Bosset, 2002). So, the amount and the performance to prevent microorganism of volatile essential oil is expected to be low at such that low temperature condition.

Therefore, cake was selected to be the product for this research study. Premix cake was selected to study in objective 2. Premix fruitcake and rice fruitcake were evaluated in objective 3 and rice jasmine butter cake was used in objective 4.

2 Study effectiveness of using cinnamon and clove oil as antifungi in IMF product by using active packaging technique.

2.1 The inhibitory effect of the volatile gas phase of combinations of cinnamon oil and clove oil

The effect of cinnamon oil under modified atmosphere conditions were tested with four fungi i.e. *Penicillium rogueforti, Aspergillus flavus, Eurotium sp and Mucor plmbeus* and four yeasts i.e. *Candida lipolytica, Debaryomy hansenii, Zygosaconaromyces roxii* and *Pichia membranaefacien* (Figure 2). The effective inhibitory period of cinnamon oil on microorganism increased with decreasing of O_2 concentration (10-<0.5%) or increasing CO_2 concentration (20-40%). At 40% CO_2 concentration could inhibit growth of *Aspergillus flavus* for 14 days, *Eurotium* sp for 18 days, *Penicillium rogueforti* for 36 days, *Candida lipolytica* and *Debaryomy hansenii* for 31days *Zygosaconaromyces roxii* for 34 days, *Pichia membranaefacien* for 25 days and *Mucor plmbeus* for 26 days

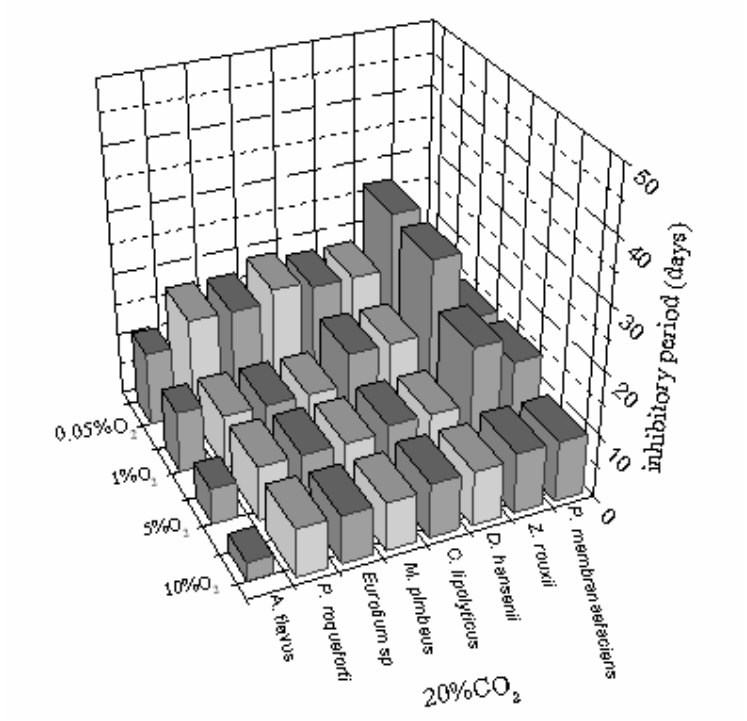
Volatile compounds from clove oil with MAP conditions also showed inhibition (Figure 3). However, the volatile compound from clove oil did not completely inhibit the yeasts and moulds. Clove oil at 1,000 μl with 40 %CO_2 could not inhibit *Aspergillus flavus*. On the other hand this inhibited *Eurotium* sp for 12 days, *Penicillium rogueforti* for 30 days, *Candida lipolytica* and *Debaryomy hansenii* for 25 days, *Zygosaconaromyces roxii* for 16 days, *Pichia membranaefacien* for 19 days and *Mucor plmbeus* for 21 days. A mixture of clove and cinnamon oil in the ratio 1:1 (500 μl: 500 μl) inhibited *Aspergillus flavus* for 18 days, *Eurotium* sp for 20

days, *Penicillium* rogueforti for 48 days, *Candida lipolytica* and *Debaryomy hansenii* for 43 days, *Zygosaconaromyces roxii* for 46 days, *Pichia membranaefacien* for 30 days and *Mucor plmbeus* for 28 days(Figure 4).

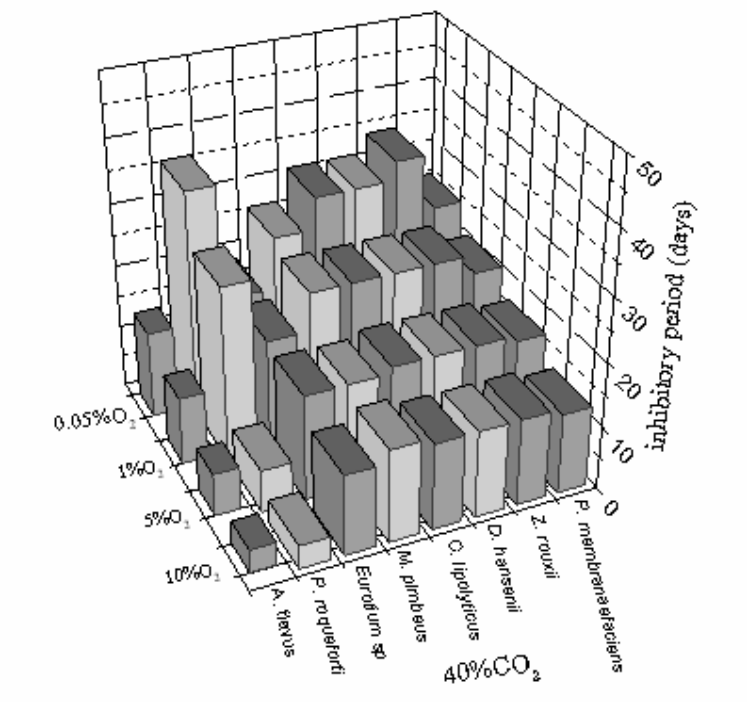
It is evident that the headspace gas composition has a synergistic influence on the inhibitory effects of the cinnamon and clove oil volatiles. As the O_2 concentration decreased from 10% to 0.05% the period of inhibition increased. Increasing the CO_2 concentration from 20 to 40% also increased the inhibitory effect of the oils. The oils were most effective in inhibiting growth of all microorganisms when used in combination with low oxygen levels (<0.05%) and high CO_2 concentrations (40%).

The effectiveness of each of cinnamon oil and clove oil as an antibacterial agent was reported by Ouattara *et al*. (1997) and Pradsad, *et al*. (2000). The main inhibitory components of cinnamon oil and clove oil are believed to be cinnamaldehyde and eugenol, respectively (Jayatilaka, *et al*., 1995; Della, *et al*., 1998) and their effectiveness against molds and yeasts has been reported by Lópenz-Malo et at. (2001). It has been proposed that cinnamaldehyde and eugenol inhibit production of an essential enzyme by the bacteria and/or cause damage to the cell wall of bacteria (Helander *et al*., 1998).

As can be seen from the results, combination of clove and cinnamon oil at 1: 1 prevented *Aspergillus flavus* only for 18 days. So, higher concentration of clove and cinnamon oil should be explored in the future for the use with IMF products. It was concluded that a high concentration of CO_2 (40%) and low concentration of O_2 (<0.05%) with the volatile gas phase of cinnamon oil and clove oil may be suitable as an active packaging system for preventing the growth of microorganisms on IMF products.

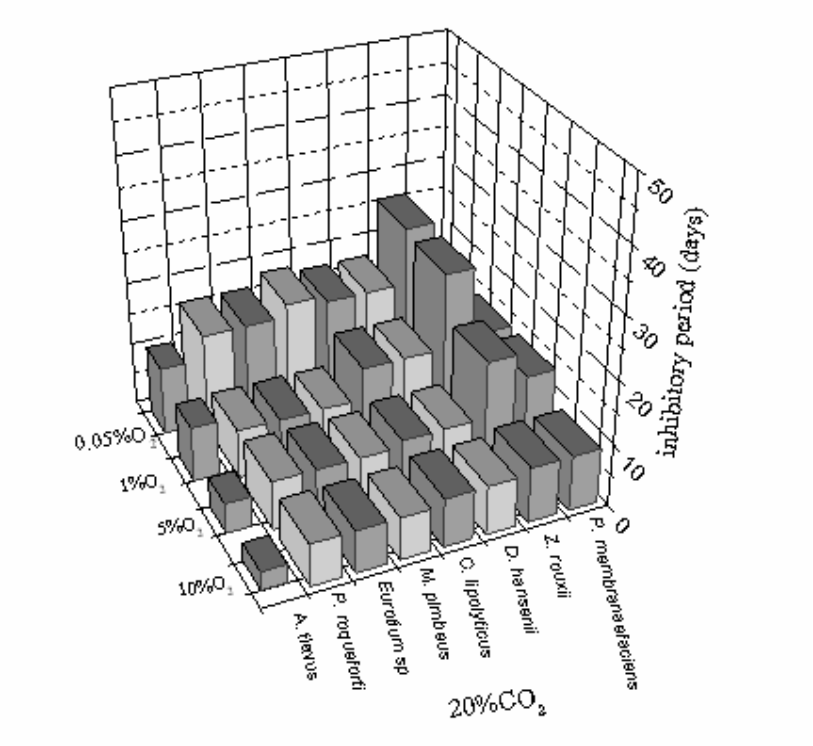


(a)

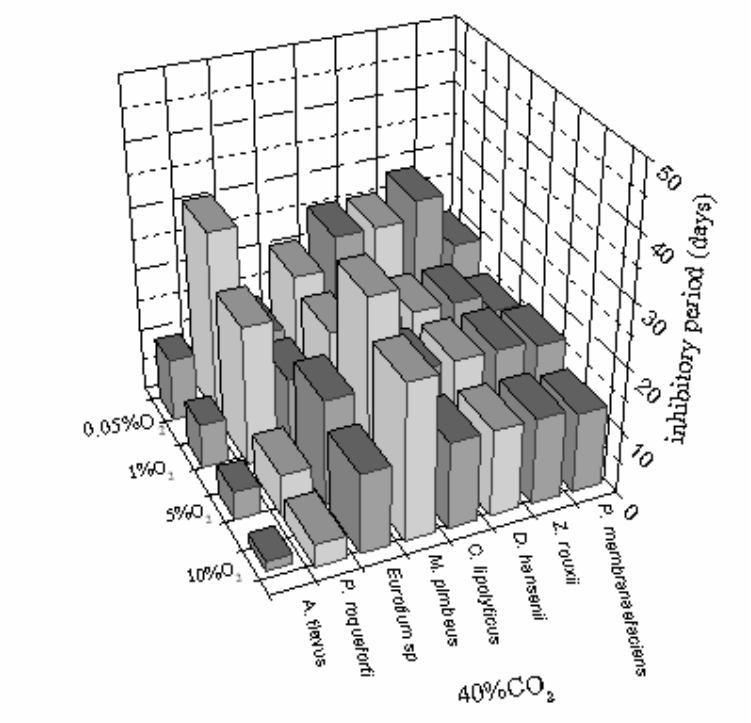


(b)

Figure 2 Days for initiation of colony growth under MAP conditions with cinnamon oil at 2,000 μl: O_2 (<0.05%-10%) and two CO_2 (a) 20% or (b) 40%

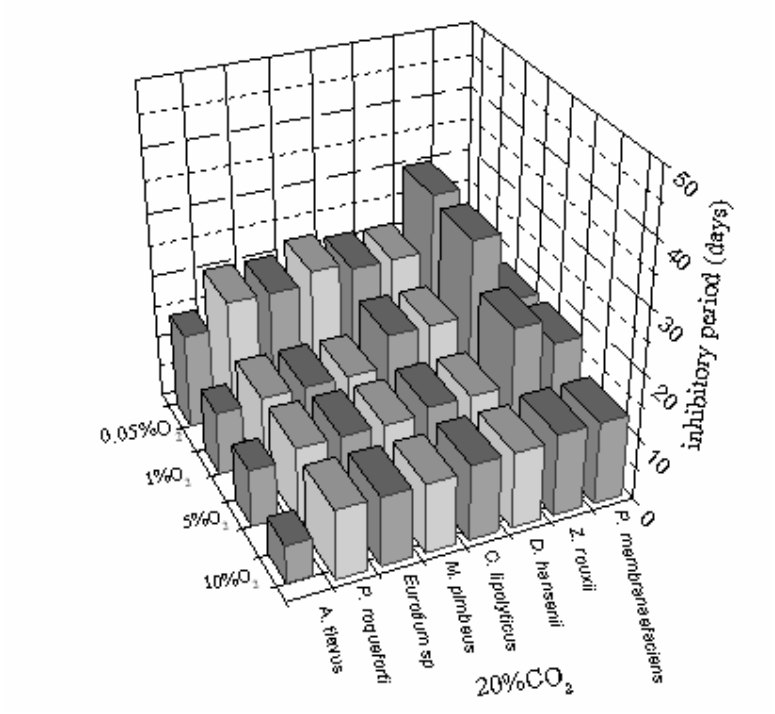


(a)

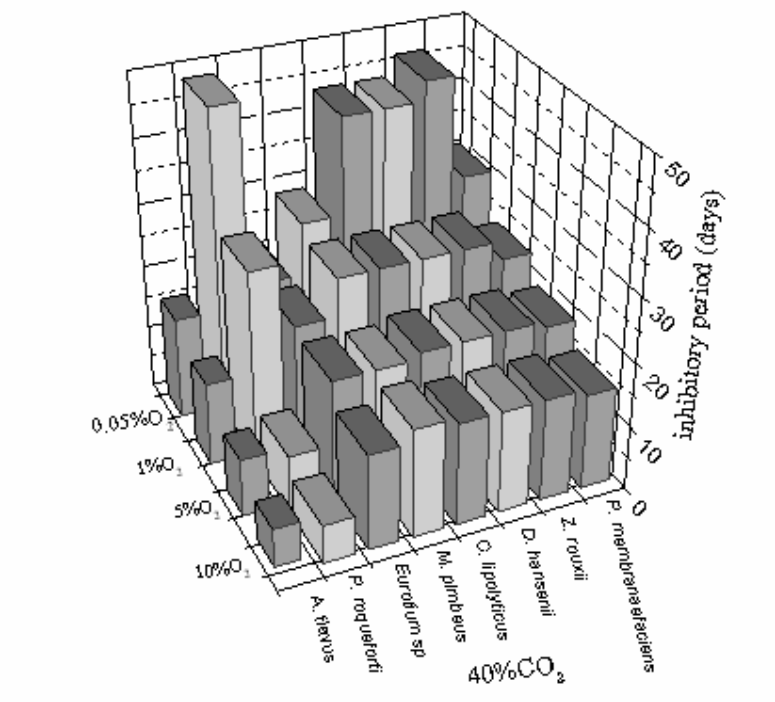


(b)

Figure 3 Days for initiation of colony growth under MAP conditions with clove oil at 2,000 μl: O_2 (<0.05%-10%) and two CO_2 (a) 20% or (b) 40%



(a)



(b)

Figure 4 Days for initiation of colony growth under MAP conditions with combination of clove and cinnamon oil at ratio 1:1: O_2 (<0.05%-10%) and two CO_2 (a) 20% or (b) 40%

The combination between cinnamon oil and clove oil with MA conditions showed stronger inhibiton than added only essential oil into agar containing those microorganisms frequently causing spoilage in the IMF products. Therefore, combination of clove oil and cinnamon oil at the ratio 1:1 was selected to use for further investigation in section 2.2.

2.2 Minimum inhibitory volume (MIV) of cinnamon and clove oil for yeasts and molds:

Further investigation was made of the inhibition of spoilage yeasts and molds by cinnamon and clove oil volatiles when the oils were used in the ratio 1:1 (Figure 5). The volatile gas phase of the essential oils added at 1,000 µL inhibited the growth of *Candida lipolyticus* and *Pichia membranaefaciens* only for 30 and 16 days, respectively; 2,000 µL could inhibit growth of *Zygosaccharomyces roxii, Penicillium roqueforti, Debaryomyces hansenii,* and *Mucor plumbeus* for more than 30 days, and could inhibit growth of *Aspergillus flavus* for 17 days and *Eurotium* sp. for 20 days. At 3,000 µL, the volatiles inhibited the growth of *Aspergillus flavus* for 19 days and 4000 µL provided completely inhibited growth of all molds and yeasts for more than 40 days. *A. flavus* appeared to be the most resistant organism requiring a high level of volatiles for satisfactory inhibition. The majority of the other yeasts and molds required the addition of 2,000 µL to achieve a significant inhibitory period. The least resistant mould was *C. lipolyticus,* which was inhibited for 30 days at a volume of 1000 µL. Because *Aspergillus flavus* is the most resistant microorganism and is capable of producing alflatoxin in IMF products (Pitt and Hocking, 1997), as a result only *A. flavus* will be examined in all following sections of this work. Moreover, higher concentrations of clove and cinnamon oil volatiles should be explored to provide adequate protection of IMF products.

Comparisons between the effectiveness of the volatile gas phases and the liquid phases of essential oils have shown that oil in the liquid phase is more effective in preventing spoilage than when added via the gas phase. Soliman and Badeaa (2000) found that ≤500 ppm of cinnamon oil can inhibit *Aspergillus flavus* on potato

dextrose agar medium. Nguefack *et al* (2004) reported that cinnamon oil at concentrations of 1,000 ppm completely inhibited the growth of *Aspergillus flavus.* The data presented in this study confirm that higher volumes are required if the essential oils only contact the contaminating microorganisms in the gas phase. However, advantages of using a volatile gas phase of essential oil for food products are that it may have a lesser influence on the final taste and aroma of the product and its release may better be able to be regulated. It is expected that the headspace volatiles will increase in concentration following their release, which might be triggered by a change in environment (e.g. increased temperature or humidity), then decline during storage (depending on the permeability of the package and length of storage) and be dispersed when the packaged is opened by the consumer.

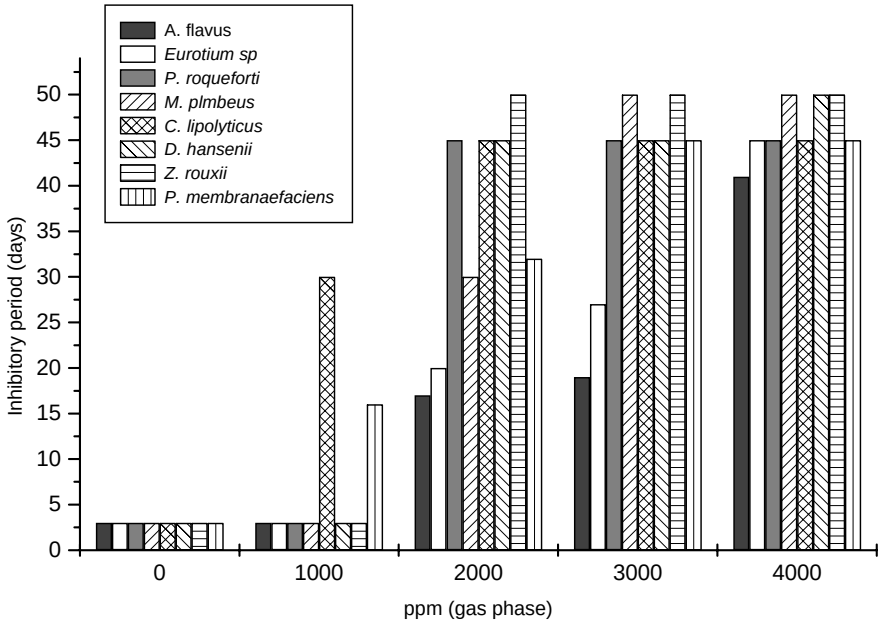


Figure 5 Days for initiation of colony growth with varying added volumes of mixtures of cinnamon and clove essential oils.

In order to inhibit the growth of *A. flavus*, the most resistant organism examined, in agar up to 40 days, 4,000 µL of cinnamon oil and clove oil at the ratio

1:1 was required. This amount of oil mixture was used in the following section (section 2.3) to examine the most suitable ratio of cinnamon oil and clove oil for preventing the growth of microorganisms.

2.3 Inhibition of *A. flavus* by mixtures of cinnamon and clove oils

The growth of *A. flavus* on MEA in the presence of the volatiles of 4,000 µL of added oil comprising various ratios of cinnamon and clove oils in combination with 40% CO_2 and less than 0.05% O_2 is shown in Figure 6. All of the mixtures used significantly reduced mycelial growth. However increasing the ratios of cinnamon oil (5:1, 7:1, and 9:1) significantly reduced the growth rate of *Aspergillus flavus* compared with lower ratios of cinnamon oils (1:1, 1:3, 1:5, 1:7, 1:9, and 3:1). Growth of *Aspergillus flavus* was clearly retarded at a higher cinnamon oil concentration.

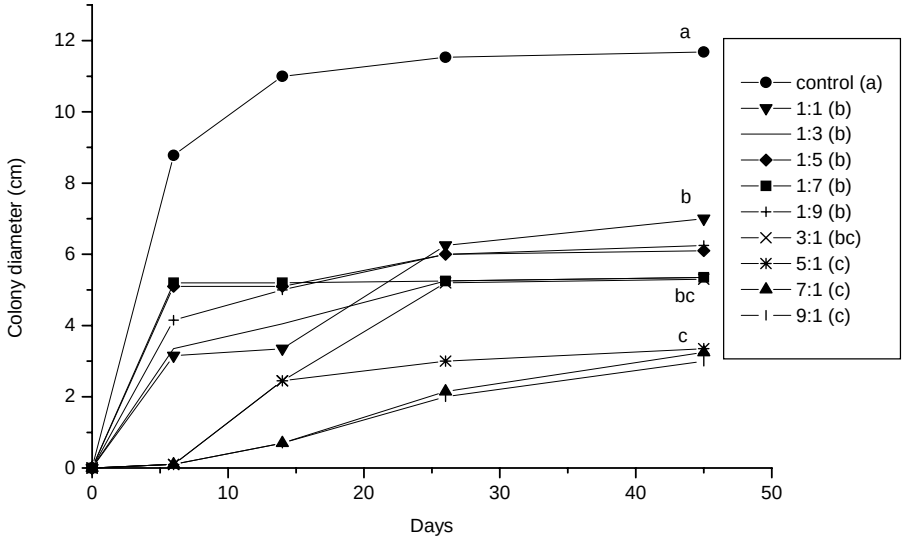


Figure 6 Growth curves of *Aspergillus flavus* incubated with 4,000 µL of combinations of cinnamon oil and clove oil in various ratios

[a, b, bc, c] Significant $P<0.05$.

The ratio of cinnamon and clove oils of 5:1 was selected for the tests in all following sections because of its effectiveness in inhibiting growth of *A. flavus* at low cost.

2.4 Effectiveness of cinnamon oil and clove oil as an inhibitor of *Aspergillus flavus* on fruitcake with modified atmosphere and normal air conditions, and sensory testing.

2.4.1 Selection of fruitcake recipe

Water activity and moisture content of the fruitcakes are shown in Figures 7 and 8. The water activity of trial fruitcake recipes was between 0.914 and 0.938, which was similar to spongy cake (0.90 to 0.95) (Abellane et.al., 2001). The moisture content of trial fruitcake recipes was 37.05 to 37.57 %. Water activity and moisture content of premix fruitcakes was lower than that the trial fruitcake recipes, ranging from 0.768 to 0.890. Water activity and moisture content of commercial fruitcake were measured at 0.740 to 0.800 and 23 to 37 %, respectively.

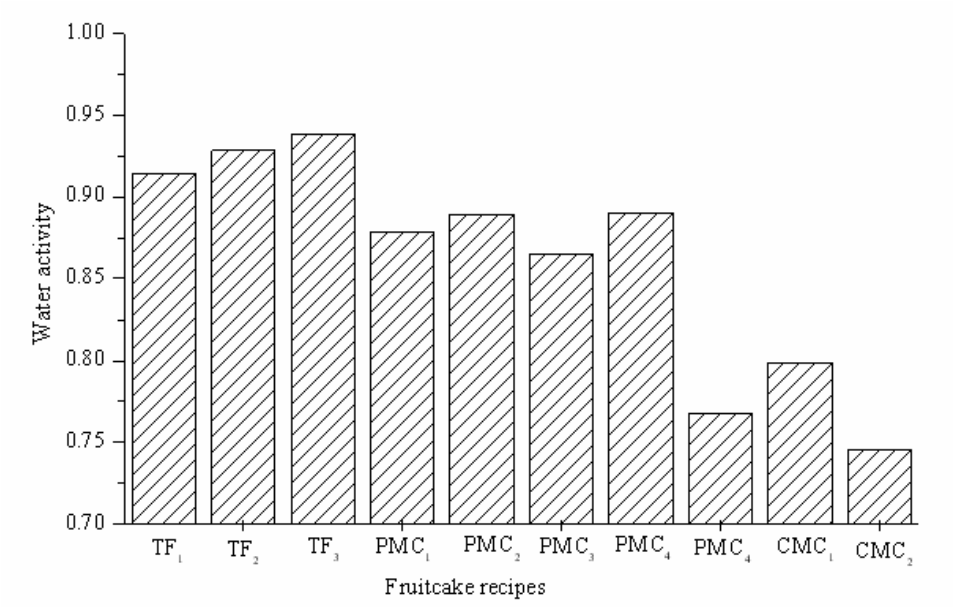


Figure 7 Comparison of water activity of fruitcake (TF_1= Trial fruitcakes 1, TF_2= Trial fruitcakes 2, TF_3= Trial fruitcakes3, PMC_1= Mixed berry muffin mix, PMC_2= Vanilla cake mix, PMC_3= Banana cake mix, PMC_4= Cake mix, PMC_5= Variety fruitcake mix, CMC_1= Jamaican ginger loaf, and CMC_2= Waikato farmhouse cake)

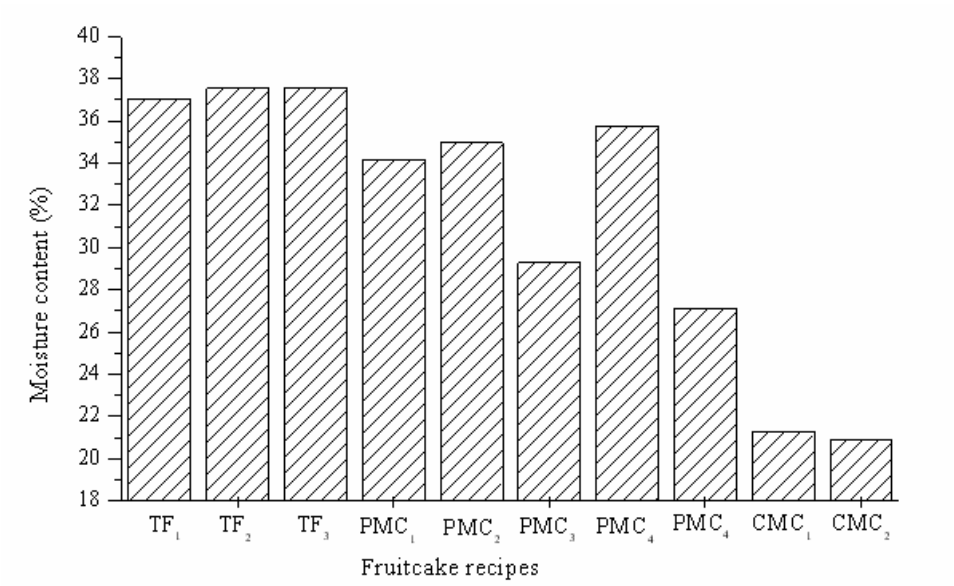


Figure 8 Comparison of moisture content of fruitcake (TF_1= Trial fruitcakes 1, TF_2= Trial fruitcakes 2, TF_3= Trial fruitcakes3, PMC_1= Mixed berry muffin mix, PMC_2= Vanilla cake mix, PMC_3= Banana cake mix, PMC_4= Cake mix, PMC_5= Variety fruitcake mix, CMC_1= Jamaican ginger loaf, and CMC_2= Waikato farmhouse cake)

A comparison of the ingredients of premix fruitcakes and commercial fruitcakes is shown in the Table 12. The results revealed that the different ingredient used produced different moisture contents and water activities. The main common ingredients found were wheat flour, sugar, emulsifiers, flavour, salt, eggs, water, and colour.

From Figure 7 and Figure 8, Banana cake mix (PMC_3) and variety fruitcake mix (PMC_5) were selected to study water activity and moisture content of fruitcake during storage in the next section (section 2.4.1.1) because the water activity and moisture content were similar to those of the commercial fruitcake.

Table 12 Comparison of ingredients of premix fruitcakes and commercial fruitcakes

Ingredients	Commercial Fruitcake		Premix Fruitcake	
	CMC_1	CMC_2	PMC_3	PMC_5
Wheat Flour	X	X	X	X
Sugar	X	X	X	X
Vegetable oil	X	-	X	X
Dextrose	-	-	X	-
Emulsifiers	X	X	X	X
Mineral salts	-	-	X	-
Flavours	X	X	X	X
Salt	X	X	X	X
Anticaking agent	-	-	X	-
Antioxidants	X	X	X	-
Food acid	X	X	X	-
Sultanas	X	X	-	X
Currants	-	X	-	-
Margarine	-	X	-	-
Egg	X	X	X	X
Coconut oil	-	X	-	-
Water	X	X	X	X
Surcose	-	X	-	-
Vegetable gum	-	X	-	-
Peel	-	X	-	-
Perservative	X	X	-	X
Nut	-	X	-	-
Gluten-containing cereal	X	-	-	-
Glace	X	-	-	-
Cherries	X	-	-	-
Butter	X	-	-	-

Table 12 (continued)

Ingredients	Commercial Fruitcake[a]		Premix Fruitcake[b]	
	CMC_1	CMC_2	PMC_3	PMC_5
Fat oil	X	-	-	-
Skim milk powder	X	-	-	-
Glucose syrup	X	-	-	X
Modified maize starch	X	-	-	-
Raising agents	X	-	-	-
Colour	X	X	X	X
Baking powder	-	-	-	X
Banana	-	-	X	-
Thickener	-	-	X	X
Caramel	-	-	-	X

[a] CMC_1= Jamaican ginger loaf, CMC_2= Waikato farmhouse cake

[b] PMC_3= Banana cake mix, PMC_4= Cake mix, PMC_5= Variety fruitcake mix

2.4.1.1 Water activity and moisture content of fruitcake during storage

Water activity and moisture content are shown in Figures 9 and 10. Water activity of fruitcake at 30 °C and 75%RH was tested, varying from 0.764 to 0.853 at 0 days and 0.796 to 0.831 for 30 days. Moisture contents of the fruitcakes ranged from 25.53 to 30.28 at 0 day and 21.78 to 29.84% at 30 days.

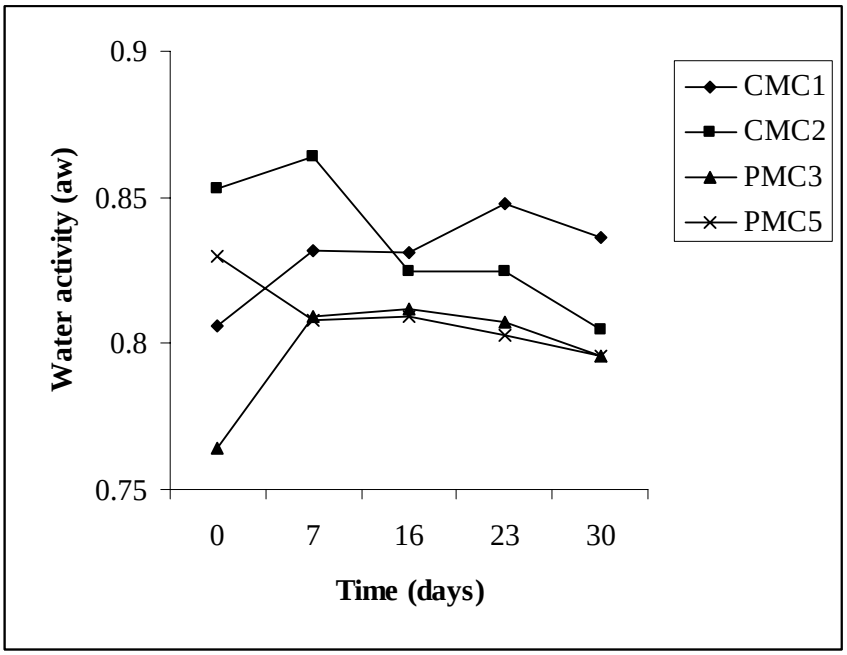


Figure 9 Water activity of fruitcakes during storage

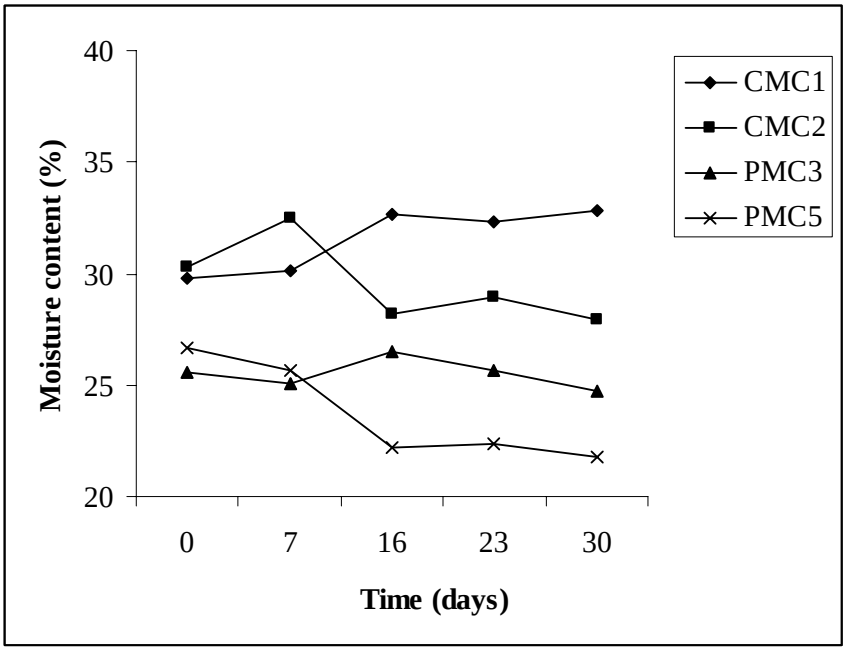


Figure10 Moisture content of fruitcakes during storage.

The results showed that water activity and moisture of the commercial Jamaican ginger loaf (CMC_1) increased over time. However, the water activity and moisture content of the Waikato farmhouse cake (CMC_2), the premix fruitcake (Banana cake mix (PMC_3) and the variety fruitcake mix (PMC_5) all decreased during storage. The variation in moisture content of premix fruitcakes over time was similar to normal cake during storage, as reported by Baik, *et al*. (2000).

According to the results above, variety fruitcake mix (PMC_5) was selected as a product to be examined in the following sections because its water activity and its moisture content was similar to those of the commercial fruitcake available in the market and the price was lower than that of the banana cake mix.

2.4.2 Identification of *Aspergillus flavus* from fruitcake

Two of *Aspergillus* sp. three of *Penicillium* sp., one of *Eurotium* sp., one of *Xerophilic* fungi and two unknowns are found on the premix cake. One *Aspergillus* sp. is found on the commercial fruitcake. Table 13 shows that the one of *Aspergillus* sp. found on premix cake was *Aspergillus flavus*, but the *Aspegillus* sp. found on the commercial fruitcake was not *Aspegillus flavus.*

Aspergillus flavus can grow up on CYA agar and showed yellow-green colonies on CYA at 30^0C, exceeding more than 35 mm. diameter, conidia with relatively thin walls, smooth or finely roughened, spherical to broadly ellipsoidal and the vesicles up to 50 μm diameter, following Pitt & Hocking (1997) key for *Aspergillus* identification. The *Aspergillus* sp. found on commercial fruitcake could not grow up on CYA agar and did not show yellow-green colonies on CYA at 30^0C, exceeding more than 35 mm. diameter, conidia with relatively thin walls, smooth or finely roughened, spherical to broadly ellipsoidal and the vesicles not up to 50 μm diameter. The characteristics of *Aspergillus* sp. found on premix fruitcake (PMC_5 No.1) and *Aspergillus flavus* identification from the environmental did not differ.

Table 13 Comparison of colony colour, conidia of *Aspergillus* sp. found on premix cake, and commercial fruitcake, and *Aspergillus flavus* identified from the environment.

Key to *Aspergillus flavus*[a]	*Aspergillus flavus* ID from environmental	PMC_5 No.1	PMC_5 No.2	CMC_2
Colonies on CYA, 7 days, exceeding 35 mm diam	70 mm.	45 mm.	80 mm.	30 mm.
Colonies white or coloured	Coloured	Coloured	Coloured	Coloured
Colonies yellow, green or brown	Green	Green, yellow	Green-red	Brown
Conidia yellow green or yellow	Conidia yellow	Conidia yellow green	Conidia red	Conidia brown
Conidia with relatively thin walls, smooth, vesicles up to 50 micrometer diam	Yes	Yes	No	No

[a] = Key from: Pitt and Hocking, 1997 (Appendix II)

CMC_2= Waikato farmhouse cake

PMC_5= Variety fruitcake mix

The *Aspergillus flavus* No.1 found on surface of Variety fruitcake mix (PMC_5) was used to study the effectiveness of oils on fruitcake in the following section.

2.4.3 Effectiveness of oils on fruitcake with MAP and normal air and preliminary sensory test:

2.4.3.1 The effectiveness of cinnamon oil and clove oil in ratio 5:1 as inhibitors of *Aspergillus flavus* on fruitcake under modified atmosphere conditions are shown in Figure 11. The results revealed that *A. flavus* grew slowly on commercial fruitcake and variety fruitcake mix under modified atmosphere condition at control condition (0 µl) up to the condition of 300 µl for 21 days. The colony diameter was between 4.0 to 5.3 mm for commercial fruitcake and 6.0-12.0 mm for variety fruitcake mix. To create the MAP condition, gases were pressurized into the high barrier bag. This was believed to enhance the amount of essential oil vaporized into the headspace. As a result, only 300 µl of essential oil used was found to completely inhibit the growth of *A. flavus* on the fruitcake up to 21 days.

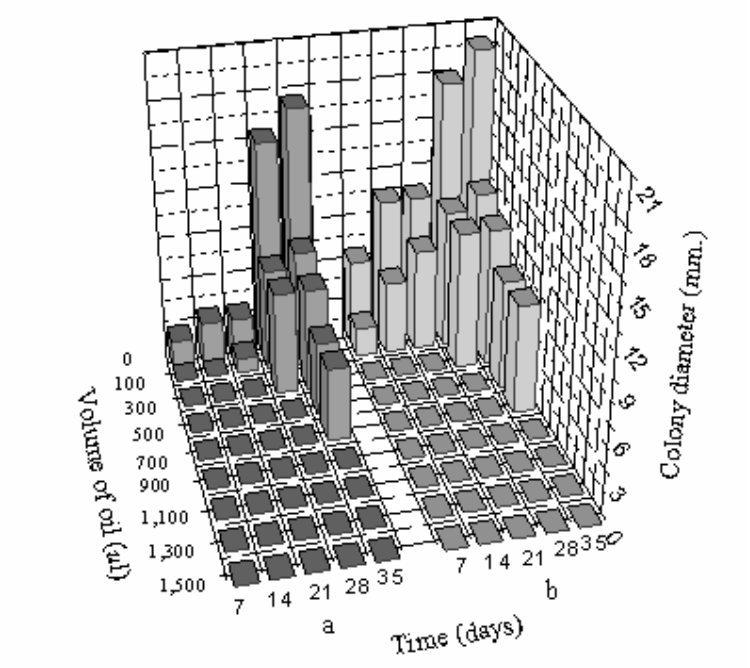


Figure 11 Growth of *Aspergillus flavus* on (a) commercial fruitcake (CMC_2) and (b) Variety fruitcake mix (PMC_5) with modified atmosphere condition

2.4.3.2 Effectiveness of oils on fruitcake with normal air: Higher concentrations of cinnamon oil and clove oil were required to inhibit the growth of *Aspergilus flavus* under normal air conditions. Figure 12 shows clearly rapid growth of *Aspergillus flavus* in the normal air. Addition oil at 700 µl to 1,500 µl was required to inhibit growth of *Aspergillus flavus* with normal air condition.

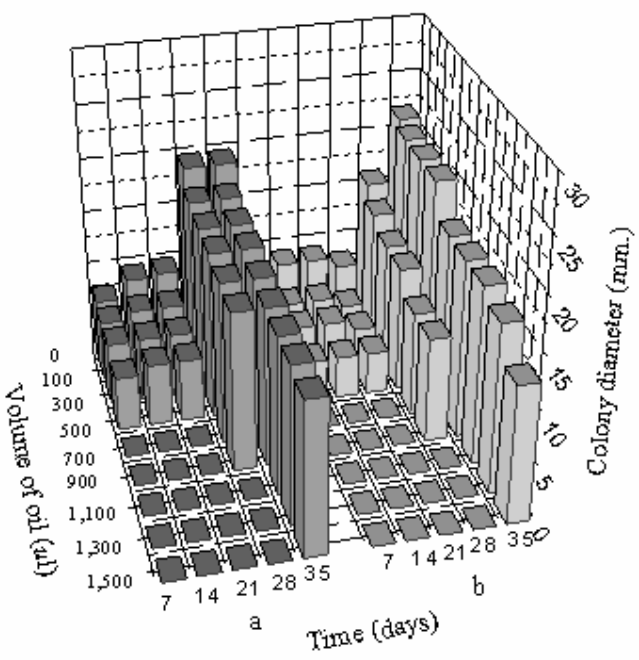


Figure 12 Growth of *Aspergillus flavus* on commercial fruitcake (a) and Variety fruitcake mix (b) with normal air condition.

It is clear from Figures 11 and 12 that a high level of CO_2 could enhance the effectiveness of volatile cinnamon and clove oils in inhibiting growth of *A. flavus*. Amount of oil required for inhibition was from 700 µl under normal air condition to 300 µl under modified atmosphere condition of 40% CO_2 to inhibit growth of fungi up to 21 days. However, since the volatiles of cinnamon and clove oils alone were capable of inhibiting growth of fungi in the normal air condition, another possibility to increase inhibition was to increase the amount of oil

vaporized into the headspace while still using the same amount of liquid oil. This could be achieved by using high temperature condition (see section 4).

According to the results above, in order to prevent the growth of *A. flavus* on the surface of fruitcake, 300 µL with high level of CO_2 or 700 µL with normal air at 30 °C were required. The amount of cinnamon oil and clove oil of of 300 µL and 700 µL at the ratio 5:1 were, therefore, used to perform the sensory test in the following section.

2.4.5 Results of sensory test are shown in Table 14. The odour and taste of cinnamon and clove oils were examined by Thai and western persons. The results showed that both odour and taste scores of fruitcake at 300 µl. and 700 µl. obtained from western consumers were higher than those obtained from Thai consumers. The average ratings for attributes ranged from “Neither like nor dislike” at 700 µl. and “Like slightly,” at 300 µl. On the other hand, the liking ratings for odour and taste from Thai person ranges from “Dislike slightly” to “Neither like nor dislike” both 300 µl. and 700 µl. However, the average score from 60 persons both Thai and Western were “Neither like nor dislike” but the average score of control was from “ Like slightly” to “Like moderately”. The sensory scores depended on the amount of cinnamon and clove oils added. The lower amount of oils are limited to use with the packaging.

It appears from Table 14 that western consumers tend to accept odour and taste of cinnamon and clove oil more than Thai consumers do. This could be because essential oil is commonly mixed into bakery products in western countries so the consumers have already used to the odour and the taste of essential oil.

Table 14 Mean hedonic scores of attributes of fruitcake at 300 μl and 700 μl by Western person and Thai person

Panellists	Attribute/sample	Control	300 μl.	700 μl.
Thai & Western	Odour	6.8±1.6[a]	5.4±1.8[b]	5.0±2.1[b]
(n=60)	Taste	6.8±1.6[a]	5.6±1.9[b]	5.2±2.1[b]
Western	Odour	6.5±1.3[a]	6.2±1.3[a]	5.6±2.1[b]
(n=30)	Taste	6.4±1.6[a]	6.1±1.6[a]	5.2 ±1.8[b]
Thai	Odour	6.8±1.7[a]	4.6±1.9[b]	4.4±1.9[b]
(n=30)	Taste	7.3±1.5[a]	5.2±2.1[b]	5.1±2.3[b]

[1]Mean within a row followed by the same letter are significantly different ($P \leq 0.05$) as determined by Fischer 's Least Significance Difference (LSD) mean separation test.

The Thai consumers did not like the cake with too much cinnamon and clove oil flavour. The amount of cinnamon oil and clove oil used possibly could be reduced by using high temperature condition (see section 4).

3 Developing the active packaging product for preservation of the selected IMF product with modified atmosphere condition.

3.1 Selection of plastic film, adsorbent material and synergistic action of inhibitory volatiles

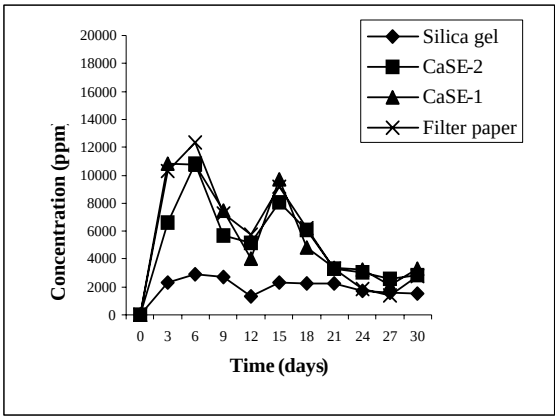
3.1.1 Selection of plastic film, adsorbent material: C

Composition of essential oil compounds of the fruitcake extract and volatile oil headspace are shown in Figures 13 to 16. The main compounds that were found in the headspace were *p*-cymene (1,343-14,790 ppm), linalool (115-9,248 ppm), cinnamaldehyde (142-2,297 ppm) and eugenol (44 – 1,047 ppm), and these showed dynamic behaviors. The concentration of p-cymene rapidly increased and

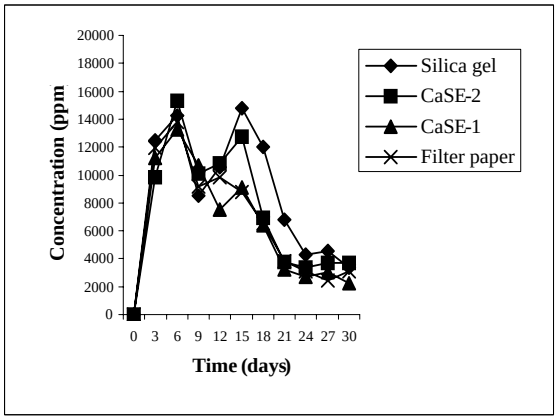
reached a maximum value by the first sixth day, followed by a gradual decline up to 30 days both in the headspace (Figures 13) and fruitcake (Figures 17).

The linalool concentration increased rapidly in the headspace from a low concentration to a peak concentration between 3 to 9days followed by a steady decrease in concentration (Figure 14). However linalool concentration in the cake increased slowly to a low concentration relative to the peak found in the headspace. (Figure18). Cinnamamaldehyde headspace concentration slowly increased up to 15 days followed by a very rapid increase in concentration (Figure 15). The concentration of cinamaldehyde in the cake extract increased slowly and there is evidence of a stable concentration although there is much noise in the data (Figure 19). Eugenol concentration in the headspace remained very low for18 days and then increased rapidly and steadily for the remainder of the month (Figure 16). Meanwhile in the cake the concentration increased steadily from 18 days to a much lower concentration relative to the headspace. Cinnamaldehyde and eugenol are main compounds in cinnamon and clove oils but p-cymene showed initial higher concentrations. Benjilali *et al*., 1984 reported the *p*-cymene was a lightest compounds and could gave higher concentration above essential oil headspace compared with heavier compounds e.g. phenolic compounds.

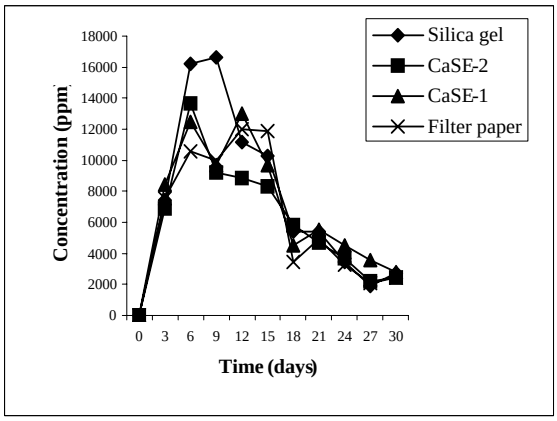
The evolution of *p*-cymene, linalool, cinnamaldehyde and eugenol released from five plastic sachets made of thyvex, PP, LLDPE, LDPE35, LDPE70 packed inside with various absorbent materials (silica get, CaSE-1, CaSE-2 and filter paper), in the headspace of plastic jar under MAP condition are shown in Figures 13 to16. PP and LLDPE films were found to favour highest concentrations of p-cymene, linalool, cinnamaldehyde and eugenol in the headspace than those created from other plastic films, but the PP film (20 Baht/kg) is cheaper than LLDPE film (50 Baht/kg) and is easily obtained in many places. For these reasons the PP films were selected to produce sachets in the next laboratory.



(a)

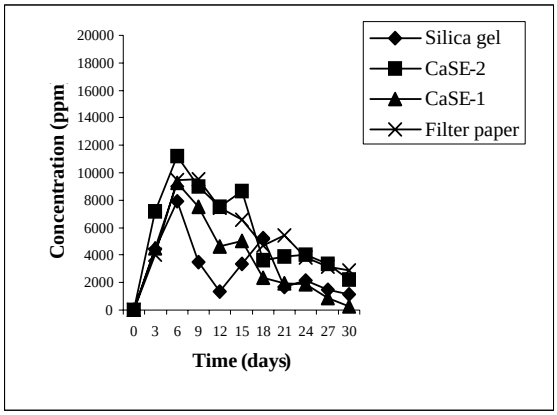


(b)

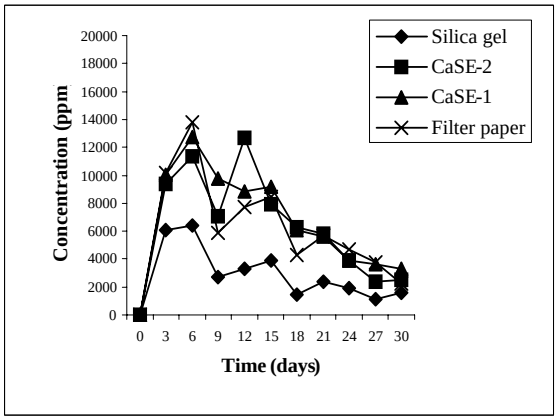


(c)

Figure13 Evolution of *p*-cymene concentration in the headspace released from 5 cm x 5 cm sachet of thyvex (a), LLDPE (b), PP (c), LDPE35 (d), LDPE70 (e) film packed inside with various absorbent materials

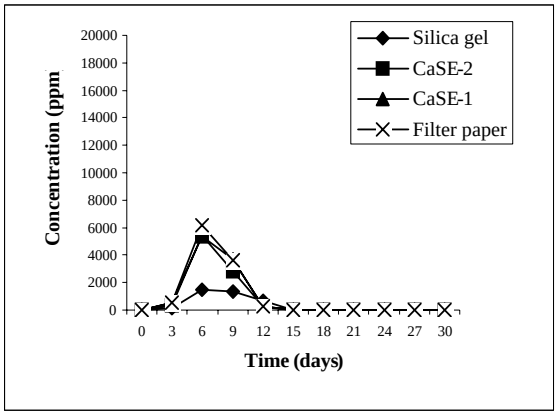


(d)

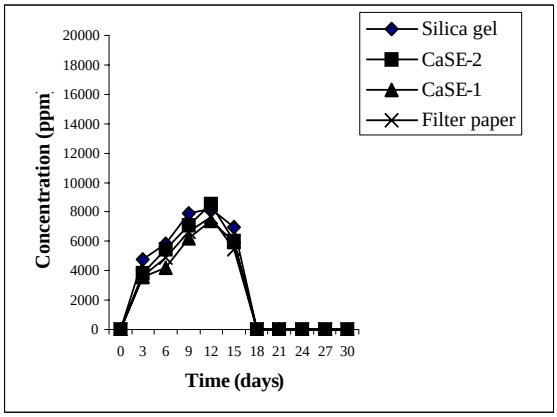


(e)

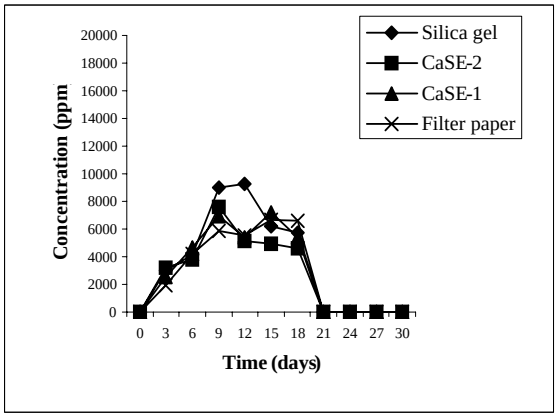
Figure 13 (Continued)



(a)

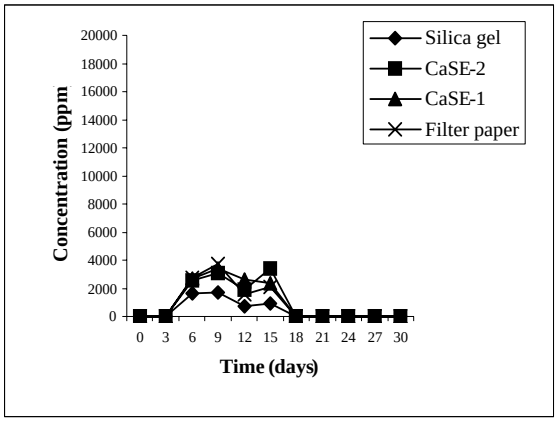


(b)

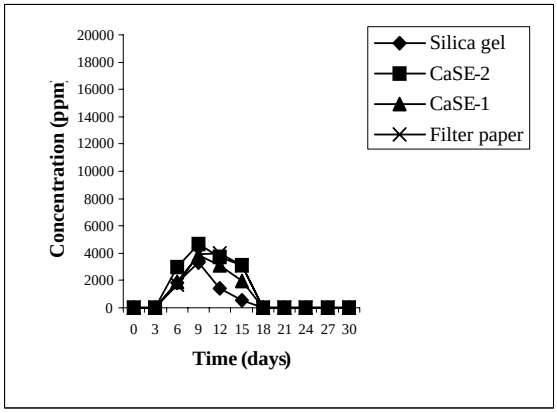


(c)

Figure14 Evolution of linalool concentration in the headspace released from 5 cm x 5 cm sachet of thyvex (a), LLDPE (b), PP (c), LDPE35 (d), LDPE70 (e) film packed inside with various absorbent materials

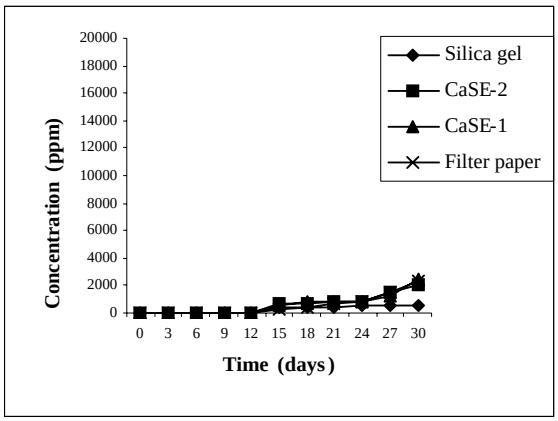


(d)

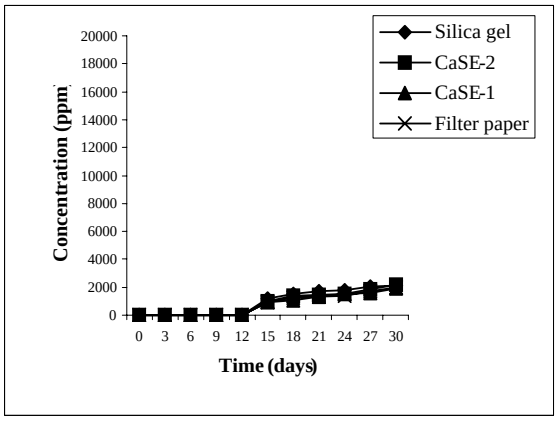


(e)

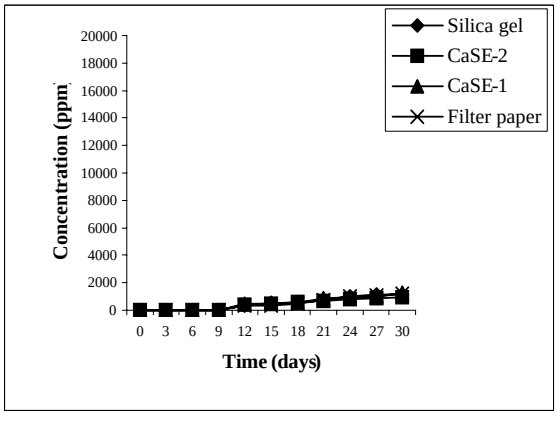
Figure 14 (Continued)



(a)

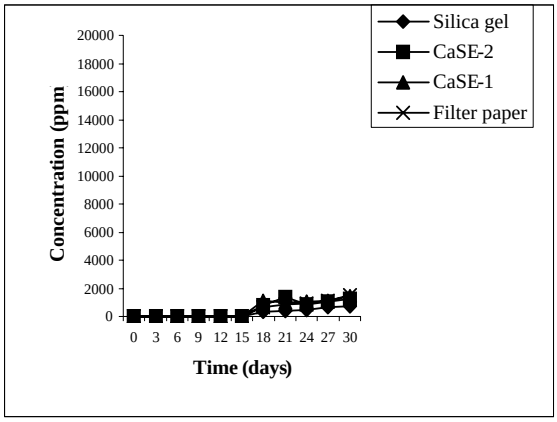


(b)

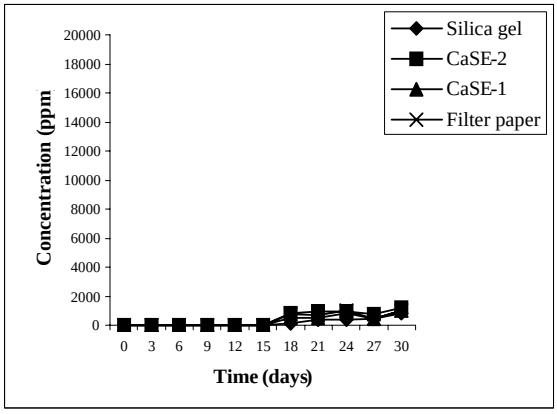


(c)

Figure15 Evolution of cinnamaldehyde concentration in the headspace released from 5 cm x5 cm sachet of thyvex (a), LLDPE (b), PP (c), LDPE35 (d), LDPE70 (e) film packed inside with various absorbent materials

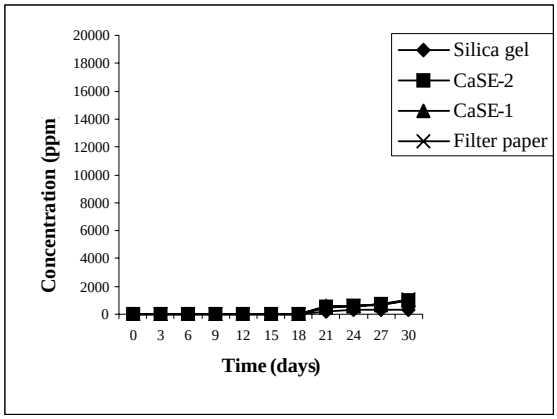


(d)

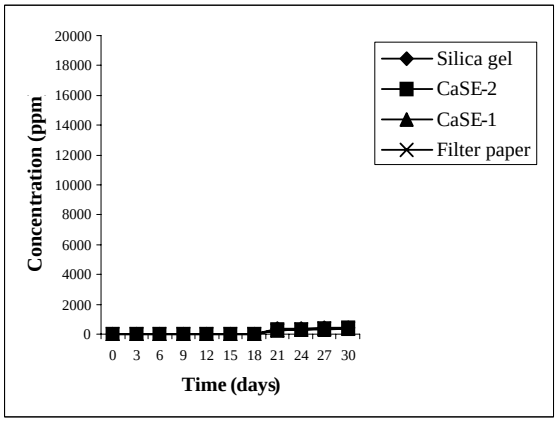


(e)

Figure 15 (Continued)



(a)



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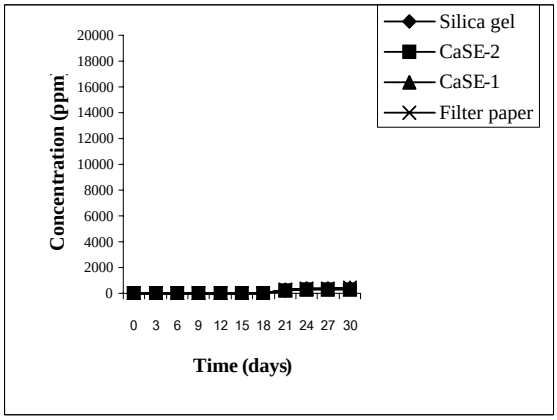
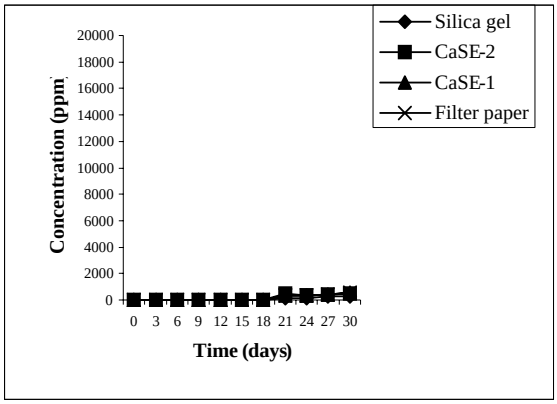
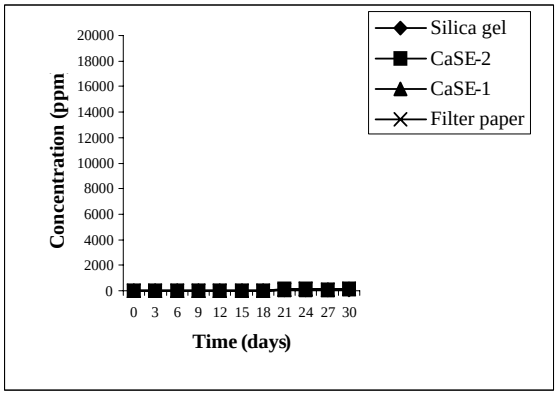


(c)

Figure16 Evolution of eugenol concentration in the headspace released from 5 cm x 5 cm sachet of thyvex (a), LLDPE (b), PP (c), LDPE35 (d), LDPE70 (e) film packed inside with various absorbent materials



(d)

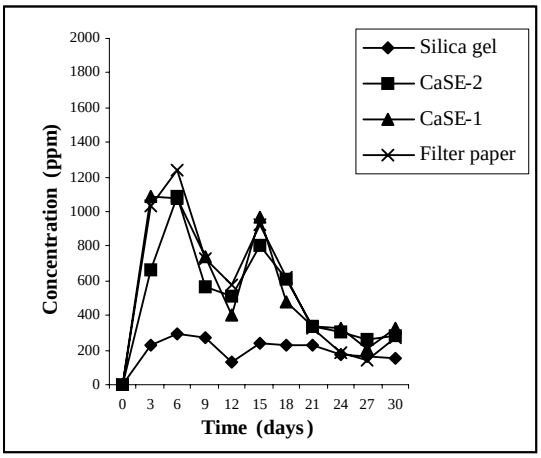


(e)

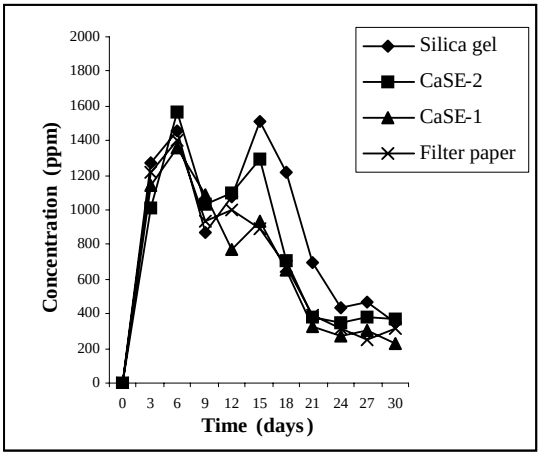
Figure 16 (Continued)

Absorbent materials i.e. CaSE-1, CaSE-2 and filter paper were capable to absorb liquid essential oil to be later released as volatile oil. CaSE-1 was selected to use in 3.2 because it was easy to pack in the plastic film. However, filter paper was selected to create volatile oil environment for the active packaging condition in the last objective because the cost of filter paper is cheaper than CaSE-1 and moreover, it was convenient for buying.

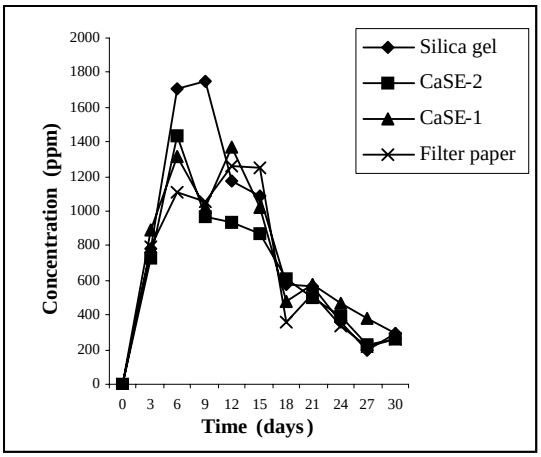
The evolution of *p*-cymene, linalool, cinnamaldehyde, and eugenol concentration in the fruitcakes after their release from 5 cm x 5 cm sachet of thyvex, LLDPE, PP, LDPE35, LDPE70 film packed inside with various absorbent materials are shown in Figures 17 to 21.



(a)

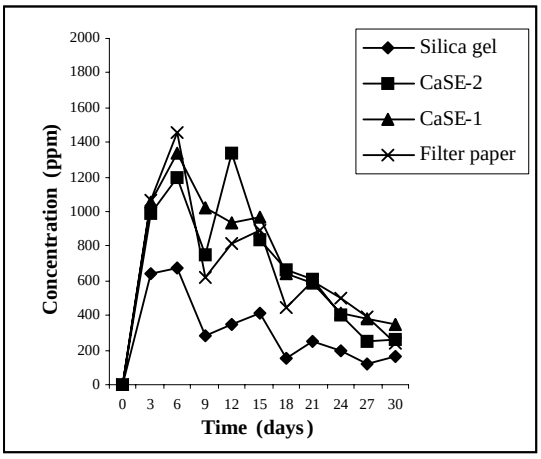


(b)

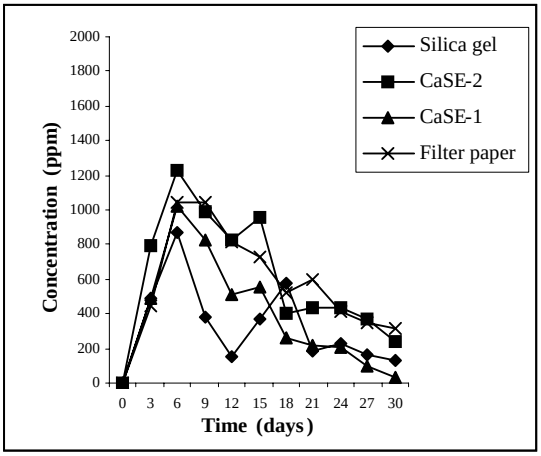


(c)

Figure17 Evolution of *p*-cymene concentration in the fruitcake released from 5 cm x 5 cm sachet of thyvex (a), LLDPE (b), PP (c), LDPE35 (d), LDPE70 (e) film packed inside with various absorbent materials

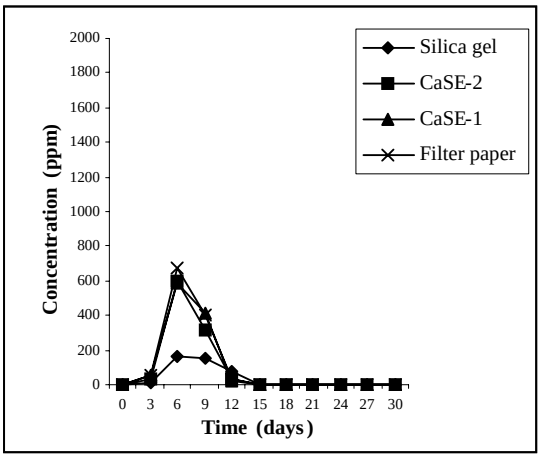


(d)

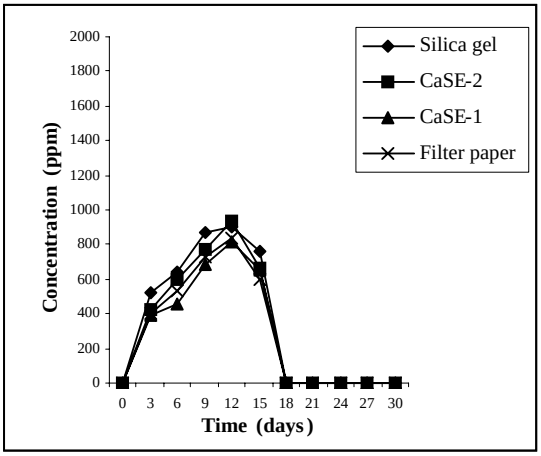


(e)

Figure 17 (Continued)



(a)



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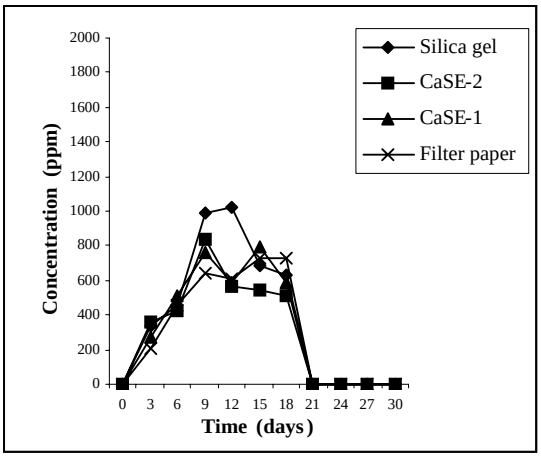
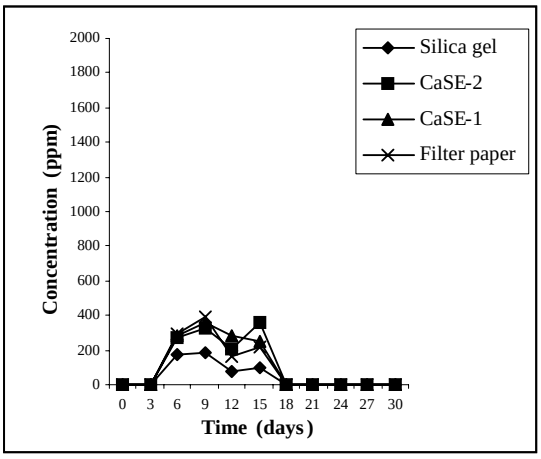
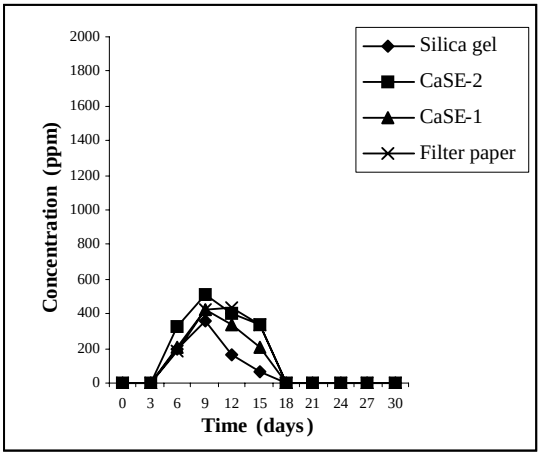


(c)

Figure18 Evolution of linalool concentration in the fruitcake released from 5 cm x 5 cm sachet of thyvex (a), LLDPE (b), PP (c), LDPE35 (d), LDPE70 (e) film packed inside with various absorbent materials

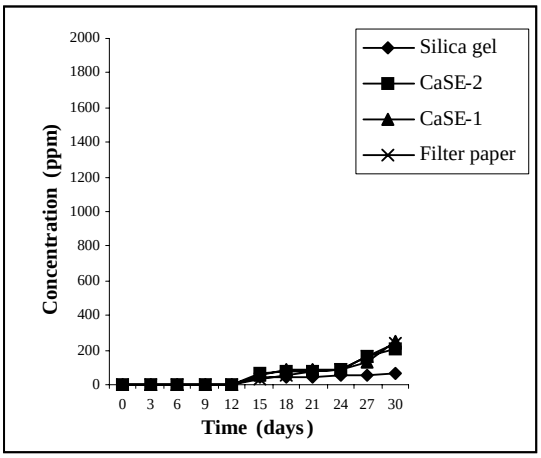


(d)

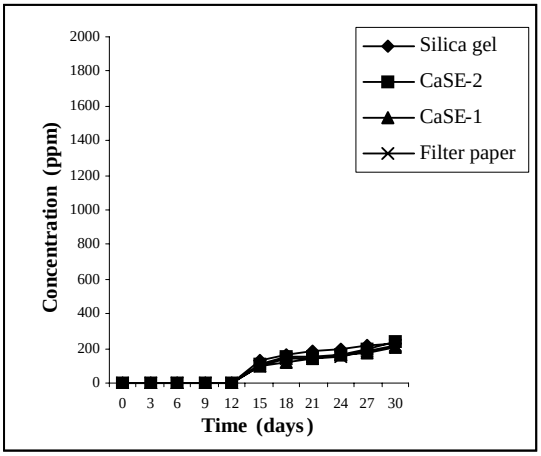


(e)

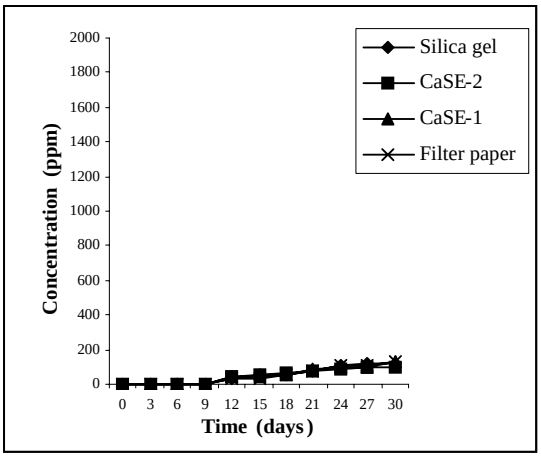
Figure 18 (Continued)



(a)

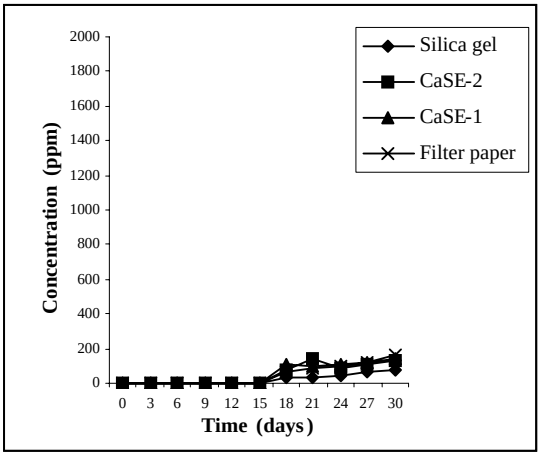


(b)

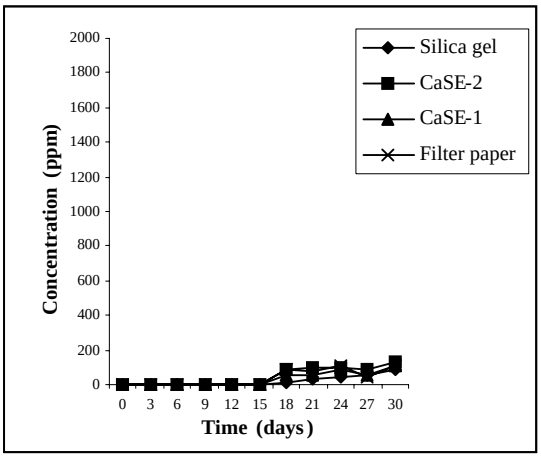


(c)

Figure19 Evolution of cinnamaldehyde concentration in the fruitcake released from 5 cm x5 cm sachet of thyvex (a), LLDPE (b), PP (c), LDPE35 (d), LDPE70 (e) film packed inside with various absorbent materials

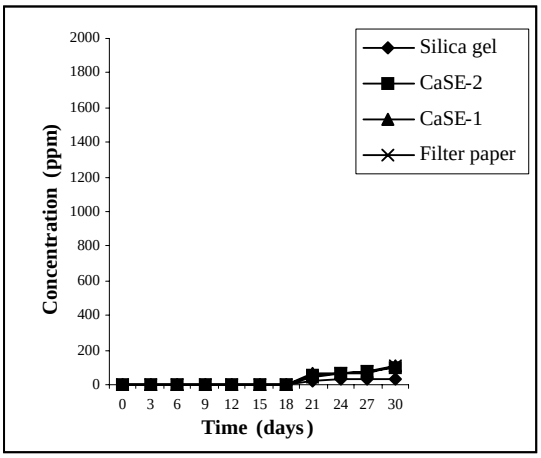


(d)

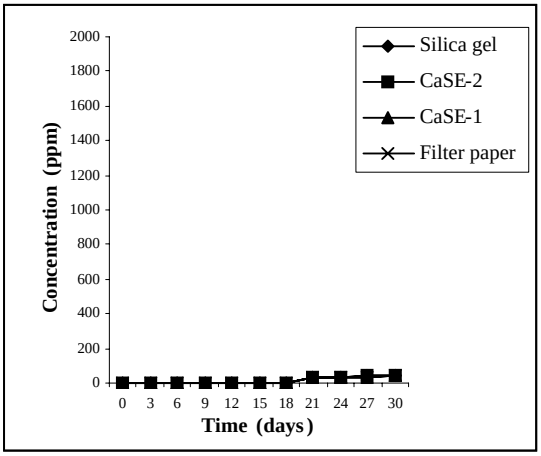


(e)

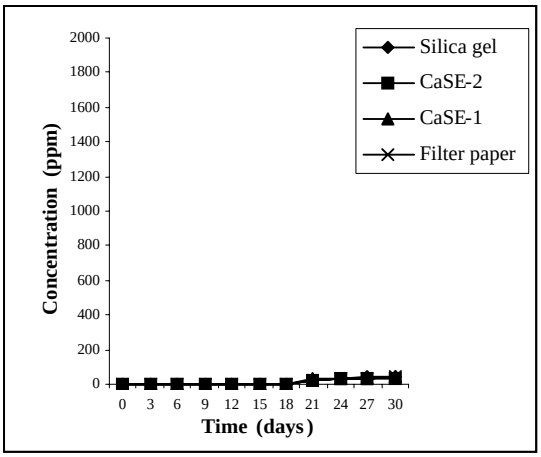
Figure 19 (Continued)



(a)

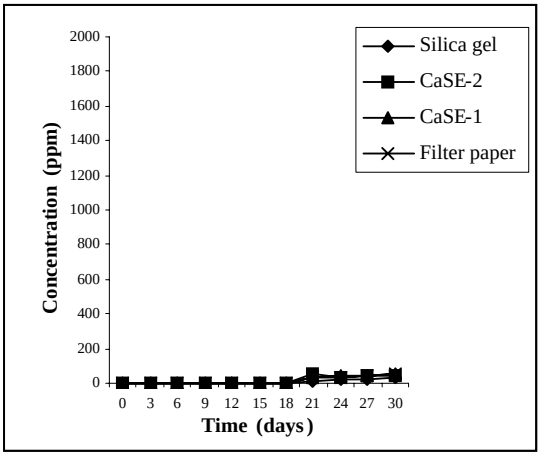


(b)



(c)

Figure 20 Evolution of eugenol concentration in the fruitcake released from 5 cm x 5 cm sachet of thyvex (a), LLDPE (b), PP (c), LDPE35 (d), LDPE70 (e) film packed inside with various absorbent materials



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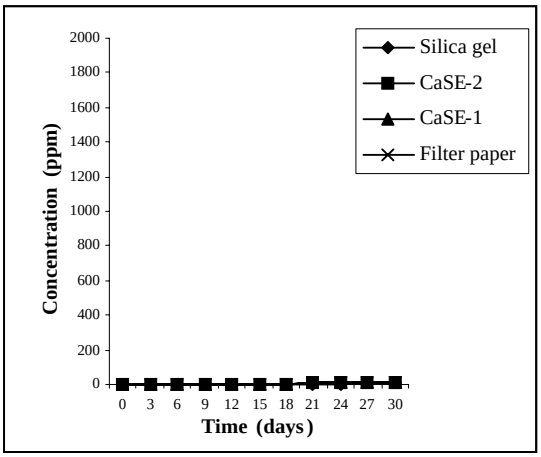

(e)

Figure 19 (Continued)

As can be seen from the result, the volatile compounds were adsorbed at the fruitcake surface at approximately 10% of the concentration of volatile compounds in the headspace. In addition, the amount of volatile compounds at the fruitcake surface was dependant on amount of volatile compound in the headspace.

From the results above, it can be seen that fruitcake could absorb oil of about 10% from the headspace. The relationship between concentration of oil

absorbed by the fruitcake (Y) and concentration of oil in the headspace (X) can be expressed by

$$Y = -0.580308 + 0.108933X - 0.000000254X^2 \text{ …….(2)}$$

with R-adjust = 0.9996. This equation was used to calculate the concentration of oil absorbed in fruitcake in section 4.

3.1.2 Synergistic action of inhibitory volatiles: The antifungal activity of cinnamaldehyde, eugenol, linalool and *p*-cymene and combinations of these compounds is summarized in Table 15. The MIC against *A. flavus* for each of cinnamaldehyde and eugenol was 300 ppm. No MIC was found for linalool or *p*-cymene added individually within the range tested (50 to 700 ppm). *p*-Cymene is commonly found in many essential oils at varying amounts depending on the type and source of the botanical, e.g. 6.5 % of thymol oil (Nickavar *et al*., 2005); 16.7 % of oregano oil (Marín *et al*., 2003). *p*-Cymene usually is not an effective antifungal agent when used alone but it strongly interacts with the cell membrane and when combined with another compound such as carvacrol, of which it is the precursor, it does exert a positive action (Ultee *et al*., 2002). Wang *et al*. (2005) also noted a much greater inhibition of wood decay fungi by cinnamaldehyde and eugenol than by linalool.

This is in agreement with the many reports that show cinnamaldehyde and eugenol are strong inhibitors for mould growth (e.g. Jayatilaka *et al*., 1995; Della *et al*., 1998;Nielsen and Rios 2000; Soliman and Badeaa 2002; Wang *et al*., 2005). The key active principle of essential oil volatiles is their hydrophobicity and it is increasingly clear that phenolic compounds with an hydroxyl (e.g. carvacrol) or aldehyde group (such as cinnamaldehyde) have good antifungal activity (Ultee *et al*. 2002, Burt 2004). Compounds having a conjugated double bond and a long CH chain outside the ring also possess strong antifungal activity (Wang, *et al*., 2005).

When food materials are exposed to antimicrobial agents in desorption (controlled release) active packaging systems, the active ingredients must

first volatilise according to their respective vapour-liquid equilibrium relationships, then be transported to and be adsorbed at the interface(s) where they act. This could be via direct adsorption by the microbial cell wall, or by adsorption into the food matrix in contact with the spoilage organism. The food matrix will in any case become a secondary reservoir for the active ingredients and volatiles may also be lost from the package to the external environment depending on their solubility in, and diffusion through, the packaging material. The concentration profile of the active ingredient(s) in the both the gas and solid phases will therefore be time dependent. When mixtures essential oils are used, interactions between the active components must also be considered (Burt 2004).

In a recent paper (Matan *et al*., 2006) demonstrated that mixtures of cinnamon and clove oils are effective against a range of fungal and bacterial food spoilage organisms. The main active components are considered to be *p*-cymene, linalool, cinnamaldehyde and eugenol and the time dependent release of these has been confirmed in a model active packaging system. Synergistic action of various oil components has previously been demonstrated by Ultee *et al*. (2002) and Burt, (2004). They showed that the combined compounds from essential oils could be more effective in inhibiting microorganisms than using single compounds alone. This work confirms that oil mixtures may be beneficial for offering increased shelf life and product safety when incorporated in food packaging systems.

In brief, the polypropylene film, filter paper and calcium silicate type 1 (CaSE-1) were found to release volatile oil into the headspace more effectively than thyvex, LLDPE, LDPE35 and LDPE 70 with silica gel and calcium silicate type 2 (CaSE-2). The results from the volatile headspace showed that major volatiles in the headspace above an inhibitory mixture of these oils were *p*-cymene, linalool, cinnamaldehyde and eugenol. Furthermore, the result from the experiment showed that 10% of compound in the headspace could be adsorbed into a surface of fruitcake inside the packaging.