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Original Article

Local Cuban bentonite clay as potential low-cost adsorbent for shark liver oil pool purification

[Bentonita cubana como adsorbente potencial de bajo costo para la purificación de un pool de aceite de aceite de hígado de tiburón]

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Abstract

Context: The shark liver of the species *Ginglimostoma cirratun*, *Carcharhinus longimanus*, and *Carcharhinus falciformis*, captured in the north-central coast of Cuba are a source of oil, that when extracted it must be purified due to its bad smell, taste and the amount of impurities it has.

Aims: To evaluate the purification of the shark liver oil (SLO) pool of species *Ginglimostoma cirratun*, *Carcharhinus longimanus*, and *Carcharhinus falciformis*, by Cuban bentonite clay.

Methods: The effects of bentonite dose, contact time and speed rate were studied using the 2^3 factorial designs. The parameters are evaluated in the experiment design: acidity value, *p*-anisidine value, peroxide value, Totox value and Bleaching performance. Response Surface Methodology modeling techniques were applied to model the process and their performance and predictive capabilities of the response (purification efficiency) was also examined.

Results: The experiments showed that the oil is suitable for consumption after the purification process. The best treatment, which could reduce the impurities in the SLO pool, was a treatment with bentonite dose at 80 g/L, time at 15 min, and speed rate at 250 rpm. Cuban bentonite clay is a promising adsorbent candidate for the removal of impurities of the SLO.

Conclusions: The local Cuban bentonite clay can be used as potential low-cost adsorbent for shark liver oil pool purification, as showed the experiments.

Keywords: adsorption; Cuban bentonite clay; factorial design; oil purification; response surface methodology; shark liver oil pool.

Resumen

Contexto: El hígado de tiburón de las especies *Ginglimostoma cirratun*, *Carcharhinus longimanus* y *Carcharhinus falciformis*, capturados en la costa centro-norte de Cuba, son una fuente de aceite, que al ser extraído debe ser purificado por su mal olor, sabor y cantidad de impurezas que contiene.

Objetivos: Evaluar la purificación del pool de aceite de hígado de tiburón de las especies *Ginglimostoma cirratun*, *Carcharhinus longimanus* y *Carcharhinus falciformis*, mediante arcilla bentonita cubana.

Métodos: Los efectos de la dosis de bentonita, el tiempo de contacto y la velocidad se estudiaron utilizando un diseño factorial 2^3. Los parámetros que se evaluaron en el diseño del experimento fueron: índice de acidez, índice de *p*-anisidina, índice de peróxido, índice de Totox y rendimiento de blanqueo. Se aplicó la técnica de Metodología de Superficie de Respuesta para modelar el proceso y también se examinó su rendimiento y capacidades predictivas de la respuesta (eficiencia de purificación).

Resultados: Los experimentos demostraron que el aceite es apto para el consumo después del proceso de purificación. El mejor tratamiento que pudo reducir las impurezas en el pool de aceite de hígado de tiburón fue un tratamiento con dosis de bentonita a 80 g/L, tiempo a 15 min y velocidad a 250 rpm. La arcilla de bentonita cubana es un candidato prometedor para la eliminación de impurezas del aceite de hígado de tiburón.

Conclusiones: La arcilla de bentonita cubana se puede utilizar como adsorbente potencial de bajo costo para la purificación del pool de aceite de hígado de tiburón, como se mostró en los experimentos.

Palabras Clave: aceite de hígado de tiburón; adsorción; arcilla de bentonita cubana; diseño factorial; metodología de superficie de respuesta; purificación de aceite.

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INTRODUCTION

In recent decades, scientists have conducted studies on the positive effects of nutrients available to different marine organisms on health, which may be the key to new treatments for diseases such as cancer (Correia et al., 2017; Gómez et al., 2019), neurodegenerative diseases (Gopal et al., 2008), human immunodeficiency virus (Güneş, 2013), or cardiovascular disease (Fidalgo et al., 2019; Norde et al., 2019). In addition, it provides other valuable components such as docosahexaenoic acid and eicosapentaenoic acid, both omega 3 (ω-3), important for optimal neurological development in children (Al-Ghannami et al., 2019; Coelho et al., 2019), diabetes (Behl et al., 2019) and epilepsy (Tejada et al., 2019).

The world production of fish oils reaches millions of tons, of which 88.5% is destined for aquaculture, and remains are destined to industrial production, human consumption, development of pharmaceutical products and dietary supplements (Rizliya and Mendis, 2013). During the heating process in the extraction, oxidative processes of the lipids can also be produced; the nature of these alterations depends on factors such as the temperature, the heating time, the degree of unsaturation of the oil, among others (Muik et al., 2005), therefore, new conditions for shark liver purification are investigated.

The use of bentonite as an adsorbent for oil impurities has been widely used and discussed in various investigations (Larouci et al., 2015). Although bentonite can be used as an adsorbent in its natural form, some researchers have proposed experimental designs and methodologies for the acid activation of these minerals in order to optimize the purification process *(*Didi et al., 2009; Palanisamy et al., 2011; Ajemba, 2012; Usman et al., 2013; Larouci et al., 2015); when applying this activation method it should be borne in mind that excessive activation can be explained in terms of a loss of porosity and acid strength (Makhoukhi et al., 2009). Also, acid treatment of bentonites has been shown to create mesoporosity depending on the acid concentrations and time of treatment involving major structural changes and partial decomposition of montmorillonite (Zheng et al., 2017).

Nadhiro et al. (2018) studied the quality of *Sardinella lemuru* oil using bentonite as an adsorbent, revealing that the addition of activated bentonite can increase the quality of this oil that is canned in industry; meanwhile Suseno et al. (2014) showed in his research that the combination of different concentrations of natural bentonite followed by centrifugation is an excellent technique for purifying this oil, where the oil bleaching process tends to decrease as the concentration of the adsorbent is decreased and centrifugation speed. Other researchers such as Maskan and Bagci (2003) have studied the use of various adsorbents for the purification of sunflower oil used in frying, checking that the chemical properties are easily improved after treatment; however, this study should be conducted to study the concentrations of decomposition compounds with low affinity to the adsorbent. Although bentonite has the ability to absorb impurities from the oil, acidic activation of the mineral leads to superior results (Taxiarchou and Douni, 2014), since it increases its specific surface area resulting in a more porous material than natural bentonite (Önal and Sarıkaya, 2007). Hardyanti et al. (2017) compares the use of bentonite with zeolite for the treatment of *Pogostemon cablin* oil, however, significant increases occur in adsorption with bentonite, due to several factors including its acid activation, the types of cations, Si/Al ratio, and pore geometry; therefore, it can be concluded that in the process of purifying *Pogostemon cablin* oil, bentonite has a higher adsorption power than that of zeolite. In this research, the purification of shark liver oil pool using the species *Ginglimostoma cirratun*, *Carcharhinus longimanus*, and *Carcharhinus falciformis*, captured in the north-central coast of Cuba, are studied through use of local Cuban bentonite clay.

MATERIAL AND METHODS

Extraction of lipids

Shark specimens of *Ginglimostoma cirratun*, *Carcharhinus longimanus*, and *Carcharhinus falciformis* were captured in the Caribbean Sea (between 23.40°160′ to 22.82°160′ N, and 81.27°145′ to 78.94°145′ W), near Villa Clara province shore in Cuba in the summer (June) of 2018. Specimens, as well as their livers were weighed for hepatosomatic index (HSI). Dissected livers were placed in polyethylene bags and frozen at -20°C for their transportation in coolers from the Empresa Pesquera Industrial de Caibarién (Villa Clara, Cuba). Livers were stored at -80°C, for no more than 2 weeks, until oil extraction. Livers were at thawed at room temperature and homogenized for 2 min using a 14-507-7 M cutter (Fisher Scientific, Pittsburgh, PA). The homogenized liver was heated at 50°C for 20 min with agitation and centrifuged at 7500 rpm for 20 min at room temperature in a centrifuge (model TG16, Yingtai Instrument Co., China), to release solid impurities from liver cells; then the oils were washed three times with hot distilled water (50- 60°C). A second centrifugation was performed at 7500 rpm in the oil that was released from the heavy fats and other impurities; these were clean and transparent with a characteristic light-yellow color. The physicochemical characterization of the extracted oil was reported previously (Quero-Jiménez et al., 2020; 2021a).

Purification process

The bentonite Cuban clays are characterized by different methods and it can be concluded that the bentonite under study is a calcium montmorillonite, with a low specific surface area and little porosity. The structural formula for one-layer unit of montmorillonite was determined as $(Na_{3.99}Al_{0.01})(Al_{1.11}Fe^{3+}_{0.49}Mg_{0.18}Ti_{0.07})(Ca_{0.24}Na_{0.15}K_{0.01})O_{10}(OH)_2$. This bentonite are characterized by Quero-Jiménez et al., 2021b; where it was shown that it had low concentrations of heavy metals and the viability of its use as an adsorbent for oil impurities. Samples of 45 mL of crude SLO and the Cuban bentonite clays at conditions stipulated (bentonite dose, time and speed rate) were taking for analysis. Each point of the experiment was placed into an Erlenmeyer flask frosted mouth while the experiment was carried out. After transferred to a 50 mL Falcon tubes and was centrifuged (2500 rpm for 15 min). The purification was performed in triplicate.

Characterization of purified SLO

Acidity value (W_{AV})

The W_{AV} was determined using the British Pharmacopoeia, (2013a) method. Thirty milliliters of ether/ethanol/water 3:3:2 (v/v/v) were used to dissolve 5 g of sample and titrated against 0.1 mol/L sodium hydroxide solution.

Peroxide value (PV)

PV was determined by titration with a 0.01 mol/L sodium thiosulfate solution (Yildiz et al., 2003). The PV is expressed in milliequivalents of active oxygen per kilograms.

p-Anisidine value (p-AV)

The *p*-AV was determined as per Bhattacharya et al. (2008) and British Pharmacopoeia (2013b). The sample (0.5–4.0 g) was dissolved and diluted to volume with iso-octane in a 25 mL volumetric flask. The absorbance (Ab) of the solution was measured at 350 nm. Exactly 5 mL of the fat solution were transferred to a test tube and 5 mL of only the solvent were added to another test tube. One millilitre of *p*-anisidine reagent (2.5 g/L solution in glacial acetic acid) was added to each tube and shaken. After exactly 10 min, the absorbance (As) of the solution in the first test tube was measured at 350 nm, using the solution in the second test tube as blank. Anisidine value was calculated with the following formula:

$$p - \mathrm{AV} = \frac{25 * (1.2 * A_s - A_b)}{m} \quad [1]$$

As is absorbance of the fat solution after reaction with the *p*-anisidine reagent, Ab is absorbance of the fat solution, m mass of the test portion in g.

Totox value

Totox value was calculated with the following formula: *p*-anisidine value + 2 peroxide value.

Bleaching performance (BP)

The bleaching efficiency of activated clays was then determined by measuring the color of the bleached oil using a UV–VIS spectrophotometer (L6S, Inesa) (Usman et al., 2013), at λ_{max} = 450 nm that corresponding to the β-carotene absorption band (Foletto et al., 2013). In this study, bleaching efficiency was express in terms of β-carotene removal.

Statistical analysis

All analyses were carried out in triplicates. The R statistical software package (version 3.6.3) was used to generate the orthogonal matrix of the design, response surface methodology modelling, statistical analysis, regression models, three-dimensional (3D) surface and two-dimensional (2D) contour plots of the independent variables, interactive effects with their corresponding responses, and the optimum values.

Experimental design

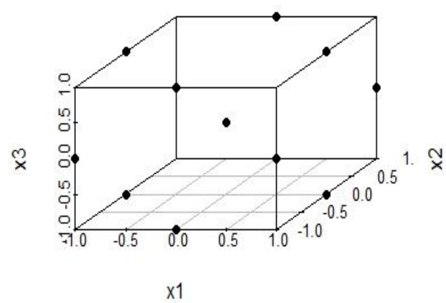
In the context of this experiment, the factorial 2^3 design are used, by varying three key variables, namely the bentonite dose (X_1), the contact time (X_2) and the speed rate (X_3). The value considered convenient, as previously reported by Suseno et al. (2014). The response variables to analyze were the W_{AV}, PV, *p*-AV, Totox value and the BP. A total of 30 experiments were performed, in which three replicates of the factorial 2^3 were considered, with two points in the center of the experiment as summarized in Table 1. Experiments were performed randomly to avoid systemic error.

Response Surface Methodology (RSM) modeling

RSM modelling technique were applied to model the process and predictive capabilities of the response (W_{AV}, PV, *p*-AV) was proposed by Igwegbe et al. (2019). Box-Behnken Design (BBD)

Table 1. Experimental range and levels of independent variables in a 2^3 factorial design and Box-Behnken Design (BBD) distribution for the optimization.

CB (%)	V (rpm)	t (min)
1	1	-1
-1	1	-1
0	0	0
-1	-1	-1
1	-1	1
-1	1	1
0	0	0
1	1	1
1	-1	-1
-1	-1	1



Factors	Symbol of coded variables	Low level (-1)	Medium level (0)	High level (+1)
Bentonite dose (g/L)	X_1	5	7.5	10
Contact time (min)	X_2	20	40	60
Speed rate (rpm)	X_3	150	175	200

CB: Bentonite dose; V: Speed rate; t: Contact time.

was used to design the experiments for the adsorption of SLO on Cuban bentonite clays. Three factors (the independent variables) including bentonite dose (%), contact time (min) and speed rate (rpm) at three levels. The operating variables were coded according to Eq. [1] (Mohamadi et al., 2016):

$$X_i = \frac{(X_i - X_0)}{\Delta X} * 100 \quad [2]$$

where X_i is the coded value of the independent variable, X_0 is the value of X_i at the center point and ΔX is the step change value.

The generated data obtained were subjected to the second-order polynomial regression model. The response, Y can be related to the independent variables as a polynomial model based on the following quadratic equation [3] (Agarry and Owabor, 2014; Ahmadi et al., 2018):

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 + \beta_{12} AB + \beta_{13} AC + \beta_{23} B \quad [3]$$

where Y is the predicted output response; A is the initial bentonite dose (%), B is the contact time (min), C is the speed rate (rpm); (β_0, β_1, β_2 and β_3), (β_{11}, β_{22} and β_{33}), and (β_{12}, β_{13} and β_{23}) are the constant regression coefficients for the linear, quadratic and interaction effects, respectively.

The analysis of variance (ANOVA) was employed to evaluate the adequacy of the developed model and the statistical significance of the constant regression coefficients. ANOVA was also used to examine the individual, the interactive and the quadratic effects of the process variables on the purification efficiency of SLO. The model terms were assessed using the *p*-value with a confidence level of 95%.

RESULTS AND DISCUSSION

Analysis of factorial design 2^3

Table 2 shows the responses achieved at each point of the applied design for all the variables analyzed.

Regarding the bleaching performance and Totox the ANOVA table shows that no effect has a p-value less than 0.05, indicating that they are not significant, with a confidence level of 95.0%; the lack of fit indicator reveals that the linear model is adequate for these data since its p-value is less than 0.05, but the coefficient of determination is very small and only manages to explain 28.0209 and 28.951%, respectively, of the variability in the tests. For the analysis of the Totox response, it must be considered that this is not directly measured but is the combination of the PV and *p*-AV). In the case of the PV, the ANOVA analysis showed that the three factors evaluated have a p-value less than 0.05, indicating that they are significant with a confidence level of 95.0%, however, the lack of fit test shows that the p-value for the lack of fit is less than 0.05, so there is a statistically significant lack of fit with a confidence level of 95.0%. Analysis of variance for acidity value yielded very different results from those seen in the other response variables, in this case, the effect of bentonite concentration has a p-value less than 0.05, indicating that it is significantly different from zero with a confidence level of 95.0%.

The Durbin-Watson (DW) statistic tests the residuals to determine if there is any significant correlation based on the order in which the data is presented in the file. Since the p-value in all cases is greater than 5.0 %, there is no indication of serial autocorrelation in the residuals with a significance level of 5.0 %.

The model used to describe the whitening process is not the optimal one as revealed by the statistical analysis, mostly due to the fact that there is no protonation of the ion exchange sites, so inevitably stronger acid sites are required than the silanol groups (Si-OH) and the remaining protonated sites ([Al-O-Metal^{2+}]H) (Azzouz et al., 2006), then, the presence of multivalent cations is an essential requirement for higher bleaching activities, according to the data reported by Güler and Tunç (1992). A possible explanation should be the polarizing effect of multivalent cations. The latter must induce not only the dissociation of water molecules and then the Bronsted sites with releasable protons, but also electrostatic fields on the bentonite surface. The occurrence of such electrostatic fields is presumably the cause of the attraction and adsorption of molecules. For all the reasons discussed above, it is necessary to broaden the range of effects to evaluate this process, as well as to carry out a bentonite activation study to obtain better results.

Various chemical and physical factors such as relative polarity, active surface sites, surface area and porosity, particle size, pH value in water, and moisture content are responsible for the BP (Didi et al., 2009). The surface concentration and intensity distribution of the active sites, that is, the acidic and basic sites, are the most important factor in the

Table 2. Full 2^3 experimental design matrix with the experimental values.

CB (g/L)	V (rpm)	t (min)	PV	Totox	p-AV	BP	W_{AV}
1	1	-1	1.77	33.08	29.54	45.04	0.45
-1	1	-1	2.66	26.72	21.38	16.03	1.51
0	0	0	3.01	37.42	31.40	40.46	0.90
-1	-1	-1	3.05	31.39	25.29	28.24	1.20
1	-1	1	0.38	28.29	27.53	13.74	0.60
-1	1	1	4.17	36.28	27.94	9.03	0.91
0	0	0	3.42	27.97	21.13	23.66	0.60
1	1	1	2.66	36.85	31.53	27.48	1.20
1	-1	-1	3.80	27.51	19.91	29.01	0.30
-1	-1	1	3.77	34.27	26.73	38.17	1.80
1	1	-1	4.13	31.95	23.69	13.74	1.21
-1	1	-1	4.15	26.08	17.78	14.50	0.58
0	0	0	2.65	27.67	22.37	15.27	1.81
-1	-1	-1	3.39	26.80	20.02	4.58	1.15
1	-1	1	2.66	33.70	28.38	17.23	0.60
-1	1	1	3.38	29.91	23.15	20.61	1.20
0	0	0	2.65	21.17	15.87	16.03	1.19
1	1	1	2.28	31.12	26.56	21.37	1.18
1	-1	-1	1.89	28.64	24.86	10.69	1.50
-1	-1	1	2.66	24.28	18.96	7.630	1.80
1	1	-1	1.89	19.29	15.51	19.42	1.20
-1	1	-1	4.12	39.05	30.81	4.580	1.51
0	0	0	2.61	31.49	26.27	15.27	1.20
-1	-1	-1	2.28	19.48	14.92	13.67	1.49
1	-1	1	2.66	33.70	28.38	17.27	0.60
-1	1	1	4.12	38.86	30.62	10.07	1.49
0	0	0	2.27	26.47	21.93	12.21	1.49
1	1	1	1.52	27.51	24.47	27.10	0.60
1	-1	-1	3.41	27.35	20.53	48.85	1.21
-1	-1	1	1.89	26.23	22.45	21.76	1.51

CB: Bentonite dose; V: Speed rate; t: Contact time; PV: Peroxide value; p-AV: *p*-anisidine value; BP: Bleaching performance; W_{AV}: Acidity value.

adsorption of polar compounds from the oil according to Alemdaroğlu et al. (2003), although Zhu et al. (1994) stated that the percentage of change in the color of the oil is related to the total acid sites. In our process, the low bleaching efficiency is attributed to the small surface area and the Si/[Al_2O_3 + Fe_2O_3 + MgO] ratio, denoting the low capacity of bentonite to adsorb pigments such as carotenoids (Pohndorf et al., 2016).

Taking into account the products of secondary oxidation, it shows a reduction expressed by the *p*-AV, denoting the ability of bentonite to adsorb these types of compounds (Sathivel et al., 2003). Despite the fact that the *p*-AV decreased considerably, the high values obtained (>20) denote the high rancidity state of the treated samples, which is why they present a high concentration of secondary oxidation products such as aldehydes, ketones and alcohols. produced by decomposition of hydroperoxides (García-Moreno et al., 2013).

The decrease in the PV could be due to the high pore volume and pore size of natural clay, allowing sufficient adsorption of trace element impurities (Palanisamy et al., 2011), in terms of oxidation products, this represents the primary oxidation in the oil. As we could see about this response, all factors affect it, so it must be thoroughly studied for its correct interpretation. García-Moreno et al. (2013) in their study reveals that including temperature among the effects causes an increase in the formation of peroxides due to the exposure of the oil to high values of the same. According to Sathivel (2010), during the bleaching process, peroxides decompose into aldehydes and ketones as a consequence of the high temperatures used and subsequently, these secondary oxidation products are adsorbed on the activated clay surface.

Yates et al. (1997) suggested that triglycerides and oleic acid are physically adsorbed by bentonite through the hydrogen bond of the carbonyl group to surface silanols (Si-OH). Oleic acid must also be chemically adsorbed through an ionic bond between the carboxylate ion (COO^-) and the metal silicate on the surface. As we verified this response, only the concentration of bentonite used has a significant impact, so the higher concentrations of the clay can be translated into errors, since large amounts of bentonite promote a loss of tocopherols and antioxidant substances, which further affects stability of oil (Patterson, 2009).

In general, the process of adsorption of impurities that can occur in the refining of SLO is binding based on physical and chemical adsorption, which is related to the configuration of the constituent molecules of bentonite. Physical bonding occurs due to van der Waals forces between the molecules that form in the bentonite (adsorbent) with the impurity molecules (adsorbate).

Because in the variable's responses Totox value and bleaching performance, there is no effect of the factors studied, these are not included in the optimization study. A design is then carried out to optimize the purification of SLO with natural bentonite. Due to the results took design 2^3, the variables analyzed were the same, but the bentonite dose, time and speed rate was expanded from 10 to 100 g/L, 10 to 20 min, and 200 to 300 rpm respectively. For this, the BBD can be used because the number of factors must be minimum 3 and maximum 7. These designs can be applied for the optimization of various chemical and physical processes, where the number of experiments is determined according to the process requirements.

Box-Behnken design

In the present work, the relationship between four criteria of the impurity removal (namely W_{AV}, PV and *p*-AV) and three controllable factors (namely Bentonite dose, time and speed rate) was studied. The mathematical equations where each response variable y is assessed as a function of Bentonite dose (X_1), time (X_2) and speed rate (X_3) and calculated as the sum of a constant, three first-order effects (terms in X_1, X_2 and X_3), three interaction effects (terms in X_1X_2, X_1X_3 and X_2X_3) and three second-order effects (X_{21}, X_{22} and X_{23}) according to the Eq. [2]. The results obtained were then analyzed by ANOVA to assess the "goodness of fit".

Only terms found statistically significant were included in the model. β_{13} and β_{23} coefficient for the PV, *p*-AV and W_{AV}, and β_{12} for the PV removal all the four responses were non-significant (p-value > 0.05). Therefore, these coefficients were dropped from the model and then a new ANOVA was performed for the reduced model. The following fitted regression models (equations in terms of coded values for the regressors) were used to quantitatively investigate the effects of Bentonite dose, time, and speed rate concentration on the characterization of the SLO process for the impurities removal:

$$PV = 3.235 - 0.0175X_1 - 0.019375X_2 - 0.021875X_3 + 0.185625X_1^2 - 0.0025X_1X_3 + 0.101875X_2^2 + 0.026875X_3^2$$

$$p\text{-}AV = 23.1533 - 0.0325\ X_1 - 0.038125\ X_2 - 0.053125\ X_3 + 0.451458\ X_1^2 + 0.03\ X_1X_2 + 0.220208\ X_2^2 + 0.0752083\ X_3^2$$

$$W_{AV} = 1.785 - 0.0275X_1 - 0.035\ X_2 - 0.0575\ X_3 + 0.48125\ X_1^2 + 0.28125\ X_3^2 + 0.05625\ X_3^2$$

In the present study, the adjusted R^2 is 0.9544 for peroxide value, 0.985 for *p*-anisidine value, and 0.982 for the acidity value. The R^2 coefficient in this study ensured a satisfactory adjustment of the quadratic model to the experimental data. High R^2 values do not necessarily mean that decrease of these pollutants, all follow the same trend and are strongly correlated. The models for PV, *p*-AV and W_{AV} were significant by the F-test at the 5% confidence level ($P_{rob} > F < 0.05$).

Response surface methodology

To study the interaction of the different process variables and their corresponding effects on the response (SLO purification efficiency), 2D contour plots and 3D response surface plots against any two independent process variables were made while keeping the other process variable at their central (0) level. Figs. 1-3 present the 3D surface and 2D contour plots made for the interactions between the process variables with their respective output responses. The nonlinear nature of all 3D response surfaces demonstrated that there were considerable interactions between each of the independent variables and the bleaching efficiency.

On the basis of the calculation steps defined for the optimization algorithm, the optimal values of the test variables in coded units were found as X_1 = 80, X_2 = 15, and X_3 = 250 with the corresponding Y = 99. The real values were then determined to be bentonite dose at 80 g/L, time at 15 min, and speed rate at 250 rpm these results confirming once again the judicious choice of the parameters ranges and the applicability and accuracy of the model developed to describe the correlation between the factors and its performances in the purification of SLO. Under these conditions, a purification efficiency value of 99% was obtained.

As shown already, the parameters bentonite dose and time are significant. According to Figs. 1, 2 and 3, low PV, *p*-AV and W_{AV}, respectively, is strongly favored when bentonite dos is kept between 70 and 90 for a time of the 15 min. At a higher bentonite dose, one can observe that SLO purification decreases, reaching a maximum level at intermediate time (level 0 or 15 min), because the adsorption of impurities on bentonite clays was found to decrease with increasing concentration owing to the adsorbent surface is saturated with the adsorbate.

Adsorption processes are significantly influenced by the functional groups present on the adsorbing material and the chemistry of solution. The adsorption process was more favorable in the medium bentonite dose because of the electrostatic attractions between the positively charged surface of the bentonite layers and the H^+ ion of the impurities of the SLO. Time of contact is a very important parameter in all processes. The adsorption of the adsorbate was improved with increasing time of contact and dosage of bentonite clays. The increase in impurities removal efficiency of SLO, with Cuban bentonite clays dose and time is due to the availability of more active adsorption sites for the trapping of the H^+, primary and second products of oxidation, and presence of enough time for the adsorption process, respectively (Ahmadi and Igwegbe, 2018; Igwegbe et al., 2019).

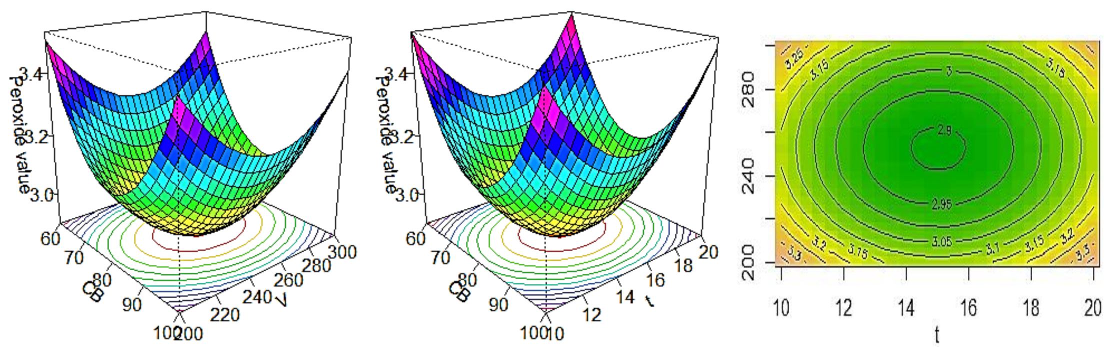


Figure 1. 3D representation and contour graphic of the purification capacity of the Cuban bentonite clays for the response peroxide value, slice at bentonite dose = 80 g/L.

CB: Bentonite dose; V: Speed rate; t: Contact time.

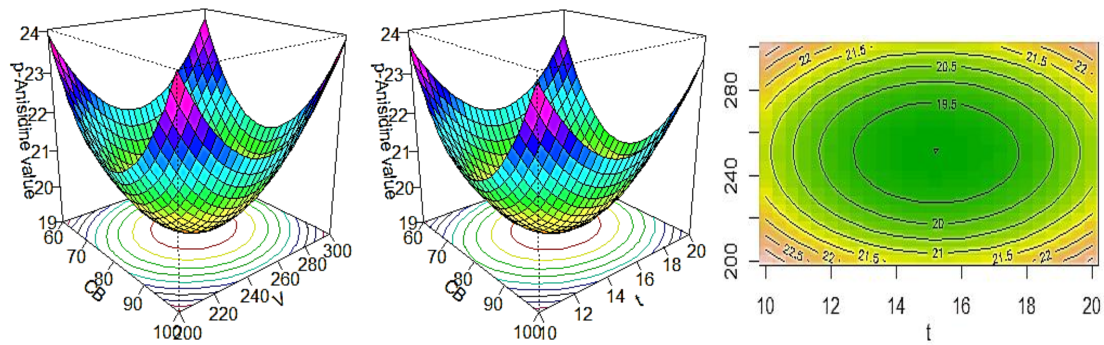


Figure 2. 3D representation and contour graphic of the purification capacity of the Cuban bentonite clays for the response *p*-anisidine value, slice at bentonite dose = 80 g/L.

CB: Bentonite dose; V: Speed rate; t: Contact time.

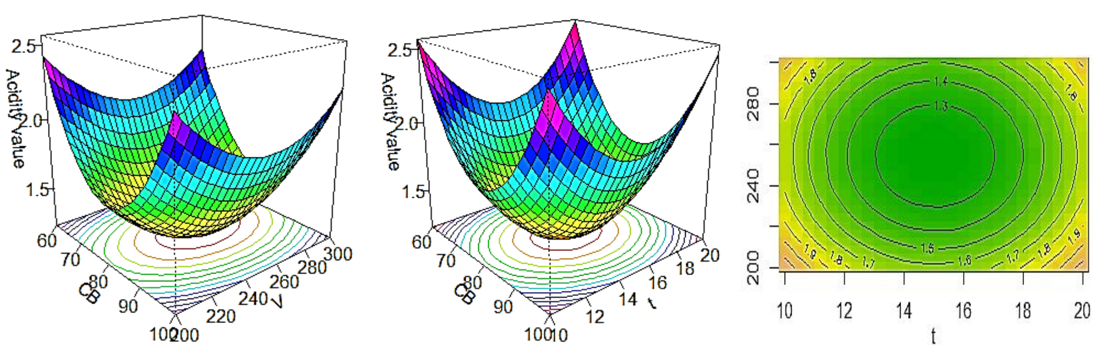


Figure 3. 3D representation and contour graphic of the purification capacity of the Cuban bentonite clays for the response acidity value, slice at bentonite dose = 80 g/L.

CB: Bentonite dose; V: Speed rate; t: Contact time.

CONCLUSIONS

This study was the evaluation of the liver oil pool of shark species *Ginglimostoma cirratun*, *Carcharhinus longimanus*, and *Carcharhinus falciformis*, purification performance by Cuban bentonite clay. The effects of bentonite dose, contact time and speed rate were studied using the 2^3 factorial design. Cuban bentonite clays did not have a significant effect on the bleaching performance and the elimination of the Totox value during the purification process, although the bleaching performance is not a critical parameter since the oil is extracted with an acceptable color. However, a significant decreased in PV, *p*-AV and W_{AV} was observed. The bentonite dose and contact time are the parameters influencing the adsorption process. Although the process of adsorption of impurities that can occur in the refining of SLO is binding based on physical and chemical adsorption, principally by due to van der Waals forces between the molecules that form in the bentonite (adsorbent) with the impurity molecules (adsorbate). RSM modeling techniques were applied to model the process and their performance and predictive capabilities of the response (purification efficiency) was also examined. The decrease of the PV, *p*-AV and W_{AV}, during the bleaching process was due to the adsorption of peroxide compounds and transformation of peroxide into secondary oxidation products and followed by the adsorption of secondary oxidation products on the organo-bentonite particles. The best treatment which could reduce the impurities in the SLO was a treatment with bentonite dose at 80 g/L, time at 15 min, and speed rate at 250 rpm. Cuban bentonite clay is a promising adsorbent candidate for the removal of impurities of the SLO.

CONFLICT OF INTEREST

The authors declare no conflicts of interests.

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Concepts or ideas	x		x	x	x		x
Design	x	x					
Definition of intellectual content	x						x
Literature search	x	x	x	x	x	x	x
Experimental studies	x	x	x				
Data acquisition	x						
Data analysis	x			x	x	x	
Statistical analysis	x				x	x	x
Manuscript preparation	x		x	x	x		x
Manuscript editing	x		x	x		x	
Manuscript review	x	x	x	x	x	x	x

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