3.2 Storage of premix fruitcake (PMC_5) and rice fruitcake (RF) with active packaging condition

Storage test of the PMC_5 and RF were investigated. Results of the PMC5 were used to develop a mathematical model describing growth of microorganism and results of the RF were used to verify the model developed.

3.2.1 Preparation of premix fruitcake and packaging

Premix fruitcake (PMC_5) was put into the 200 mm wide x 200 mm long high barrier bags (permeability of 4 cc/m^2/day, MVTR 11 g/m^2/day at 25 °C; Sealed Air, New Zealand). The small sachet (5 x5 cm. of polypropylene film) was made by adding 300 µL of a 5:1 mixture of cinnamon and clove oils into 0.8 g of calcium silicate and this was placed inside the high barrier bag. The bags were gas flushed with 40% CO_2, <0.05 O_2 with the balance being N_2 and sealed. The composition of gas was confirmed using a miniature infrared CO_2 transducer (Analytical Development Company, UK), with O_2 free N_2 as a carrier gas. The packaging was kept at 20 °C 68%RH and 30 °C, 75%RH for 1 month.

3.2.2 Preparation of rice fruitcake (RF) and packaging

Rice fruitcake was composed of rice flour 15.29 g, wheat flour 15.29 g, egg 24.47 g, butter 10 g, fine sugar 30 g, baking power 0.26 g, preserved egg plant 6.41 g, dried banana 5.77 g, sultana 5.42 g, and cashew nut 5 g. The ingredients were mixed using a beater combine on low speed and then mixed at top speed for 5 minutes. Then, the fruit was folded through the batter with the beater. Dough was placed into a foil lined tin and baked in an oven at 170 °C for 30 minutes. After cooling, the rice fruitcakes were put into a high barrier bag. The high barrier bag was 200 mm wide x 200 mm long, with permeability of 4 cc/m^2/day, MVTR 11 g/m^2/day at 25 °C (Sealed Air, New Zealand). The small sachet (5 x5 cm. of polypropylene film) was made by adding 300 µL of a 5:1 mixture of cinnamon and clove oils into

0.8 g of calcium silicate and was placed inside a high barrier bag. The bags were gas flushed with 40% CO_2, <0.05 O_2 with the balance being N_2 and sealed. The composition of gas was confirmed using a miniature infrared CO_2 transducer (Analytical Development Company, UK), with O_2 free N_2 as a carrier gas. The packaging was kept at 20 °C 68%RH and 30 °C, 75%RH for 1 month.

3.2.3 Total bacteria, total yeast and mould

Total bacteria, yeast and mould were analyzed according to Association of Official Analytical Chemists (AOAC, 1995) procedure numbers 933.11 and 2002.11, respectively.

3.2.4 Moisture content and pH

Moisture content and pH method were described by Baik, *et al*. (2000).

3.2.5 Water activity

Water activity was measured using a CX2, AquaLab (Decagon Devices, Inc. USA).

3.2.6 Texture analyzer

Texture was measured using a texture analyser (TA-XT plus, 1 kg model: Stable Micro Systems, Godalming, Surrey, UK). Rice fruitcake was cut into 3 x 3 x 3 cm. A double cycle was programmed and the texture profile was determined using Xrad software (Stable Micro systems). A plunger (5 mm dia) penetrated the samples by 1 cm at a cross head speed of 30 cm/min.

3.2.7 Fruitcake extract and GC, GC/MS conditions

The volatile components of essential oil taken up in PMC_5 and RF were extracted using ethyl acetate. The method to extract fruitcake was explained by Friedman *et al* (2000). GC analysis was carried out on a GC 6000 VEGA Series2 (Carlo Erba Instruments) gas chromatograph equipped with a FID and a ZB-5 (Phenomenex®NZ, Ltd, Newzealand) capillary column (30mx0.25mmx0.25 μm). The oven temperature was held at 60 °C for 0.50 min then increased at a rate of 40 °C/min to 150 °C and then increased at a rate of 2 °C/min to 260 °C. Other operating conditions were as follows: carrier gas, N_2 with a flow rate of 1 ml/min; injector temperature, 250 °C; detector temperature, 260 °C; split ratio, 50:1. Rice fruitcake extract (1 μl) was then injected into the gas chromatograph using a10 μl Hamilton syringe (Hamilton Gastight®, USA). The samples were injected into the GC every 7 days until 1 month.

GC/MS analysis was conducted using a Shimadzu QP-5050A equipped. The mass range was from 10-900m/z at 6000m/z mass spectrometer and a DB-5 (J&W Scientific, USA) column (30mx0.25mmx0.25 μm) was used. Carrier gas was used H_2 with a flow rate of 1 ml/min. The operating conditions were the same conditions as described above. References standards were purchased from Sigma-Aldrich (Australia) for developing the standard curve.

3.2.8 Sensory evaluation

Untrained panelists were used for sensory testing. Seventy-one consumers (aged between 20-40) selected from students and staff at the Massey University, Palmerston North, New Zealand were participated in the study. Evaluation was conducted in taste panel booths.

One piece of the PMC_5 and RF, cut into 5x5 cm, was presented in a small size of high barrier bag, 200 mm wide x 200 mm long, and was evaluated by

consumer by liking ratings on the nine-point hedonic scale. At day 0, the consumers were asked to evaluate control and three treated samples packed with 0, 300, 700 µl of cinnamon oil and clove oil at ratio 5:1 in the order they were presented to them. The liking ratings used the standard nine-pointed hedonic scale “dislike extremely” (scored as 1) to “like extremely” (scored as 9). The same premix fruitcake samples were stored at 20°C, 68%RH and 30°C, 75 %RH for 21 days and were evaluated again by the consumers on day 21 using the same evaluation protocol. The control at 21 days was baked fresh to compare with the fruitcake in storage.

3.2.9 Data Analysis

The data obtained from quality evaluation of premix fruitcake during storage were analyzed for statistical significance using the Statistical Analysis System (SAS V8, 2001) at Massey University, Palmerston North, New Zealand.

3.3 Growth modeling of microorganisms

3.3.1. Cultures

Aspergillus flavus used in this study was isolated from fruitcake. Codes refer to strains held in the culture collection of The Institute of Food, Nutrition and Human Health (IFNHH) of the Massey University, Plamerston North, New Zealand.

3.3.2 Preparation of spore suspensions

Spores was obtained from mycelium grown on MEA medium at 30 °C, for 7 days, collected by flooding about 5 ml at the surface of the plates with sterile saline solution (NaCl, 8.5 g/l water) containing Tween 80 (0.1% v/v). After counting the spores, the solution was standardized. Mould growth was observed after inoculation of $1x\ 10^{6}$- 10^{7}at the center of the plates (10 µl of a standardized suspension $1x\ 10^{6}$- 10^{7}spores ml^{-1})

3.3.3 Preparation and inoculation of the fruitcake

Fruitcake (PMC_5) was used in this experiment. The fruitcake was cut into 5 cm x 5 cm square, placed into 9-cm sterile plastic plates containing water-glycerol agar (1.5% agar and different amount of glycerol depending on the desired water activity) at a_w=0.75, 0.80 and 0.85 and then sealed with Para film. Fruitcake was allowed to equilibrate at 20, 25, 30, 37 °C for 1 week. Finally, water activity of the cake was measured using a water activity meter (CX2, AquaLab).

3.3.4 Growth of *Aspergillus flavus* on fruitcake with MAP conditions

Aspergillus flavus was inoculated on the surface of fruitcake (PMC_5) and this was placed it into the pouch bags (Cryovac, Thailand). The pouch is 200 mm wide x 200 mm long, with permeability of 4 cc/m^2/day, MVTR 11 g/m^2/day at 25 ^{0}C. Different volumes (100, 300, 500, and 700 µL) of a 5:1 mixture of cinnamon and clove oils were absorbed into Whatman paper placed inside of a pouch bag along with the inoculated plates. The bags were gas flushed with 40% CO_2, <0.05 O_2 with the balance being N_2 and sealed. The composition of gas was confirmed using a miniature infra-red CO_2 transducer (Analytical Development Company, UK), with O_2 free N_2 as a carrier gas. The *Aspergillus flavus* were incubated at 20 ^{0}C, 25 ^{0}C, 30^{0}C, and 37^{0}C for 42 days. The colony diameter was recorded in millimeters and the mean of six colony measurements was used for data analysis. The control was done using filter paper without cinnamon oil and clove oil. The experimental variables and levels are shown in Table 7.

Table 7 Full factorial experimental designs for growth of *Aspergillus flavus*

Variable	levels
Temperature (^{0}C)	20, 25, 30, 37
Time (days)	7, 14, 21, 28, 35, 42
Volume of essential oil (µl)	0, 100, 300, 500, 700
Water activity (Aw)	0.75, 0.80, 0.85

3.3.5 The modeling analyses

The data from 5.2.4 were used to create the model by using a (4x6x5x3) full factorial design. The variables and their levels selected for the study of PGML synthesis were: volume of essential oil (0,100, 300, 500, 700 µl), time (7, 14, 21, 28, 35, 42 days), temperature (20, 25, 30, 37) and water activity (0.75, 0.80, 0.85). Statistical analyses: A surface response model was used to evaluate the effect of the water activity, temperature, volume of essential oil, and time on growth rate (mm.day^{-1}). Statistical analyses were computed using the Statistical Analysis System (SAS, 1999). All graph contour plots were done using Sigma plot, 2002.

3.4 Verification of modeling of microorganisms

3.4.1 Cultures

Aspergillus flavus used in this study was isolated from fruitcake. Codes refer to strains held in the culture collection of The Institute of Food, Nutrition and Human Health (IFNHH) of the Massey University, Plamerston North, New Zealand.

3.4.2 Preparation of spore suspensions

Spores was obtained from mycelium grown on MEA medium, at 30 °C, for 7 days, collected by flooding about 5 ml at the surface of the plates with sterile saline solution (NaCl, 8.5 g/l water) containing Tween 80 (0.1% v/v). After counting the spores, the solution was standardized. Mould growth was observed after inoculation of $1x\ 10^{6}$- 10^{7} at the center of the plates (10 µl of a standardized suspension $1x\ 10^{6}$- 10^{7} spores ml^{-1})

3.4.3 Preparation and inoculation of the rice fruitcake

Rice fruitcake was composed of Rice flour 15.29 g, wheat flour 15.29 g, egg 24.47 g, butter 10 g, fine sugar 30 g, baking power 0.26 g, preserved egg plant 6.41 g, dried banana 5.77 g, sultana 5.42 g, and cashew nut 5 g. The ingredients mixed using a beater combine on low speed and then mixed at top speed for 5 minutes. Then, the fruit was folded through batter with the beater. Dough was baked in an oven at 170 °C for 30 minutes. The water activity of rice fruitcake was measused by the CX2, AquaLab (Decagon Devices, Inc. USA).

3.4.4 Growth of *Aspergillus flavus* on rice fruitcake with MAP conditions

Aspergillus flavus inoculated on the surface of rice fruitcake and this was placed into the pouch bags (Cryovac, Thailand). The pouch is 200 mm wide x 200 mm long, with permeability of 4 $cc/m^2/day$, MVTR 11 $g/m^2/day$ at 25 °C. Different volumes (100, 300, and 400 µL) of a 5:1 mixture of cinnamon and clove oils were absorbed into Whatman paper placed inside of a pouch bag along with the inoculated plates. The bags were gas flushed with 40% CO_2, <0.05 O_2 with the balance being N_2 and sealed. The headspace gas analysis were measured by gas analyzer a miniature infrared CO_2 transducer (Analytical Development Company, UK), with O_2 free N_2 as a carrier gas. The *Aspergillus flavus* were incubated at 30°C, and 37 °C for 42 days. The colony diameter was recorded in millimeters and the

mean of six colony measurements was used for data analysis. The control was done using filter paper without cinnamon oil and clove oil

3.4.5 Statistical analyses

Statistical analyses were made using the Statistical Analysis System (SAS, 1999). All graph contour plots were done using Sigma plot, 2002.

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

4.1 Temperature and volatile compound with normal air

4.1.1 Dessicator preparation

Sachets were made by adding 50, 100, 200 and 300 µl of cinnamon oil and clove oil mixed in the volume ratio 5:1 to filter paper No 6 (Whatman International Ltd, England) and sealing this within a 5x 5 cm. polypropylene film (Naraipak Co., Ltd, Thailand). One sachet was placed into 800 ml desiccators. The desiccators were placed in an incubator at 30, 40, 50 and 60 °C. A 15 cm glass line was fixed on the top of the desiccators and 1 mL gas samples were removed for analysis using a gas-tight syringe (Hamilton Gastight®, USA) at 1, 10, 20 and 30 mins via a rubber septum fitted to the lid.

4.1.2 GC analysis

GC analysis was carried out on a 3380 GC (Varian, Inc, USA) gas chromatograph equipped with a FID and a DB-5 (J&W Scientific, USA) capillary column (30mx0.25mmx0.25 µm). The oven temperature was held at 60 °C for 0.50 min then programmed at 40 °C/min to 150 °C, and was subsequently next set at 2 ^{0}C/min to 260 °C. Other operating conditions were as follows: carrier gas, N_2 at a

flow rate of 1 ml/min; injector temperature, 250 °C; detector temperature, 260 °C; split ratio, 50:1. Standard curves were separately prepared for cinnamaldehyde (Merk Co.,LTD, Thailand)

4.2 Cinnamaldehyde headspace modeling

4.2.1 Experimental design

A 3-level-4-factor fractional factorial design was employed in this study, requiring 27 experiments (Table 7). The variables and their levels selected for the study of essential oil were: temperature (40-80 °C), time (10-50 mins), Volume of oils (50-700 μl), volume of desiccators (800-3,800) ml. Table.8 shows the independent factors (x_i), levels and experimental design.

4.2.2 Controlled-release sachets

Controlled-release sachets were made by adding 100μl, 300 μl and 700 μl of cinnamon oil and clove oil mixed in the volume ratio 5:1 to 5x5 cm filter paper and sealing this within a 5x5 polypropylene film (Naraipak Co., Ltd, Thailand). One sachet was placed into 800, 2,200 and 3,800 ml desiccators. The desiccators were placed in an incubator at 40, 60 and 80 °C. A 15 cm glass line was fixed on the top of the desiccators and 1 mL gas samples were removed for analysis using a gas-tight syringe (Hamilton Gastight®, USA) at varying times via a rubber septum fitted to the lid.

4.2.3 GC analysis

GC analysis was carried out on a 3380 GC (Varian, Inc, USA) gas chromatograph equipped with a FID and a DB-5 (J&W Scientific, USA) capillary column (30mx0.25mmx0.25 μm). The oven temperature was held at 60 °C for 0.50 min then programmed at 40 °C/min to 150 °C, and was subsequently next set at 2

°C/min to 260 °C. Other operating conditions were as follows: carrier gas, N_2 at a flow rate of 1 ml/min; injector temperature, 250 °C; detector temperature, 260 °C; split ratio, 50:1. Standard curves were separately prepared for cinnamaldehyde (Merk Co.,LTD, Thailand)

4.2.4 Statistical analysis

The experimental data (Table 8) were analyzed by a response surface regression (RSREG) procedure to fit the following second-order polynomial equation (SAS, 1990)

$$Y = \beta_0 + \sum_{j=1}^{4} \beta_j X_j + \sum_{j=1}^{4} \beta_{jj} X_j^2 + \sum_{i<j} \beta_{ij} X_i X_j \qquad (1)$$

Where Y is predicted response (concentration of cinnamaldehyde in the headspace); β_{k0} is the intercept coefficient, β_{ki} are the linear terms, β_{kii} are the squared term, and β_{kij} are the interaction terms, and x_i and x_j represent the uncoded independent variables. For each experimental factor the variance was partitioned into components, linear quadratic and interaction, in order to assess the adequacy adequacy of the second order polynomial function and the relative importance or significance of the terms.

Three-dimensional response surfaces and contour plots were used for facilitating a straightforward examination of the influence of experimental variables on the responses. The individual response surface and the contour plots were created by holding two of the four variables constant at their center points. The coefficients of the models for the three responses were estimated with multiple regression analysis. The fit of the models was judged from their coefficients of correlation and determination. The adequacy of each model was checked with the analysis of variance (ANOVA) using F-test. The significance of the equation parameters for each response was assessed by the p-value. The significance test of