CHAPTER 6

Development of multi-core microencapsulation (II): Optimization of gelatin and gum arabic for *Michelia alba* D.C. flavor powder

Abstract

This research aimed to optimize the multi-core encapsulated flavor powder (MEFP) using gelatin (3–7% w/v), gum arabic (3–7% w/v), pandan flavor (5–10% w/w of gelatin-gum arabic solid), and *Michelia alba* D.C. (MAD) flavor powder as the core material at 2.5% w/w. The gelatin and gum arabic (GGA) system was employed using response surface methodology (RSM). The optimized formula of the MEFP in the GGA system was 3.00% w/v gelatin, 3.73% w/v gum arabic, and 5.26% w/v pandan flavor. This ratio of GGA system provided high yield recovery (46.45%) and high encapsulation efficiency (70.04%) with low moisture content (3.12%) and low water activity (0.165). The released pandan flavor content from the MEFP was 394.92 μg/ml. The MAD flavors from the MEFP in simulated artificial saliva fluid (SSF) demonstrated the release rate of linalool at the slowest release (1.23 min^{-1}), followed by verbenone (0.51 min^{-1}) and 2-methyl butanoic acid (0.23 min^{-1}). In conclusion, the optimized MEFP can retain the pandan flavor within the multi-core microcapsule, resulting in a slight increase in the release of pandan flavor in high moisture and temperature conditions within 40 min. Finally, the MAD flavor powder inside the microcapsules was retained within the product, and it can be released through SSF, exhibiting excellent multi-core microencapsulation of both the pandan flavor and the MAD flavor powder.

Keywords: optimization, multi-core encapsulation, controlled release, pandan, *Michelia alba* D.C.

Publication:

Samakradhamrongthai R., Thakeow, P., Kopermsub, P., and Utama-ang N. 2015. Development of multi-core microencapsulation (II): Optimization of gelatin and gum arabic for *Michelia alba* D.C. flavor powder. *LWT-Food Science and Technology,* (Submitted and Reviewing)

6.1 Introduction

Flavor engaged an important role in consumer satisfaction and influences toward food consumption (Teixeira, Andrade, Farina, & Rocha-Leão, 2004). Flavor and aroma are applied on food, beverages and sweets in many processed. Even though, there are increasing interests on stability of flavor and aroma because of its relationship with quality and acceptability, however, it still has limitation and difficulty to control. (Lubbers, Landy, & Voilley, 1998). The encapsulation system plays a dominant part and is one of the most efficient ways to convey flavor into product and can keep most of the specific properties until the product has delivered to consumer (Venskutonis, 1997; Lastwiley, 2007). In the last decades, the encapsulation flavors have become very appealing process and the encapsulation of flavor ingredients is one of the most consideration processes in the food industry. The main purpose of microencapsulation is to entrap sensitive ingredients, such as volatile and unstable flavors into carriers increasing their protection, reduce evaporation, boosted easier handling. In addition, it can be applied for controlling the release of flavors during food processing and storage. (Gouin, 2004; Reineccius, 2006).

Controlled-release can be defined as a system that active ingredients are made presentable at a desired place and time at the specific rate (Pothakamury & Barbosa-Canovas, 1995). Researchers have been studied to find a better understanding of effects that influence the releasing of active ingredients from complex matrix as these represents an selected target in many applications (Guichard, 2000). The complex systems of encapsulated volatile compounds and its releasing depends on variation of dependent processes such as type and geometry of the particle, diffusion of volatile compound through the matrixes, degradation of matrix material, and transfer from

matrix to environment (Pothakamury & Barbosa-Canovas, 1995). There are potential product applications for the controlled release from nanosphere or microsphere system, for example, baked goods, refrigerated/frozen dough, microwaveable entrees, confectionery, chewing gum, and steamed desserts (Shefer, 2012).

There were many researches involving complex coacervation in GGA system to entrap active ingredients. Those materials were chosen because of desirable properties which are complex-forming, heat-resistance, and moisture-resistance. Microcapsules from the coacervation provides excellent heat resistance and moisture-resistance property with toleration of high-temperature and high-moisture dispersing medium (Graf & Soper, 1996). The effect of dispersing medium from gelatin/gum Arabic on the release of coacervate microcapsules was very significant (Chang, Kimura, Yamamoto, Nobe, & Dobashi, 2003: Prata, Menut, Leydet, Trigo, & Grosso, 2008). To develop a microcapsule system that satisfies consumer requirements, complex coacervation microcapsule was considered within GGA system (Burgess & Singh, 1993; Singh &Burgess, 1989; de Jong & Hoskam, 1942). The GGA system was investigated because of its potential in high water solubility, biocompatibility and low cost.

Gelatin is a well subjugated natural protein with biomedical properties for example biodegradability, biocompatibility, non-immunogenicity and safety. It is vastly applied in pharmaceutical and food industry (Sarika & James, 2015). Silva, Fávaro-Trindade, Rocha, & Thomazini (2012) encapsulated lycopene by complex coacervation using gelatin and pectin as the encapsulants which did not improve the stability of the pigment. Shu, Yu, Zhao, & Liu (2006) and Rocha, Fávaro-Trindade, & Grosso (2012) microencapsulated lycopene by spray drying using gelatin/sucrose and modified starches together which showed that the stability of microencapsulated was significantly higher than individual wall material. Gum arabic also selected to improve poor mechanical properties and increase strength and aqueous stability of only gelatin system using cross-linking. These plant polysaccharide had complex branched structure with rhamnose, galactose and glucuronic acid residues which provided back bone and side chain consisted of 1,3 linked β-D-galactopyranosyl. Avadi *et al*. (2010) developed gum arabic and chitosan nanoparticle system for oral delivery of insulin. The release model

of gum arabic microparticles were investigated with vettiver essential oil and camphor oil as models.

Yeo, Bellas, Firestone, Langer, & Kohane (2005) studied flavor encapsulation using GGA system for thermally sensitive controlled release of flavor compounds. The results showed that GGA effectively encapsulate flavor compounds. The morphology of particle was depended on the homogenization rate and concentrations of GGA solution. The higher homogenization rate, the smaller particle size and the higher encapsulated of flavor. The effect of temperature toward oil release was investigate using heat responsive kinetic. The temperature was set at 100°C and 200°C. The higher temperature effect oil release more than lower temperature but there was not statistically significant. However, the researches about release of coacervate microcapsules in high-temperature medium are still rarely investigated. Blending wall material with specific properties like heat-resistance, moisture-resistance, and wide range of pH usage promote coacervation with inclusion encapsulation in baking, frying, microwaving and heat-involved with high moisture processing (Dong *et al*., 2011). The morphology and release profile of microcapsules encapsulating peppermint oil by complex coacervation from GGA was investigated by Dong *et al*. (2011). The results showed that suitable ratio of gelatin/gum arabic was 2:1 which provide spherical shape and peppermint oil was concentrated in the center of matrix. Comunian *et al*. (2013) also suggested that microcapsules from GGA preparation to entrap ascorbic acid showed spherical, multinucleate with slightly soluble and hygroscopic. The encapsulation efficiency was approximately 98%. Moreover, Alvim & Grosso (2010) investigated release profile of paprika oleoresin encapsulated which showed the release of 65.6% of oleoresin after 120 min in anhydrous ethanol. Cho, Shim, & Park (2003) evaluated the release of fish oil in microparticles with wall materials of soy protein isolate crosslinked with tranglutaminase. The release profile for the oil was slow in the first 60 min increasing significantly from there until reaching total release after 240 min.

In conclusion, complexation coacervation was applied together to encapsulate flavor and aroma in form of multi-core encapsulation to serve as an efficient platform for controlled release of aroma through production processes and oral cavity

mechanisms. The optimized encapsulating condition was determined by RSM using central composite design (CCD). The effects of gelatin, gum arabic and pandan flavor on the yield recovery, moisture content, water activity, color value (L^*, a^*, b^*), solubility and encapsulation efficiency were assessed. The characteristics of microencapsulated flavor powder were investigated including morphology, glass transition temperature and crystallinity. The aroma release from the multi-core encapsulated MAD flavor powder in high moisture and temperature condition were analyzed then inner aromas were analyzed in SSF was also investigated to suggest the kinetic aroma release model of from the multi-core encapsulated MAD flavor powder.

6.2 Materials and Methods

6.2.1 Materials

The MAD flavor powder was prepared from the MAD extract (15% w/w of dry starch) and Octenyl Succinic Anhydride starch (OSA starch) (963.20 g per 1000 ml of water) using spray drying process. The spray dryer (March Cool Industry Co., Ltd., Bangkok, Thailand) was operated at an inlet temperature of 150°C and outlet temperature of 50°C with blower speed at 50 rpm (Samakradhamrongthai & Utama-Ang, 2008). The wall materials for the multi-core encapsulation were gelatin and gum arabic which were purchased from Union Science Co., Ltd. (Chiang mai, Thailand). The pandan flavor (Winner Brand, Greathill, Co., Ltd., Bangkok, Thailand) was purchased from Yok intertrade Co., LTD. All standard chemicals (2-methyl butyric acid, (-)-linalool, and (1s)-verbenone) were purchased from Sigma-Aldrich Co., LLC. (MO, USA). The analyzed organic chemicals were of analytical grade.

6.2.2 Preparation of MEFP from gelatin and gum arabic infusing with pandan flavor

The experiment was set to find the optimal percentages of gelatin, gum arabic and the pandan flavor as wall materials with the core material for the multi-core encapsulation. The gelatin solution and the gum arabic solution were used as wall material with variation from 3–7% w/v. The pandan flavor was infused to gelatin and gum arabic solution with variation from 5–10% w/w of gelatin-gum arabic solid. The MAD flavor powder at 2.5% w/w was used as core materials (Alvim & Grosso, 2010). The RSM was employed to optimized content of gelatin (X_1), gum arabic (X_2) and the pandan flavor (X_3) on the multi-core encapsulation process in term of yield recovery, moisture content, water activity, solubility, encapsulation efficiency of the pandan flavor, encapsulation efficiency of the MAD flavor powder and the pandan aroma release profile in heated water. The five coded levels (-1.6818, -1, 0, 1, 1.6818) of three factors were incorporated in the CCD design with three center points leading to 17 experiments (Table 6.1). All experiments were carried out in triplicate. The quadratic polynomial regression model was assumed for predicted all responses.The mixtures were prepared according to a method described by Alvim & Grosso (2010), Zhang, Pan, & Chung (2011) and Butstraen & Salaün (2014) with modifications. The aqueous phase was prepared by dissolving gelatin and gum arabic separately in deionized water at 50°C while stirring for 30 min until the solution dissolved into homogenous mixture. The pandan flavor was infused into gelatin mixture. The MAD flavor powder was dispersed into gelatin mixture at 2.5% w/w under magnetic stirring condition (1000 rpm). The solution of gum arabic was then added into gelatin mixture to GGA system. The pH of GGA mixture was adjusted to 4.0±0.2 using 10% v/v acetic acid and then slowly cooled to 0°C to create complexes of the multi-core complexes in GGA system (Fig. 6.1).

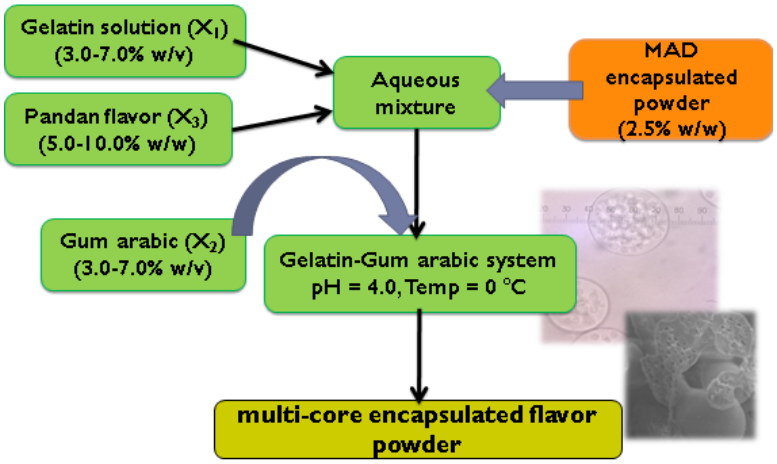


Fig. 6.1 The preparation of multi-core encapsulated flavor powder using gelatin and gum arabic infused with pandan flavor**.**

Table 6.1 Treatment variation of gelatin, gum arabic and pandan flavor using CCD

Treatment	**Coded**			**Actual**		
	X_1	**X_2**	**X_3**	**gelatin (%w/v)**	**gum arabic (%w/v)**	**pandan flavor (%w/w gelatin-gum arabic solid)**
1	-1	-1	-1	3.00	3.00	5.00
2	+1	+1	-1	7.00	3.00	5.00
3	-1	+1	-1	3.00	7.00	5.00
4	+1	+1	-1	7.00	7.00	5.00
5	-1	-1	+1	3.00	3.00	10.00
6	+1	-1	+1	7.00	3.00	10.00
7	-1	+1	+1	3.00	7.00	10.00
8	+1	+1	+1	7.00	7.00	10.00
9	-1.6818	0	0	1.64	5.00	7.50
10	+1.6818	0	0	8.36	5.00	7.50
11	0	-1.6818	0	5.00	1.64	7.50
12	0	+1.6818	0	5.00	8.36	7.50
13	0	0	-1.6818	5.00	5.00	3.30
14	0	0	+1.6818	5.00	5.00	11.70
15	0	0	0	5.00	5.00	7.50
16	0	0	0	5.00	5.00	7.50
17	0	0	0	5.00	5.00	7.50

Note: X_1 = Gelatin, X_2 = Gum arabic, X_3 = Pandan flavor

The mixture was stirred for another 15 min to allow a complete formation of multi-core complexes. The precipitated microspheres were washed twice by decanting with distilled water and collected by centrifugation at 5000 rpm for 5 min. (Universal 320R, Hettich, Germany). The microspheres were dehydrated using freeze dryer (Model 494830, Labconco, USA). The sample was frozen at -20°C immediately after preparation. The frozen sample was then dried in freeze dryer for 48 hr at -45°C under pressure of less than 0.120 mbar. The microcapsules obtained from freeze drying were directly weighed and stored in desiccator for further analysis. Yield recoveries from freeze drying have been calculated using Eq. (6.1).

$$\%Y = (\text{mass of collected dry solid (g)}/ \text{mass of solid in the feed (g)}) \times 100 \qquad (6.1)$$

6.2.3 Morphology of MEFP

The MEFP obtained from freeze drying were examined for their morphology using optical microscope (BX51M, Olympus, Corp., Japan) and scanning electron microscope (SEM, JSM5410-LV, JEOL, Japan). The samples were placed on the SEM stubs using a two-sided adhesive tape and subsequently coated with gold using an electrically conductive of 60 kV in a vacuum chamber. Photographs were taken at an excitation voltage of 10 kV (Ferreira, Rocha, & Coelho, 2007). The particle of collected microcapsules was also measured using Image Processing and Analysis in Java: ImageJ (National Institutes of Health, Maryland, USA) to define the size of MAD encapsulated flavor powder.

6.2.4 Release profile of pandan flavor from MEFP

Twenty ml of the MEFP was incubated in heated water bath (WB22, Memmert GmbH + Co.KG, Germany) at 98±2°C. Incubation was set in glass vial sealed by a screw cap covered with an aluminum foil filled with distilled water under continues stirring with shaker at 12 rpm (SV 1422, Memmert GmbH + Co.KG, Germany). The 5

µl of evaporated pandan flavor from static head space was taken for analysis at 0, 5, 7.5, 12.5, 15 min toward 45 min. The extent of aroma released from static head space following the incubation was measured from the reaction medium. The gas chromatograph analysis (GC) was performed on gas chromatography (GC-2010, Shimadzu, Corp., Japan). Column and carrier gas use for both analyses were DB-1column (30 x 0.25 mm ID and 0.25 µm film thickness) (Model 122-1032, Agilent Technologies, Inc., USA) and 1.0 ml/min. The oven temperature was held at 40°C for 3 min and increased to 250°C at 4°C/min and held for 5 min at 250°C (applied from Ades, Kesselman, Ungar, & Shimoni, 2012; Samakradhamrongthai, 2011). All data were recorded to create release profile. The maximum released pandan flavor content was considered at 15 min then taken to optimize the MEFP together with other responses. The pandan flavor content was calculated from standard calibration curves in term of µg/ml. The pandan release profile was presented between aroma release amount (µg/ml) and releasing time (min).

6.2.5 Physical properties of MEFP

Moisture content. Five grams of encapsulating powder were dried in hot air oven (FD 115, Serial 08-836864, Binder, Germany) for five hr at 105°C for five hr. Afterwards, samples were weighed and the moisture contents were calculated (AOAC, 2000, NO. 934.01). All samples were measured in triplication.

Water activity. One gram of encapsulating powder was analyzed with water activity analyzer (AquaLab LITE, DECAGON Devices Inc., USA). All samples were kept in sealed packages prior the analysis. The sample was poured in a analyzed plastic cup with cover before analyzed. All samples were measured in triplication.

Color measurement. The color was analyzed using Hunter LAB (Colorquest XE, Hunter Lab, USA). The light source was Illuminant D65. The CIELab color values were used with L^* (Lightness), a^* (negative value means green and positive value

means red), *b** (negative value means blue and positive value means yellow). All samples were measured in triplicate.

Solubility. The solubility of the MEFP was examined according to the method described in Fernandes *et al*. (2014). The 2.5 g of powder were dissolved in 250 ml of boil water in 600 ml beaker for 5 min. Aqueous solution was filtered all solution with dried and weighted on filter paper (Whatman™ No. 1, Buckinghamshire, UK). The filter paper was dried in hot air oven at 105°C for 24 hr. The solubility (%) was calculated as the percentage of dried supernatant in relation to the amount of powder. All samples were measured in triplicate.

6.2.6 Encapsulation efficiency (%EE)

Determination of surface extract content

Free core content refers to aroma that is associated on the outside of wall material. One gram of MEFP was dispersed in 50 ml dichloromethane and washed for 5 min. One µl of surface extract content was analyzed for surface content using gas chromatography flame ionization detector (GC-FID). The GC analysis condition was followed the method from 6.2.4.

Determination of total extract content in complexes

Total aroma content was determined by disassembly of the complex. One gram of MEFP was incubated with 50 ml dichloromethane for 15 min to ensure that matrix complexes were fully dissolved. The solution was cooled and filtered. One µl of total extract content was determined amount of extract content using GC-FID. The GC analysis condition was followed the method from 6.2.4.

Encapsulation efficiency of pandan content

The encapsulation efficiency (%EE) of the pandan content was obtained from the calculation of total pandan content and surface pandan content from equation (Eq. 6.2). The pandan content from total content and surface content were obtained from the calculated standard calibration curve in term of µg/ml. The pandan content was

presented between aroma release amount (µg/ml) at sampling time (min) (modified from Jafari, He, & Bhandari, 2007; Dong *et al*., 2011)

$$\%EE = \{[\text{total aroma content (µg/ml)} - \text{surface content (µg/ml)}]/\text{total aroma content (µg/ml)}\} \times 100 \quad (6.2)$$

6.2.7 Microstructural characterization of optimized MEFP

Morphology of optimized MEFP

The optimized MEFP obtained from freeze drying were examined using scanning electron microscope followed the method from 6.2.3.

Glass transition temperature (T_g) of optimized MEFP

The optimized MEFP was stored at 25% relative humidity in desiccator for 24 hr prior the glass transition temperature (T_g) measurement. The samples were weighed (5±0.2mg) in and aluminium pan and sealed. The measurement was conducted by differential scanning calorimeter (Diamond DSC, Perkin Elmer, Inc., OH, USA) using liquid nitrogen cooling system (Intracool 2P, TA instruments, NC, USA). The operating conditions were under nitrogen flow rate at 20 ml/min and temperature ramping from 20°C to 120°C at the rate of 10°C/min. A sealed empty aluminum pan was used as reference. All measurements were performed in triplicate (Chen, Zhong, Wen, McGillivray, & Quek, 2013).

X-Ray diffraction of optimized MEFP

The formation of the optimized MEFP was verified using X-ray diffraction (XRD). The experiment was carried out by a Miniflex II Desktop X-ray Diffractometer equipped with a graphite crystal monochromator (Miniflex II, Rigaku Corporation, Japan) providing the Cu Kα radiation (λ = 0.154 nm). The diffractograms were obtained under the condition of 40 kV and 30 mA with scanning angle 2θ set from 5 - 30° with a scanning rate of 0.02°/sec. The crystalline nature of the complexes was determined by the position of the X-ray diffraction peaks (Bhosale & Singhal, 2007).

6.2.8 Determination of aroma and flavor of optimized MEFP

6.2.8.1 Determination of pandan aroma from outer shell

The MEFP was analyzed for the pandan flavor. The evaporated pandan flavor from static head space was taken for analysis at 0, 5, 7.5, 12.5, and 15 min. The extent of aroma released from static head space following the incubation was measured from the reaction medium and quantification by GC. The condition of GC-FID was followed from the method in 6.2.4 (Dong *et al.*, 2011). The suspension from this experiment was filtered and then keeps for MAD aroma release in further experiment.

6.2.8.2 Determination of MAD aroma from inner encapsulated powder in SSF

MAD aroma release of MEFP from 6.2.8.1 was analyzed in SSF (Ades *et al*., 2012). The incubation was carried out in a 2 ml glass vial sealed by a screw cap covered with an aluminum foil. The pH of SSF was adjusted to 7.2 by potassium hydroxide. The α-amylase activity used was 100 unit/ml as the average activity found while chewing (Watanabe & Dawes, 1988; Yamaguchi *et al*., 2004; Ades *et al*., 2012). The suspension from MEFP was then filtered and incubated in 2 ml of SSF at 37±2°C in controlled temperature water bath (WB22, Memmert GmbH + Co.KG, Germany) under continues stirring with shaker at 12 rpm (SV 1422, Memmert GmbH + Co.KG, Germany). The evaporated volatile compound from static head space was taken for analysis at 0, 1, 2, 3, 4, and 5 min toward 300 min. The main aromas of MAD were analyzed as 2-methyl butanoic acid and linalool following identification aroma from Pensuk *et al.* (2007) investigation. In addition, verbenone also analyzed as other main aroma following identification aroma from Samakradhamrongthai Thakeow, Kopermsub, Chansakoaw, & Utama-ang (2012). All samples were analyzed in triplicate. Gas chromatograph analysis was performed on gas chromatography (GC-2010, Shimadzu Corp., Japan). Column and carrier gas for released aroma analysis were DB-1column (30x0.25 mm ID and 0.25 μm film thickness) (Model 122-1032, Agilent Technologies, Inc., USA) and 1.0 ml/min. The oven temperature was held at 40°C for 3

min and increased to 250°C at 4°C/min and held for 5 min at 250°C (Samakradhamrongthai, 2011). Standards calibration curve of aroma were in order to calculate amount of each aroma.

6.2.9 Statistical analysis

All data were carried out in triplicate and reported as mean±standard deviation of mean (S.E.M.). The optimization of the MEFP was employed using Analysis of variance (ANOVA) (Design Expert 7.0, Stat-Ease, Inc., MN, USA). The Statistic analysis was conducted using SPSS 17.0 (SPSS Inc., IBM Corp., IL, USA) using the Duncan's multiple range test (DMRT) with significant level determined at 95% confident limit ($p < 0.05$).

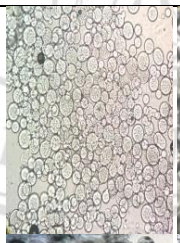
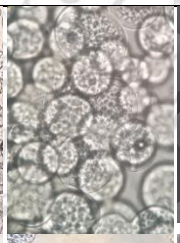
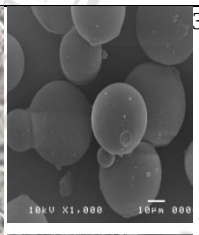
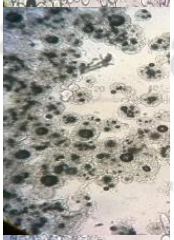
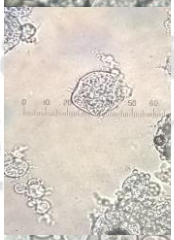
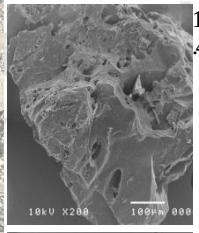
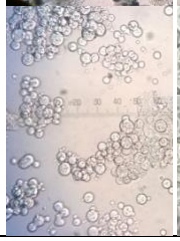
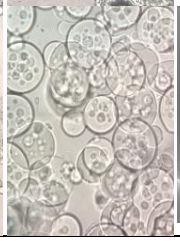
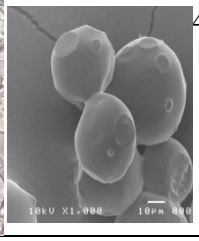
6.3 Results and discussion

6.3.1 Morphology of MEFP

The microcapsules were shown to be in the form of a reservoir in which the core was perfectly surrounded by GGA system as suggested in Zhang *et al*. (2011), Dong *et al*. (2011) and Comunian *et al.* (2013). The optical microscopy showed that the microcapsules acquired were successfully formed sphered-shape in most of treatment except the treatment 2 and 6 that had exceeded amount of gelatin that cannot form spherically shape due to high moisture absorption of gelatin to create irregular structure (Zhang *et al*., 2011). The higher core and flavor loading affected the thickness of microcapsules membrane to be decreased. This was because of the higher loading affected the quantity of emulsion droplets in the suspension, therefore, the more emulsion droplets with coacervate tended to create larger spherical multinuclear microcapsules (Dong *et al*., 2011). In addition, the freeze drying process also maintained the wall reliability of microcapsules. The freeze-dried coacervated were objected to create complex coacervation easier and conferring greater stability on microcapsules.

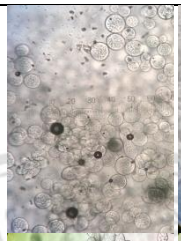
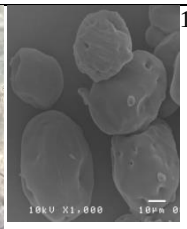
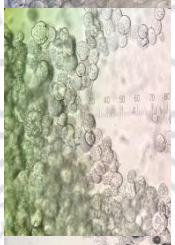
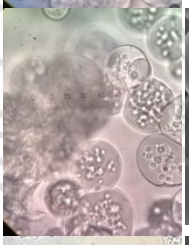
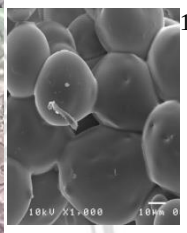
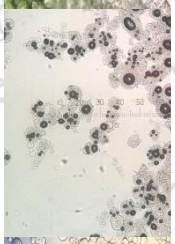
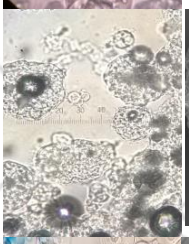
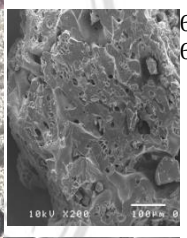
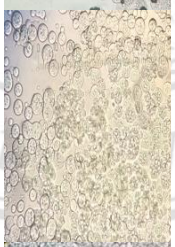
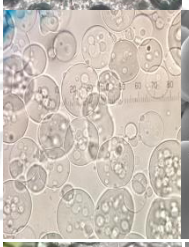
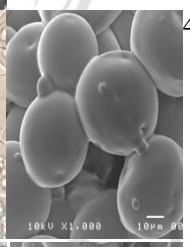
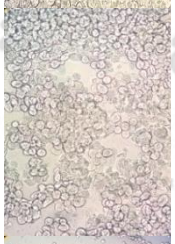
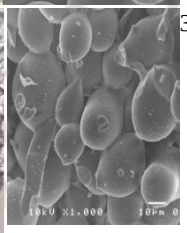
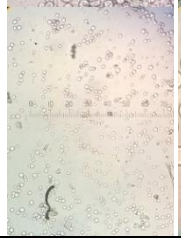
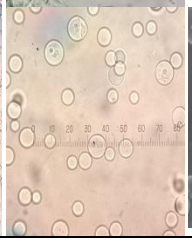
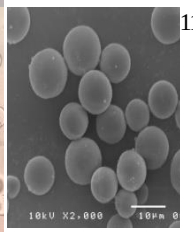
The microparticle demonstrated a multinuclear distribution of a core material with defined wall protection. It was desirable to observe the morphology of particles and the results were shown in Table 6.2. These results were mostly similar to Alvim & Grosso (2010), Dong *et al*. (2011) and Comunian *et al*. (2013) investigation which previously studied by Guo & Zhao (2008). The particles were spherical with some deformation associated to the shrinking of the matrix during drying including solid bridges between the particles but without fractures. Those microcapsules were formed in various of sizes with significant different. The particle size of microcapsules was varied from 8.63–120.48 μm. The result suggested that the size distribution depended on the mixture of shell material. The ratio of gelatin and gum arabic at 1:1 affected higher uniformity and created more stable microcapsule whereas the exceeded amount of gelatin or gum arabic can lead to irregular form of shell material.

Table 6.2 Optical microscopic and SEM micrographs of freeze-dried MEFP

TRT	Gelatin (%w/v)	Gum Arabic (%w/v)	Pandan (%w/w of gelatin-gum arabic solid)	Optical microscopic (X10)	Optical Microscopic (X100)	SEM	Particle size (μm)
1	3.00	3.00	5.00				39.02±2.39de
2	7.00	3.00	5.00				1037.67±120.48a
3	3.00	7.00	5.00				45.39±0.94d

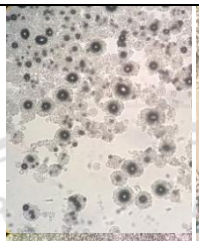
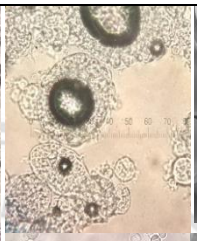
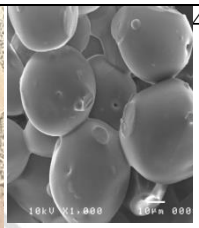
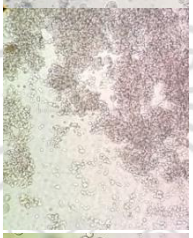
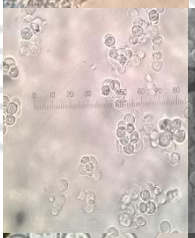
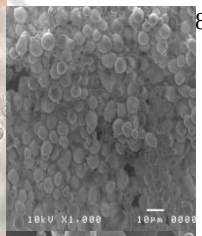
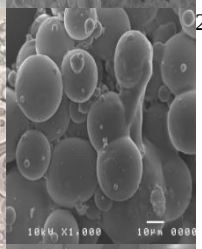
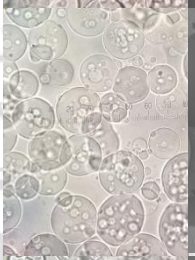
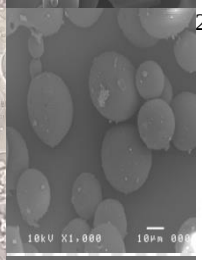
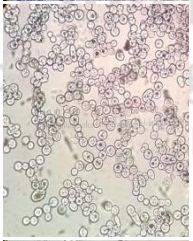
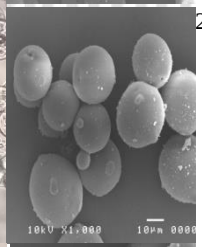
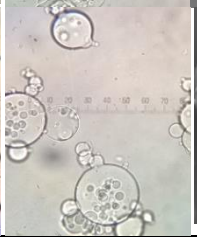
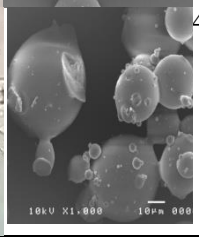
Note: The different letters in the same column mean significant difference ($p \leq 0.05$)

Table 6.2 (cont'd.) Optical microscopic and SEM micrographs of freeze-dried MEFP

TRT	Gelatin (%w/v)	Gum Arabic (%w/v)	Pandan (%w/w of gelatin-gum arabic solid)	Optical microscopic (X10)	Optical Microscopic (X100)	SEM	Particle size (µm)
4	7.00	7.00	5.00				101.73±6.12c
5	3.00	3.00	10.00				120.48±9.98c
6	7.00	3.00	10.00				600.15±59.36b
7	3.00	7.00	10.00				45.39±0.94d
8	7.00	7.00	10.00				32.67±3.64de
9	1.64	5.00	7.50				11.09±1.39de

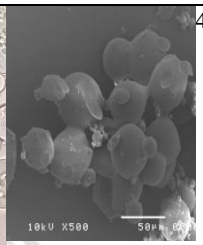
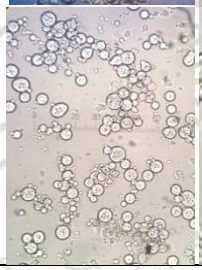
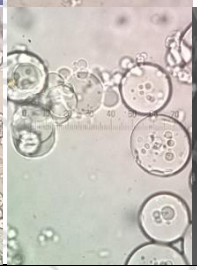
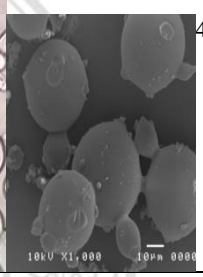
Note: The different letters in the same column mean significant difference ($p \leq 0.05$)

Table 6.2 (cont'd.) Optical microscopic and SEM micrographs of freeze-dried MEFP

TRT	Gelatin (%w/v)	Gum Arabic (%w/v)	Pandan (%w/w of gelatin-gum arabic solid)	Optical microscopic (X10)	Optical Microscopic (X100)	SEM	Particle size (µm)
10	8.36	5.00	7.50				45.83±2.26d
11	5.00	1.64	7.50				8.63±0.69e
12	5.00	8.36	7.50				25.32±2.46de
13	5.00	5.00	3.30				28.42±1.59de
14	5.00	5.00	11.70				28.88±1.32de
15	5.00	5.00	7.50				42.60±1.36de

Note: The different letters in the same column mean significant difference ($p \leq 0.05$)

Table 6.2 (cont'd.) Optical microscopic and SEM micrographs of freeze-dried MEFP

TRT	Gelatin (%w/v)	Gum Arabic (%w/v)	Pandan (%w/w of gelatin-gum arabic solid)	Optical microscopic (X10)	Optical Microscopic (X100)	SEM	Particle size (μm)
16	5.00	5.00	7.50				42.57±2.86de
17	5.00	5.00	7.50				42.62±2.78de

Note: The different letters in the same column mean significant difference ($p \leq 0.05$)

6.3.2 Kinetic study of the release of pandan flavor

The release profile of pandan flavor from the MEFP in hot water conditions was determined in the water bath at 98±2°C as shown in Fig. 6.2. The results showed that the cumulative release amount of the pandan flavor was increased over time within 20 min and then started to be stabled. This indicated that the MEFP was capable of releasing the encapsulated active ingredients slowly over time and condition. The results were conformed to Dong *et al*. (2011) which indicated that the higher core/wall ratio exhibited better heat-resistant property and possessed strong microcapsules membrane with three dimensional structure. The higher core/wall ratios of MEFP were also remained intact structure in hot water with slowly releasing interior core material. In addition, the MEFP can be retained flavor compound in thermal condition more than single core microcapsule which suggested that the MEFP demonstrated more stable and controllable that the single core microcapsules (Yeo *et al*., 2005*)*.

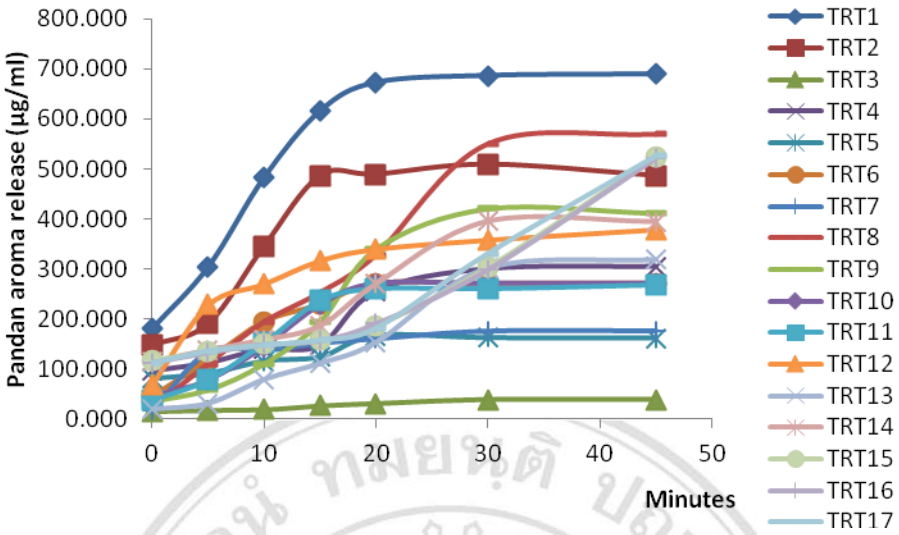


Fig. 6.2 The release of pandan flavor during incubation in hot water at temperature of 98±2°C, Aroma release was presented as the amount from equilibrium head space.

Note: The different treatments were marked as TRT1– TRT17 in Table 6.1.

The rate of aroma release was evaluated using Avrami's equation (Weibull distribution function) as suggested from previous chapter. Avrami's equation was chosen to employ in this experiment because of the fitted model that was applied to describe the shelf-life failure and was suitable to describe the release time-course of the encapsulated flavor powder as shown in Eq. (6.3) (Soottitantawat *et al*, 2004; Szente & Sejtli, 2004).

$$R = 1\text{-}\exp[-kt]^{n} \qquad (6.3)$$

The encapsulation of MAD flavor powder of treatment 1 was selected due to its ratio of gelatin and gum arabic as 1:1 as suggested in Dong *et al.* (2011) to be suitable model. The parameters *k* and *n* were achieved by taking a logarithm of both sides of equation (6.3). The released pandan content was analyzed from 0 min toward 45 min. The detected content was initiated from 181.67–689.25 µg/ml (Fig. 6.3).

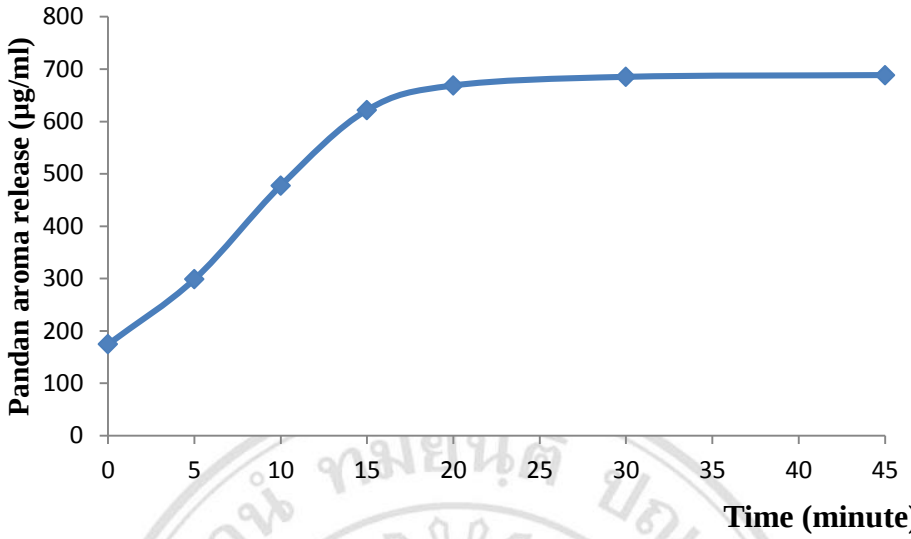


Fig. 6.3 The release of pandan flavor during incubation in hot water at temperature of 98±2°C, Aroma release was presented as the amount from equilibrium head space.

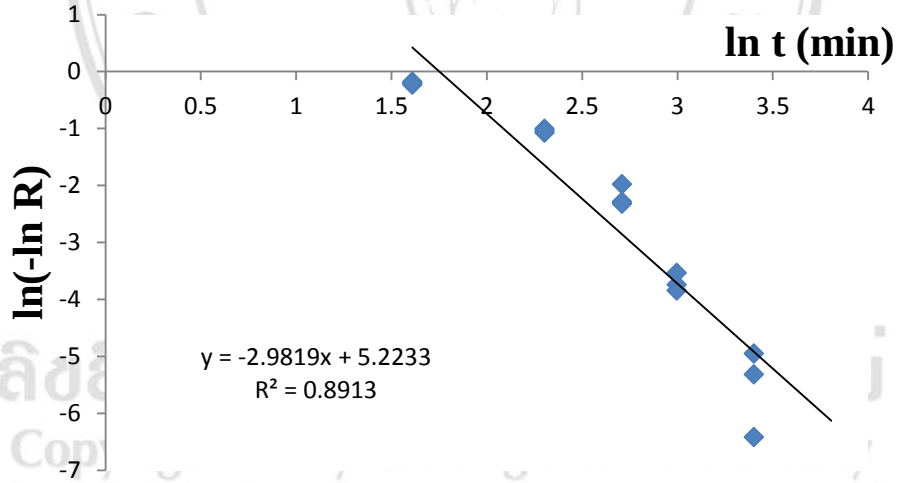


Fig. 6.4 Correlation of release time-course of pandan flavor incubation in hot water at temperature of 98±2°C by Avrami's equation.

The release time-course of the encapsulated flavor powder was then calculated for the correlation of release time-course of released aroma content using Eq. (5.3). The correlation of release time-course of released aroma content was created from the plotting between ln[-ln*R*] and ln*t* to reveal *k* and *n* parameter as shown in Fig 6.3. The parameters *k* and *n* were achieved by taking a logarithm of both sides of equation, provided *k* parameter from the interception at *ln t* = 0 and *n* parameter as slope by plotting ln[-ln*R*] vs. ln*t*. The parameter *k* and *n* of released pandan content shown as *5.22* and *2.98* (Fig. 6.4).

The release rate constant of pandan was in range of 0.82 min^{-1}–6.64 min^{-1} as shown in Table 6.3. The value of *n* was in range of 0.25–3.74 which suggested that release mechanism of MEFP can be identified as first-order mechanism, also suggested the MEFP showed diffusional release mechanism as the *n* was higher than 0.89 as Soottitantawat, Partanen, Neoh, & Yoshii (2015) and Dash, Murphy, Nath, & Chowdhury (2010) investigation. The release time-course of aroma compounds were analyze using response surface. The regression equation of pandan flavor was suggested that gelatin (X_1), gum arabic (X_2) and the pandan flavor (X_3) affected the released pandan flavor content constant. The pandan release rate increase when decreased X_1, X2, and X_3, separately while the increasing all factors in term of exponential affected the pandan release rate to be increased separately as well (Table 6.4). The contour plot from regression equation of responses revealed response surface of pandan release rate as shown in Fig. 6.5.

Table 6.3 Kinetic parameter of pandan flavor from Avrami's equation

Factors			Pandan flavor		
X_1 (%w/w)	X_2 (%w/v)	X_3 (%W/W)	k (min^{-1})	n	R^2
3.00	3.00	5.00	5.2230	2.9819	0.8913
7.00	3.00	5.00	6.4249	3.7419	0.8837
3.00	7.00	5.00	6.6171	2.8786	0.8687
7.00	7.00	5.00	3.4154	2.0070	0.8049
3.00	3.00	10.00	4.9673	3.0805	0.9022
7.00	3.00	10.00	2.4308	1.4360	0.9804
3.00	7.00	10.00	4.8001	2.5509	0.8300
7.00	7.00	10.00	1.7535	0.7546	0.9510
1.64	5.00	7.50	6.6493	2.7005	0.8878
8.36	5.00	7.50	3.1302	1.7302	0.8928
5.00	1.64	7.50	4.5075	2.5454	0.8251
5.00	8.36	7.50	1.5434	1.2561	0.9231
5.00	5.00	3.30	2.1721	0.8084	0.9792
5.00	5.00	11.70	2.8041	1.2210	0.8309
5.00	5.00	7.50	0.8859	0.2773	0.8117
5.00	5.00	7.50	0.9464	0.3044	0.8082
5.00	5.00	7.50	0.8206	0.2465	
	p-value		0.0067		

Note: X_1 = Gelatin, X_2 = Gum arabic, X_3 = Pandan flavor

Table 6.4 Regression equation of released pandan flavor content rate constant from MEFP using RSM

Responses	Regression equation	Adjusted R^2	*p-value*
Released pandan flavor content rate constant (min^{-1})	$0.83-0.99X_1-0.56X_2-0.49X_3 +1.60X_1^2+0.95X_2^2 +0.75X_3^2$	0.7000	0.0067

Note: X_1 = Gelatin, X_2 = Gum arabic, X_3 = Pandan flavor

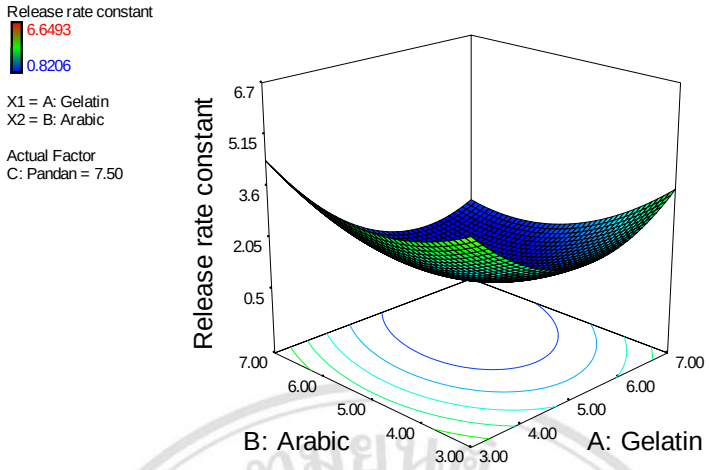


Fig. 6.5 The response surface demonstrated regression of released pandan flavor content rate constant model between gelatin and gum arabic at 7.50% pandan flavor.

6.3.3 Physical and encapsulation properties of MEFP

The results of physical and encapsulation properties were significantly different. The yield recovery was in range of 23.56–83.40%. The moisture content and water activity were in range of 0.32–0.77% and 0.168–0.463, respectively. The results shows that color of *L**, *a**, and *b** were in range of 88.97–96.42, 0.44–1.20, and 4.94–10.41, respectively. The encapsulation properties of the MEFP were measured as solubility, surface content, encapsulation efficiency of the pandan flavor, encapsulation efficiency of MAD flavor powder. The result of solubility was in range of 97.64 – 99.74%. The surface content of encapsulation powder was in range of 1.16–5.54%. The encapsulation efficiency of the pandan flavor was in range of 38.56–95.15%. The encapsulation efficiency of MAD encapsulated flavor powder was in range of 10.28–94.33%. The pandan aroma release from outer shell was in range of 26.93–615.62 µg/ml (Table 6.5).

Table 6.5 Physical and encapsulation properties of MEFP

T[1]	Gelatin (%w/v)	Gum arabic (%w/v)	Aroma (%v/v)	Yield recovery (%)	Moisture content (%)	Water activity	*L**	*a**	*b**	Solubility (%)	Surface content (%)	[2]Encapsulation Efficiency1(%)	[2]Encapsulation Efficiency 2(%)	Released pandan flavor content (µg/ml)
1	3.00	3.00	5.00	45.38±0.35j	0.32±0.01hi	0.168±0.001j	91.39±0.46e	1.20±0.02	8.69±0.13d	97.81±0.02hi	3.02±0.12ef	92.52±0.35a	87.49±0.70abc	615.62±12.48a
2	7.00	3.00	5.00	55.15±0.40g	0.66±0.03c	0.430±0.002c	89.12±0.65f	0.87±0.05	9.93±0.08b	98.62±0.20c	1.16±0.12j	95.15±0.45a	55.37±8.11g	486.51±7.66b
3	3.00	7.00	5.00	42.89±0.07k	0.35±0.02gh	0.185±0.002i	95.27±0.39c	0.44±0.02	5.44±0.03h	99.74±0.02a	3.13±0.04e	44.59±4.41h	10.28±0.80j	26.93±1.56i
4	7.00	7.00	5.00	57.19±0.26f	0.70±0.02b	0.421±0.001c	89.57±0.82f	1.04±0.02	9.32±0.04c	99.07±0.08c	1.91±0.04i	59.81±2.61ef	36.86±3.17h	148.29±13.16g
5	3.00	3.00	10.00	31.41±0.23m	0.37±0.01g	0.170±0.001j	92.23±0.01de	1.02±0.01	8.70±0.03d	97.64±0.07i	2.12±0.03h	62.37±1.62ef	94.33±2.99a	124.04±1.77h
6	7.00	3.00	10.00	49.68±0.05i	0.61±0.01d	0.447±0.023b	88.97±0.46f	0.85±0.01	10.41±0.16a	99.08±0.07c	3.53±0.02d	61.83±0.42ef	36.66±2.61h	228.91±4.87e
7	3.00	7.00	10.00	45.35±0.08j	0.52±0.01d	0.187±0.002i	96.41±0.51b	0.73±0.02	5.66±0.11h	98.36±0.10de	2.69±0.30gh	64.14±2.70de	65.59±3.95f	151.92±3.12g
8	7.00	7.00	10.00	50.44±0.06h	0.72±0.03b	0.403±0.002d	89.87±1.27f	1.07±0.03	9.88±0.19b	97.67±0.07ij	1.25±0.09j	38.56±1.25i	93.28±2.73ab	252.78±3.05d
9	1.64	5.00	7.50	23.56±0.32n	0.77±0.02j	0.136±0.002k	95.47±0.06bc	0.76±0.10	6.16±0.06g	99.30±0.03b	1.29±0.07j	50.42±1.66g	28.06±1.27i	194.16±8.02f
10	8.36	5.00	7.50	67.13±0.14e	0.43±0.01a	0.462±0.002a	90.08±0.07f	1.14±0.03	10.43±0.13a	98.30±0.14e	5.54±0.04b	67.78±1.45d	67.76±5.69ef	228.91±4.87e
11	5.00	1.64	7.50	11.82±0.07h	0.64±0.03f	0.463±0.002a	93.02±1.57d	0.40±0.06	7.10±0.43f	99.19±0.01b	1.33±0.17i	83.81±2.15b	32.44±0.48hi	237.53±4.12e
12	5.00	8.36	7.50	50.68±0.98f	0.54±0.02cd	0.349±0.013f	91.95±0.18e	1.18±0.01	8.74±0.03d	98.43±0.04d	3.80±0.13c	18.70±2.08j	62.80±1.28fg	317.15±17.69c
13	5.00	5.00	3.30	69.76±0.20d	0.76±0.01e	0.266±0.001g	91.18±0.45e	0.77±0.01	8.02±0.05d	97.85±0.33gh	3.96±0.03c	42.88±5.52hi	85.79±1.44bc	111.29±5.36h
14	5.00	5.00	11.70	37.12±0.12l	0.64±0.03a	0.362±0.003e	91.34±0.21e	0.64±0.02	8.69±0.10e	98.00±0.09fg	7.47±0.28a	73.02±1.78c	80.09±3.73cd	186.79±8.06f
15	5.00	5.00	7.50	80.85±.025cd	0.63±0.02cd	0.217±0.002h	97.58±0.21a	0.78±0.02	5.07±0.08i	98.12±0.02fg	2.55±0.04h	62.92±1.48de	75.16±0.60de	158.04±0.99g
16	5.00	5.00	7.50	81.28±0.14b	0.63±0.02cd	0.217±0.001h	97.44±0.10a	0.77±0.02	4.94±0.06i	98.16±0.04ef	2.46±0.10h	60.86±6.47ef	73.72±1.60de	157.79±1.42g
17	5.00	5.00	7.50	83.40±0.20a	0.63±0.02cd	0.217±0.001h	97.50±0.17a	0.79±0.01	4.94±0.06i	98.44±0.62fg	2.87±0.07fg	57.37±2.12f	74.43±1.74de	157.29±2.45g
p-value				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Note: The different letters in the same column mean significant difference ($p \leq 0.05$)

[1]T = Treatments

[2]Encapsulation efficiency1 = the pandan flavor encapsulation efficiency

[2]Encapsulation efficiency2 = the MAD flavor powder encapsulation efficiency

6.3.4 Response surface of physical and chemical properties of MEFP

The physical and encapsulation properties of the MEFP were submitted to generate a response surface to determine the optimized formulation. There were 8 responses that fitted to create regression model which were yield recovery, water activity, color value (L^* and b^*), encapsulation efficiency of the pandan flavor, encapsulation efficiency of the MAD flavor powder and the released pandan flavor content. The relationship of gelatin (X_1), gum arabic (X_2) and the pandan flavor (X_3) was explained using regression equations as shown in Table 6.6.

The increasing of X_1 and X_2 increased yield recovery until 5% w/v, where the yield recovery started to be decreased. The increasing of the pandan flavor also increased yield recovery until 7.5% w/w of gelatin-gum arabic solid, where the yield recovery started to be decreased (Fig. 6.6a). The positive effect of $X_1$and X_2 was considered from influences on both emulsification and interfacial complex coacervation. The occurrence of larger content of biopolymer induced more stable emulsion droplets that can be formed faster and showed thicker complex coacervate layer which created surrounding the emulsion droplets (Yeo *et al*., 2005; Dong *et al*., 2011; Zhang *et al*., 2011).

The increasing of X_1 and X_2 was affected moisture content to be decreased until reaching 5% w/v, where the moisture content started to be increased (Fig. 6.6b). The increasing of X_1 and X_3 increased water activity whereas the increasing of X_2 decreased water activity (Fig. 6.6c). The moisture content and water activity were influenced by polymer content which capability on water absorption (Sarika & James, 2015). The ratio of polymer between gelatin and gum arabic was also affected toward moisture content and water activity. As suggested in research from Dong *et al*. (2011). The increasing the core/wall weight ratio from 1:2 to 4:1, at the same time the particle size and the loading (the oil content in the microcapsules) gradually increased, but the membrane thickness of coacervate microcapsules decrease. When the core/wall ratio increased from 1:1 to 2:1, the quantity of emulsion droplets in the suspension

significantly increased, therefore more emulsion droplets with coacervate on the surface were easy to stick into big spherical multinuclear microcapsules.

The response surface of the MEFP showed that X_1, X_2 and X_3 had effected on color value L^* and b^*. The response surface of lightness (L^*) indicated that the increasing of X_2 increased the lightness as well as the decreasing of X_3 (Fig. 6.6d). The response surface of yellowness (b^*) indicated that the increasing of X_1, X_2 and X_3 increased color value b^* (Fig. 6.6e).

The encapsulation efficiency of the pandan flavor (EE1) showed that the decreasing X_2 increased EE1 as well as interaction from X_1 and X_3 (Fig. 6.6f). The encapsulation efficiency of MAD flavor powder (EE2) was affected by percentages of X_2 and X_3 which was increased until reaching 5% w/v and 5% w/w, the encapsulation efficiency then established to be stabled whereas the increasing of X_3 affected encapsulation efficiency to be decreased after exceeded amount of X_3 (Fig. 6.6g).

The released pandan flavor content affected by X_2 and X_3. The increasing of X_2 affected the released pandan flavor content to be decreased as well as the increasing of X_3. The interaction of X_2 and X_3 also affected the released pandan flavor content to be increased as the increasing of X_2 and X_3 (Fig. 6.6h). The reason that increasing gum arabic and pandan flavor affected to decrease released pandan flavor content because of the limitation of wall material to encapsulate all core material which normally not exceeded over 10% w/v of flavor. As Sutaphanit & Chitprasert (2014) suggested that the gelatin less than 4% w/v not only produced small amount of the microcapsules, but also provided low encapsulation efficiency whereas the gelatin concentrations of greater than 16% w/v caused as excessively viscous solution leading to irregular clusters.

The regression model of EE1, EE2, and the released pandan flavor content which considered being major responses in this experiment were affected by X_2 and X_3, whereas yield recovery, moisture content and water activity were considered being

minor responses were affected by X_1 and X_2. This suggested that gum arabic and pandan flavor were affected toward major responses more than gelatin.

Table 6.6 Regression equations of significant responses from MEFP

Responses	Regression equation	Adjusted R^2	*p-value*
Yield recovery (%)	$81.44+8.84X_1+5.83X_2-11.53\ X_1^2-16.52X_2^2$ $-8.67X_3^2$	0.7125	0.0013
Moisture content (%)	$3.82+1.01X_1+0.43X_2+1.04X_2^2+0.68X_1^2X_2$ $+0.61X_1X_2^2$	0.8892	0.0001
Water activity	$0.22+0.11X_1-0.015X_2+0.012X_3-0.01X_1X_2$ $+0.019X_1^2+0.053X_2^2+0.024X_3^2$	0.8825	0.0001
Color value *L**	$97.52-1.97X_1+0.56X_2+0.17X_3-0.84X_1X_2$ $-0.23X_1X_3+0.095X_2X_3-1.73X_1^2-1.83X_2^2-2.26X_3^2$	0.8976	0.0015
Color value *b**	$4.98+1.33X_1+0.34X_2+0.011X_3+0.64X_1X_2$ $+0.10X_1X_3+0.0037X_2X_3+1.20X_1^2+1.06X_2^2$ $+1.22X_3^2$	0.8019	0.0002
Encapsulation Efficiency pandan flavor (%)	$60.99-15.69X_2-5.50X_1X_3+7.72X_2X_3$	0.6199	0.0013
Encapsulation Efficiency of MAD powder (%)	$81.44+8.84X_1+5.83X_2+5.76X_3-11.53X_1^2-16.52X_2^2-$ $8.67X_3^2$	0.7951	0.0006
Released pandan flavor content (µg/ml)	$187.81+122.33X_2X_3+43.29X_2^2-109.40X_1^2X_2$ $-64.96X_1^2\ X_3$	0.8072	0.0001

Note: X_1 = Gelatin, X_2 = Gum Arabic, X_3 = Pandan flavor

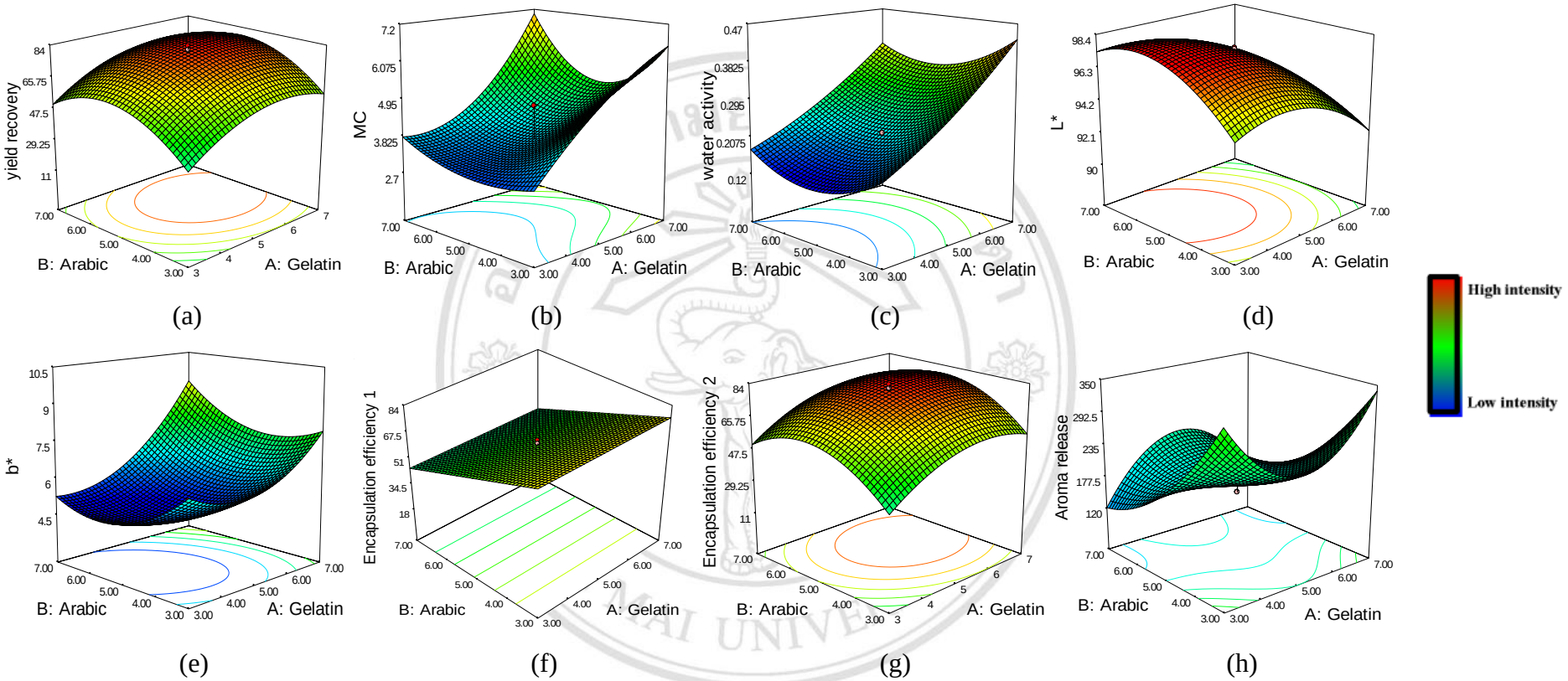


Fig. 6.6 The response surfaces demonstrate regression model between gelatin, gum arabic and fixed pandan flavor at 7.50%; (a) yield recovery (b) moisture content, (c) water activity,(d) color value *L** and (e) color value *b**, (f) encapsulation efficiency of pandan flavor, (g) encapsulation efficiency of flavor powder, and (h) released pandan flavor content at 15 min.

6.3.4 Optimization and validation on MEFP

The optimization of the MEFP was then concentrated on gum arabic and pandan flavor. The gelatin at more than 5% w/v was affected yield recovery to be decrease, also affected moisture content and water activity to be increase which lead to the undesirable optimization of the MEFP. The robust regression model was overlaid to fabricate optimized formulation for the MEFP. The predicted value with constrained of the highest and the lowest responses provided the highest yield recovery (47.27%), encapsulation efficiency of the pandan flavor (70.04%), and the released pandan flavor content (395.92 µg/ml) with the provided lowest moisture content (3.14%) and water activity (0.163). The predicted response values that the optimized MEFP was consisted of gelatin 3.00% w/v, gum arabic 3.73% w/v, and pandan flavor 5.26% w/w of gelatin-gum arabic solid (Fig. 6.7). The optimized MEFP was produced, validated and analyzed for all responses (Table 6.7). The predicted and validated values were summited to calculate for the percentage of approximated error which should not be over 10% of proximity error. The percentage of approximated error was in range of 0.64–7.77. This indicated that the results of validation were in perfect agreement between the predicted and measured values (Hu, 1999).

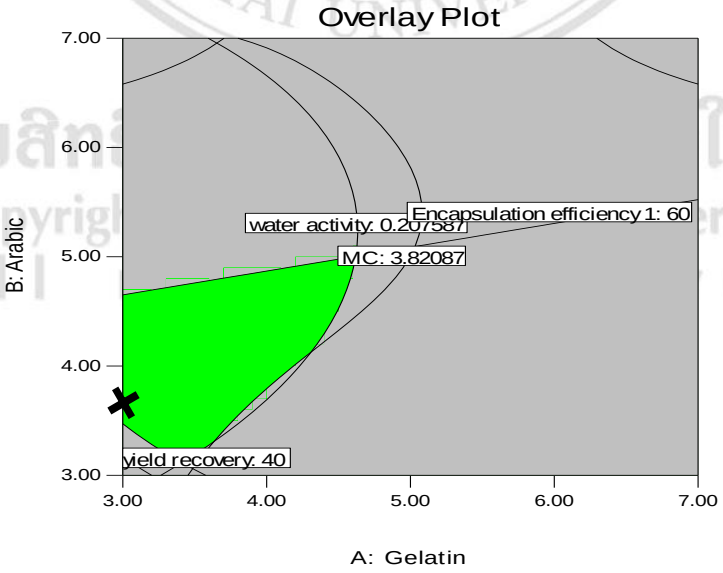


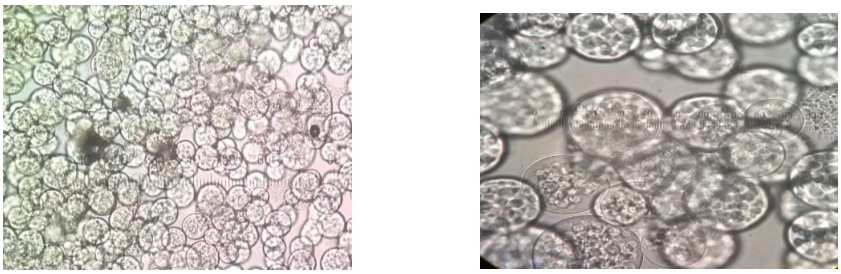
Fig. 6.7 The overlay plot from response surface demonstrated the optimum formula of the MEFP using pandan flavor at 5.26%.

Table 6.7 Comparison of prediction and validation value of MEFP with approximated error

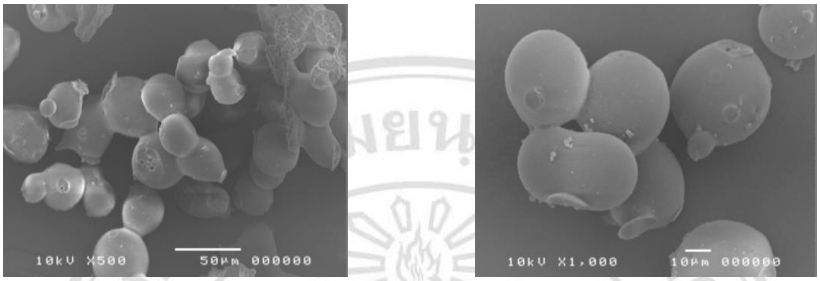
Responses	Prediction value	Validation value	Approximation error (%)
Yield recovery (%)	47.27	46.45±0.03	1.77
Moisture content (%)	3.14	3.12±0.09	0.64
Water activity	0.163	0.165±0.007	1.21
Color value *L**	95.16	93.89±0.68	1.35
Color value *a**	0.96	0.95±0.02	1.05
Color value *b**	6.20	6.54±0.54	5.20
Solubility (%)	98.28	97.54±0.57	0.76
Surface content (%)	0.13	0.16±0.01	
Encapsulation Efficiency of pandan flavor (%)	70.04	70.54±0.62	0.71
Encapsulation Efficiency of MAD flavor powder (%)	51.21	47.52±0.98	7.77
Released pandan flavor content (µg/ml)	395.92	394.92±2.02	0.25

6.3.6 Microstructural characterization of optimized MEFP

The internal and external structure of the optimized MEFP was observed using light microscopy and SEM as shown in Fig. 6.8. The image from multi-core flavor powder from complex coacervates showed spherical and smooth surface were successfully formed. The optimized MEFP exhibited transparent multinuclear structure. For coacervate as wall material was transparent, this showed that MAD flavor powder were encapsulated by GGA system and formed multinuclear microcapsules as concurring to many complex coacervate encapsulation (Thies, 1995; Dong *et al*., 2011; Comunian *et al*., 2013).



(a)



(b)

Fig. 6.8 Microstructural characterization of optimized MEFP; (a) optical microscopic micrographs of MEFP, (b) SEM micrographs of MEFP.

The T_g from the optimized MEFP from freeze drying was 63.54°C at 3.12% moisture content (Fig. 6.9). The results showed that drying methods did not affect the glass transition temperature, which was conformed to the result of Chen *et al*. (2013). Analyzed T_g of encapsulated powder was above 60°C which indicated that the material was transformed to rubbery state when temperature of sample reached over 60°C and can be stored under temperature 60°C at 25% ambient relative humidity (Desobry, Netto, & Labuza, 1997).

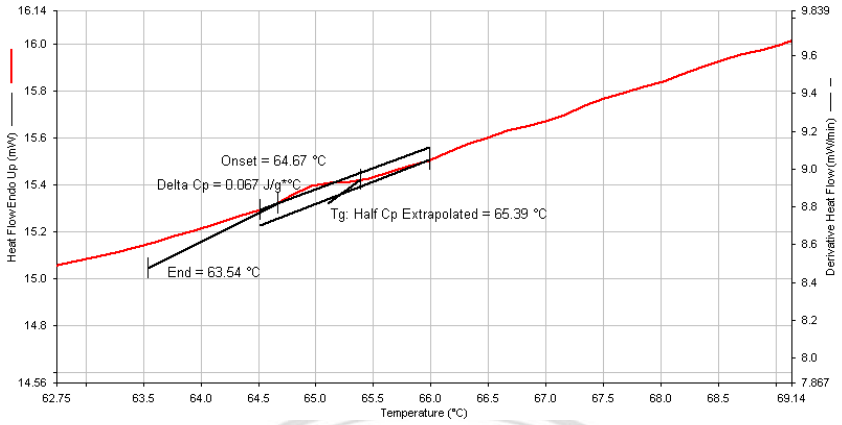


Fig. 6.9 The differential scanning calorimetry result of optimized MEFP.

The changes of degree of crystallinity of samples were analyzed using X-ray diffraction. The results of XRD analysis showed that samples were in amorphous form (Fig. 6.10). It is involved many parameters such as wall material content and core material content which provided amorphous or crystalline ratio of the produced material (Da Silva-Junior *et al*., 2009). The results of XRD pattern was compared GGA system without MAD flavor and with MAD flavor powder. The pattern showed characteristic peak around 8° and 20°. Therefore, GGA system without MAD flavor powder showed higher crystalline structure than GGA system with MAD flavor powder which indicated that the surface of multi-core flavor powder exhibited more amorphous and spherical structure than GGA system without MAD flavor powder. The XRD pattern of GGA system showed crystalline structure corresponding to the characteristic peak at 2θ around 8° and 20° similar to results from Ki *et al*. (2005) and Sutaphanit and Chitprasert (2014). Those researches indicated that GGA system powder provided typical XRD pattern of crystalline material because of α-helix and triple-helical structure which mainly happen from the formation of inter-chain hydrogen bond between gelatin, gum arabic and pandan flavor, then create formation of shell material as the result conformed to Rivero, García, & Pinotti (2010), who investigated correlations between structural, barrier, thermal and mechanical properties of plasticized gelatin films.

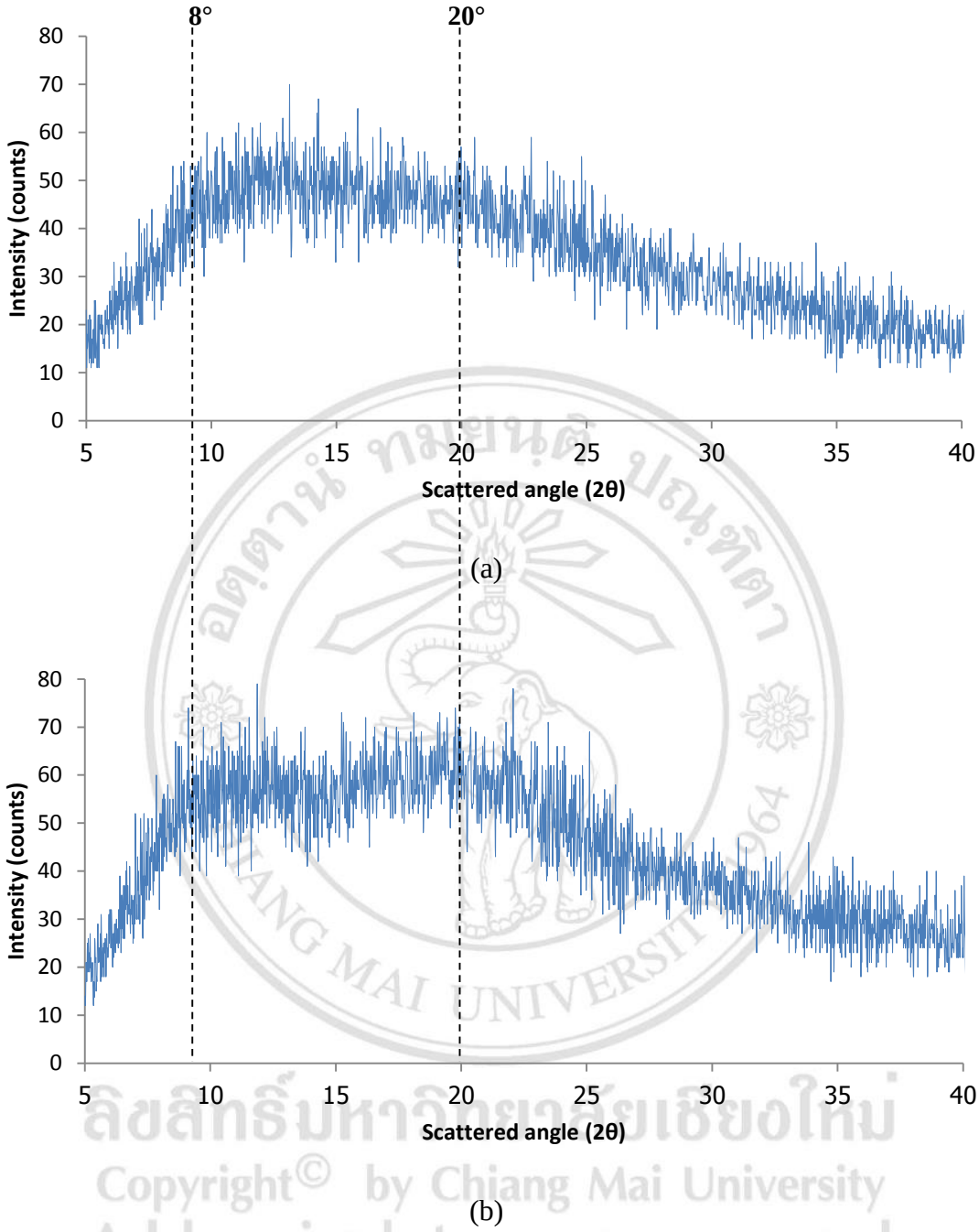


Fig. 6.10 X-ray diffraction scans of (a) Optimized GGA without MAD, (b) optimized GGA with MAD.

6.3.7 Determination of release profile of optimized MEFP from GGA system

6.3.7.1 Kinetic release of pandan flavor of optimized MEFP

The optimized MEFP from GGA system showed the released pandan flavor content profile was release within 20 min before that pandan flavor started to be stable toward 40 min as shown in Fig. 6.11. The release profile of the pandan flavor was conformed to Dong *et al*. (2011) investigation which discovered that the release content of peppermint MEFP was slightly increased over time from 40 min to 60 min. The release rate constant of the pandan flavor was calculated from Avrami's equation was at 4.17 min^{-1} with R^2 at 0.8694 as shown in Fig. 6.12. The value of *n* was at 2.39 indicated that the release mechanism of MEFP was diffusional mechanism as the *n* was higher than 0.89 as suggested in researches of Soottitantawat, Partanen, Neoh, & Yoshii (2015) and Dash, Murphy, Nath, & Chowdhury (2010).

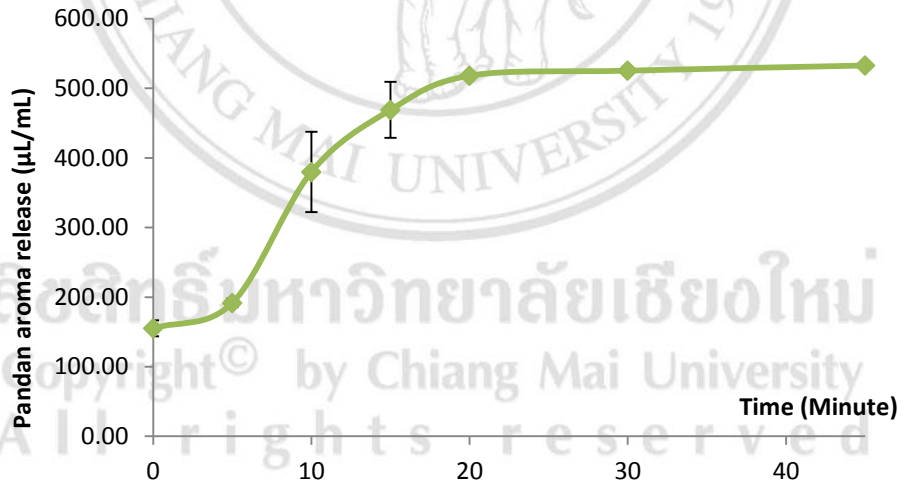


Fig. 6.11 The release of pandan flavor during incubation in hot water (98±2°C), Aroma release was presented as the amount from equilibrium static head space.

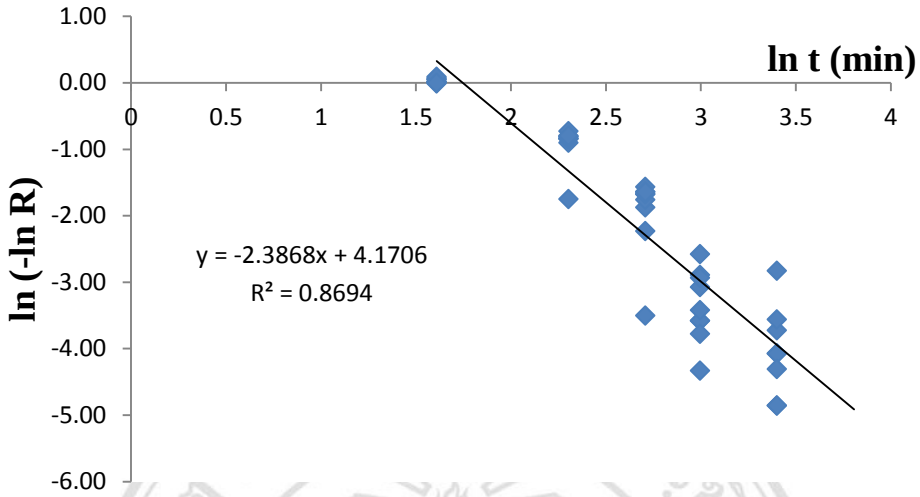


Fig. 6.12 Correlation of release time-course of pandan flavor from MEFP incubated in hot water at temperature of 98±2°C by Avrami's equation.

6.3.7.2 Kinetic release of MAD flavor from optimized MEFP

The optimized multi-core encapsulated powder encapsulated powder was further analyzed for MAD main aroma release. The results showed he content of linalool was the highest, followed by 2-methyl butanoic acid and verbenone as shown in Fig. 6.13. The release rate constant of three main compounds followed Avrami's equation calculation suggested that linalool had the slowest release rate constant (1.23 min^{-1}), followed by verbenone (0.51 min^{-1}) and 2-methyl butanoic acid (0.23 min^{-1}) (Fig. 6.14 and Table 6.8). According to the results, 2-methyl butanoic acid was the fastest to be released from the complex in SSF, followed by verbenone and linalool. The result was agreed on many studies (Kim & Maga, 1994; Anantha & Milford, 1997; Jouquand, Ducruet, & Bail, 2006) that suggested volatile compounds higher molecular weight comprised lower release rate constant. Since, molecular weight of 2-methyl butanoic acid, verbenone and linalool were shown as 102.13, 150.22 and 154.25, respectively. This result showed the same direction as Naknean & Meenune (2010) reviews. This showed that linalool and verbenone were retained longer in the

encapsulated matrix than 2-methyl butanoic acid resulting from higher molecular weight and those volatile compounds created encapsulated complex better than 2-methyl butanoic acid.

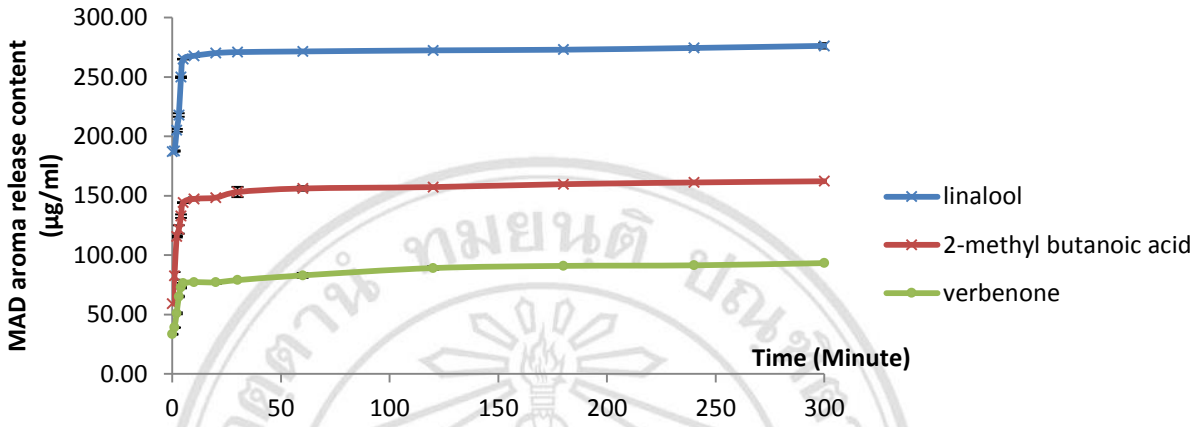


Fig. 6.13 The release of main aroma compounds during incubation in SSF (pH 7.0±0.2, 37 ˚C). Aroma release was presented as the amount from static head space; linalool (blue line), 2-methyl butanoic acid (red line), and verbenone (green line).

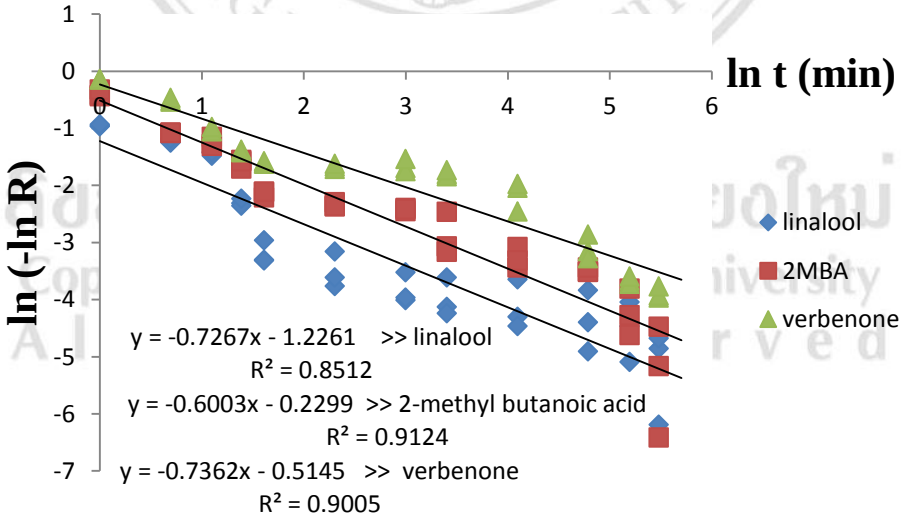


Fig. 6.14 Correlation of release time-course of main aroma compounds during incubation in SSF (pH 7.0±0.2, 37 °C) by Avrami's equation.

Table 6.8 The release rate constant and the kinetics parameters of the Weibull model under SSF condition

Main aroma volatile compounds	k (min^{-1})	n	R-square
linalool	1.23	0.7267	0.8512
2-methyl butanoic acid	0.23	0.7362	0.9005
verbenone	0.51	0.6003	0.9124

6.4 Conclusion

The finding from the optimization of the MEFP in GGA system provided the highest in yield recovery, encapsulation efficiency, pandan release content, linalool aroma release, 2-methyl butanoic acid and verbenone with lowest water activity. The optimized formula of MEFP was gelatin 3.00% w/v gum arabic 3.73% w/v and pandan flavor 5.26% w/w of gelatin-gum arabic solid with MAD flavor powder as a core material at 2.5% w/w of total gelatin-gum arabic solid. The released pandan flavor content slightly increased over time in hot water condition with the release rate constant at 4.14 min^{-1}. The amount of MAD main aroma showed that the content of linalool was the highest, followed by 2-methyl butanoic acid and verbenone. The release rate constant of three main compounds in SSF suggested that linalool had the slowest (1.23 min^{-1}), followed by verbenone (0.91 min^{-1}) and 2-methyl butanoic acid (0.51 min^{-1}). The results suggested that the pandan flavor can be retained within MEFP and slightly released in high temperature condition. In addition, the MAD flavor powder inside MEFP was also retained within product and can be release through SSF.

6.5 References

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