Optimization of Bleaching Conditions in Refining Process of Camellia Oil with Response Surface Method

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Summary: The objective of this study was to optimize bleaching conditions of camellia oil by minimizing the lovibond yellow color and maximizing the polyphenol content. The experiments of bleaching were conducted using RSM with Box-Behnken design for the camellia oil. RSM was used to investigate the effects of active clay amount (2-6 wt%), heating temperature (30-60 ºC) and stirring speed (60-140 rpm) on lovibond yellow color and polyphenol content. An analysis of variance (ANOVA) revealed that active clay amount, heating temperature and stirring speed significantly effect on lovibond yellow color and polyphenol content ($p < .05$), while the quadratic (X_2^2, X_3^2) and interaction effect of X_1X_2, X_2X_3 hardly influence the polyphenol content ($p > .05$). In addition, the optimized conditions were active clay amount of 4 g/hg, the heating temperature of 40 ºC, and the stirring speed of 60 rpm. Under these conditions, the experimental lovibond yellow color and polyphenol content were 8.5 (133.4mm) and 26.74 mg/kg respectively, which were well matched with the predictive values.

Keywords: Camellia oil, Bleaching conditions, Optimization, Response surface methodology.

Introduction

With the rapid growth in world over population, high quality seed oils are increasingly becoming a demand. Apart from the increase in production of major oilseed crops, there is a pressing need to explore niche areas for novel oils. Among dietary oils, one that is common in East and Southeast Asia, where camellia oil (also known as tea seed oil) is widely available given its source from camellia tea seeds [1, 2]. Camellia oil shares similarities with olive oil, containing a high percentage of oleic acid (75–80%) [3, 4] making it beneficial for human health [3, 5, 6]. Crude camellia oil is rich in certain minor components, such as polyphenols, renowned for their antimicrobial activity against specific bacteria, as well as for their anti-inflammatory and antioxidant effects [7]. Additionally, crude camellia oil contains impurities that not only impart unpleasant flavors and tastes but can also pose health risks to consumers. Hence, oil refining is crucial for removing impurities and undesirable compounds while retaining valuable components like polyphenols.

Bleaching stands out as the most crucial process within the refining procedures [8]. This process helps improve the quality of the oil, making it more appealing to consumers by enhancing its taste, color, and overall health benefits. Adsorbent materials like active clay are used to absorb impurities, such as color pigments, soap, phosphates, trace metals and pro-oxidant metals from oil [2]. The valuable bioactive compounds, such as polyphenols, could degrade easily during the absorption process under inappropriate operating conditions [9]. Thus, it is imperative to minimize losses of bioactive compounds while effectively eliminating undesirable components from the crude camellia oil simultaneously. In this article, polyphenols and color are chosen to evaluate the quality of bleaching oil. To the best of our knowledge, there is no research on the effect of bleaching process parameters on beneficial compounds of camellia oil [10-12].

Response Surface Methodology (RSM) has been a useful model to study the influence of different factors on the end response variable [13]. With the

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advantages of providing suitable mathematical models with a minimum number of experimental runs, RSM has been extensively employed for modeling and analyzing issues where the studied subject is influenced by multiple factors [14]. In the absorption process of camellia oil, the influencing factors affecting the quality of camellia oil are composition in 4 aspects including active clay dosing amount, heating temperature, stirring speed, and time. Our previous study [15] showed that the physicochemical properties of camellia oil do not change much with different stirring time. Therefore, In this study, RSM is employed to identify the optimal parameter combination of the active clay amount, heating temperature, and stirring speed for the bleaching process and therefore minimizing lovibond yellow color compounds while maximizing polyphenol content.

The rest of the article is organized as follows. In Section 2, the materials and methods used for this study are elaborated, starting with the materials and experimental procedure then presenting the analytical design and design of experiment, and finally introducing the the quadratic polynomial equation of RSM. Section 3 describes the results and discussion, encompassing the optimization of bleaching conditions. Finally, Section 4 provides concluding remarks and offers perspectives.

Experimental

Materials and Experimental procedure

In this study, the crude camellia oil selected for bleaching was sourced from Fengge Ecological Agriculture Co. Active clay was chosen as the adsorbent material and was procured from Macklin Biochemical in Shanghai, China. The bleaching process was carried out using a jacket reaction vessel with an overhead stirrer (illustrated in Fig. 1), consisting of a stirring paddle, inner glass bottle, and outer glass bottle. Prior to the bleaching process, the crude camellia oil was preheated to a temperature range of 30 to 60 ºC, while the circulating water between the inner and outer glass bottles was heated by a thermostatic heater to match the preheated oil's temperature. The mixture of preheated oil and active clay (ranging between 2 and 6 g/hg) underwent heating under continuous stirring at speeds ranging from 60 to 140 rpm, at temperatures between 30 and 60 ºC. The entire bleaching treatment lasted for 10 minutes. Subsequently, the treated oil was cooled to 30 ºC through nitrogen flushing. Additionally, the supernatant oil used for physicochemical property analysis was obtained by centrifuging the bleached camellia oil that stored in a sealed centrifuge tube at a speed of 8000 rpm for 20 minutes using a frozen centrifuge (Velocity 18R, Dynamica, UK). Note that the frozen centrifuge refers to a centrifuge that is used in laboratories to separate substances based on density differences in a frozen state.

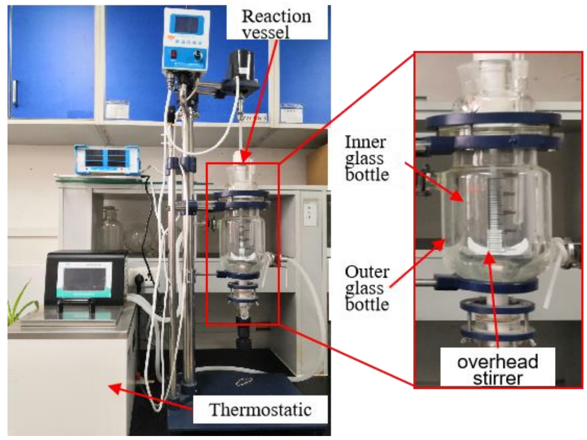

Fig. 1: Oil bleaching experimental device.

Analytical design

The yellow unit of oil samples was determined manually by Lovibond Universal Tintometer (PFXi 880/L, Tintometer Ltd., Amesbury, UK) according to ISO 15305:1998 [16]. The spectrophotometric analysis is performed according to LS/T 6119 - 2017 to quantify the polyphenol content in the camellia oil samples.

Design of Experiment

The effects of three processing parameters namely active clay, heating temperature, and stirring speed on the oil lovibond yellow color and polyphenol content were investigated in this study. A 3-factor-3-level factorial Box-Behnken Design (BBD) of response surface methodology (RSM) was applied to the initial studies, which made a total of 17 experimental runs. However, there is a scarcity of literature in terms of the optimization of bleaching conditions as far as color pigments and polyphenols are concerned. Based on our one-factor experiments and studies of others with different seeds, ranges for each individual variable were set. Three levels of active clay (2, 4, and 6 g/hg), heating temperature (30, 45, and 60 ºC), and stirring speed (60, 100, and 140 rpm) were chosen.

Response surface methodology (RSM)

Linear or quadratic polynomial functions are possibly the most widely used approximate model in RSM that allows building a relationship between independent variables and end response variables. When there are three or more levels for each parameter, a second-order model should be employed [17]. The two responses (lovibond yellow color and polyphenol content) were separately represented as the function of independent variables using the quadratic polynomial equation (1). This model is fit by estimating the unknown coefficients in a least square procedure.

$$Y_m = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum_{i=1}^{k} \sum_{j=i+1}^{k} \beta_{ij} X_i X_j \quad (1)$$

where Y_m (m=1, 2 in this study) represents the response; X_i and X_j denote variables (with *i* and *j* ranging from 1 to *k*); β_o is the intercept coefficient of the model; β_j, β_{jj} and β_{ij} represent linear, quadratic and second-order terms interaction coefficients, respectively; *k* is the number of independent factors (*k*=3 in this work). In this study, the Design Expert (Trial Version 13, Stat-Ease) was used to create an experimental design, statistically analyze and develop a model.

Results and discussion

Model fitting and validation

Table-1 shows the structure of this BBD designed test and subsequent responses of the tested factors. Lovibond yellow color is from 4.0 to 14.0 (133.4mm) and polyphenol content is in the range of 18.47 to 33.03 mg/kg, respectively. Under different experimental conditions, the minimum Lovibond yellow color (4.0 units on a scale of 133.4mm) and the maximum polyphenol content (33.03 mg/kg) were observed to be obtained, respectively. Therefore, a balance should be established through the RSM to obtain the minimum lovibond yellow color and maximum polyphenol content under the same experimental conditions.

Table-1: The physichemical properties of camellia oil at various bleaching conditions.

Run	Active clay (g/hg)	Temperature (ºC)	Stirring speed (rpm)	Lovibond yellow color (133.4mm)	Polyphenol content (mg/kg)
1	2	30	100	13.2	31.59
2	6	30	100	7.6	21.10
3	4	30	60	10.0	29.26
4	4	30	140	8.0	20.15
5	2	45	60	14.0	33.03
6	6	45	60	6.1	22.26
7	2	45	140	8.2	24.62
8	6	45	140	6.5	20.18
9	4	45	100	6.7	23.83
10	4	45	100	6.8	22.41
11	4	45	100	6.8	22.94
12	4	45	100	6.7	23.67
13	4	45	100	6.7	23.26
14	2	60	100	8.7	26.45
15	6	60	100	5.0	18.47
16	4	60	60	7.9	25.44
17	4	60	140	4.0	19.57

The quadratic polynomial models representing lovibond yellow color and polyphenol content in terms of process variables are expressed as Equations (2) and (3), respectively:

$$Y_1 = 69.18150 - 7.62237X_1 - 0.27268X_2 - 0.28216X_3 + 0.020917X_1X_2 + 0.019781X_1X_3 + 0.001350X_2X_3 + 0.32475X_1^2 - 5.26667E\text{-}004X_2^2 + 3.13438E\text{-}0.04X_3^2 \quad (2)$$

$$Y_2 = 38.70500 - 6.94125X_1 - 0.22617X_2 - 0.12781X_3 + 0.015833X_1X_2 + 0.019375X_1X_3 - -7.91667E\text{-}004X_2X_3 + 0.38875X_1^2 + 1.46667E\text{-}003X_2^2 + 2.53125E\text{-}004X_3^2 \quad (3)$$

where Y_1 and Y_2 are dependent variables for lovibond yellow color and polyphenol content, respectively, X_1, X_2, and X_3 are independent variables for active clay amount, heating temperature, and stirring speed.

Table-2 presents the analysis of variance (ANOVA) results from the fitted RSM models of Lovibond yellow color and polyphenolic content. The statistical significance of the RSM-based model was assess ed using the F-test and p-values. The results indicate the significance of the model, as evidenced by the high F-values (458.46 for lovibond yellow color and 31.22 for polyphenol content) and low p-values ($p < .0001$). The goodness of fit was measured by the determination coefficient (R^2) and adjusted determination coefficient (Adj-R^2) of the RSM model

[18]. The high values of R^2 (.9983 for lovibond yellow color and .9757 for polyphenol content) and Adj-R^2 (0.9961 for lovibond yellow color and 0.9444 for polyphenol content) approach 1, indicating a perfect fitting between the predicted value and measured one.

Response surface analysis of lovibond yellow color

Table 2 lists the F and p values for all significant terms in the attempted quadratic model for lovibond yellow color. It can be seen that three linear parameters (X_1, X_2, X_3), three quadratic terms (X_{12}, X_{22}, X_{32}), and three interaction effects of X_1X_2, X_1X_3, X_2X_3 have significant influences on the lovibond yellow color ($p < .05$). The results also indicate that the first-order effect of clay amount (X_1), heating temperature (X_2), and stirring speed (X_3), quadratic terms of X_1^2, and interaction effect of X_1X_3 were highly significant ($p < .0001$).

Additionally, 3D surface plots of the response, along with their corresponding contour plots (Fig 2(a) - (f)), were generated based on Equation (2) to examine the relationship between processing conditions and lovibond yellow color. The data in these plots were obtained by changing values of two variables from all combinations of their respective design ranges, while keeping the third variable at its central value within the design range. From Fig 2(a) - (d), an increase in active clay amount from 2 - 6 g/hg resulted in the decrease of lovibond yellow color. This observation agrees with the findings in bleaching of carp oil [11] and kenaf seed oil [19]. This could likely be due to the fact that, the higher the concentration of active clay used, the larger the surface area of the active clay that can be used to adsorb the color pigments, resulting in lower lovibond yellow color. It was observed from Fig 2(a), (b), (e), and (f) that the lovibond yellow color decreased with the increase of heating temperature from 30 - 60 ºC. The same has also been reported for the kenaf seed oil bleaching as well [19]. Fig 2(a) and (b) showed that an increase in the interaction effect between active clay and heating temperature decreased lovibond yellow color. It is suggested that the rise of temperature caused the reduction of the oil viscosity and enhances oil flow, which facilitated the interaction between oil and active clay and flow ability [20]. Fig 2(c), (d), (e), and (f) showed that the lovibond yellow color decreased with the increase of stirring speed. This can be probably because that the higher stirring speed would get more color pigments in touch with active clay and then, causing more color pigments to be absorbed.

Based on the above research, the least oil lovibond yellow color could be attained toward the higher range of operating parameters, namely active clay amount, heating temperature, and stirring speed.

Response surface analysis of polyphenol content

Table 2 shows the effects and interactions of clay amount, heating temperature and stirring speed on polyphenol contents. Significant effects on polyphenol content were observed due to three linear parameters (X_1, X_2, X_3), one quadratic term (X_1^2), and one interaction effect of X_1X_3, ($p < .05$). Furthermore, the clay amount (X_1) and stirring speed (X_3) showed very significant effect with $p < .0001$. On the other hand, the quadratic (X_2^2, X_3^2) and interaction effects of X_1X_2 and X_2X_3 hardly affect the polyphenol content, which can be verified through a higher p-values ($p > .05$).

Table-2: ANOVA for response surface model.

Source	***Y₁***			***Y₂***		
	Sum of squares	***F* value**	***p* value**	**Sum of squares**	***F* value**	***p* value**
Model	**105.81**	**458.46**	**<0.0001**	**264.09**	**31.22**	**< 0.0001**
X_1	**44.65**	**1741.27**	**<0.0001**	**141.79**	**150.87**	**< 0.0001**
X_2	**21.78**	**849.36**	**< 0.0001**	**18.51**	**19.70**	**0.0030**
X_3	**15.96**	**622.44**	**< 0.0001**	**81.09**	**86.28**	**< 0.0001**
X_1X_2	**0.90**	**35.19**	**0.0006**	**1.58**	**1.68**	**0.2365**
X_1X_3	**9.61**	**374.76**	**<0.0001**	**10.02**	**10.66**	**0.0138**
X_2X_3	**0.90**	**35.19**	**0.0006**	**2.62**	**2.79**	**0.1386**
X_1^2	**10.18**	**397.04**	**<0.0001**	**7.10**	**7.56**	**0.0285**
X_2^2	**0.46**	**17.88**	**0.0039**	**0.059**	**0.063**	**0.8092**
X_3^2	**0.69**	**26.93**	**0.0013**	**1.06**	**1.13**	**0.3237**
	R^2=0.9983 Adj-R^2=0.9961			**R^2=0.9757 Adj-R^2=0.9444**		

Note. X_1 represents active clay amount; X_2 represents heating temperature; and X_3 represents stirring speed.

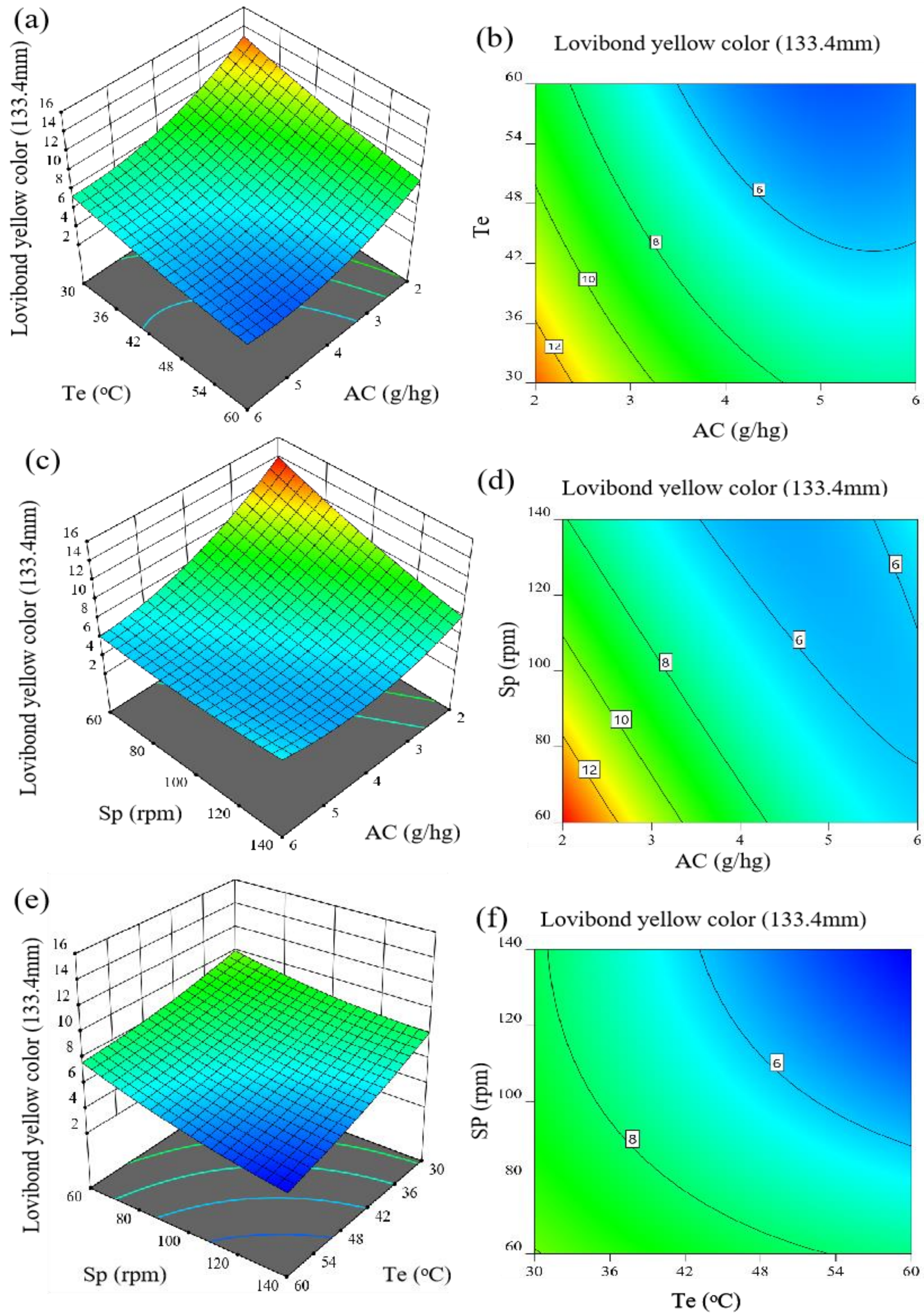

Fig. 2: Response surface plots and contour plots for lovibond yellow color as a function of: (a) and (b) active clay and heating temperature; (c) and (d) stirring speed and active clay; (e) and (f) stirring speed and heating temperature.

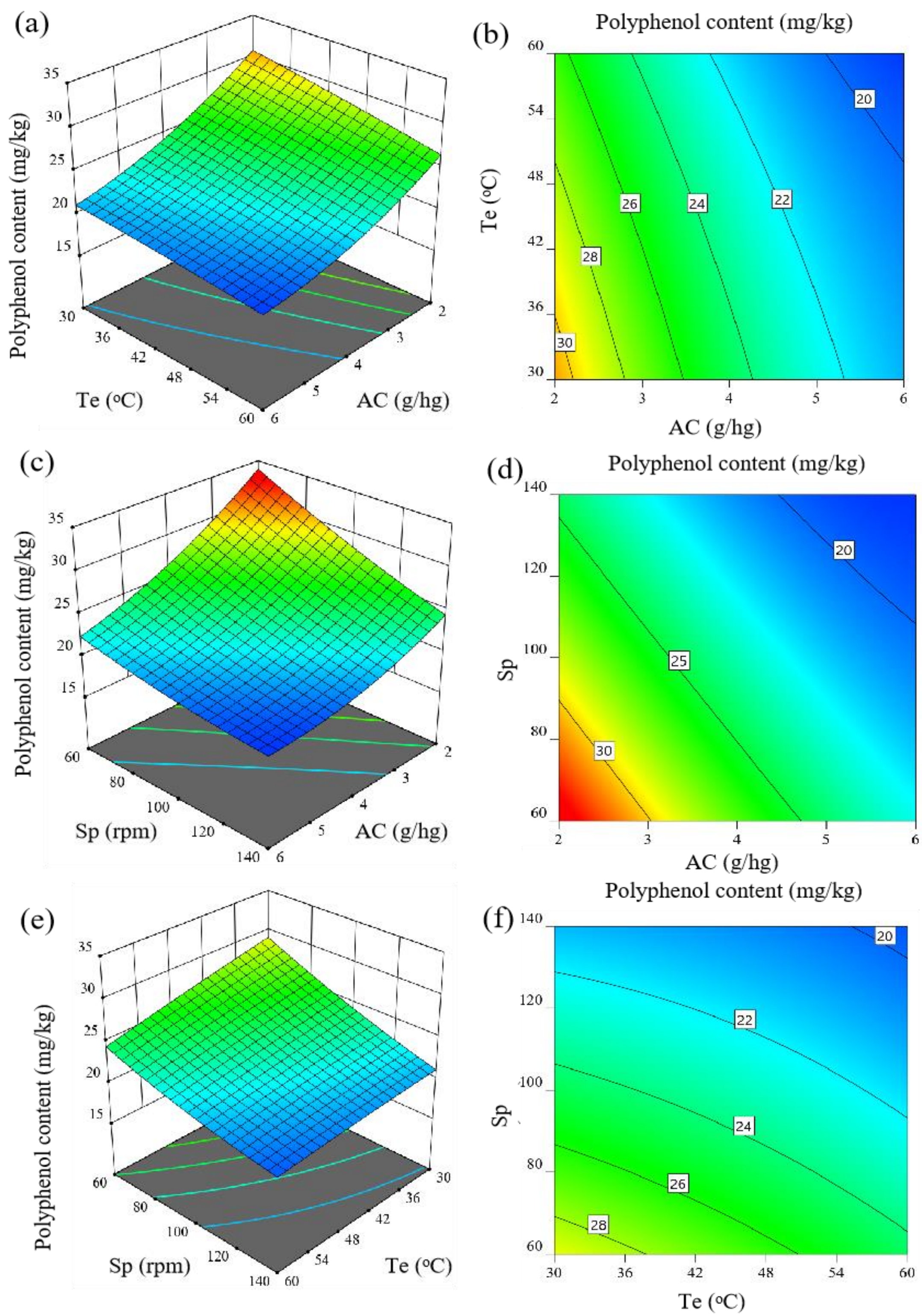

Fig. 3: Response surface plots and contour plots for polyphenol content as a function of: (a) and (b) active clay and heating temperature; (c) and (d) stirring speed and active clay; (e) and (f) stirring speed and heating temperature.

The 3D response surface plots and 2D contour plots for polyphenol content are shown in Fig 3(a) – (f). Fig 2(a) - (d) showed that increase in active clay amount decreased in polyphenol content. A similar trend was also noted by Hew and coworkers who have extracted the polyphenols from chestnut tree wood [9]. An increase in heating temperature from 30 - 60 ^{o}C resulted in a decrease in polyphenol content (Fig 2(a), (b), (e), and (f)). This trend was also observed during polyphenol extraction from chestnut tree wood [21]. This may be attributed to the fact that high temperatures can result in degradation or evaporation of volatile phenolic/flavonoid compounds, as polyphenols are commonly considered heat-labile compounds [22]. It is observed from Fig 2(c) and (d) that, the interaction effect between active clay and stirring speed significantly decreased polyphenol content. This phenomenon is caused due to the fact that, with an increase in stirring speed, there may be better interaction and improved contact with active clay. Accordingly, the optimal points for the most polyphenol content were approximately determined to be active clay amount of 2 g/hg, heating temperature of 30 ^{o}C, and stirring speed of 60 rpm.

Optimization

The aim of this work was to obtain the optimal bleaching conditions of camellia oil by minimizing the lovibond yellow color and maximizing the polyphenol content. Although three-dimensional plots and their corresponding contour plots were analyzed, it was not feasible to identify conditions that simultaneously obtain two conflict objectives (minimum lovibond yellow color and maximum polyphenol content). Therefore, a numerical optimization technique embedded in Design-Expert software (Trial Version 13) was carried out in present study to achieve a balance between the minimum lovibond yellow color and maximum polyphenol content. The results showed that the best operating parameter levels were active clay amount of 4.297 g/hg, the heating temperature of 39.804 ^{o}C, and the stirring speed of 60.000 rpm. Under the conditions, the lovibond yellow color was 8.52 and the polyphenol content was 26.99. In order to consider the practical operability industrial production, the optimal conditions were adjusted to active clay amount of 4 g/hg, heating temperature of 40 ^{o}C, and stirring speed of 60 rpm. The experimental lovibond yellow color and polyphenol content obtained under these modified conditions were 8.5 (133.4mm) and 26.74 mg/kg, respectively, closely approximating the predicted values.

Conclusions

In this study, the effects of three factors and the interactions between them on the lovibond yellow color and the polyphenol content in bleaching stage were determined by Box-Behnken design response surface methodology (BBD-RSM). The results showed that the active clay amount, heating temperature, stirring speed have highly significant influence on the lovibond yellow color ($p < .0001$), while the clay amount and stirring speed exhibit very significantly effects on the polyphenol content ($p < .0001$). Furthermore, the optimal bleaching conditions, including an active clay amount of 4 g/hg, a heating temperature of 40°C, and a stirring speed of 60 rpm, yielded the minimum lovibond yellow color (8.5 (133.4mm)) and maximum polyphenol content (26.74 mg/kg), closely aligning with the experimental results.

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Conflict of interest

The authors have declared no conflict of interest.

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