35], which are replaceable by other positively charged species through an ionexchange 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 1246–1261 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 K are presented in Fig. 1a, while the specific BET surface area and the total pore volume deduced from these isotherms are given 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 of	organoclays.

Clay	CEC [†] (mequiv/100 g)	Textural Parameters		$d_{001}(nm)$	Interlayer expansion $^{\ddagger}\left(nm ight)$
		$S_{BET}(m^2/g)$	$V_{\rm T}({\rm cm}^3/{\rm g})$		
UB	30.1 ± 3.6^a	86.8	0.69	1.39	0.47
RSB-0.2	27.1 ± 3.2^{a}	99.1	0.71	1.43	0.51
RSB-1	22.7 ± 2.4^b	99.4	0.85	1.46	0.54
RSB-2 RSB-10	$\begin{array}{c} 19.1 \pm 4.4^{b} \\ 19.6 \pm 8.0^{ab} \end{array}$	99.5 99.0	0.85 0.86	1.50 1.52	0.58 0.60

[†] Different letters among groups indicate a statistically significant difference (Student's *t*-test, *P* < 0.05).

[‡] Calculated as the difference between the basal distance (d_{001}) and the thickness of dry montmorillonite 2:1 (tetrahedral-octahedral-tetrahedral) layer (~9.5 Å).



Fig. 1. Characteristic of the as-prepared UB and RSB bleaching clays. (a) N_2 adsorption (solid circles) and desorption (empty circles) isotherms measured at 77 K. (b) XRD patterns of Cu K radiation, and (c) transmission FTIR spectra recorded in the 4000–500 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 ~2350 cm⁻¹ for rarasaponin is due to absorption by atmospheric CO₂. (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 N₂ 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 0.45–0.85 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 BET surface area (S_{BET}) and total pore volume than the UB sample. The S_{BET} values are 86.8, 99.1, 99.4, 99.5, and 99.0 m² 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 N₂ 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 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 2θ values of 6.34° (001), 19.94° (011), 20.95° (101), and 39.49° (210), along with impurity phases attributed to quartz (SiO₂, 2θ = 26.69°, 28.50°, and 36.11°) and K-feldspar (KAlSi₃O₈, 2θ = 21.80° and 24.53°) 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 2θ angles of 6.19° and 5.97°, 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 cm⁻¹), asymmetric and

symmetric C-H stretching of the CH2 and groups (2800-2950 cm⁻¹), C=O stretching (1718 cm⁻¹), C–O–C stretching of the acetyl group (1215 cm^{-1}), and O–H bending modes (923 cm^{-1}). Meanwhile, the UB clay shows the stretching vibration of the OH groups at 3700 and 3358 cm^{-1} , which corresponds to the siloxane-bound water and the interlayer water molecules, respectively. The former band at 3700 cm⁻¹ can also be ascribed to the stretching mode of H-bonded of silanol groups. The IR bands at 1644 cm^{-1} and 1525 cm^{-1} are associated with the H–O–H bending mode of water molecules bound to the interlayer Ca²⁺ cations and N–H stretching modes. Additionally, the stretching vibrations of the siloxane bond (Si-O-Si) and Si-O of the surface silanol (SiOH) groups appear at 1050 and 958 cm⁻¹, respectively. The low-frequency bands in the 800–1000 cm⁻¹ region can be assigned to the O-H deformation bands from the octahedral sheet of montmorillonite clay, such as Al-Mg-OH at 844 cm⁻¹, Al-Fe-OH at 863 cm⁻¹, and Al-Al-OH at 917 cm⁻¹. Upon modification of bentonite with rarasaponin, additional IR bands at 2800-2900 (C-H stretch) and 1292 cm⁻¹ (C=O stretch) are observed, implying the presence of intercalating rarasaponin compound. Moreover, the δH_2O bending vibration band at 1620 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 C=O stretch of the rarasaponin carbonyl group at ~1250 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 nm), crumpled micro-islands or flakes (100-300 nm), and nanocrystallites (<50 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 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 N₂ 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 Ca²⁺ 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 β -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). (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 β -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 interlayer structure of nonintercalated bentonite with rarasaponin affords an enhanced bleaching power. More specifically, the β -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 β -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 β -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 β -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 CH₂ scissoring and C–O–C stretching modes, respectively. (c) Schematic illustration of the oxidation of β -carotene by the oxygen radicals and its resulting oxidized species. (d) Adsorption of β -carotene on the rarasaponin-intercalated bentonite clay (RSB-2). (For interpretation of 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.

204

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 β -carotene (355 mg/kg) under the same test conditions applied in this work, while the enhanced reduction in β -carotene content are obtained at 105 (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 β -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 m² g⁻¹ also displays a lower removal capacity of β -carotene in rice bran oil at 100 °C [44]. Those results clearly suggest that the asprepared 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 β -carotene. This can be explained by the RSB organoclays could be 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 mechanism of β -carotene onto the RSB clay can be proposed as follows:

(i) During the bleaching process at 90 °C, β -carotene is oxidized into some oxidized-species such as β -cyclocitral, crocetindial, and β -apo-8'-carotenal (Fig. 4c) [10,11]. The oxidation of β -carotene occurs as a result of attack by peroxyl radicals (OO) contained in DPO. Furthermore, the high bleaching temperature can promote a radical attack on β -carotene [45]. The oxidation phenomena of β -carotene can be evidenced by the appearance of the FTIR peak (Fig. 4b) at 1462 cm⁻¹, which corresponding to CH₂ scissoring at the hydrocarbon chain [46]; this peak might occur due to the deformation of β -carotene. (ii) The adsorption of β -carotene and its oxidized species on RSB occurs through the interaction between the radical-damaged sites of β -carotene and the carbonyl group of rarasaponin (Fig. 4d). This interaction is evidenced by the alteration of the FTIR band at 1153 cm⁻¹ (Fig. 4b), which corresponds to the C—O-C out-of-plane stretch of rarasaponin. Furthermore, the adsorption of β -carotene on RSB promotes by the physical interaction with the adsorptive sites of bentonite [47,48].

The changes in the FFA content and PV of oil after bleaching treatment were also examined for quality evaluation associated with the oxidative stability and shelf-life of the product. As can be seen from Fig. 4a (blue and green bars), the FFA level in DPO decreased significantly from 4.9 wt% to 0.8-1.5 wt% upon adsorptive bleaching of β -carotene. The RSB organoclays show superior performance than the UB in terms of the FFA reduction ability, where the bleached oil 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 undesirable saponification reaction or significant deactivation of the solid catalysts [49]. The PV, which is an indicator of the oxidation degree of oils and fats, exhibits a generally decreasing trend following bleaching treatment with RSB organoclays. Unlike the reported earlier studies, the use of acid-activated bleaching clays may increase the PV, thus rendering the bleached oils easier to become rancid and reducing its life. These results indicate that FFA and peroxide compounds may also be simultaneously removed with β -carotene during the bleaching operation. Such the adsorption of FFA (as oleic acid) and peroxide-based compounds onto the RSB surface is facilitated by the edge aluminol (Al-OH) and silanol (Si-OH) functional groups through either electrostatic or attraction forces.

3.3. Equilibrium adsorption isotherms of β -carotene onto RSB-2 organoclay

The investigation of the adsorption isotherms is of crucial importance for both the fundamental study of sorption mechanism and the scale-up design considerations of a sorption-based system since it reflects the equilibrium distribution of the adsorbate species between liquid and solid adsorbed phases under given experimental conditions. In this regard, the equilibrium adsorption data of β -carotene onto RSB-2 organoclay were correlated with three classical adsorption isotherm models, namely Langmuir [50], Freundlich [51], and Redlich–Peterson [52]. The mathematic expressions of these isotherm models are presented in Eqs. (3)–(5).

$$Q_e = Q_{max} \frac{K_L C_e}{1 + K_L C_e} \tag{3}$$

$$Q_e = K_F C_e^{\alpha} \tag{4}$$

$$Q_e = Q_{max} \frac{b_{R-P} C_e}{1 + b_{R-P} C_e^{\alpha}}$$

$$\tag{5}$$

The characteristic parameter values for each isotherm are determined by the nonlinear least-squares fitting method using SigmaPlot 12.5 software (Systat Software, Inc.), and the results are given in Table 2. Nonlinear curve fitting has been proven to be superior to the linearized one, due to the inclusion of error parameter and it can avoid the overestimation of values. The modeling analysis reveals that both the Langmuir and Redlich–Peterson isotherms provide good fits to the experimental equilibrium data over the whole concentration range with the correlation coefficient (R^2) of 0.97 and 0.99, respectively. On the other hand, the Freundlich adsorption model fails to adequately describe the adsorption behavior of β -carotene onto RSB-2 organoclay, which is reflected by a lower R^2 value of 0.91.

Table 2

The fitted equilibrium isotherm parameters of Freundlich, Langmuir, and Redlich–Peterson models for β -carotene adsorption onto RSB-2 organoclay.

Model Parameter		Error analysis [†]					
Symbol	Value	Symbol	Value				
Langmuir							
$Q_{\rm max}$ (mg/g)	97.58	R^2	0.968				
$K_L(L/mg)$	0.010	SS	178.3				
R_L	0.117						
Freundlich							
$K_F(mg/g)$	3.888	R^2	0.927				
α	0.954	SS	403.6				
Redlich-Peterson							
$Q_{\rm max} ({\rm mg/g})$	78.09	R^2	0.987				
$b_{R-P}(L/g)$	1.423	SS	65.28				
α	1.098						

A closer look at the model fitting shows the apparent discrepancies between experimental data and model fit, particularly at both lowand high-concentration regimes. This phenomenon can be ascribed to the intrinsic characteristic of the empirical Freundlich model, which is less thermodynamically and lacks both Henry's law and saturated adsorption behaviors in the low- and high-concentration regimes, respectively [53]. Thus, a deeper investigation of the most appropriate isotherm models between Langmuir and Redlich–Peterson isotherms is focused on interpreting the physical meaning of each fit parameter, albeit the latter model producing a slightly better fit to the data for the low-end regions.

The equilibrium isotherm curve of β -carotene adsorption on RSB-2 organoclay (Fig. 5a) shows typical L-shaped isotherm, more specifically the L3-type according to the Giles et al. classification [54,55]. A gradual increase in the adsorbed amount occurs at low concentrations, followed by a slower uptake before reaching saturation at high concentrations. Such the L3-type isotherm suggests that the adsorption of β -carotene on the surface of the clay adsorbent occurs through relatively weak attraction forces (i.e., van der Waals force or hydrogen bonding) rather than strong covalent bonding, also in good agreement with the earlier studies. The existence of a plateau region at high equilibrium concentrations ($C_e > 150 \text{ mg/L}$) of the isotherm plot may reflect the saturation coverage of β -carotene on the organoclay surface, which is also in accordance with the essential characteristic of the Langmuir isotherm. Moreover, the L3-type curve is also an indication that not all the available adsorption sites on the solid surface are occupied by the adsorbate molecules. In the case of more adsorption sites coverage by the adsorbate is expected, a higher energy supply will be required to form the multilayer [54]. The fitted q_{max} value (i.e., the theoretical maximum adsorption capacity) of 78.09 mg g⁻¹ obtained from the Redlich–Peterson isotherm model agrees well with the experimentally measured value obtained at 90 ° C (76.98 mg/g). Compared to the Langmuir fitting for the $q_{\rm max}$ obtained from fitting, a significantly larger value of 119.54 mg g^{-1} was obtained. The fit values of q_{max} for RSB-2 are also superior to those of commercial bleaching earths and other adsorbents (Fig. 5b), thus suggesting the higher potential of as-synthesized organoclay. The constant α of the Redlich–Peterson model shows a near-unity value (1.098), suggesting that this model tends to approach the original Langmuir isotherm and may further indicate the monolayer adsorption behavior. The relatively low value of the fit parameter $b_{R} = P$ (or K_{L} in the Langmuir model) may reflect weak affinity between the adsorbate molecules and the surface, which agrees well with the typical L3-shaped behavior. Moreover, the calculated parameter $1/(b_{R-P} \bullet Q_{max})$ is found to be significantly lower than 0, further confirming the monolayer adsorption system. The favorable adsorption of β -carotene onto the RSB-2 surface is further verified by





Fig. 5. (a) Equilibrium isotherms (symbol) for the adsorption of β -carotene onto RSB-2 organoclay at 90 °C and the model fits of Freundlich (red dashed line), Langmuir (blue dotted line), and Redlich–Peterson isotherms (green solid line). The experimental data were fitted using nonlinear regression with SigmaPlot software. (b) Comparison of the maximum adsorbent capacities (q_{max}) of the as-prepared RSB-2 organoclay and commercial acidic bleaching earths obtained from the Langmuir isotherm fit toward β -carotene removal. *Sample abbreviations: AAS = acid-activated sepiolite, AAB = acid-activated bentonite, MCCM = mesoporous carbon coated monolith. (For 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 (R_L), 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 Red-lich–Peterson isotherm appears to be the best-fitted model for describing the equilibrium adsorption behavior of β -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 are presented in Fig. 6. As can be seen from the figure, the effectiveness of the solvent regeneration toward β -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 β -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 removal rate of β -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 β -carotene removal efficiency from 87.4 \pm 1.6% (54.24 mg g⁻¹) in the fresh sample to 43.0 \pm 0.6% (31.19 mg g⁻¹) after four cycles. The loss of the removal capacity was attributed to the incomplete removal of the β -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 β -carotene remains as high as 91.1 \pm 1.1% after the fourth cycle. This result may suggest the endothermic desorption behavior of the β -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 β -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 β -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 letters above the error bars represent statistically significant differences among treatments based on the one-way ANOVA with Tukey's posthoc test (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 surfaceadsorbed 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., $\bullet OH$, $HO_2 \bullet$, and $O_2 \bullet^-$) 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⁻¹. 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 increase in the basal spacing, surface area, and pore volume of bentonite clay, all benefited features toward the enhanced adsorptive removal of β -carotene. The RSB-2 organoclay prepared with an optimum rarasaponin concentration of 2 wt% exhibits the best bleaching performance with a β -carotene removal efficiency of 91.2%, representing a competitive performance to that of commercial acid-activated bleaching earths. The equilibrium adsorption data of β -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.

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.

Supplementary materials

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

References

 Karpiński B, Szkodo M. Clay minerals - mineralogy and phenomenon of clay swelling in oil & gas industry. Adv Mater Sci 2015;15:37–55.

- [2] Swai RE. A review of molecular dynamics simulations in the designing of effective shale inhibitors: application for drilling with water-based drilling fluids. J Petrol Explor Prod Technol 2020;10:3515–32.
- [3] Ghaleh SP, Khodapanah E, Tabatabaei-Nezhad SA. Experimental evaluation of thiamine as a new clay swelling inhibitor. Pet Sci 2020;17:1616–33.
- [4] Ramli MR, Siew WL, Ibrahim NA, Hussein R, Kuntom A, Razak RAA, Nesaretnam K. Effects of degumming and bleaching on 3-mcpd esters formation during physical refining. J Am Chem Soc 2011;88:1839–44.
- [5] Wiedermann LH. Degumming, refining and bleaching soybean oil. J Am Chem Soc 1981;58:159–66.
- [6] Goh SH, Khor HT, Gee PT. Phospholipids of palm oil (*Elaeis guineensis*). J Am Chem Soc 1982;59:296–9.
- [7] Ong KK, Fakhru'l-Razi A, Baharin BS, Hassan MA. Degumming of crude palm oil by membrane filtration. Artif Cell Blood Sub 1999;27:381–5.
- [8] Oey SB, Fels–Klerx HJvd, Fogliano V, Leeuwen SPJv. Mitigation strategies for the reduction of 2– and 3–MCPD esters and glycidyl esters in the vegetable oil processing industry. Compr Rev Food Sci Food Saf 2019;18:349–61.
- [9] Silva SM, Sampaio KA, Ceriani R, Verhé R, Stevens C, Greyt WD, Meirelles AJA. Effect of type of bleaching earth on the final color of refined palm oil. Food Sci Technol 2014;59:1258–64.
- [10] Fiedor J, Burda K. Potential role of carotenoids as antioxidants in human health and disease. Nutrients 2014;6:466–88.
- [11] Martin HD, Jäger C, Ruck C, Schmidt M, Walsh R, Paust J. Anti– and prooxidant properties of carotenoids. J Prakt Chem 1999;341:302–8.
- [12] Aung LL, Tertre E, Suksabye P, Worasith N, Thiravetyan P. Effect of alumina content and surface area of acid-activated kaolin on bleaching of rice bran oil. J Am Oil Chem Soc 2015;92:295–304.
- [13] Kheok SC, Lim EE. Mechanism of palm oil bleaching by montmorillonite clay activated at various acid concentrations. J Am Oil Chem Soc 1982;59:129–31.
- [14] Poku K. Small-Scale palm oil processing in africa. Rome, Italy: Food and Agriculture Organization of the United, Agricultural Support Systems Division; 2002 FAO Agricultural Services Bulletin 148. Vol. 148.
- [15] Ribeiro JAA, Almeida ES, Neto BAD, Abdelnur PV, Monteiro S. Identification of carotenoid isomers in crude and bleached palm oils by mass spectrometry. LWT 2018;89:631–7.
- [16] Sabah E, Çelik MS. Sepiolite: an effective bleaching adsorbent for the physical refining of degummed rapeseed oil. J Am Oil Chem Soc 2005;82:911–6.
- [17] Silva SM, Sampaio KA, Ceriani R, Verhé R, Stevens C, Greyt WD, Meirelles AJA. Adsorption of carotenes and phosphorus from palm oil onto acid activated bleaching earth: equilibrium, kinetics and thermodynamics. J Food Eng 2013;118:341–9.
- [18] Heyding RD, Ironside R, Norris AR, Prysiazniuk RY. Acid activation of monmorillonite. Can J Chem 1960;38:1003–16.
- [19] Krupskaya VV, Zakusin SV, Tyupina EA, Dorzhieva OV, Zhukhlistov AP, Belousov PE, Timofeeva MN. Experimental study of montmorillonite structure and transformation of its properties under treatment with inorganic acid solutions. Minerals 2017;7:49. (15 pages).
- [20] Kurniawan A, Sutiono H, Ju Y-H, Soetaredjo FE, Ayucitra A, Yudha A, Ismadji S. Utilization of rarasaponin natural surfactant for organo-bentonite preparation: application for methylene blue removal from aqueous effluent. Micropor Mesopor Mater 2011;142:184–93.
- [21] Amari A, Gannouni H, Khan MI, Almesfer MK, Elkhaleefa AM, Gannouni A. Effect of structure and chemical activation on the adsorption properties of green clay minerals for the removal of cationic dye. Appl Sci 2018;8:2302. (18 pages).
- [22] Guégan R. Intercalation of a nonionic surfactant (C10E3) bilayer into a na-montmorillonite clay. Langmuir 2010;26:19175–80.
- [23] Ismadji S, Soetaredjo FE, Ayucitra A. Clay materials for environmental remediation. In: Sharma SK, editor. Springerbriefs in green chemistry for sustainability. New York: Springer International Publishing; 2015.
- [24] Gunawan NS, Indraswati N, Ju Y-H, Soetaredjo FE, Ayucitra A, Ismadji S. Bentonites modified with anionic and cationic surfactants for bleaching of crude palm oil. Appl Clay Sci 2010;47:462–4.
- [25] Bundjaja V, Santoso SP, Angkawijaya AE, Yuliana M, Soetaredjo FE, Ismadji S, Ayucitra A, Gunarto C, Ju Y-H, Ho M-H. Fabrication of cellulose carbamate hydrogeldressing with rarasaponin surfactant for enhancing adsorption of silver nanoparticles and antibacterial activity. Mater Sci Eng, C 2021;118:111542.
- [26] Ray PC, Yu H, Fu PP. Toxicity and environmental risks of nanomaterials: challenges and future needs. J Environ Sci Health 2009;27:1–35.
- [27] Rahardjo AK, Susanto MJJ, Kurniawan A, Indraswati N, Ismadji S. Modified Ponorogo bentonite for the removal of ampicillin from wastewater. J Hazard Mater 2011;190:1001–8.
- [28] Medina-Meza IG, Aluwi NA, Saunders SR, Ganjyal GM. GC–MS Profiling of triterpenoid saponins from 28 quinoa varieties (*Chenopodium quinoa* Willd.) grown in Washington State. J Agric Food Chem 2016;64:8583–91.
- [29] Kurniawan A, Effendi C, Ong LK, Ju Y-H, Lin CX, Ismadji S. Novel, integrated biorefinery approach of *Ceiba pentandra* (Kapok) seed and its secondary waste. ACS Sustainable Chem Eng 2013;1:473–80.
- [30] PORIM. PORIM test methods: carotene content. Palm oil research institute of Malaysia, Kuala Lumpur; 1990.
- [31] Angkawijaya AE, Nguyen VC, Gunawan F, Nakamura Y. A pair of arabidopsis diacylglycerol kinases essential for gametogenesis and endoplasmic reticulum phospholipid metabolism in leaves and flowers. Plant Cell 2020.

- [32] Jia K-P, Dickinson AJ, Mi J, Cui G, Xiao TT, Kharbatia NM, Guo X, Sugiono E, Aranda M, Blilou I, Rueping M, Benfey PN, Al-Babili S. Anchorene is a carotenoid-derived regulatory metabolite required for anchor root formation in *Arabidopsis*. Sci Adv 2019;5 eaaw6787.
- [33] Santoso SP, Kurniawan A, Soetaredjo FE, Cheng K-C, Putro JN, Ismadji S, Ju Y-H. Eco-friendly cellulose–bentonite porous composite hydrogels for adsorptive removal of azo dye and soilless culture. Cellulose 2019;26:3339–58.
- [34] Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 2006;15:473–97.
- [35] Farajzadeh R, Guo H, Winden Jv, Bruining J. Cation exchange in the presence of oil in porous media. ACS Earth Space Chem 2017;1:101–12.
- [36] Muñoz H-J, Blanco C, Gil A, M-Á Vicente, Galeano L-A. Preparation of Al/Fe-pillared clays: effect of the starting mineral. Materials (Basel) 2017;20:1364.
- [37] Cychosz KA, Guillet-Nicolas R, García-Martínez J, Thommes M. Recent advances in the textural characterization of hierarchically structured nanoporous materials. Chem Soc Rev 2017;46:389–414.
- [38] Tanev PT, Liang Y, Pinnavaia TJ. Assembly of mesoporous lamellar silicas with hierarchical particle architectures. J Am Chem Soc 1997;119:8616–24.
- [39] Condon JB. An overview of physisorption. Surface area and porosity determinations by physisorption - measurements and theory. Oxford, UK: Elsevier; 2006. p. 1–27.
- [40] Rutherford DW, Chiou CT, Eberl DD. Effects of exchanged cation on the microporosity of montmorillonite. Clay Clay Miner 1997;45:534–43.
- [41] Bundjaja V, Sari TM, Soetaredjo FE, Yuliana M, Angkawijaya AE, Ismadji S, Cheng K-C, Santoso SP. Aqueous sorption of tetracycline using rarasaponin-modified nanocrystalline cellulose. J Mol Liq 2020;301:112433.
- [42] Sabah E, Çinar M, Çelik MS. Decolorization of vegetable oils: adsorption mechanism of β-carotene on acid-activated sepiolite. Food Chem 2007;100:1661–8.
- [43] Boki K, Sakamoto N, Minami K. Inhibitory effect of 1,3-diglycerides during adsorption of β -carotene onto attapulgite and sepiolite. J Am Chem Soc 2001;78:733–6.
- [44] Pohndorf Jr. RS, TRSC, and Pinto LAA. Kinetics and thermodynamics adsorption of carotenoids and chlorophylls in rice bran oil bleaching. J Food Eng 2016;185:9– 16.
- [45] Qiu D, Chen Z-R, Li H-R. Effect of heating on solid β -carotene. Food Chem 2009;112:344–9.
- [46] Larkin PJ. IR and Raman spectra-structure correlations: characteristic group frequencies. In: Larkin PJ, editor. Infrared and raman spectroscopy: principles and spectral interpretation. Stamford, CT, United States: Elsevier; 2018. p. 85–134.
- [47] Christidis GE, Kosiari S. Decolorization of vegetable oils: a study of the mechanism of adsorption of β-carotene by an acid-activated bentonite from cyprus. Clay Clay Miner 2003;51:327–33.
- [48] Tong J, Wu Z, Sun X, Xu X, Li C. Adsorption kinetics of β -carotene and chlorophyll onto acid-activated bentonite in model oil. Chin J Chem Eng 2008;16:270–6.
- [49] Degfie TA, Mamo TT, Mekonnen YS. Optimized biodiesel production from waste cooking oil (WCO) using calcium oxide (CaO) nano-catalyst. Sci Rep 2019;9:18982.
- [50] Langmuir I. The constitution and fundamental properties of solids and liquids. Part II. Liquids. J Am Chem Soc 1917;39:1848–906.
- [51] Freundlich H. Of the adsorption of gases. Section II. Kinetics and energetics of gas adsorption. Introductory paper to section II. T Faraday Soc 1932;28:195–201.
- [52] Wu F-C, Liu B-L, Wu K-T, Tseng R-L. A new linear form analysis of Redlich-Peterson isotherm equation for the adsorptions of dyes. Chem Eng J 2010;162:21-7.
- [53] Hindarso H, Ismadji S, Wicaksana F, Mudjijati, Indraswati N. Adsorption of benzene and toluene from aqueous solution onto granular activated carbon. J Chem Eng Data 2001;46:788–91.
- [54] Piccin JS, Cadaval TRSA, LAAd Pinto, Dotto GL. Adsorption isotherms in liquid phase: experimental, modeling, and interpretations. In: Bonilla-Petriciolet, editor. et al, 2017, editor. Adsorption processes for water treatment and purification. Springer International Publishing AG; 2017. et al.
- [55] Giles CH, MacEwan TH, Nakhwa SN, Smith D. Studies in adsorption. Part XI. A system of classification of solution adsorption isotherms, and its use in diagnosis of adsorption mechanisms and in measurement of specific surface areas of solids. J Chem Soc 1960:1960:3973–93.
- [56] Weber TW, Chakravorti RK. Pore and solid diffusion models for fixed-bed adsorbers. AIChE 1974;20:228-38.
- [57] Muhammad Khan MA, Choong TSY, Chuah TG, Yunus R, Yap YHT. Desorption of β-carotene from mesoporous carbon coated monolith: isotherm, kinetics and regeneration studies. Chem Eng J 2011;173:474–9.
- [58] Wu Z, Li C. Kinetics and thermodynamics of β-carotene and chlorophyll adsorption onto acid-activated bentonite from Xinjiang in xylene solution. J Hazard Mater 2009;171:582–7.
- [59] Baek KH, Kim HS, Oh HM, Yoon BD, Kim J, Lee IS. Effects of crude oil, oil components, and bioremediation on plant growth. J Environ Sci Heal A Tox Hazard Subst Environ Eng 2004;39:2465–72.
- [60] Hodge S, Merfield CN, Bluon A, Berry NA, O'Connell DM. The potential of culinary vegetable oils as herbicides in organic farming: the effect of oil type and repeated applications on plant growth. Org Agric 2019;9:41–51.
- [61] Gong Z, L P, Wilke BM, Alef K. Effects of vegetable oil residue after soil extraction on physical-chemical properties of sandy soil and plant growth. J Environ Sci 2008;20:1458–62.