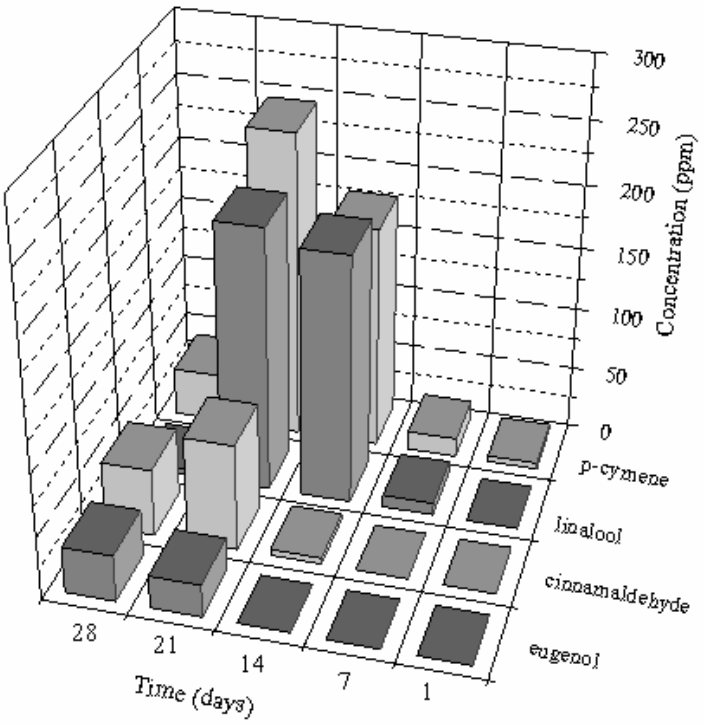


Figure 27 Main compound of premix fruitcake extract

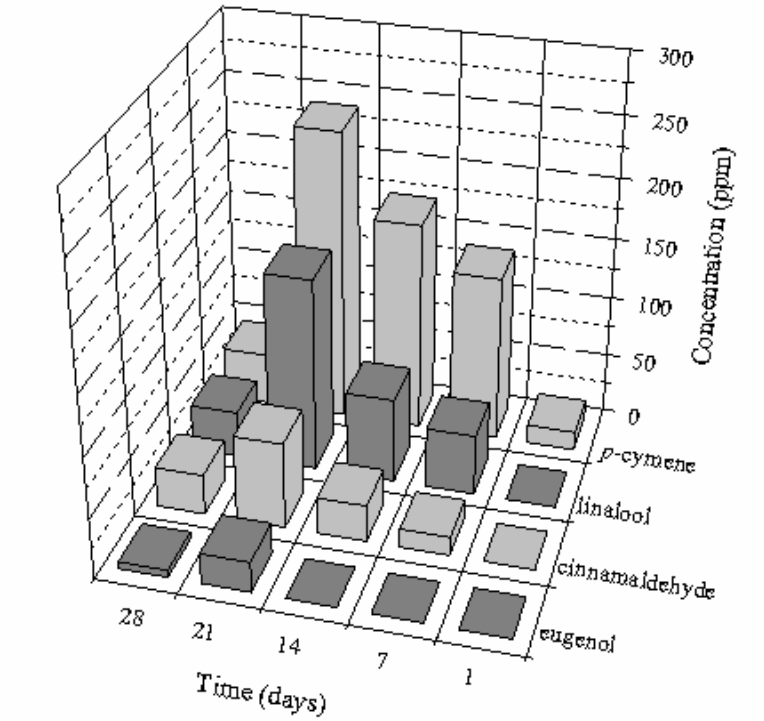


Figure 28 Main compound of premix rice fruitcake extract

3.2.7. Sensory test

The results of consumer testing of premix fruitcake and rice fruitcake before and after storage are demonstrated in Table 20. There were significant differences in term of liking from any of the attributes, using a 9 pointed hedonic scale, between control and the cake packaged with essential oils storage. Therefore it can be concluded that the use of essential oils to increase the shelf life of premix fruitcake had some effects on consumers' liking. The sensory test was performed at day one and after 21 days storage for premix cake and rice fruitcake kept at 20 °C and 30 °C.

Table 20 Hedonic scores of premix fruitcake (PMC_5) and rice fruitcake (RF)

Days	Attributes	Control	20 ^{o}C, 68%RH	30 ^{o}C, 75%RH
		PMC_5		
1	Odour	7.1±1.9a	6.6±2.1b	6.6±1.8b
	Color	7.0±2.1a	6.6±1.8a	6.7±1.9a
	Texture	7.8±1.5a	6.1±1.3b	6.1±2.4b
	Flavour	7.1±1.3a	6.1±1.6b	6.4±2.3b
	Overall	8.0±2.2a	6.4±2.2b	7.0±1.5b
21	Odour	6.8±1.4a	6.0±2.1b	5.0±1.4c
	Color	6.8±1.6a	5.0±1.6c	5.8±1.6b
	Texture	6.1±1.6a	4.2±1.9c	5.2±1.7b
	Flavour	7.2±1.7a	7.1±1.4a	5.5±2.1b
	Overall	7.2a±1.8	6.3±1.3b	4.9±2.3c

Table 20 (Continued)

Days	Attributes	Control	20 ^{o}C, 68%RH	30 ^{o}C, 75%RH
		RF		
1	Odour	7.1±1.4a	6.8±1.5a	6.6±1.5a
	Color	7.2±1.5a	6.7±1.4a	6.6±1.7a
	Texture	6.1±1.8a	6.1±1.6a	6.1±1.8a
	Flavour	7.1±1.7a	7.0±1.8a	6.1±1.6b
	Overall	7.1±1.3a	7.0±1.4a	6.4±1.7b
21	Odour	6.6±1.6a	6.0±1.4a	5.0±1.7b
	Color	6.7±1.4a	5.2±1.8b	5.8±1.4b
	Texture	6.1±1.5a	4.3±2.1c	5.2±1.2b
	Flavour	6.8±1.8a	6.1±1.4a	5.5±1.5b
	Overall	7.0±1.4a	6.1±1.5b	4.9±1.8c

[a,b,c] Means scores with different superscript in the same row are significantly difference ($P \leq 0.05$).

Although, the mean hedonic scale for premix fruitcake with essential oil was lower than control (premix fruitcake without essential oil), all attributes of premix fruitcake and rice fruitcake at 20 °C were between like slightly to like moderately. The hedonic scores of fruitcake at 30 °C were lower than control and fruitcake at 20 °C because of higher concentration of released volatile concentration in the bag headspace at 30 °C.

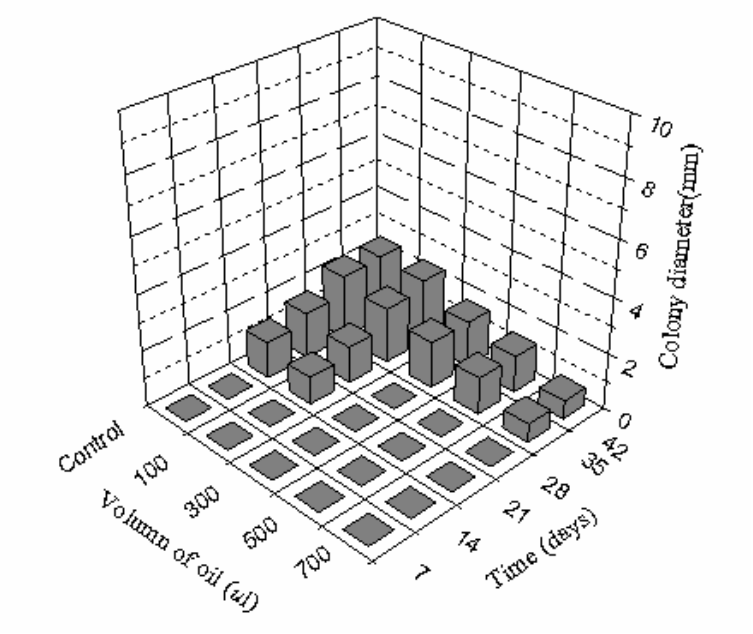
Storage of premix fruitcake and rice fruitcake were investigated under the active packing condition. High level CO_2 (40%) and low concentration of O_2 (< 0.05%) could help to extent shelf life of fruitcake from 3 days to 14 days. In addition, the combined effect between volatile compound of cinnamon oil and clove

oil at 300 μl and high CO_2 could extend shelf life of fruitcake at 20 °C, 68% RH and 30 °C, 75%RH up to 21 days.

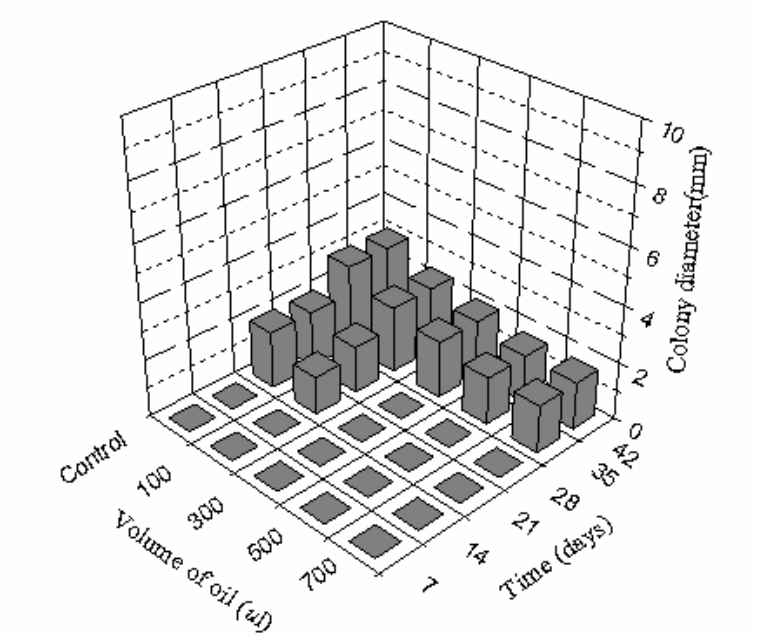
3.3 Growth modeling of *A.flavus*

3.3.1 Growth of *Aspergillus flavus* on premix fruitcake (PMC_5):

Growth of *Aspergillus flavus* on premix fruitcake incubated at several of temperature (20 ºC to 37ºC), water activities (0.75 to 0.85), and volume of oil (100 to 700 μl) are shon in Figure 29 to 32. *A. flavus* could not grow on premix fruitcake at 0.75 and grew after 14 days for all others conditions examined. The high level of CO_2, reported to be necessary to prevent growth of mould (Nielsen and Rios, 2000 and Guynot *et al.*, 2003), could be responsible for this. Furthermore, growth of *A. flavus* was also found to be water activity dependent. For example *A. flavus* showed slow growth after 14 days at 0.85, 30 °C but showed a grow after 21 days at 0.80, 30 °C. Figures 29 to 32 also show that *A. flavus* grew more slowly at 37 °C than that at 25 and 30 °C. The combined effect between temperature and volatile oil at high temperature are shown in these figure also. The effect of temperature and water activity against mould on cake analogue was explained by Abellana *et al*., (2001). The presence of 300 to 500 μl of cinnamon oil and clove oil in the head space with ratio 5:1 was found in this work to further inhibit the growth of *Aspergillus flavus* on fruitcake up to 28 days for all conditions examined. A lower amount of cinnamon oil and clove oil (100 μl) was, however, able to inhibit the growth of *Aspergillus flavus* only 14 days which was similar to that of control.

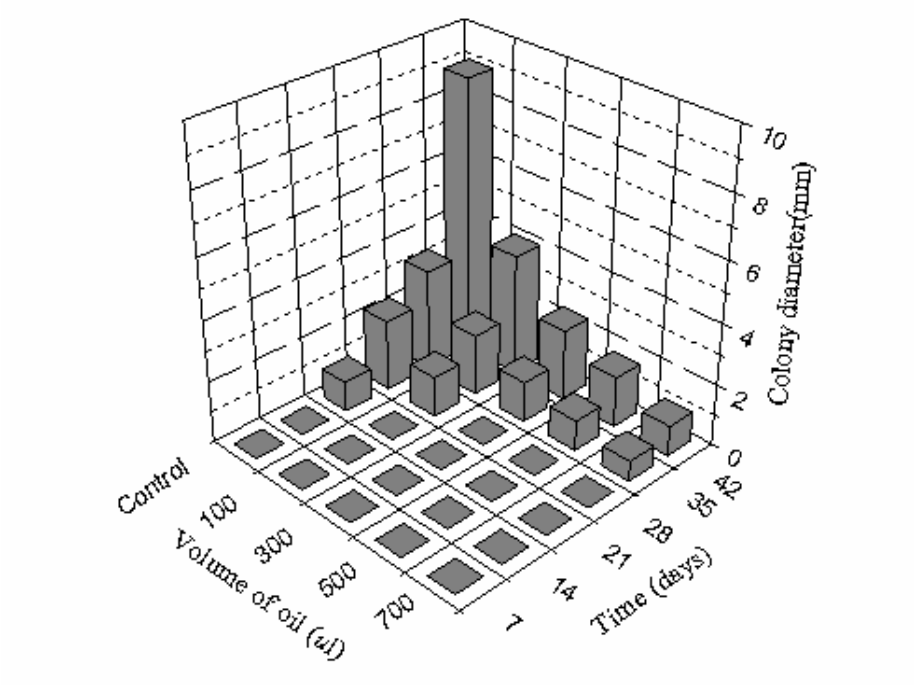


(a)

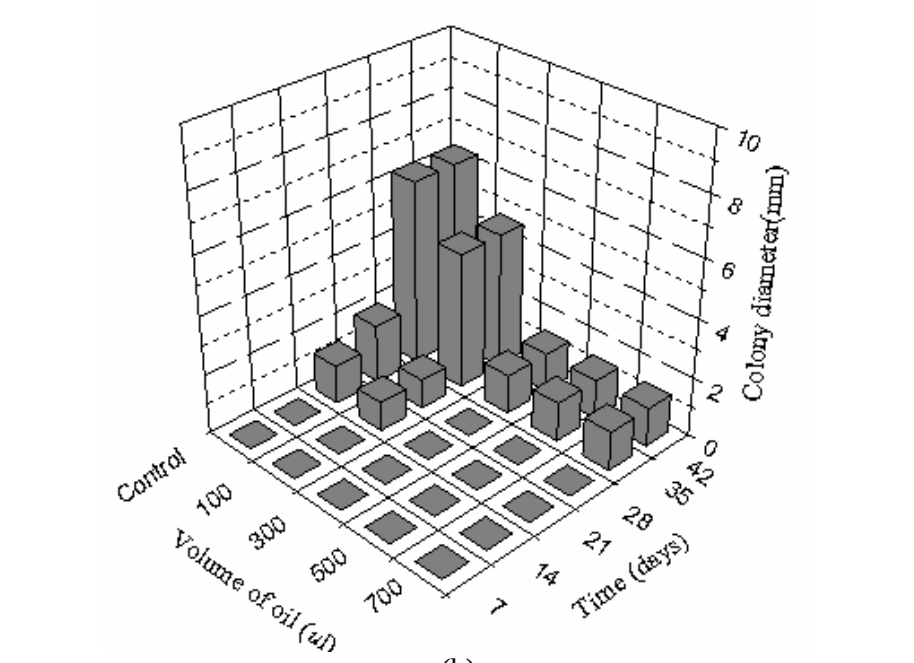


(b)

Figure 29 Growth of *Aspergillus flavus* on premix fruitcake incubated at temperature 20 ^{0}C and water activities at 0.80 (a) and 0.85 (b)

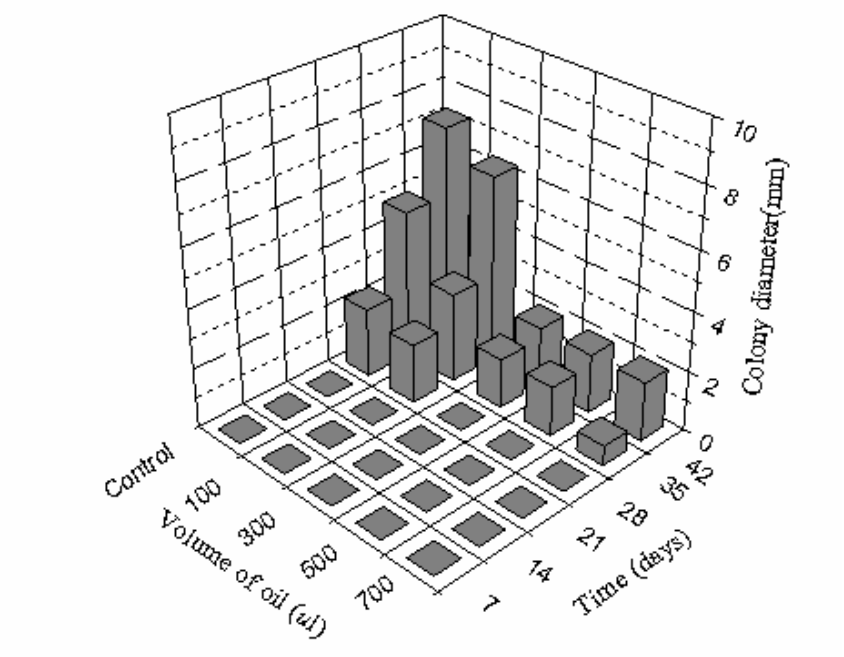


(a)

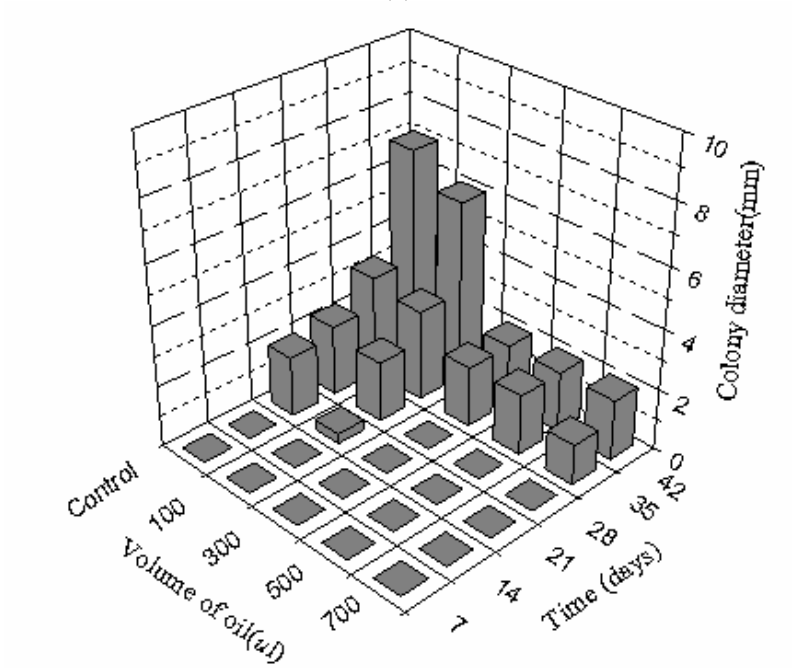


(b)

Figure 30 Growth of *Aspergillus flavus* on premix fruitcake incubated at temperature 25 ^{0}C and water activities at 0.80 (a) and 0.85 (b)

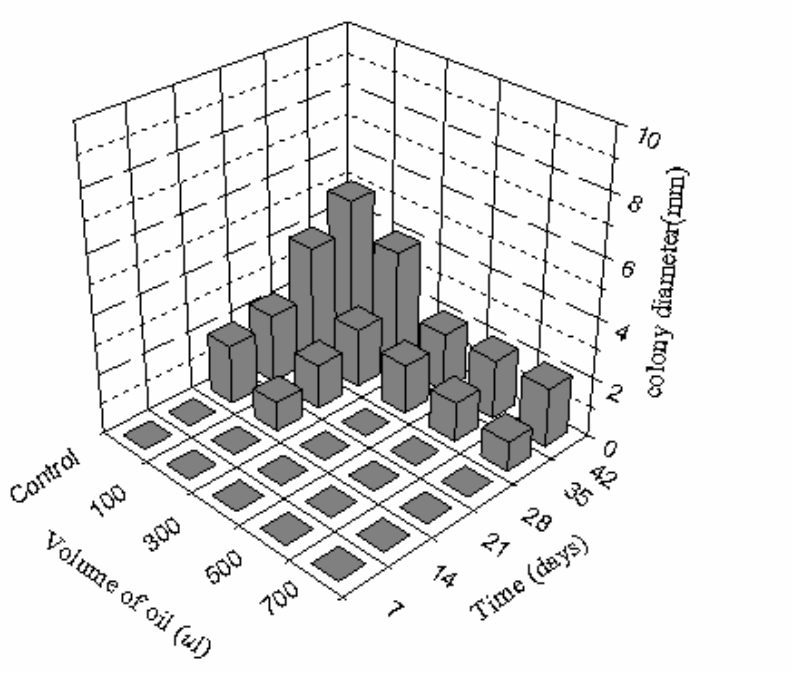


(a)

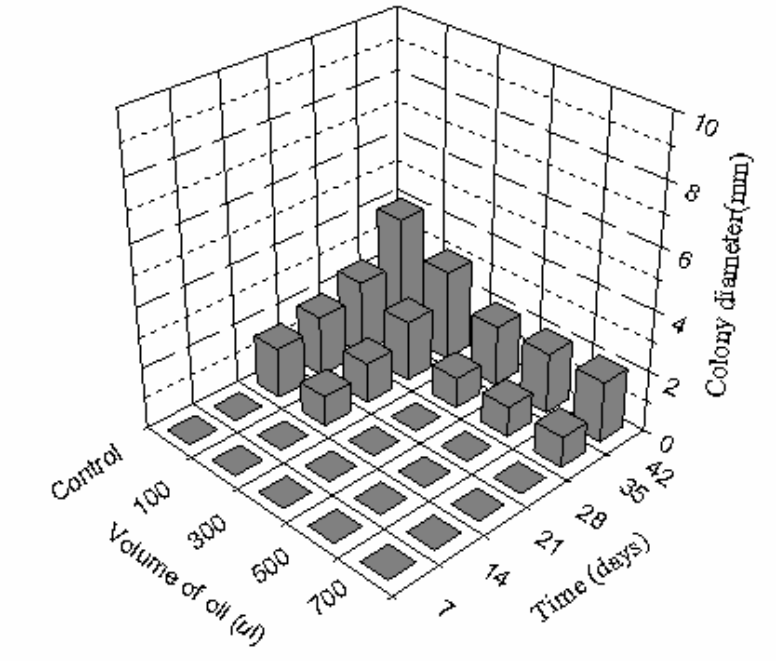


(b)

Figure 31Growth of *Aspergillus flavus* on premix fruitcake incubated at temperature 30 ^{0}C and water activities at 0.80 (a) and 0.85 (b)



(a)



(b)

Figure 32 Growth of *Aspergillus flavus* on premix fruitcake incubated at temperature 37 ^{0}C and water activities at 0.80 (a) and 0.85 (b)

Guynot, *et al* (2004) also reported a combined effect of different level of water activity and weak acid preservatives as significant effect to mould.

3.3.2 Modeling by using RSM

Based on the ratio presented above, an analysis of the F-values indicating the variable of significance is shown in Table 21. The efficiency of the fit of the model was assessed through the coefficient of determination (R^2). Water activity, volume of oil and time are significant factors whereas temperature is not significant factor on the growth rate model.

Table 21 ANOVA analysis for growth of *Aspergillus flavus* model

Factor	Degrees of freedom	F-Ratio	prob >F[a]
Water activity (x_1)	5	203.7	0.0000*
Temp (x_2)	5	3.264	0.0062
Volume of oil (x_3)	5	172.7	0.0000*
Time (x_4)	5	281.8	0.0000*

* ($P< 0.0001$)

Analysis of variance (ANOVA) for the model is given in Table 22. The coefficient of determination (R^2) of the predicted model was 0.8981, suggesting a good fit, the predicted model seemed to reasonably represent the observed values. Thus, the response was sufficiently explained by the model.

Table 22 Variance analysis of the second-order regression model on growth rate of *Aspergillus flavus*

Source	Adjust R^2	F-value	p-value
Model[a]	0.7143	190.2	0.0000*
Linear	0.4617	430.3	0.0000*
Quadratic	0.0796	74.186	0.0000*
Cross-product	0.1730	107.5	0.0000*

[a] The coefficient of determination (R^2) of the predicted model was 0.8981

* ($P < 0.0001$)

A regression analysis (Table 23) was carried out to fit mathematical models to the experimental data aiming at an optimal region for the responses studied. Some insignificant terms, such as x_2, x_3x_2, x_2^2 were neglected, and the predicted model was not refitted. Meanwhile, the term x_1, x_2 and x_4 were kept in the model to preserve model hierarchy.

Table 23 Estimated regression model of relationship between response variables (growth rate of *Aspergillus flavus*) and independent variables (x_1, x_2, x_3, x_4)

Variables	P-value
Intercept	0.0000*
x_1	0.0000*
x_2	0.3918
x_3	0.0000*
x_4	0.0000*
x_1^2	0.0000*
x_2x_1	0.4519
x_2^2	0.0012
x_3x_1	0.0000*
x_3x_2	0.5479
x_3^2	0.0000*
x_4x_1	0.0000*
x_4x_2	0.0730
x_4x_3	0.0000*
x_4^2	0.0000*

* ($P < 0.0001$)

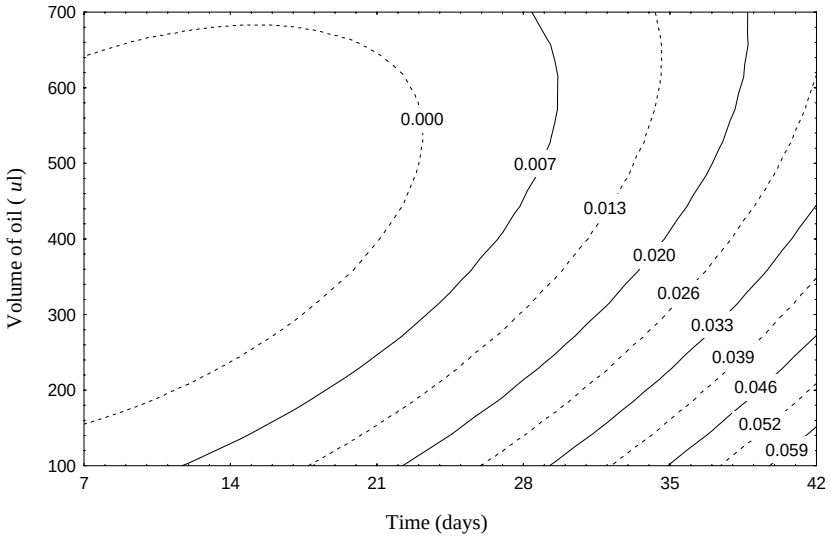
* x_1= water activity (a_w), x_2=temperature (°C), x_3= volume of oil (µl), and x_4= time (days)

The RSREG procedure from SAS was employed to fit the second-order polynomial (Eq.3) to the experimental data (grow rate of *Aspergillus flavus*). From the SAS output of RSREG, the second-order polynomial (predicted model) can be described by the following equation in terms of uncoded values:

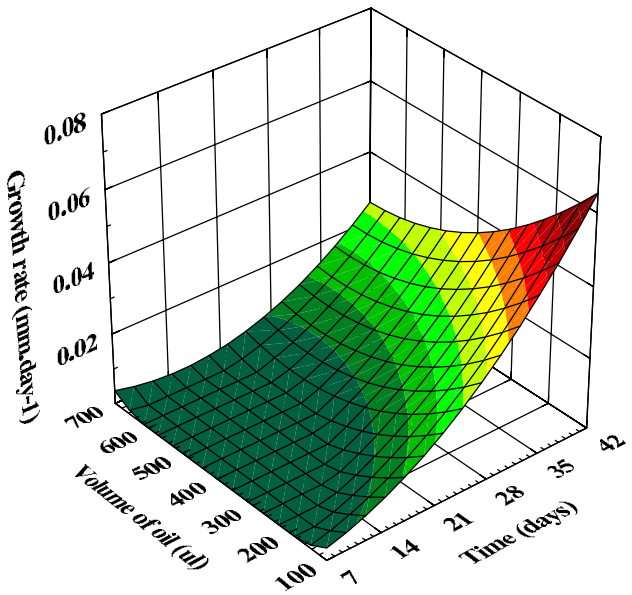
$$Y= -4.001046+ 9.965236x_1+ 0.000508x_3-0.016523x_4-6.259153x_1^2-0.000713x_3x_1+0.000000124x_3^2+0.021440x_4x_1-0.000002643x_4x_3+0.000026783x_4^2 \quad (3)$$

Where, x_1= water activity (a_w), x_2=temperature (°C), x_3= volume of oil (μl), and x_4= time (days)

3.3.2.1 Analysis of response surface: The regression model Eq.3 allowed the prediction of the effects of the four parameters on the growth rate of *Aspergillus flavus*. 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 growth rate of *Aspergillus flavus* (Figure 33 to 34).

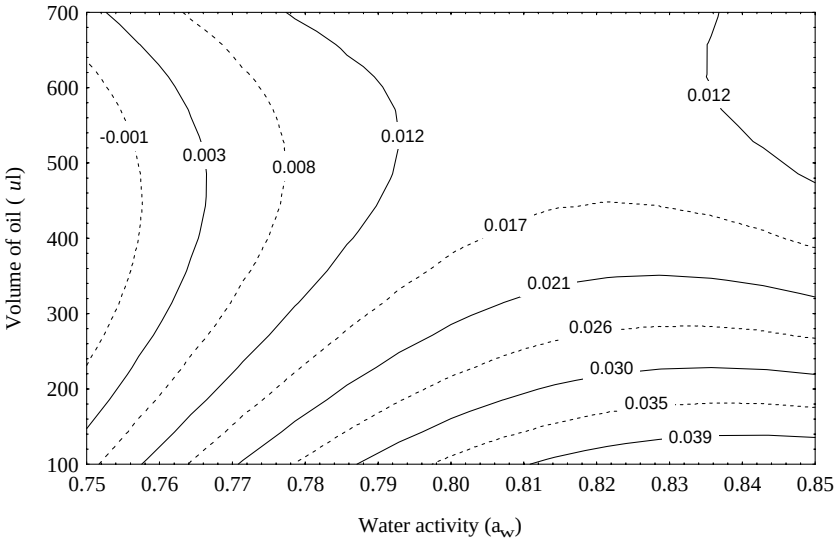


(a)

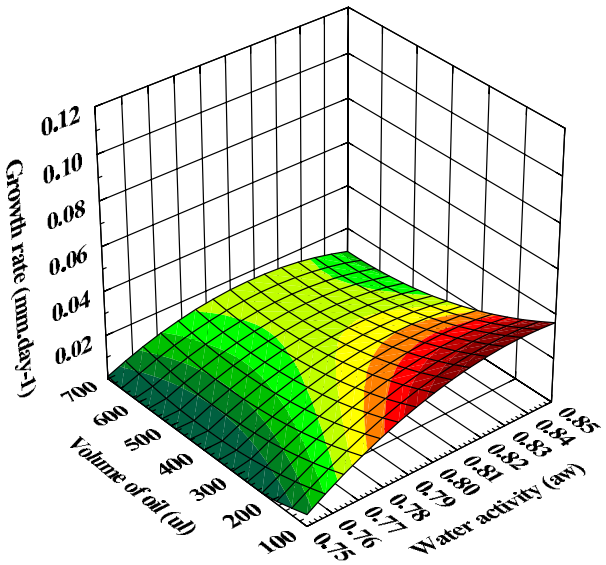


(b)

Figure 33 Contour plot (a) and response surface plot (b) of growth rate of *Aspergillus flavus* on premix fruitcake by volume of oil (µl) and time (days) at 30 °C



(a)



(b)

Figure 34 Contour plot (a) and response surface plot (b) of growth rate of *Aspergillus flavus* on premix fruitcake by volume of oil (µl) and water activity (a_w) at 30 °C

The location of the minimum for growth rate is well shown in the 3 D plot reported in Figures 33 to 34 for the combination time and volume of oil. The growth rate of *A. flavus* increased with time and depended on volume of oil. The *A. flavus* mycelium slowly expanded on premix fruitcake at 700 µl. For the combination of water activity and volume of oil, the growth rate of *A. flavus* was strongly dependent on water activity. The growth rate decreased at low water activity. The minimum a_w for growth of *A. flavus* was 0.80 to 0.85 depending on volume of oil present.

Graphs such as these can help the manufacturer in achieving the desired response, i.e., the desired product shelf life. Of particular importance are volumes of essential oil and time, which can be adjusted and controlled more readily by the manufacturer than storage water activity and temperature. A shelf life of 28 days could be expected at 20-37 °C, with aw of 0.80 to 0.85.

3.4 Verification of growth modeling of microorganisms on rice fruitcake (RF): The suitability of the model equation for predicting the optimum response values was tested using volume of oil at 300 to 400 µl, temperature at 30 to 37 ° C, the initial water activity of RF at 0.80 The growth rates were measured every 7 days until 42 days. The experimental growth rate of *A. flavus* on rice fruitcake was found to be in reasonable agreement with the predicted one (Table 24).

Table 24 Predicted and experimental growth rate of *Aspergillus flavus*

Water activity (a_w)	Temperature (°C)	Volume of oil (μl)	Time (days)	Predict ($mm.day^{-1}$)	Experiment ($mm.day^{-1}$)
0.8	30	300	7	-0.0959	0.0000
0.8	30	300	14	-0.0564	0.0000
0.8	30	300	21	-0.0313	0.0000
0.8	30	300	28	-0.0206	0.0000
0.8	30	300	35	0.0203	0.0360
0.8	30	300	42	0.0424	0.0367
0.8	37	400	7	-0.1284	0.0000
0.8	37	400	14	-0.0876	0.0000
0.8	37	400	21	-0.0612	0.0000
0.8	37	400	28	-0.0492	0.0000
0.8	37	400	35	0.0510	0.0350
0.8	37	400	42	0.0410	0.0410

From table 24, in order to evaluate the goodness of the response surface model for growth of *Aspergillus flavus* on fruitcake, the experimental values of 0.80 water activity, (30 to 37 ^{0}C) temperature, (300 to 400 μl) essential oil and (7 to 42 days) time storage were obtained for growth of *Aspergillus flavus* on rice fruitcake. The models predicted for the growth rate were from –0.0590 to 0.0510 Experimental responses obtained were 0.0000 to 0.0410. The errors can be considered small as the observed values are within the 5% level of significance. The standard deviation obtained from the ANOVA table is used to derive the confidence intervals. In addition, the statistical model is useful in the accurate prediction.

The RSM model was successfully employed to describe the growth rate of *A. flavus*. Water activity, volume of oil and time were the most significant parameters influencing growth of *A. flavus*. The rice fruitcake was used to verify the model describing the growth rate of *A. flavus*. The results showed that the predicted growth

rate of *A. flavus* was in agreement with the experimental values measured one on the rice fruitcake.

4. Applying the active packaging technique developed to preserve the IMF products under normal air condition.

4.1 Effect of temperature on the concentration of headspace volatile compound

Effect of temperature on the concentration components of volatile oil in the headspace under normal air condition is shown in Figures 35 to 38. Linalool, cinnamaldehyde and eugenol were not detected in the headspace at 30°C up to 30 minutes. This is in good agreement with the results reported in section 3.1 for the MAP condition at 30°C in which no peak of cinnamaldehyde and eugenol were observed. Increasing the temperature was found to strongly enhance the vaporization of linalool, cinnamaldehyde and eugenol into the headspace. At a temperature of 40°C within 10 minutes, the significant amounts of linalool, cinnamaldehyde and eugenol (>1,000 ppm) were found to vaporize into the headspace. There is a possibility of using this technique to preserve food products that have to keep at temperature higher than 40°C under the normal air condition. This could reduce cost of pressurizing gas into the package which have to be done in the MAP technique.