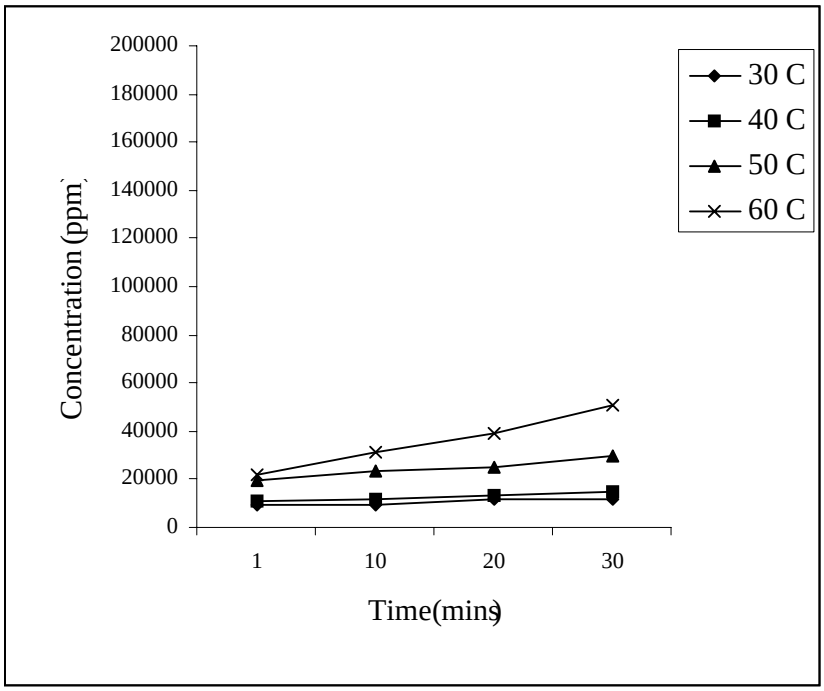
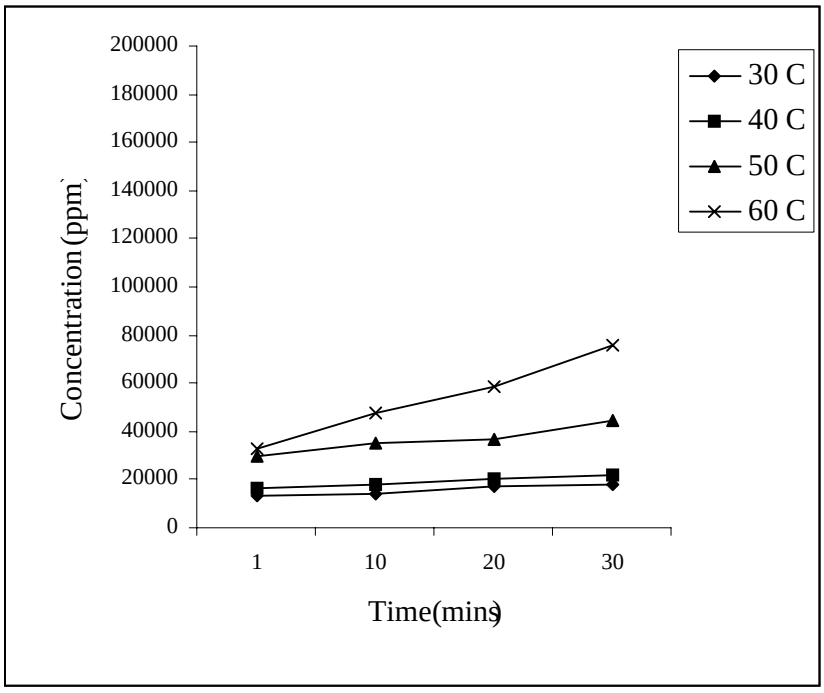
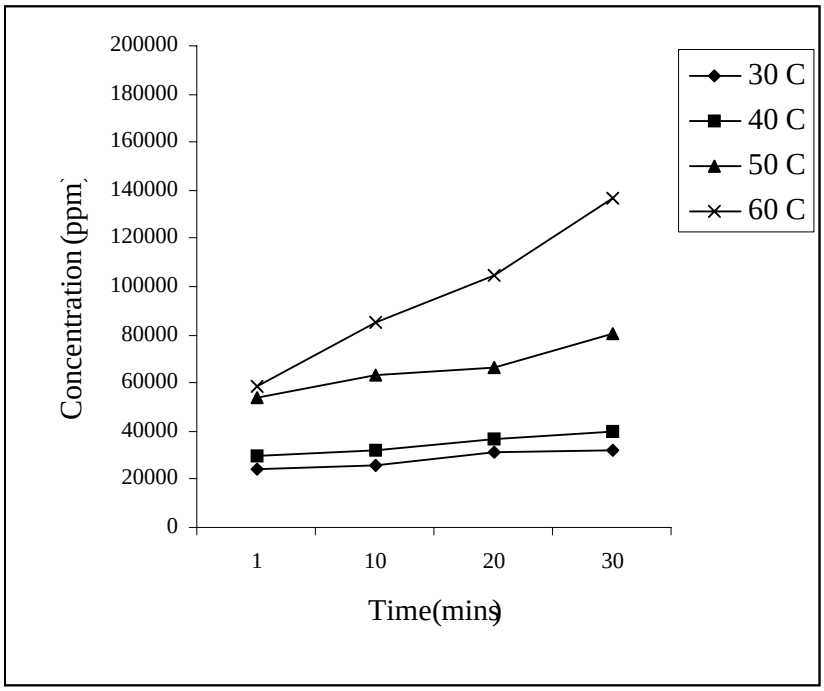


(a)

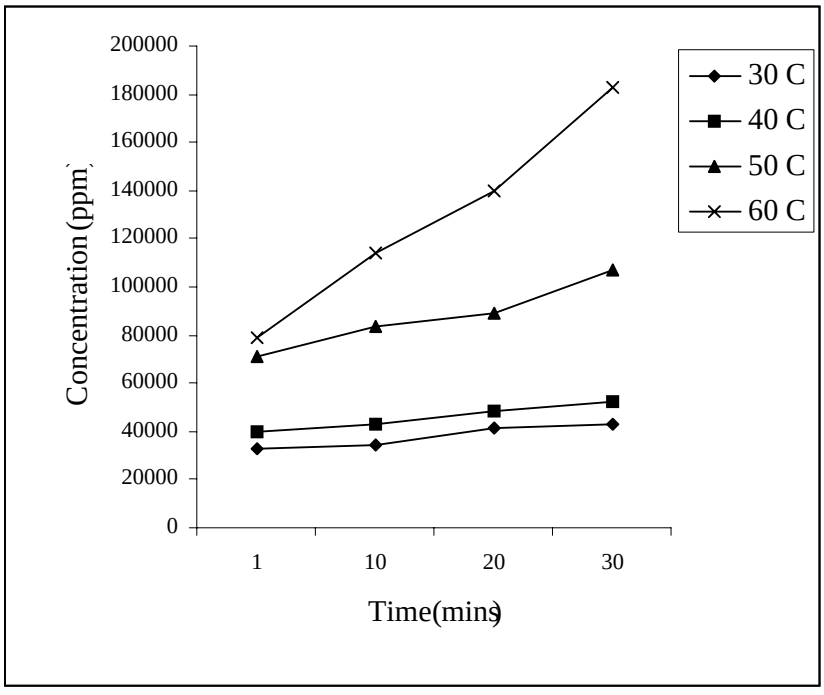


(b)

Figure 35 Concentrations of *p*-cymene in headspace with various temperatures and times at 50 (a),100 (b), 200 (c), and 300 (c) μl.

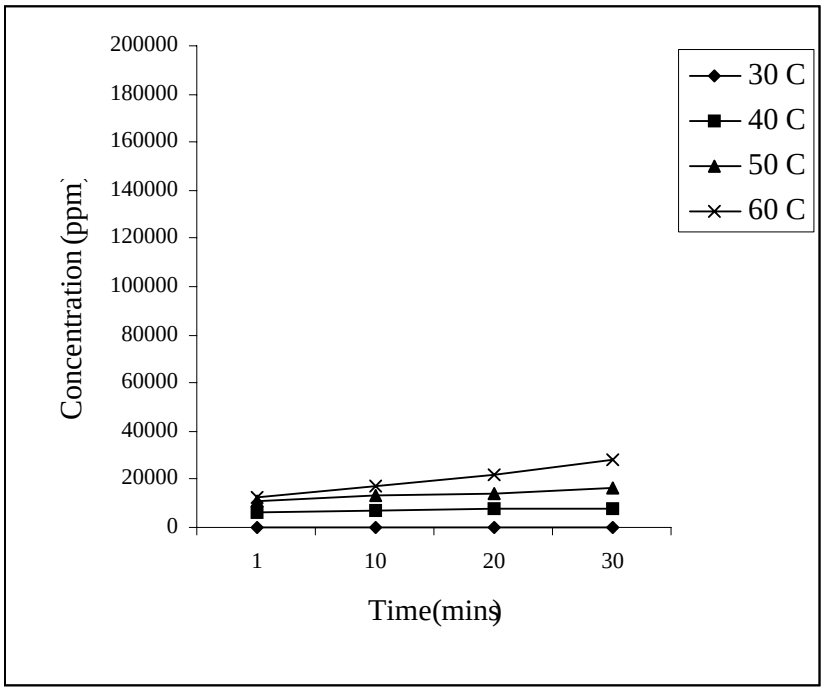


(c)

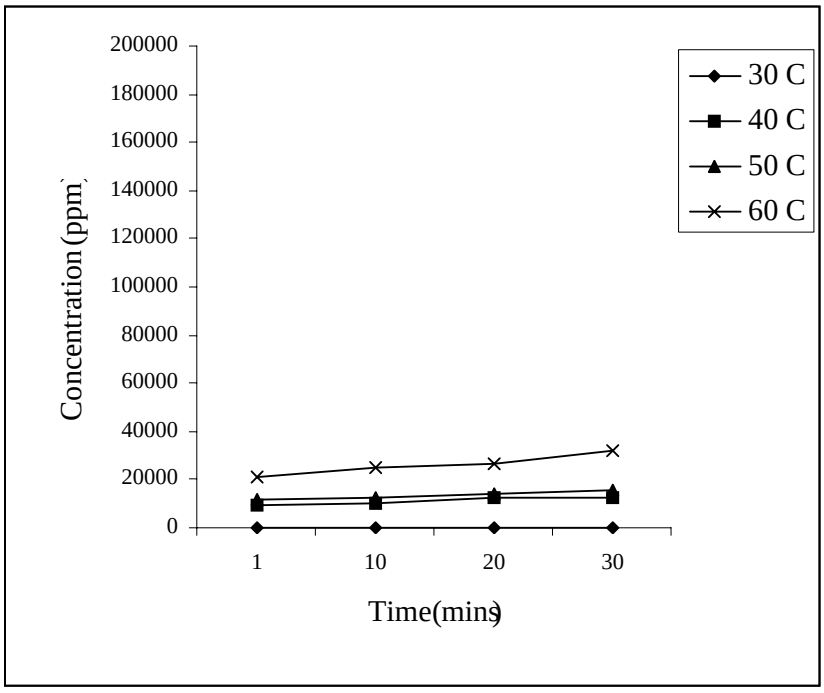


(d)

Figure 35 (Continued)

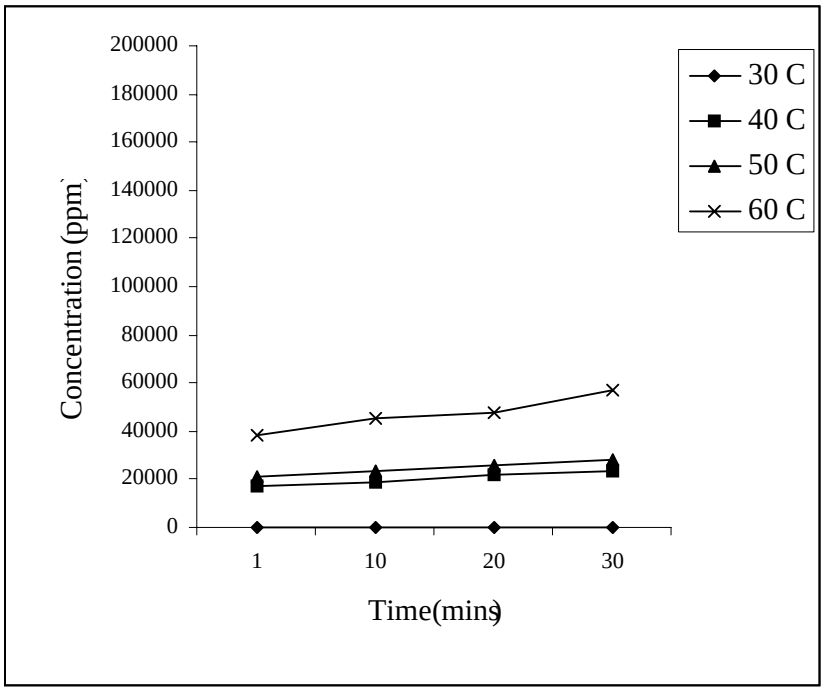


(a)



(b)

Figure 36 Concentrations of linalool in headspace with various temperatures and times at 50 (a),100 (b), 200 (c), and 300 (c) μl.



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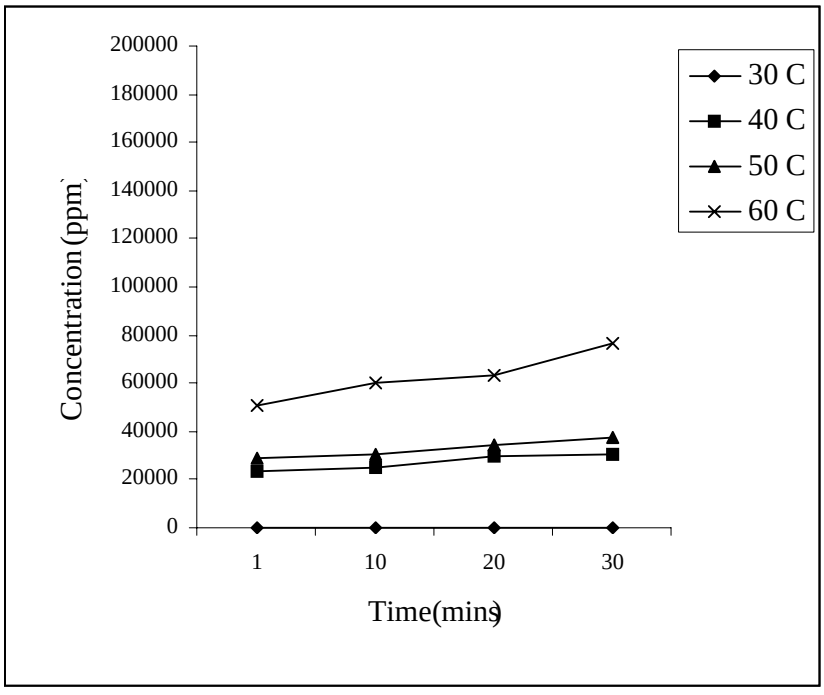
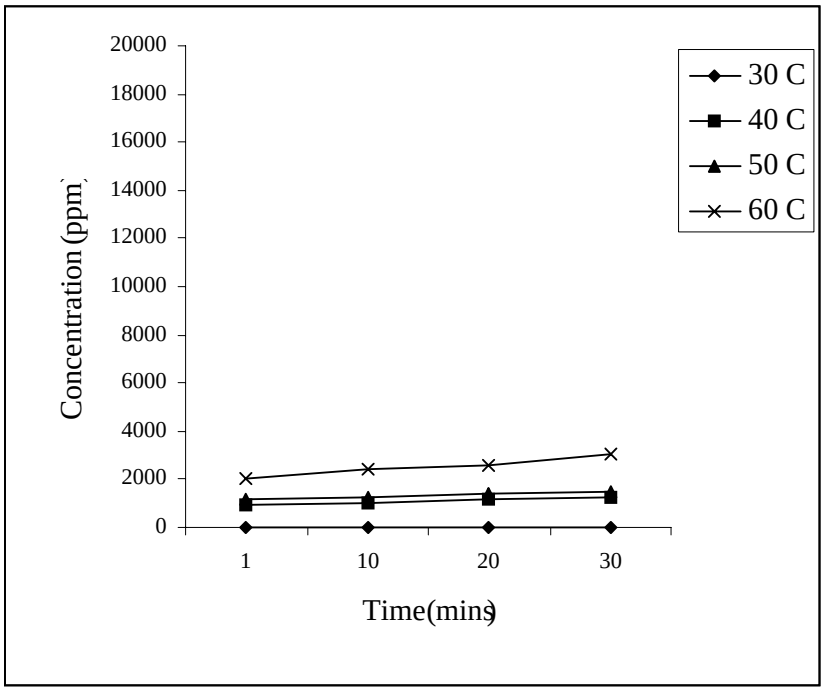
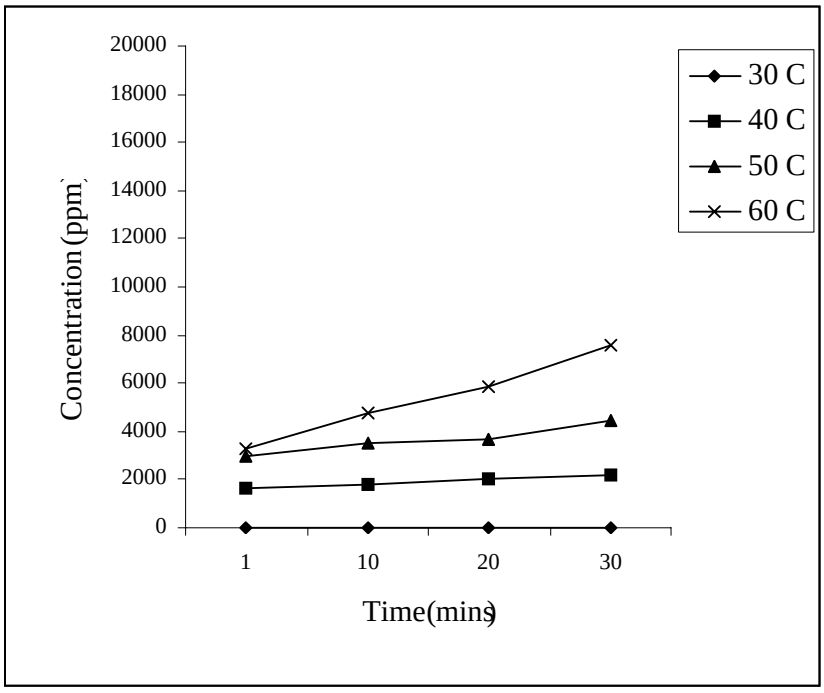


(d)

Figure 36 (Continued)

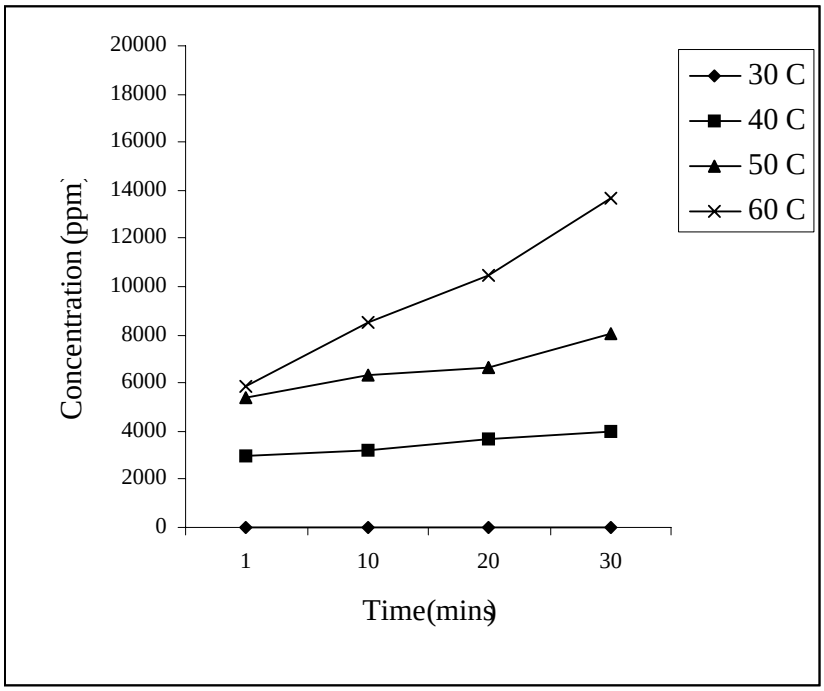


(a)



(b)

Figure 37 Concentrations of cinnamaldehyde in headspace with various temperatures and times at 50 (a),100 (b), 200 (c), and 300 (c) µl.



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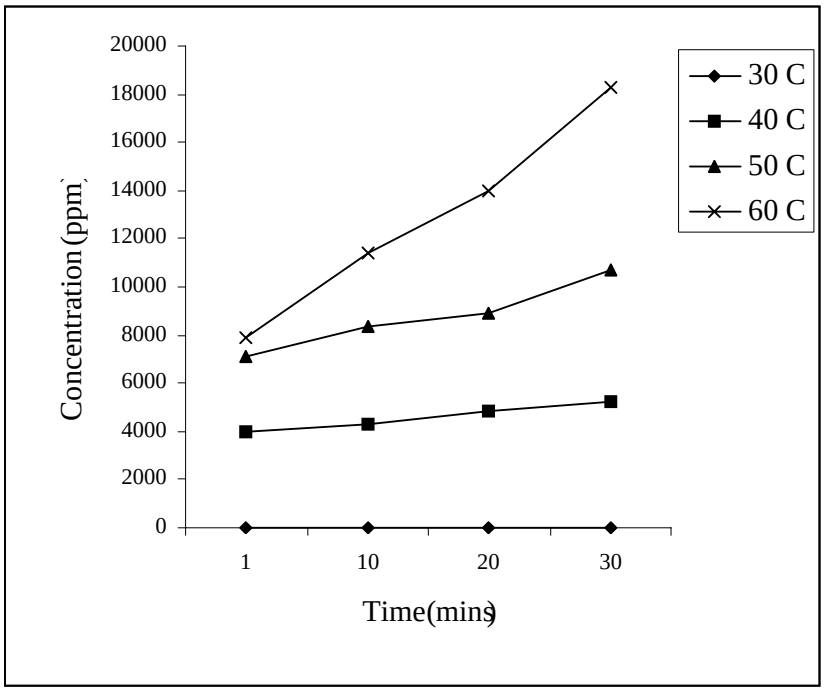
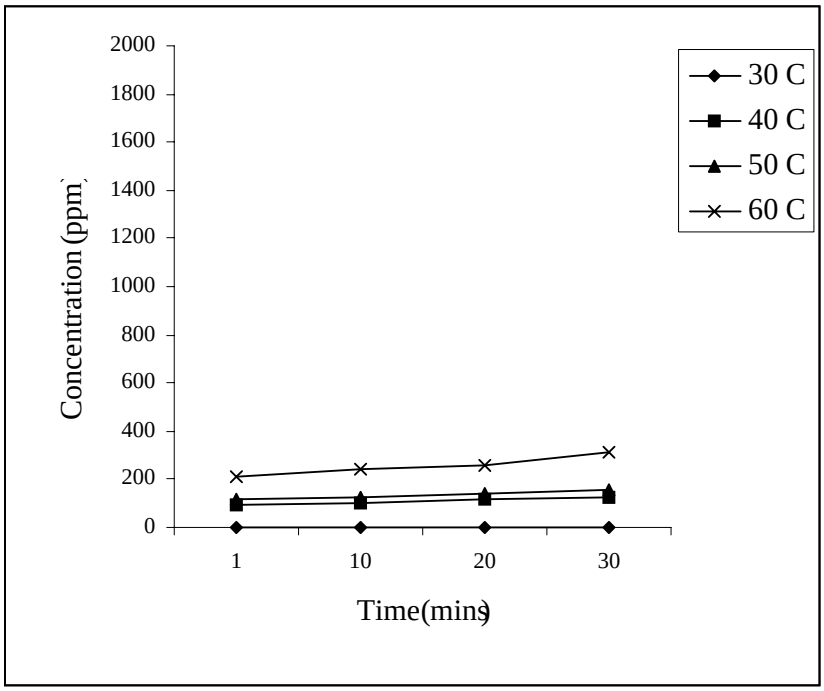
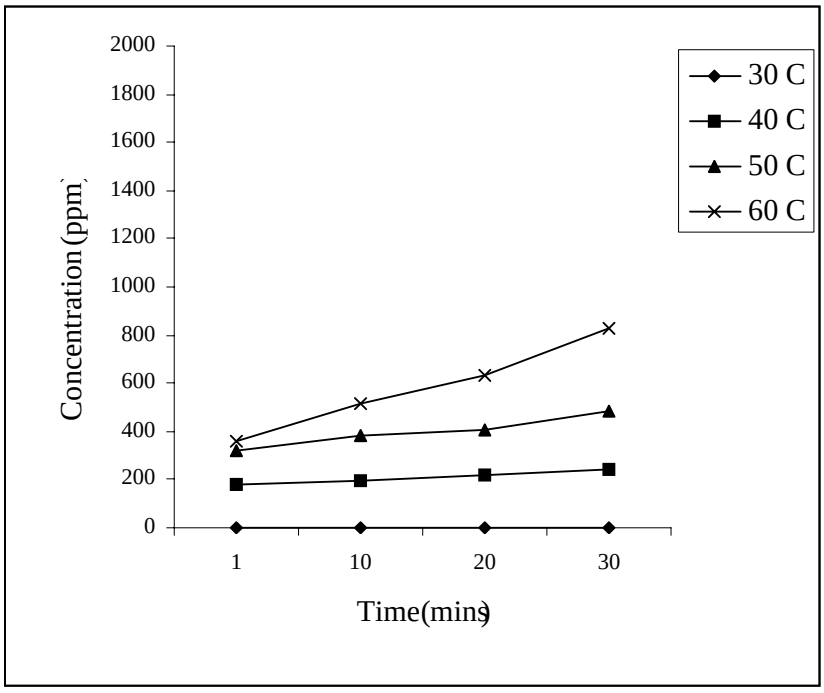


(d)

Figure 37 (Continued)

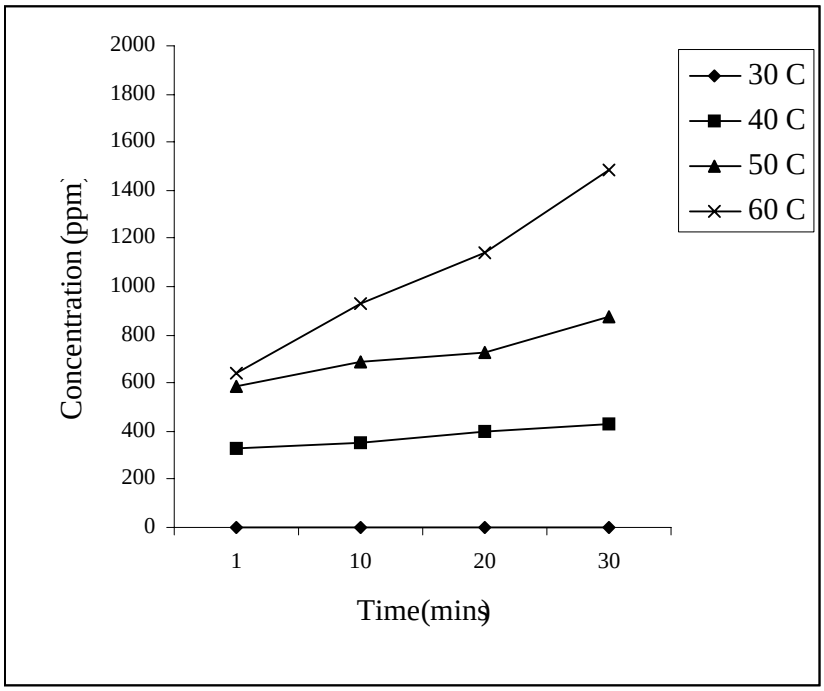


(a)



(b)

Figure 38 Concentrations of eugenol in headspace with various temperatures and times at 50 (a),100 (b), 200 (c), and 300 (c) μl.



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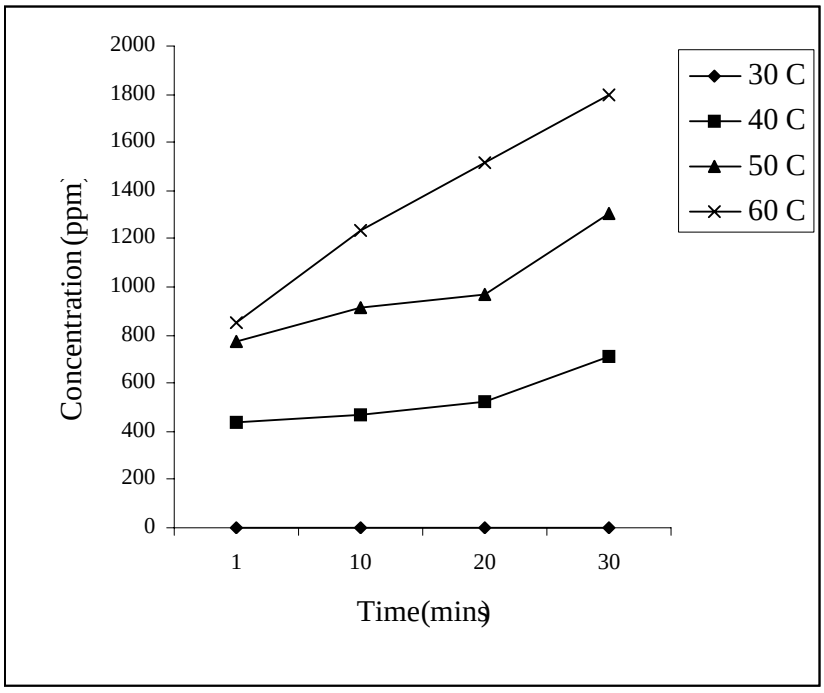


(d)

Figure 38 (Continued)

The results indicate that concentration of cinnamaldehyde increased up to ≥ 1,000 ppm in the headspace at the condition of 40 °C and at 10 minutes.

4.2 Headspace modeling

4.2.1 Fitting the model

The RSREG procedure for SAS was employed to fit the second-order polynomial Eq.(1) to the measured concentration of cinnamaldehyde in the headspace (Table 8). Cinnamaldehyde was noted as an indicator compound but the inhibitory effect will be done to both cinnamaldehyde and the other volatile components doing together. From the SAS output of RSREG, the second-order polynomial (predicted model) can be described by the following equation in terms of uncoded values:

$$Y= 2560.422387+ 5.2132\ x_1+ 8.79415\ x_2+ 4.407325\ x_3- 2.209462\ x_4- 0.018545\ x_1^2+ 0.078225\ x_2x_1- 0.083993\ x_2^2+ 0.001\ x_3x_1+ 0.002033\ x_3x_2- 0.002684\ x_3^2- 0.000059354\ x_4x_1- 0.000983\ x_4x_2- 0.00044\ x_4x_3+ 0.000356\ x_4 \quad (4)$$

A regression analysis (Table 25) was carried out to fit mathematical models to the experimental data aiming at an optimal region for the responses studied (assuming an inhibitory concentration of >100 ppm. in the fruitcake is required. Some insignificant terms, such as $x_1, x_2, x_1^2, x_4x_3, x_2^2$ were neglected, and the predicted model was not refitted. Meanwhile, the term x_3 and x_4 were kept in the model to preserve model hierarchy.

With very small P-value (0.0001) from the analysis of variance (ANOVA) and a satisfactory coefficient of determination ($R^{2=}0.9958$), the second-order polynomial model (Eq.4) was highly significant and adequate to represent the actual relationship between the concentration of cinnamaldehyde in the headspace and the significant variables. Furthermore, the overall effect of the four synthesis variables

on the concentration of cinnamaldehyde in the headspace was further analyzed by SAS (Table 26).

Table 25 Estimated regression model of relationship between response variables (concentration of cinnamaldehyde in the headspace) and independent variables (x_1, x_2, x_3, x_4)

Variables	P-value
Intercept	0.0005
x_1	0.6848
x_2	0.3842
x_3	0.0000*
x_4	0.0000*
x_1^2	0.8477
x_2x_1	0.4947
x_2^2	0.4177
x_3x_1	0.8841
x_3x_2	0.7675
x_3^2	0.0000*
x_4x_1	0.9686
x_4x_2	0.6153
x_4x_3	0.0004*
x_4^2	0.0000*

* ($P < 0.0001$)

The results revealed that the volume of oil (x_3), volume of desiccators (x_4) were the important factors, exerting a statistically significant overall effect ($P<0.0001$) on the response concentration of cinnamaldehyde in the headspace; but temperature (x_1) and time (x_2) were less significant ($P > 0.05$) for the concentration of cinnamaldehyde in the headspace.

Table 26 ANOVA analysis for the concentration of cinnamaldehyde in the headspace

Factor	Degrees of freedom	SS	MS	F-Ratio	prob >F[a]
Temp (x_1)	5	151047	30209	3.826	0.0264
Time (x_2)	5	244432	48886	6.192	0.0046
Volume of oil (x_3)	5	3294563	658913	83.459	0.0000*
Volume of desiccators (x_4)	5	17627322	3525464	446.5	0.0000*

* ($P < 0.0001$)

The significance of each coefficient was determined using the *F*-test and *p*-value in Table 3. The corresponding variables would be more significant if the absolute *F*-value becomes greater and the *p*-value becomes smaller. It can be seen that the variables with the largest effect were the linear terms of extraction volume of oil (x_3) and volume of desiccators (x_4) and the quadratic term of volume of oil (x_3^2) and volume of desiccators (x_4^2), followed by the interaction effects of volume of oil and volume of desiccators (x_4x_3). The result suggested that the change of volume of oil and volume of desiccators had highly significant effects on the concentration of cinnamadehyde ($P < 0.0001$).

Analysis of variance (ANOVA) for the model is shown in Table 27. The coefficient of determination (R^2) of the predicted model was 0.9958, suggesting a good fit, the predicted model seemed to reasonably represent the observed values. Thus, the response was sufficiently explained by the model.

Table 27 Variance analysis of the second-order regression model on concentration of cinnamaldehyde in the headspace

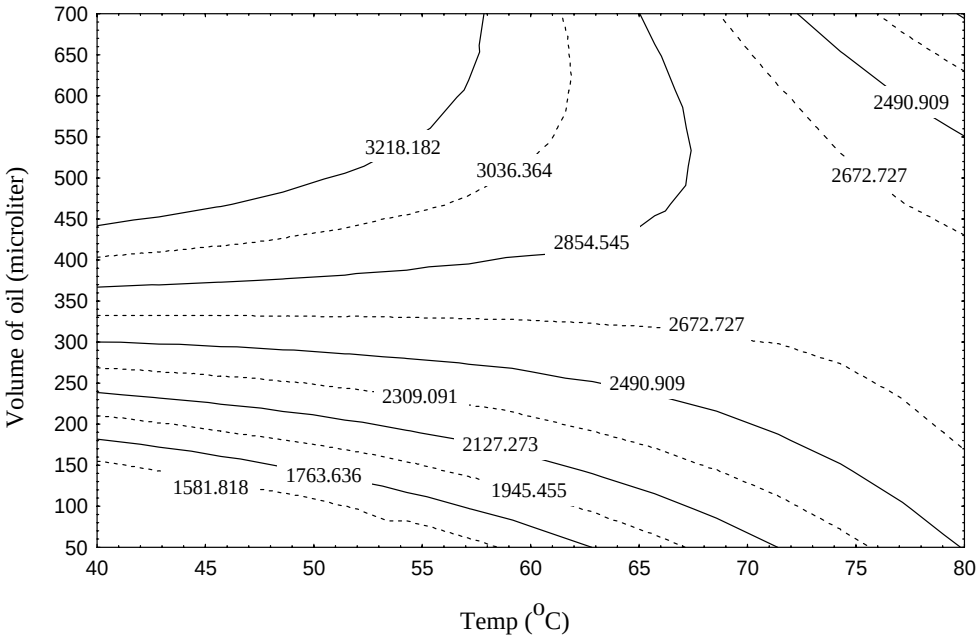
Source	R-Square	F-value	P-value
Model[a]	0.9958	201.0	0.0000*
Linear	0.7652	540.7	0.0000*
Quadratic	0.0088	156.7	0.0174
Cross-product	0.9958	4.141	0.0000*

[a] The coefficient of determination (R^2) of the predicted model was 0.9958

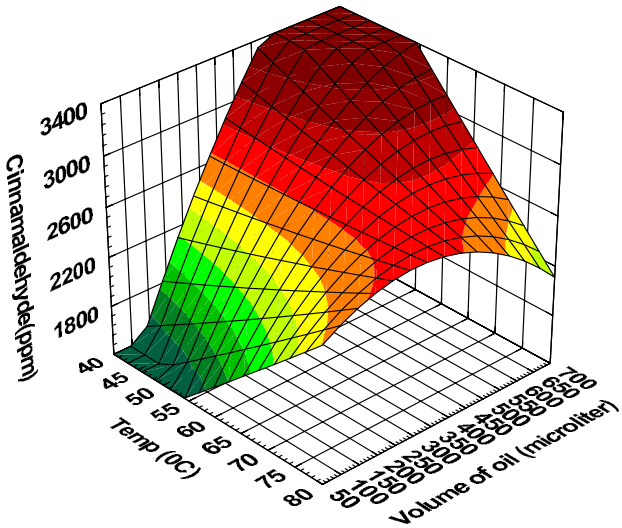
* ($P < 0.0001$)

4.2.2 Analysis of response surface: The regression model Eq.3 allowed the prediction of the effects of the four parameters on concentration of cinnamaldehyde in the headspace. The relationship between independent and dependent variables is illustrated in tri-dimensional representation of the response surfaces and two-dimensional contour plots generated by the model for concentration of cinnamadehyde (Figures 39 to 44). Two variables were depicted in one tri-dimensional surface plots while the one variables kept constant.

From the results in section 3.1, the cinnamaldehyde was adsorbed at the fruitcake surface approximately 10% of the concentration of cinnamaldehyde in the headspace. For example, the concentration of cinnamaldehyde in the fruitcake surface at 100 ppm could be obtained by using the concentration of cinnamaldehyde in the headspace at 1,000 ppm. The result from the MIC test showed that the combination of cinnamaldehyde and p-cymene at 100 ppm could completely prevent growth of microorganism for 30 days. Therefore, the level of independent factors should be set to give the concentration of cinnamaldehyde in the headspace of at least between 500 to 1,000 ppm.

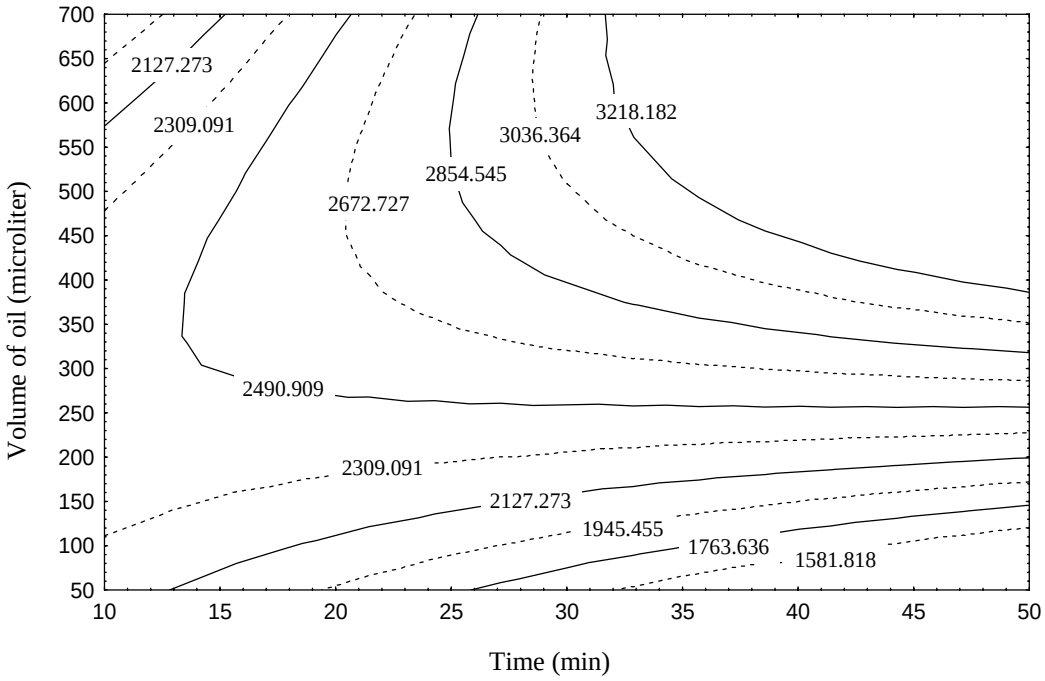


(a)

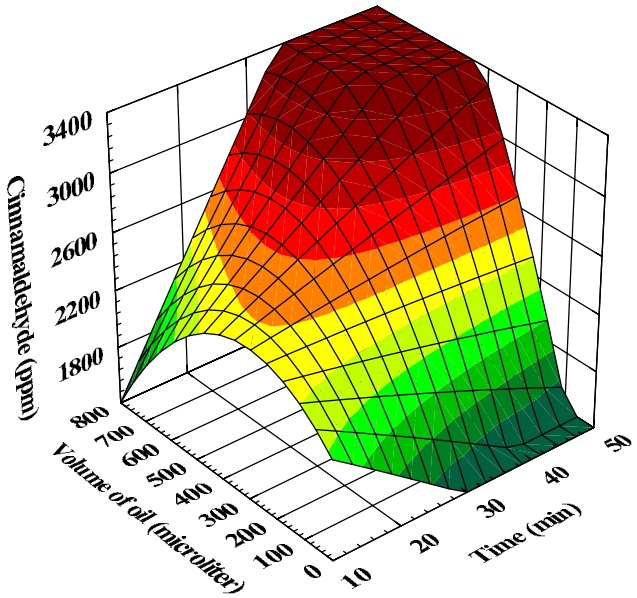


(b)

Figure 39 Contour plot (a) and response surface (b) of cinnamaldehyde headspace concentration for the volume of oil (µl) and temperature (°C) at 800 ml volume of desiccators

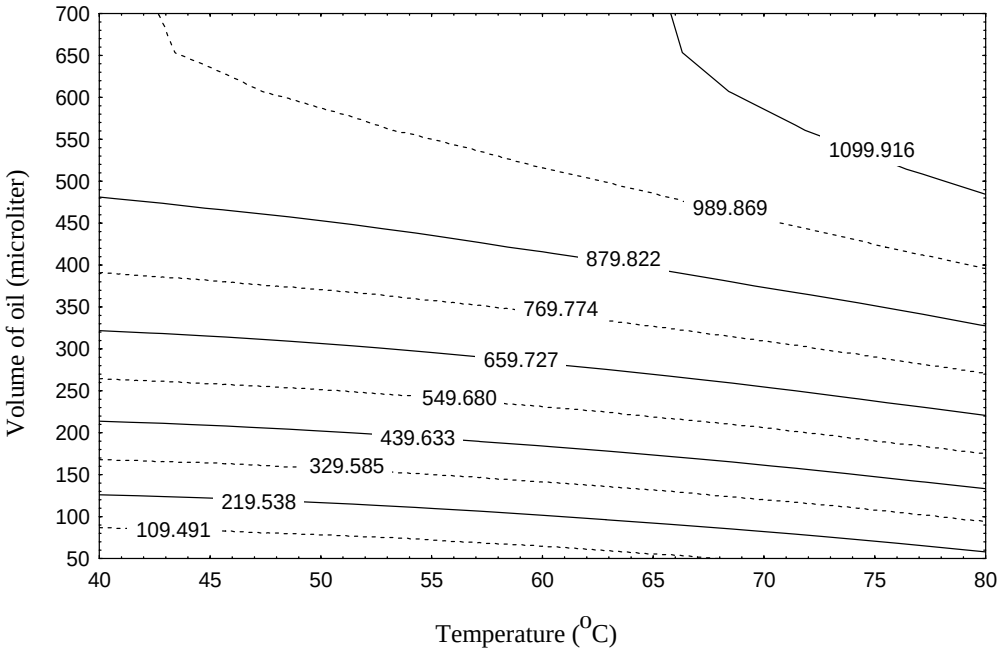


(a)

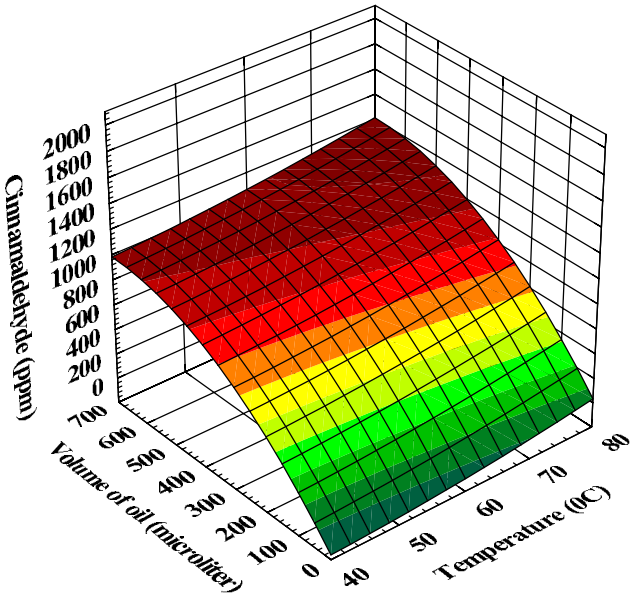


(b)

Figure 40 Contour plot (a) and response surface (b) of cinnamaldehyde headspace concentration for the volume of oil (µl) and time (min) at 800 ml volume of desiccators

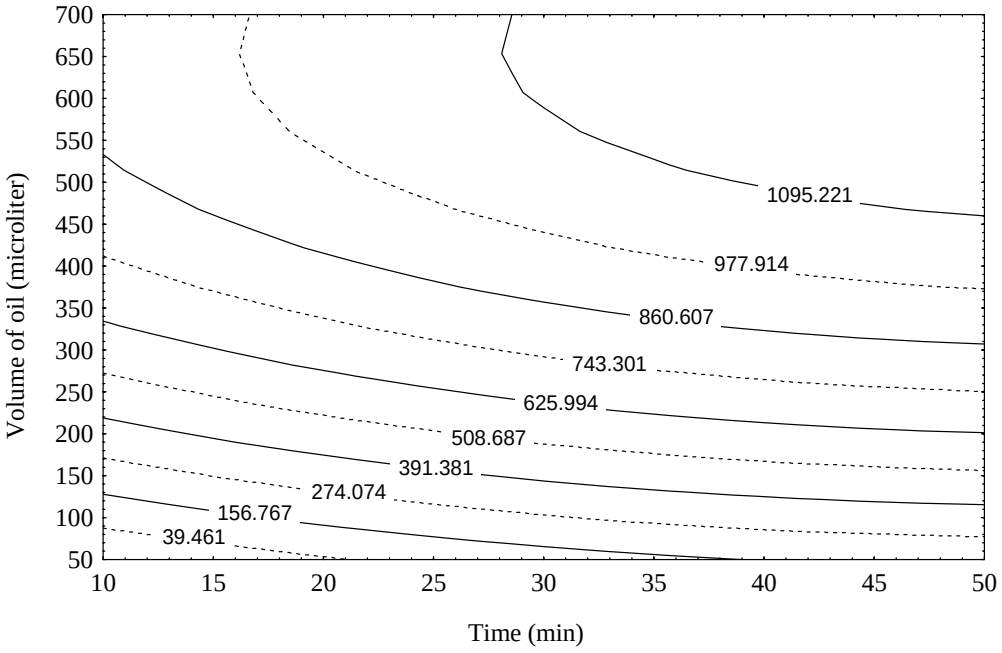


(a)

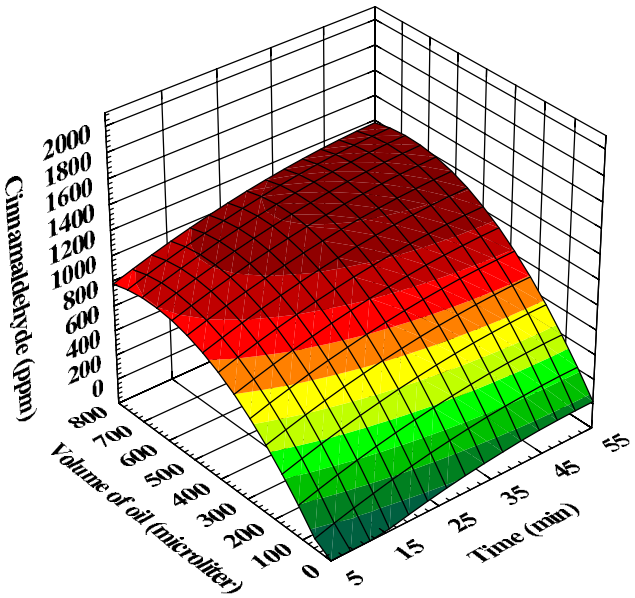


(b)

Figure 41 Contour plot (a) and response surface (b)of cinnamaldehyde headspace concentration for the volume of oil (µl) and temperature (°C) at 2,200 ml volume of desiccators

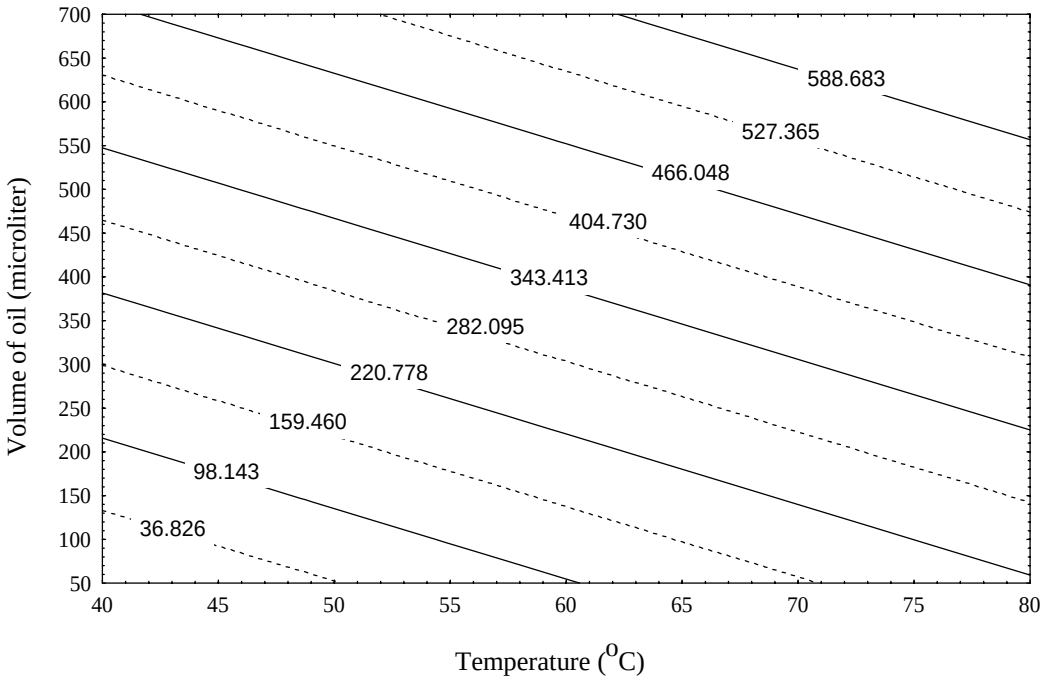


(a)

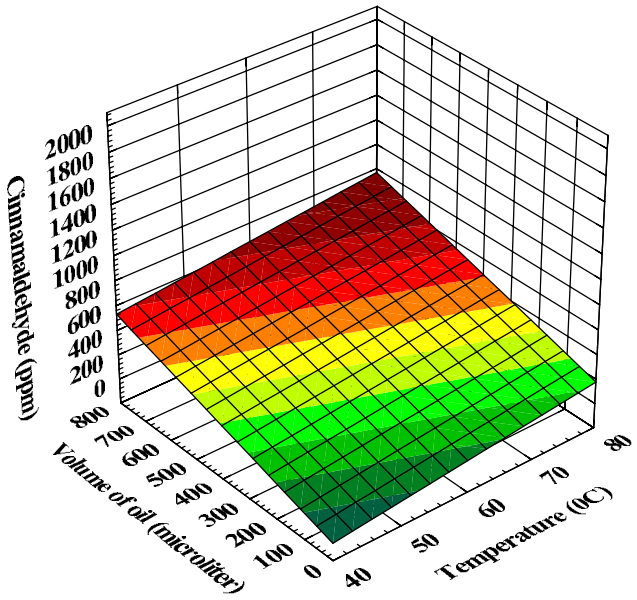


(b)

Figure 42 Contour plot (a) and response surface (b)of cinnamaldehyde headspace concentration for the volume of oil (µl) and time (min) at 2,200 ml volume of desiccators

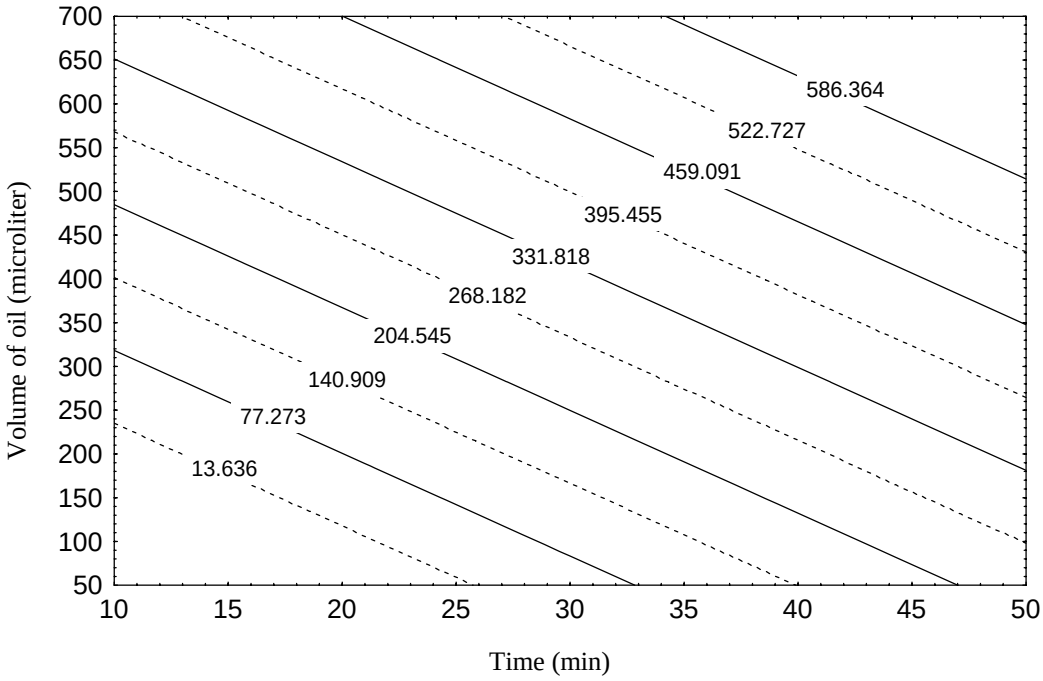


(a)

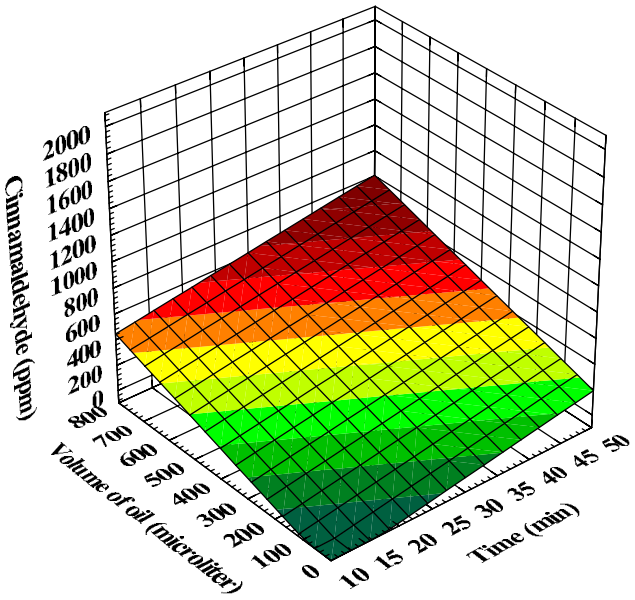


(b)

Figure 43 Contour plot (a) and response surface (b) of cinnamaldehyde headspace concentration for the volume of oil (µl) and temperature (°C) at 3,800 ml volume of desiccators



(a)



(b)

Figure 44 Contour plot (a) and response surface (b) of cinnamaldehyde headspace concentration for the volume of oil (μl) and time (min) at 3,800 ml volume of desiccators

In this study, the levels of independent factors were optimized to obtain the concentration of cinnamaldehyde in the headspace of at least 1,000 ppm. It was found that by using the volume of desiccators at 800ml, the optimized levels of independent factors were obtained at temperature of 40 °C, time 10 minutes and volume of oil 100 µl (Figure 39 and Figure 40). The concentration of cinnamaldehyde in the headspace decreased when the larger volume of desiccators was used. At the volume of desiccators 2,200 ml, the optimized levels of factors were temperature and volume of oil at 80 (°C) /550 µl, 65(°C) /700 µl and time and volume of oil at 50 min/500µl, 25 min/700µl (Figure 41 and Figure 42). On the other hand, at the volume of desiccators of 3,800 ml, it was found that all levels of independent factors within the range studied gave the concentration of cinnamaldehyde in headspace of lower than 1,000 ppm (Figure 43 and Figure 44).

4.2.3 Verification of headspace modeling: The RSM model: The suitability of the model equation for predicting the optimum response values was tested using the selected optimal conditions. The experimental yield of cinnamaldehyde in the headspace was found to be in agreement with the predicted one (Table 28).

Table 28 Predicted and experimental concentration at optimum conditions (700 ml)

Treatment No.	Optimum conditions	Predicted concentration	Experimental concentration[a]
1	Temp (40 ^{0}C) Time (10 min) Volume of oil (100 µl) Volume of jar (700 ml)	1,858.71	1,784±205
2	Temp (40 ^{0}C) Time (10 min) Volume of oil (50 µl) Volume of jar (700 ml)	1,670.77	1,546±116

[a] Mean ± standard deviation of sixth plicate determinations

From table 28, the adequacy of the predicted model was examined by additional independent experiments under the suggested optimal synthesis conditions. The predicted value was 1858.71 and 1,670.77, obtained by minimum conditions. The experimental yield was statistically the same as the predicted yield or the experimental yield was close to the predicted yield. Therefore, the RSM modeling base on a second-order polynomial equation could be used to predict cinnamaldehyde headspace. The cinnamaldehyde in the headspace was successfully developed by fractional factorial design and RSM.

From this study, the optimum amount of essential oil to be used with the package size between 800-3,800 ml could be calculated for other products. Optimum temperature and time to enhance vaporization of essential oil into the headspace at sufficient amount to inhibit growth of fungi on food surface could be obtained.

The RSM model was successfully employed to describe the concentration of cinnamaldehyde in the headspace at high temperature activation. It was found that volume of desiccators and volume of oil were the most significant parameters influencing the amount of cinnamaldehyde in the headspace.

4.3 Storage of rice jasmine butter cake (RJBC) with normal air condition: Results of the rice fruitcake kept inside the 700 ml plastic jar at temperature of 40 ^{0}C for 10 minutes to create high concentration of cinnamaldehyde (≥1,000 ppm) in the headspace before storage at 30 °C for a month are shown in Table 29. While the control was spoiled within 7 days, the addition of 100 μl of essential oils was strongly shown to be a good preservation system for the rice butter cake. Yeast, mould and bacteria were not found on the rice butter cake for up to a month. A lower level of essential oils of 50 μl added and this also inhibited growth of microorganisms for up to 21 days (Table 29 and 31). Cinnamaldehyde in the headspace was found to decrease from 1,784±205 ppm to 1,339±154 ppm within one month at 100 μl of essential oils. Cinnamaldehyde was expected to adsorb at the rice butter cake surface at approximately 10% of the headspace concentration. Therefore,

amount of cinnamaldehyde adsorbed at the rice butter cake surface at the fouth week of storage was 125 ppm (higher than 100 ppm) which was enough to protect the rice butter cake against growth of microorganisms. On the other hand, at 50 μl of essential oils added the concentration of cinnamaldehyde in the headspace at the fourth week of storage was between 1,115 to 759 ppm. The concentration of cinnamaldehyde at the rice butter cake surface was therefore between 93 to 77 ppm (lower than 100 ppm) which was not enough to prevent the growth of microorganism. Results of sensory test were illustrated in Table 30 and 32. The hedonic scores of all attributes of the control sample were higher than those of the rice butter cake preserved with essential oils. The hedonic scores of odour and flavour of the rice butter cake preserved with 50-100 μl of essential oils increased with time of storage whereas that of texture decreased with time of storage. The hedonic score of rice fruitcake preserved with essential oils was between “like slightly” to “like moderately”.