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Optimization of deacidification process for *Morinda citrifolia* extracts using packed column of calcium carbonate

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Abstract: A study was carried out to optimize the deacidification process for noni (*Morinda citrifolia* L.) extract using packed column of calcium carbonate. The experiments were based on a 3-level factorial design to study the optimum process of deacidification for *M. citrifolia* extract. The *M. citrifolia* extract was treated with $CaCO_3$ packed in different column diameter (20, 25 and 30 mm), height of calcium carbonate (0, 0.5 and 1 cm) and feed rate (10, 30 and 50 ml/min). Physico-chemical characteristics which include pH, titratable acidity, turbidity, total polyphenol content and total soluble solids were measured. Results showed that only pH, titratable acidity and turbidity could be well represented using statistical models. For pH, only the effect of height of $CaCO_3$ was found to be significant. While for titratable acidity and turbidity, effects of diameter column and height of $CaCO_3$ were significant. The optimum conditions for the deacidification of *M. citrifolia* extract was by using a column diameter of 30 mm, $CaCO_3$ height of 1 cm, and a feed rate of 50 ml/min.

Keywords: ***Morinda citrifolia L*., deacidification process, calcium carbonate, response surface methodology (RSM)**

Introduction

Noni or Morinda citrifolia is a small evergreen tree or shrub in the *Rubiaceae* family. *M. citrifolia* is a native plant in Southeast Asia throughout Australia and is being cultivated in Polynesia, India, the Caribbean and central and northern South America (Dixon et al., 1999). Extract of *M. citrifolia* has been used for generations in traditional therapy (Goh et al., 1995). Although *M. citrifolia* is widely used in traditional therapy, many people avoid consuming *M. citrifolia* because of its odor. Fruits have various odors, with some varieties being virtually odorless (McClatchey, 2002). The ripe fruit of *M. citrifolia* was reported to exude strong rancid-like unpleasant odor (Morton, 1992; Dixon et al., 1999). The unpleasant odor of *M. citrifolia* extract was reported to have been contributed by medium chain fatty acids such as capric, caproic and caprylic acids (Norma et al., 2004). Farine et al. (1996) reported that volatile components of *M. citrifolia* extract consist of carboxylic acid (83%), alcohol (5%) and ester (3%). As the reported contributing compound for the unpleasant odor of *M. citrifolia* is acidic in nature, manipulation of the acid content may provide an opportunity to improve the undesirable odor of *M. citrifolia* extract. Deacidification is a process that has been used to reduce the level of acid in food systems. Currently, there are three common chemical methods of deacidification, which consist of addition of calcium carbonate, ion exchange and electrodialysis (Vera et al., 2003). It is possible that the increase in pH may be due to the neutralization of acids and producing salt as has been reported by Vera et al. (2003). Norma et al. (2004) suggested the use of activated charcoal powder was able to reduce the levels of caproic, caprylic and capric acid in *M. citrifolia* extract. The addition of calcium carbonate was reported to have reduced the titratable acidity level and increased the pH of *M. citrifolia* extract. In addition, using $CaCO_3$ also reduced the undesirable odor and improved panelists' perception of *M. citrifolia* extract (Sharmella et al., 2005). Thus, the aim of this study is to optimize the column diameter, height of calcium carbonate and feed rate during deacidification of *M. citrifolia* extract using packed column of $CaCO_3$.

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Materials and Methods

Juice extraction

Fresh *M. citrifolia* fruits were obtained from Serdang, Selangor, Malaysia. The maturity stage chosen was stage 4 where the fruits were 80 % mature, yellowish and pale in color. Whole fruits were washed using running tap water. The washed fruits were cut into small pieces approximately 3 cm thick, added with distilled water at a ratio of 1:1 (water: fruit) and blended using a food blender (National, Malaysia) for 3 mins. The blend was filtered using a cotton cloth and centrifuged at 10,000 x g for 30 mins at 5^{o}C (Rivera et al., 2005) followed by a second filtration also using a cotton cloth. The *M. citrifolia* extract was stored at 4^{o}C prior to deacidification.

Preparation of $CaCO_3$ packed column

Glass columns with different diameter sizes of 20, 25 and 30 mm with a length of 30 cm were used. Each column was fitted with glass wool at the bottom to act as a support for $CaCO_3$. $CaCO_3$ (Sigma Aldrich, USA) with a purity of 99.9% was placed in the column at different heights (0, 0.5 and 1 cm). *M. citrifolia* extract was pumped into the column using a peristaltic pump (Model SP311, VELP) from the top of the column. Three feed rates (10, 30 and 50 ml/min) were used to pump the extract into the column. The deacidified *M. citrifolia* extract was subsequently analyzed for pH, titratable acidity, turbidity, total polyphenol content and total soluble solids.

pH value

The pH values of the treated *M. citrifolia* extract were measured by pH meter (Model PHM 210, Radiometer Analytical) which was calibrated using pH 7 and pH 4 buffers. Measurement of pH value was done at room temperature using 10 ml of sample extract (Kirk and Sawyer, 1991).

Titratable acidity

Titratable acidity was measured using the method of titration. Acidity was expressed as g of citric acid per 100g of extract. These tests were done with 5 ml of sample using NaOH 0.1N and phenolphthalein as indicator (Kirk and Sawyer, 1991).

Turbidity

Turbidity was determined using Spectrophotometer (Model Spectronic 20 PRIM, Secomam) by measuring the absorbance at 580 nm. Distilled water was used as a reference (Sin et al., 2006).

Total polyphenol content

Total polyphenol content was determined using the Folin-Ciocalteu reagent (Shahidi and Naczk, 1995) using epicatechin acid equivalents (EAE) µg/ml, which contains sodium phosphomolibdate and sodium tungstat. The epicatechin stock solution was prepared prior to use.

Total soluble solid

Total soluble solid was measured using a hand-held refractometer (Model ATAGO, Japan) with a range of 0-50% Brix.

Experimental design

The experimental design and statistical analysis were performed using Design Expert Version 6.0.10 (Stat-ease, Inc) software. The experiments were based on a 3-level factorial design. A total of 32 experiments including four replications of the center point and replication of other points were carried out in random order. The level of factors used in the experiment is shown in Table 1. Models were considered a good fit if the model was significant ($p<0.05$), had an R2 value of more than 0.75 and had an insignificant lack of fit. The optimum condition for the deacidification of *M. citrifolia* extract using packed column of calcium carbonate was determined using numerical optimization. The optimization criteria used were minimum titratable acidity and turbidity while the pH value was set for maximum.

Results and Discussion

The model and analysis of variance for five response variables i.e pH, titrable acidity, turbidity, total polyphenol content and total soluble solids are presented in Table 2. From Table 2, it is shown that only the response surface model developed for pH, titrable acidity and turbidity, fits the model well. The high R^2 values (>0.75) for pH, titratable acidity and turbidity indicate a good agreement between the experimental results and the theoretical values predicted by the model (Weisberg, 1985). Whereas the other two responses i.e total polyphenol content and total soluble solids showed low R^2 values. The lack-of-fit test showed insignificant lack-of-fit ($p>0.05$) for pH, titratable acidity, turbidity, total polyphenol content and total soluble solid. Based on the results in Table 2, models of the other two parameters, total polyphenol content and total soluble solid showed that they did not satisfactorily fit the model and thus did not represent the experimental data adequately. The coded and actual models for pH, titratable acidity and turbidity are presented in Table 3.

pH value

Table 4 shows the model coefficient for pH

Table 1. Actual and coded ( ) experimental points using a 3-level factorial design for the deacidification of *M. citrifolia* extract using calcium carbonate ($CaCO_3$).

Std	Column diameter (mm)	Height of $CaCO_3$ (cm)	Feed rate (ml/min)
1	20.00 (-1)	0.00 (-1)	10.00 (-1)
2	25.00 (0)	0.00 (-1)	10.00 (-1)
3	30.00 (+1)	0.00 (-1)	10.00 (-1)
4	20.00 (-1)	0.50 (0)	10.00 (-1)
5	25.00 (0)	0.50 (0)	10.00 (-1)
6	30.00 (+1)	0.50 (0)	10.00 (-1)
7	20.00 (-1)	1.00 (+1)	10.00 (-1)
8	25.00 (0)	1.00 (+1)	10.00 (-1)
9	30.00 (+1)	1.00 (+1)	10.00 (-1)
10	20.00 (-1)	0.00 (-1)	30.00 (0)
11	25.00 (0)	0.00 (-1)	30.00 (0)
12	30.00 (+1)	0.00 (-1)	30.00 (0)
13	20.00 (-1)	0.50 (0)	30.00 (0)
14	25.00 (0)	0.50 (0)	30.00 (0)
15	30.00 (+1)	0.50 (0)	30.00 (0)
16	20.00 (-1)	1.00 (+1)	30.00 (0)
17	25.00 (0)	1.00 (+1)	30.00 (0)
18	30.00 (+1)	1.00 (+1)	30.00 (0)
19	20.00 (-1)	0.00 (-1)	50.00 (+1)
20	25.00 (0)	0.00 (-1)	50.00 (+1)
21	30.00 (+1)	0.00 (-1)	50.00 (+1)
22	20.00 (-1)	0.50 (0)	50.00 (+1)
23	25.00 (0)	0.50 (0)	50.00 (+1)
24	30.00 (+1)	0.50 (0)	50.00 (+1)
25	20.00 (-1)	1.00 (+1)	50.00 (+1)
26	25.00 (0)	1.00 (+1)	50.00 (+1)
27	30.00 (+1)	1.00 (+1)	50.00 (+1)

Table 2. Statistical analysis of models representing the response surface of pH, titratable acidity, turbidity, total polyphenol content and total soluble solids during the deacidification of *M. citrifolia* extract using calcium carbonate ($CaCO_3$).

Responses	Model significance	R^2	Lack-of-fit Test
pH value	<0.0001*	0.9860	0.8753
Titratable acidity	<0.0001*	0.7609	0.0517
Turbidity	<0.0001*	0.9164	0.1275
Total Polyphenol Content	<0.0001*	0.2553	0.6733
Total Soluble Solids	<0.0001*	0.3690	0.3783

Table 3. Model representing the equation of pH, titratable acidity and turbidity during the deacidification of *M. citrifolia* extract.

Responses	Equation
pH value	Coded: $Y = 6.18 - 7.778 \times 10^{-3} x_1 + 0.76 x_2 + 0.04 x_3 - 0.066 x_1^2 - 0.65 x_2^2 - 0.019 x_3^2 - 0.048 x_1 x_2 - 7.5 \times 10^{-3} x_1 x_3 + 0.05 x_2 x_3$ Actual: $pH = 2.82 + 0.143 X_1 + 4.431 X_2 + 0.061 X_3 - 2.639 \times 10^{-3} X_1^2 - 2.604 X_2^2 - 8.578 \times 10^{-3} X_3^2 - 0.019 X_1 X_2 - 1.000 \times 10^{-3} X_1 X_3 + 0.067 X_2 X_3$
Titratable acidity	Coded: $Y = 0.14 - 9.244 \times 10^{-3} x_1 - 0.029 x_2 + 4.267 \times 10^{-3} x_3 + 3.716 \times 10^{-3} x_1^2 + 0.025 x_2^2 - 0.015 x_3^2 - 6.4 \times 10^{-3} x_1 x_2 - 8.533 \times 10^{-3} x_2 x_3$ Actual: $TA = 0.236 - 8.001 \times 10^{-3} X_1 - 0.066 X_2 + 0.043 X_3 + 1.486 \times 10^{-4} X_1^2 + 0.100 X_2^2 - 6.882 \times 10^{-3} X_3^2 - 2.56 \times 10^{-3} X_1 X_2 + 7.745 \times 10^{-18} X_1 X_3 - 0.011 X_2 X_3$
Turbidity	Coded: $Y = 2.87 - 0.63 x_1 + 0.85 x_2 + 0.079 x_3 - 0.81 x_1^2 - 0.77 x_2^2 + 6.538 \times 10^{-3} x_3^2 - 0.49 x_1 x_2 + 0.049 x_1 x_3 + 0.064 x_2 x_3$ Actual: $Tur = -17.849 + 1.572 X_1 + 9.464 X_2 - 0.167 X_3 - 0.032 X_1^2 - 3.063 X_2^2 + 2.906 \times 10^{-3} X_3^2 - 0.197 X_1 X_2 + 6.478 \times 10^{-3} X_1 X_3 + 0.086 X_2 X_3$

Table 4. Model coefficient for pH, titratable acidity and turbidity during the deacidification of *Morinda citrifolia* extract.

Model coefficient	pH	Titrable acidity (% citric acid, wt/vol)	Turbidity (absorbance)
b_0	6.18	0.14	2.87
b_1	-7.778×10^{-3}	-9.244×10^{-3}*	-0.63*
b_2	0.76*	-0.029*	0.85*
b_3	0.040	4.267×10^{-3}	0.079
b_{11}	-0.033	3.716×10^{-3}	-0.81*
b_{22}	-0.65*	0.025*	-0.77*
b_{33}	-0.019	-0.015*	6.538×10^{-3}
b_{12}	-0.048	-6.4×10^{-3}	-0.49*
b_{13}	-0.75×10^{-3}	0.00	0.049
b_{23}	0.050	-8.533×10^{-3}	0.064

after the deacidification process. From Table 4, it is obvious that only b*2* and b*22* are significant for pH value. This shows that only the height of $CaCO_3$ has a significant ($p<0.05$) effect on pH. The value of the linear coefficient (b_2) has a positive value, where it shows that pH increased with increasing height of $CaCO_3$. At the same time, the significant ($p<0.05$) quadratic coefficient (b_{22}) suggests that there is a maximum point for this model. Three-dimensional plots obtained for pH are shown in Figure 1 and 2. Figure 1 shows that pH of *M. citrifolia* extract as a function of column diameter and medium height at 30 ml/min of feed rate. At fixed column diameter, pH of *M. citrifolia* extract increased with increasing height of $CaCO_3$. Increased height of $CaCO_3$ corresponds with an increase in the amount of $CaCO_3$. Sharmella et al. (2005) reported that calcium carbonate was able to decrease the level of acidity and increase the pH of *M. citrifoli*a extract. Thus, a higher amount of $CaCO_3$ resulted in a higher value of pH. Figure 2 shows the pH of *M. citrifolia* extract as a function of feed rate and height of $CaCO_3$ using a 25 mm diameter column. At fixed feed rate, pH of *M. citrifolia* extract increased with increasing height of medium. The increase in pH with increasing medium height was due to the increase in the deacidifying capacity with increased amount of $CaCO_3$.

Titratable acidity

From Table 4, model coefficients for titratable acidity that were significant were b_1 (column diameter), b_2 ($CaCO_3$ height), b_{22} and b_{33}. Coefficients b_1 and b_2 (linear coefficients) showed negative values, which indicate that titratable acidity level decreased with increasing column diameter and height of $CaCO_3$. Figure 3 shows the titratable acidity of *M. citrifolia* extract as a function of medium height and column diameter at a feed rate of 50 ml/min. At fixed column diameter, titratable acidity of *M. citrifolia* extract decreased with increasing medium height. Increasing the medium height at fixed column diameter will result in an increase in the amount of $CaCO_3$ in the column, which subsequently increased the deacidifying capacity. Similarly at fixed medium height, increase in column diameter produced an increased amount of $CaCO_3$ resulting in the decrease of titratable acidity for the *M. citrifolia* extract as shown in Figure 3. Figure 4 shows the titratable acidity of *M. citrifolia* extract as a function of feed rate and column diameter at 0.5 cm of medium height. From Figure 4, tiratable acidity level was observed to increase with increasing feed rate up to approximately 40 ml/min. Increasing the feed rate reduced the contact time of the *M. citrifolia* extract with the ion exchange resin thus resulting in a higher titratable acidity. Nevertheless, it is not suggested to decrease the flow rate because the production of the final product may be interrupted. Figure 5 shows the titratable acidity level of *M. citrifolia* extract as a function of feed rate and height of medium using a 25 mm column. Titratable acidity decreased with increasing height of $CaCO_3$ as shown by its negative value of the linear coefficient (b_2). Increasing the height of $CaCO_3$ in the column subsequently increased the deacidifying capacity of the process producing lower titratable acidity values.

Turbidity

From Table 4, model coefficients that were significant for turbidity are b_1, b_2, b_{11}, b_{22} and b_{12}. The

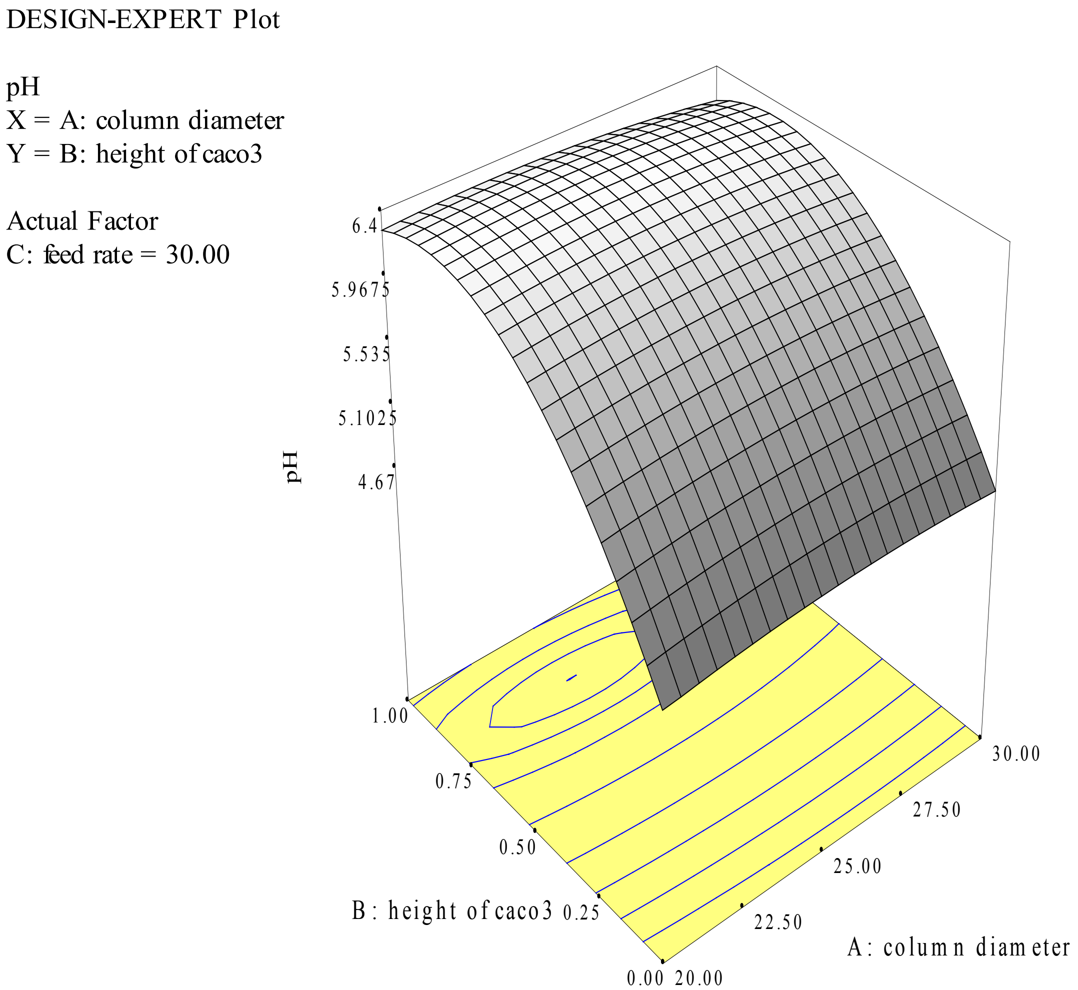

Figure 1. pH value of *M. citrifolia* extract as a function of column and height of medium using a feed rate of 30 ml/min.

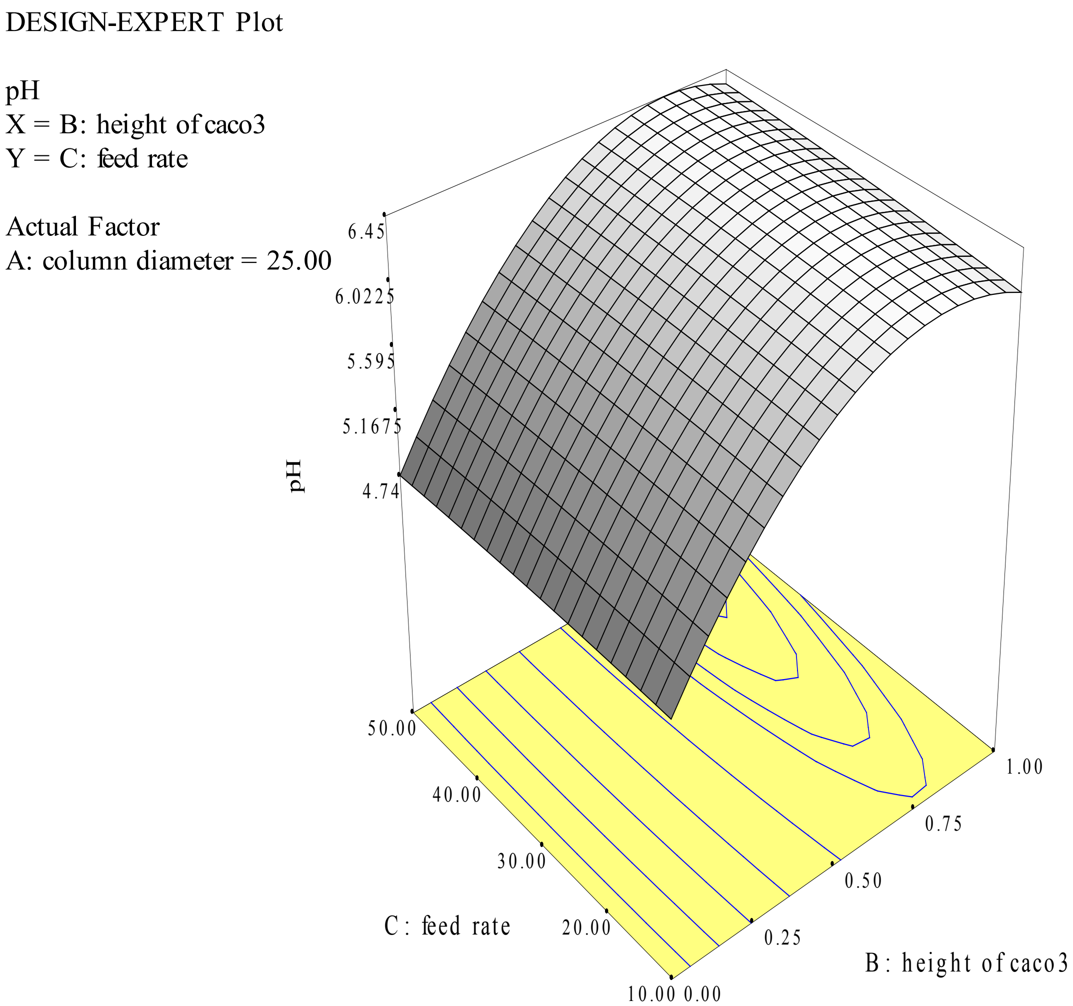

Figure 2. pH value of *M. citrifolia* extract as a function of feed rate and height of medium using 25 mm column.

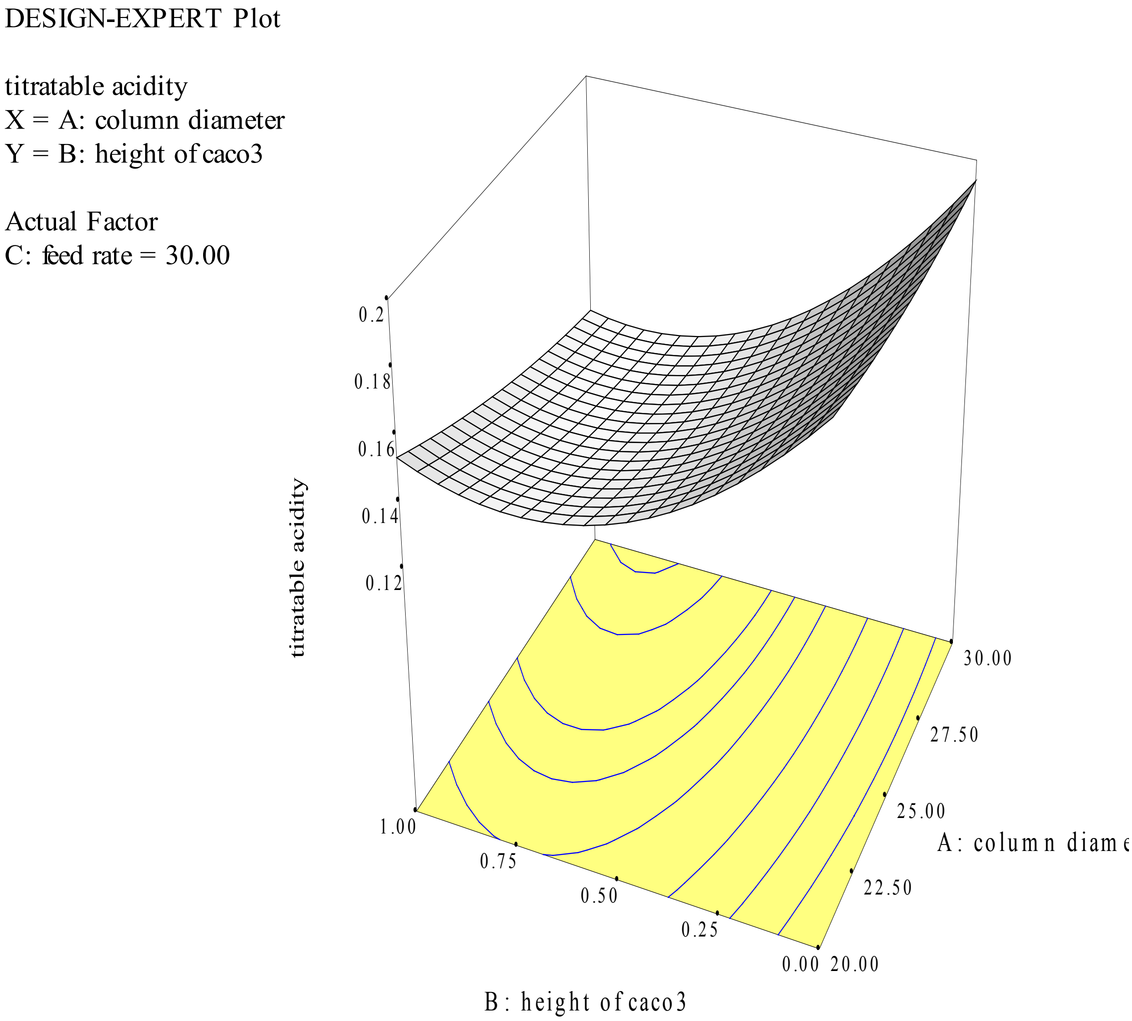

Figure 3. Titratable acidity level of mengkudu extract as a function of height of medium and column diameter using a feed rate of 50 ml/min.

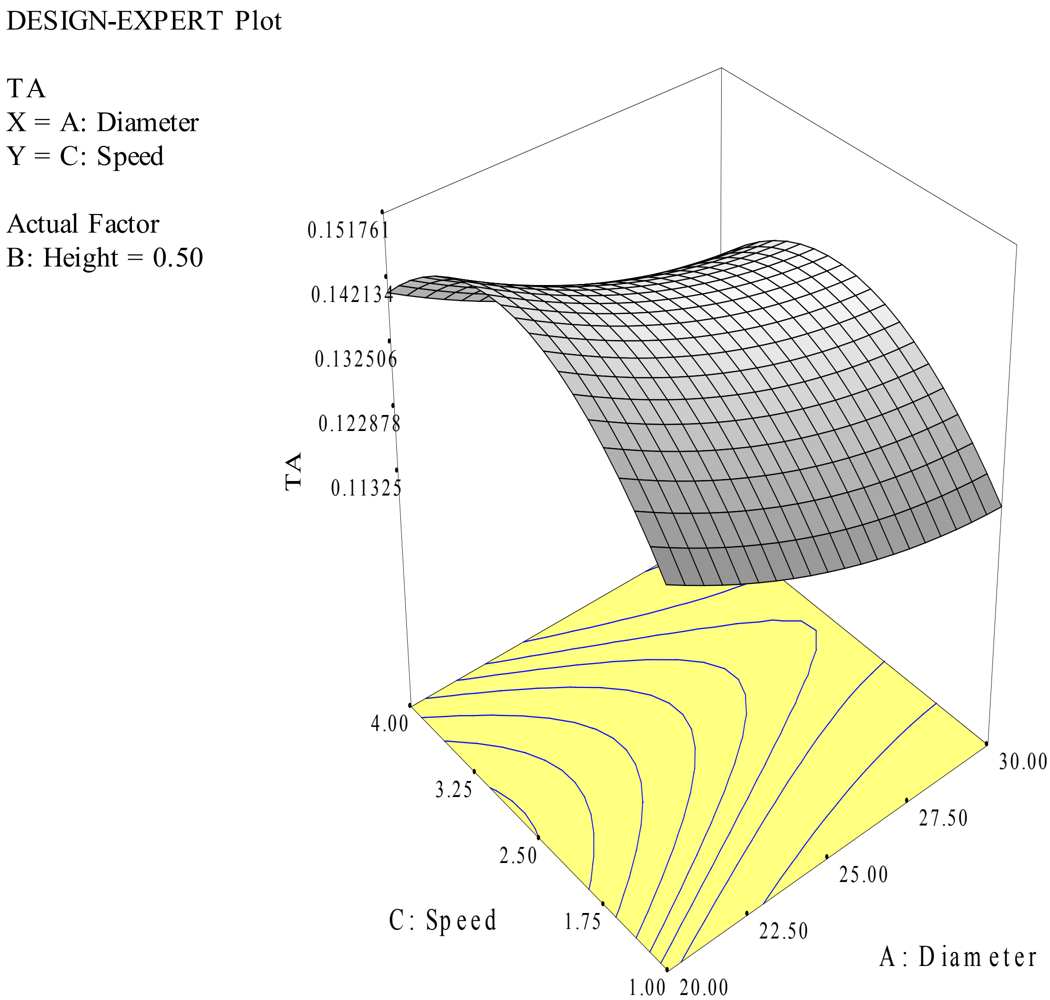

Figure 4. Titratable acidity level of *M. citrifolia* extract as a function of feed rate and column diameter using a medium height of 0.5 cm.

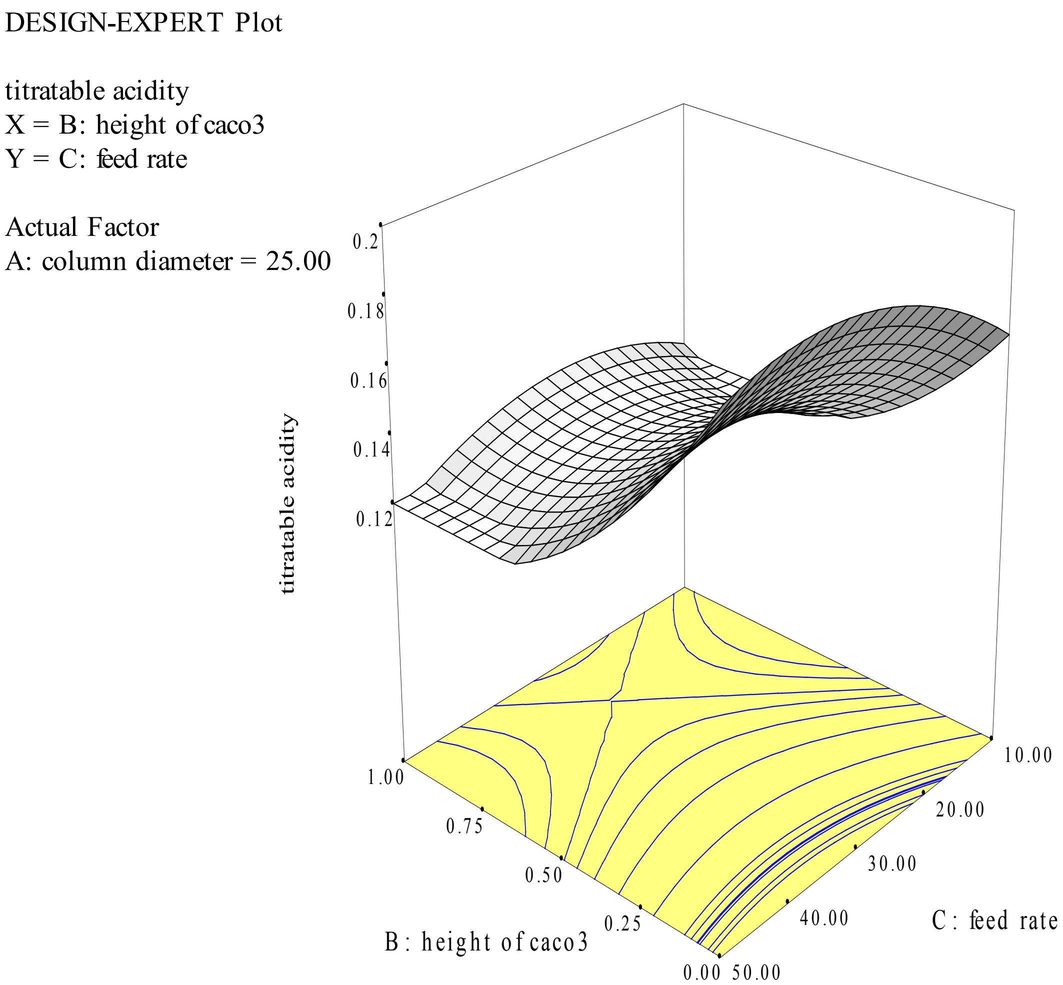

Figure 5. Titratable acidity level of *M. citrifolia* extract as a function of feed rate and height of medium using a 25 mm diameter column.

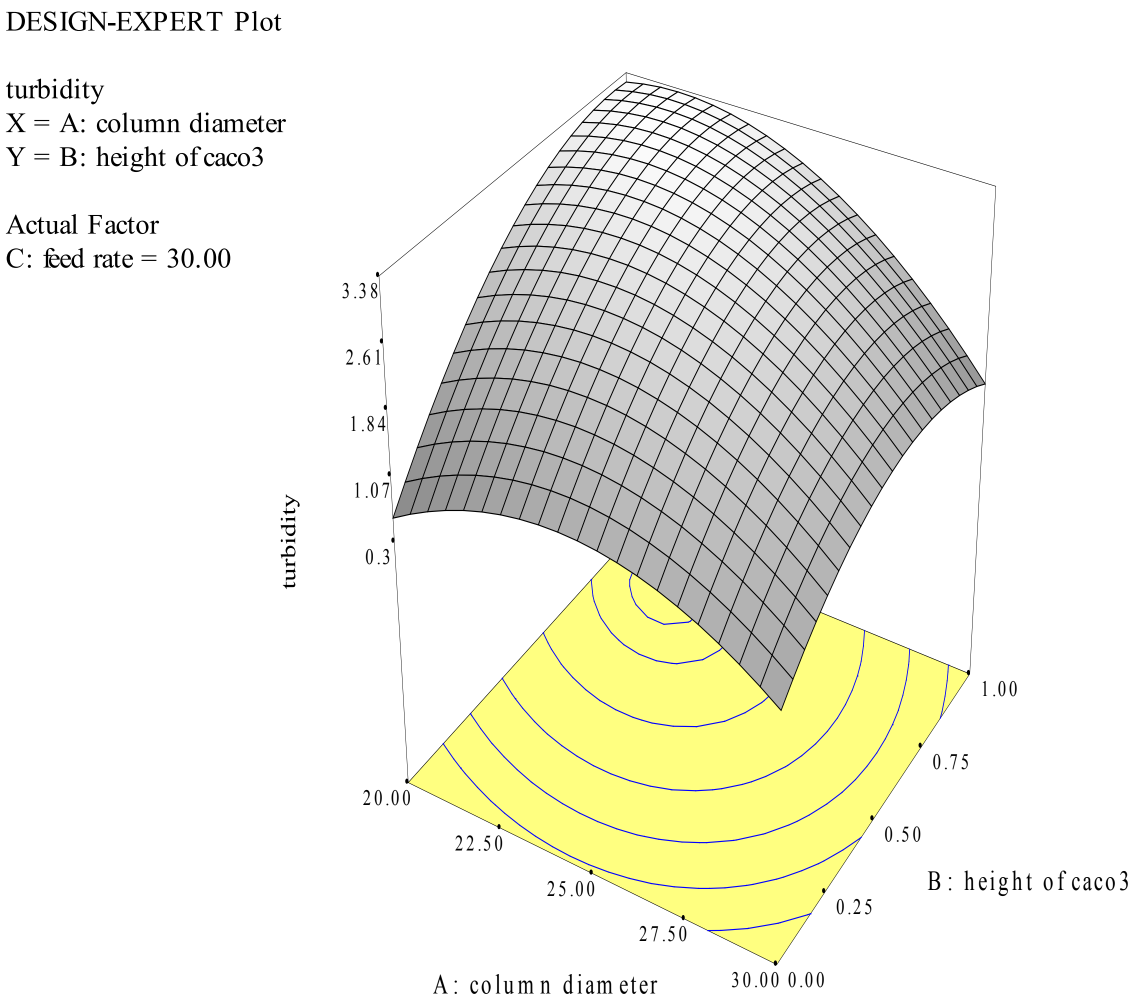

Figure 6. Turbidity of *M. citrifolia* extract as a function of medium height and column diameter at 30 ml/min feed rate.

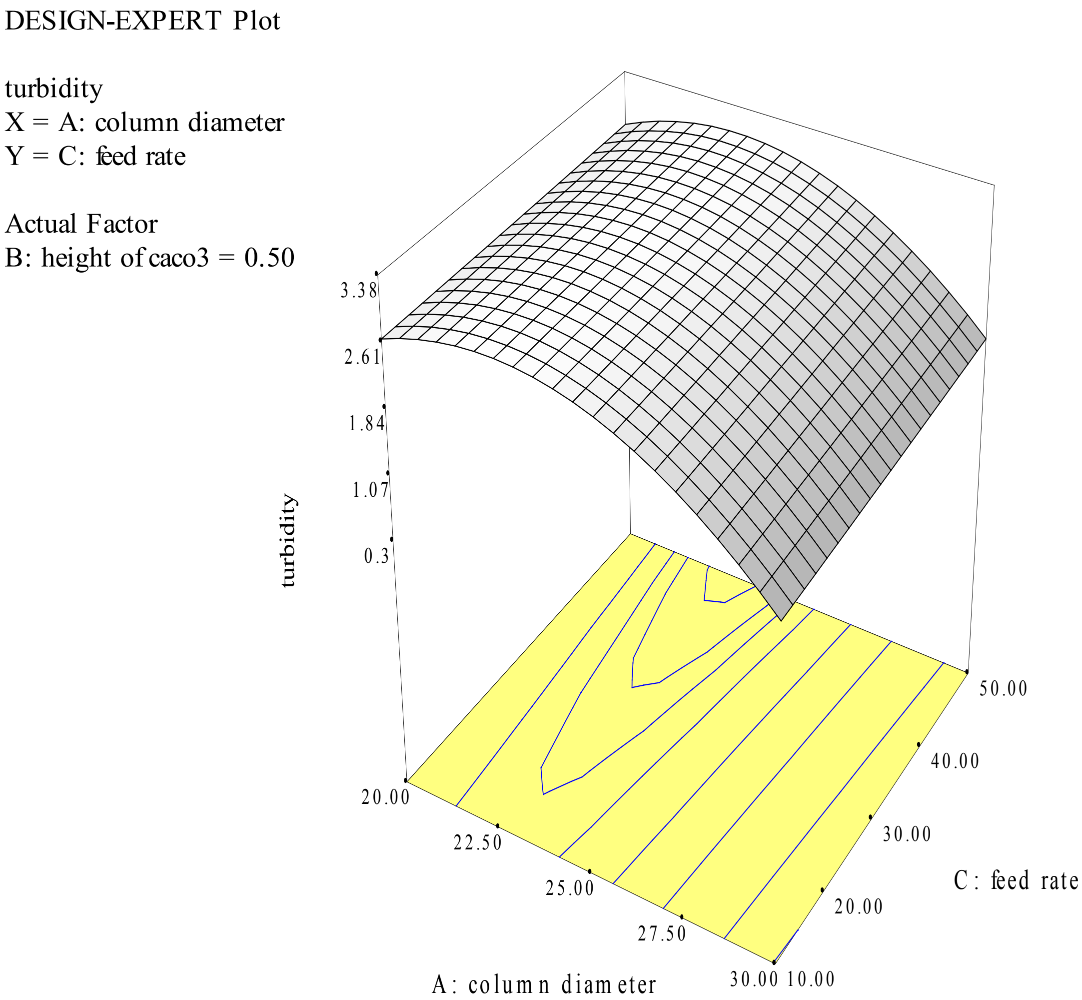

Figure 7. Turbidity of *M. citrifolia* extract as a function of feed rate and column diameter at 0.5 cm medium ($CaCO_3$) height.

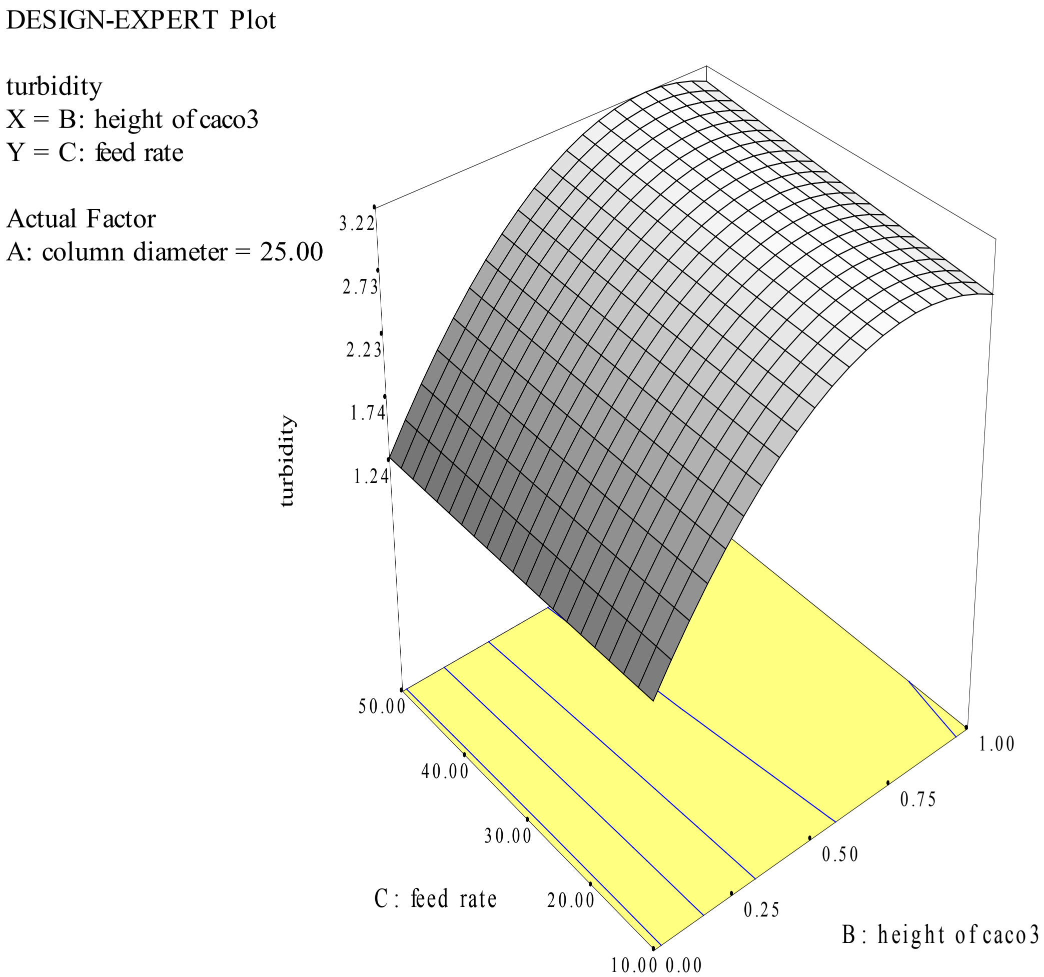

Figure 8. Turbidity of *M. citrifolia* extract as a function of pump speed and medium height using a 25 mm column.

linear coefficients for column diameter (b1) showed a negative value, which shows that turbidity level increased with decreasing column diameter. However, the linear coefficient for medium height (b_2) has a positive value which shows turbidity level increased with increasing height of $CaCO_3$. Three-dimensional plots obtained from the turbidity level are shown in Figure 6, 7 and 8. Figure 6 shows the turbidity level of *M. citrifolia* extract as a function of height of $CaCO_3$ and column diameter at 30 ml/min of feed rate. It was observed that turbidity value increased with decreasing column diameter and increasing medium height. This observation suggests that when the path that the *M. citrifolia* extract has to travel was further, as in the case for lower column diameter and higher medium height, turbidity was increased. Using higher column diameter and lower medium height resulted in a lower turbidity. Figure 7 shows the turbidity of *M. citrifolia* extract as a function of feed rate and column diameter at 0.5 cm of medium height. At fixed feed rates, turbidity decreased with increasing column diameter. Increasing the column diameter while maintaining the medium height resulted in increased amount of $CaCO_3$. The results suggest that turbidity of the *M. citrifolia* extract was influenced by the amount of $CaCO_3$ used. By using $CaCO_3$ and $Ca(OH)_2$, it increases the calcium content in the juice, which reached 48 and 25% respectively, compared with 1% in the passion juice that was not treated (Vera et al., 2002). However, in Figure 8 which shows the turbidity of *M. citrifolia* extract as a function of feed rate and medium height using 25 mm column, turbidity was found to increase with increasing height of $CaCO_3$ at fixed feed rates. Increased medium height at fixed column diameter corresponds to higher amount of $CaCO_3$ in the column. The time of sampling used after treatment was not constant. So, it was not so affected rather than the factors studied. The seemingly conflicting results of Figure 7 and 8 need further investigation to explain the effects of $CaCO_3$ amount on turbidity. It is possible that the ratio between amount of $CaCO_3$ and the volume of the extract may play a role in the resulting turbidity.

Optimization

Based on preliminary study, the used of $CaCO_3$ can reduce the unpleasant odors. So this study was monitored based on pH, titratable acidity and turbidity of the *M. citrifolia* extract. Based on the numerical optimization, the optimum condition for the deacidification process of *M. citrifolia* extract was determined to be at feed rate of 50 ml/min, using a 30 mm column and packed with a medium (CaCO3) height of 1 cm.

Conclusion

From the results, only pH, titratable acidity and turbidity data were successfully fitted with mathematical models. No acceptable mathematical models were found for total polyphenol content and total soluble solid content. Thus, optimization was carried out based on pH, titratable acidity and turbidity data. By using response surface methodology, the optimum operating conditions for the deacidification of *M. citrifolia* extract was determined to be at feed rate of 50 ml/min, using a 30 mm diameter column and packed with a medium ($CaCO_3$) height of 1 cm.

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