CHAPTER 5

Development of multi-core microencapsulation (I): Optimization of *Michelia alba* D.C. extract with octenyl succinic anhydride starch

Abstract

This study was intended to optimize *Michelia alba* D.C. (MAD) extract (5–10% w/w of dry starch) using octenyl succinic anhydride starch (OSA starch) ratio (250–1000 g per 1000 ml of water) as the wall material. The central composite design with two center points was employed in this experiment. The optimized formula for MAD encapsulated powder using the MAD extract and the OSA starch were 15% w/w of dry starch and 963.2 g per 1000 ml of water, respectively. The optimum formula also provided the highest in yield recovery (40.65%), encapsulation efficiency (68.55%), linalool aroma release (662.45 µg/ml), 2-methyl butanoic acid (360.74 µg/ml), and verbenone (190.81 µg/ml) with the lowest moisture content (3.04%) and water activity (0.230). The aroma release from the encapsulated MAD in simulated artificial saliva fluid (SSF) suggested that linalool can be retained in microcapsules higher than verbenone and 2-methyl butanoic acid. The release rate constant of the three main compounds suggested that linalool had the slowest release rate constant (1.26 min^{-1}), followed by verbenone (0.53 min^{-1}) and 2-methyl butanoic acid (0.25 min^{-1}). The findings revealed that the optimized MAD encapsulation with OSA starch can create encapsulated powder with controlled-release property. The slower rate of aroma release is considered to be indicative of its great ability to retain selective aroma at desirable conditions.

Keywords: *Michelia alba* D.C., octenyl succinic anhydride starch, encapsulation, optimization, controlled-release aroma

Publication:

Samakradhamrongthai R., Thakeow, P., Kopermsub, P., and Utama-ang N. 2015. Development of multi-core microencapsulation (I): Optimization of *Michelia alba* D.C. extract with octenyl succinic anhydride starch**.** *LWT-Food Science and Technology,* (Submitted and Reviewing)

5.1 Introduction

Michelia alba D.C. (MAD; champee) is commonly called white champaca which has spindle shape and formed in clusters, star-like shape and have a pleasant, sweet floral scent (Sanimah *et al*., 2008). Dried champee petals are used in Thai traditional medicine for heart and nerve maintenance and anti-motion sickness. It can also act as a cough suppressant and expectorant in subjected animals (Bunyapraphatsara, 1996; Subcharoen, 1999). In addition there is a monoterpene alcohol which is linalool, occurring naturally in MAD that can act as a flavoring agent which is used in many flavor industry and contributes to the characteristic aroma of a vast number of natural product such as fruits and spices as well as beverage and sweets, for instance, tea and chocolates (Kumar *et al*., 2012).

Encapsulation is the techniques the material or mixture system entrapped other active ingredients inside. It is applied to retain flavor and aroma between storage periods in food products (Green & Scheicher, 1995). Encapsulation can be applied to retain flavor and aroma food products. It can protect flavor/aroma from undesirable interaction as well as allow controlled-release and increase storage period (Reineccius, 1991: Tari & Singhal, 2002). Complexation inclusion can extend storage time of food flavoring using reduction of evaporation (Ades, Kesselman, Ungar, & Shimoni, 2012). There are many processes presently applied for flavor and aroma encapsulation. Spray drying, freeze drying, extrusion, coacervation, co-crystallization are some of the most selected techniques for encapsulating flavor in food industries (Saikia, Mahnot, & Mahanta, 2015). The advantages of encapsulation are protecting degradative reaction, preventing of loss of flavor and also controlled-release function of flavors during food processing (Soottitantawat, Yoshii, Furuta, Ohkawara, & Linko, 2003). The spray

drying is the most common, interesting and applicable process which widely apply for retaining and protecting flavor compounds for flavor and aroma encapsulation in commercial food flavor industries (Beristain, Garcia, & Vernon-Carter, 2001). There are numbers of researches have reported on encapsulation of flavors using spray drying (Beristain *et al*., 2001; Liu *et al*., 2001; Desai & Park, 2005; Dalgleish, 2006). It is known widely that wall material in encapsulation is one of the main factors that influenced flavor retention during spray drying and on the release of encapsulated flavor powder (Sosa, Zamora, Chirife, & Schebor, 2011 and Penbunditkul *et al*., 2012). The common wall materials for spray drying microencapsulation usually have low molecular weight carbohydrates (maltodextrin, modified starches, saccharose and cellulose), lipid and proteins (soy protein, whey protein and gelatin), gum (gum arabic, gum acacia). These materials improve in reduction of moisture, protect core substances from oxidative reaction and control their release during process and application (Ades *et al*., 2012; Borrmann, Pierucci, Leite, & Leão, 2013). Presently, the most interesting carrier materials for controlled-release are starch, protein, chitosan, and sodium alginate (Wang *et al*., 2011).

Starch is widely applied in food flavor microencapsulation which provides controlled-release properties to be suitable for specific application (Ades *et al*., 2012; Bhosale & Singhal, 2007). There are modifications on starches using dicarboxylic acid anhydride to generate starches with hydrophilic and hydrophobic groups. The modification of starch into octenyl succinic anhydride starch dislocates the hydrogen bonding and reduces retrogradation while increasing its hydrophobicity. In this way, Octenyl succinic anhydride starch (OSA starch) has been used to stabilize emulsions and to encapsulate flavor ingredients (Rodriguez, Wilderjans, Sosa, & Bernik, 2013). Some study indicated that OSA starch has shown considerably vulnerability to selected enzymes such as alpha-amylase and amyloglucosidase which provided controlled-release function due to the hydrolyzed of the starch-aroma complex and release entrapped flavor (Wang *et al*., 2011). Moreover, OSA starch has been permitted to apply in foods by USFDA among these alkenylsuccinic acid (Bhosale & Singhal, 2007). Parameters related with the releasing of aroma determine the instability of the flavor components. Suitable choice of conformation and microstructures provide controlled-

release of aroma during preparation and consumption. This is when encapsulation comes to be the proper choice to support and be the tool in aroma encapsulation. Flavor and aroma in products can be encapsulated to improve aroma functionality and constancy (Ades *et al*., 2012).

This study was intended to optimize MAD encapsulated flavor powder from the MAD extract using the OSA starch as a wall material. The optimized encapsulating condition was determined employing RSM with CCD experiment. The effects of the MAD extract and the OSA starch 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 MAD encapsulated flavor powder in simulated artificial saliva fluid (SSF) was also investigated to suggest the kinetic aroma release model of microencapsulated flavor powder.

5.2 Materials and Methods

5.2.1 Materials

White Champaca (*Michelia alba* D.C., MAD) was purchased from flower orchard in Nakorn Pathom and delivered from wholesale flower market (Pak Klong Talad, Bangkok, Thailand). All fresh blossoms were collected during June 2013 and the collection time was early in the morning within 5-8 am. All standards chemicals (2-methyl butyric acid, (-)-linalool, and (1s)-verbenone) were purchased from Sigma-Aldrich, Co., LLC. (MO, USA). The α-amylase from *Aspergillus oryzae* for analyzing aroma release was purchase from Sigma-Aldrich, Co., LLC. (MO, USA). Octenyl Succinic Anhydride starch (OSA starch) for wall material was purchased from National Starch & Chemical (Thailand), Co., Ltd. (Bangkok, Thailand). The analyzed organic chemicals were of analytical grade.

5.2.2 MAD extract preparation

The MAD flower was rinsed through fresh water and then drained under cool shade. The washed MAD petals were then separated before taken through drying process. The fresh MAD petals were dried using tray dry hot air oven (464CHMU, NAVALOY CO., LTD., Bangkok, Thailand) with temperature at 45±5°C for 24 hr (Samakradhamrongthai, 2011). The dried MAD petals were ground using the hammer mill grinder (C31896, Armfield, Christy&Norris LTD., Ipswich, England) with 0.5 mm mesh for consistency of sample and increase the surface on MAD extraction. All of the dried MAD was collected in the vacuum foil packages at (-20)°C. The MAD extract was prepared from the dried MAD petals in solvent extraction using 70% v/v ethanol under ambient temperature (25°C) for 12 hr with sample and solvent ratio at 1:10. After time lapse, the solvent was drained and filtered. The filtrate was evaporated under reduce pressure at the 40°C (R-200, Buchii, Switzerland). The extract was weighed and contained in amber vial less than 4°C for further experiment (Paibon *et al*., 2011).

5.2.3 Experimental design of MAD extracts microencapsulation

The procedure of emulsion preparation was modified from Ades *et al*. (2012). The MAD extracts percentages variations were 5–10% w/w of dry starch. The OSA starch solution ratio was used as wall material with variation from 250–1000 g per 1000 ml of water. The RSM was employed to optimize content of the MAD extract (X_1) and the OSA starch (X_2) on encapsulation process in term of yield recovery, moisture content, water activity, solubility, encapsulation efficiency and aroma release profile in SSF. This experiment was designed in CCD with two center points. The five coded levels (-1.414, -1, 0, +1, +1.414) of the two factors were incorporated in the design with two center points leading to 10 experiments (Table 5.1). All experiments were carried out in triplicate. The quadratic polynomial regression model was assumed for predicted all responses.

The infeed emulsion was prepared according to methods described by Flores-Martínez, Osorio-Revilla, & Gallardo-Velázquez (2004) and Ferreira, Rocha, & Coelho (2007) with modification. The aqueous phase OSA starch of was prepared in deionized water at 50°C while stirring for 30 min until the solution temperature reached 90°C. The MAD extract was added into the OSA solution and stirred vigorously. The solution was left to stand in room temperature for 30 min to ensure complete dispersion. The emulsion were dehydrated using spay drying. 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 microcapsules obtained from spray drying were directly weighed for each tests and stored in desiccators for further analysis. The yield recoveries (%Y) of spray drying have been calculated using Eq. (6.1).

$$\%Y = (\text{mass of solid in the collecting vessel (g)} / \text{mass of solid in the feed (g)}) \times 100 \quad (6.1)$$

Table 5.1 Treatment variation of MAD extract and OSA starch using CCD

Treatment	Coded		Actual	
	X_1	X_2	MAD extract of dry starch (%w/w, g)	OSA starch (%w/v, g per 1000 ml solution)
1	-1	-1	5.00 (12.50)	25.0 (250.00)
2	+1	+1	15.00 (150.00)	100.0 (1000.00)
3	+1	-1	15.00 (37.50)	25.0 (250.00)
4	0	+1	10.00 (100.0)	100.0 (1000.00)
5	0	-α	10.00 (9.47)	9.47 (94.70)
6	0	+α	10.00 (115.53)	115.53 (1155.30)
7	-α	0	2.93 (18.31)	62.5 (625.00)
8	+α	0	17.07 (106.69)	62.5 (625.00)
9	0	0	10.00 (62.50)	62.5 (625.00)
10	0	0	10.00 (62.50)	62.5 (625.00)

Note: X_1 = MAD extract and X_2 = OSA starch

5.2.4 Morphology of MAD encapsulated flavor powder

The microcapsules obtained from spray drying were examined for their morphology using 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 *et al*., 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.

5.2.5 Release profile of main aroma compounds from MAD encapsulated flavor powder

The release profiles of main aroma compounds from MAD encapsulated flavor powder were determined by incubating in SSF. The complex (20 mg) was incubated in SSF with the adjusted pH adjusted at 7.2 by potassium hydroxide. The α-amylase activity amount was 100 unit/ml as the average activity found while chewing (Watanabe & Dawes, 1988; Yamaguchi *et al*., 2004). The incubation was taken place in a 20 ml glass vial sealed by a screw cap covered with an aluminum foil filled with 2 ml SSF at 37±2°C in controlled temperature water bath (WB22, Memmert GmbH + Co.KG, Germany) under continuously stirring shaker at 12 rpm (SV1422, Memmert GmbH + Co.KG, Germany). Sample was sampling at 0, 1, 2, 3, 4, 5 toward 300 min. The extent of aroma released following the incubation in SSF was measured released aroma using the static head space from the reaction medium and quantification by GC (applied from Ades *et al*., 2012). The aroma content analysis was performed on gas chromatography (GC-2010, Shimadzu, Corp., Japan). The 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 per min and held for 5 min at 250°C. All data were recorded to create release profile. The maximum aroma release in the oral

cavity was considered at 5 min then the aroma release content at 5 min was taken to optimize MAD extract and OSA starch together with other responses from MAD encapsulated flavor powder. The aroma content of three main compounds was calculated from standard calibration curves in term of µg/ml. The aroma profile was presented between aroma release amount (µg/ml) and releasing time (min).

5.2.6 Physical properties of MAD encapsulated flavor powder

Moisture content. Five grams of encapsulating powder were dried in hot air oven (FD 115, Serial 08-836864, Binder, Germany) at 105°C for 5 hr. Afterwards, samples were weighed and the percentage of 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 an 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 triplication.

Solubility. The solubility of the encapsulated powders was examined according to the method described in Fernandes, Borges, & Botrel. (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 triplication.

5.2.7 Encapsulation efficiency (%EE)

The encapsulation efficiency of encapsulated powder was analysed followed method from Carneiro *et al*. (2013) with modification. The MAD extract was trapped in the microcapsule and adhered on the surface; therefore, to examine the microencapsulation efficiency, the quantities of surface and total content of MAD extract were determined. Five grams of encapsulated powder from spray drying and freeze drying were soaked in 50 ml of absolute ethanol using magnetic stirrer at 50 rpm. The mixing time for surface content was 5 min while the mixing time for total content was 15 min. The extracted solvent was transferred to Büchner funnel with 125 mm diameter filter paper (Whatman^{TM} No. 4, Buckinghamshire, UK) which was dried in hot air over for 24 hr and then weighted before use. The filtrates of extracted solvent was taken to evaporate and eliminate all the solvent using rotary evaporator (V800, Buchi, Switzerland) at 40°C with pressure at 175 mbar for ethanol and 72 mbar for water. After the evaporation, the pear-shaped evaporating flask with extract was taken to get rid of excessed moisture using hot air over (FD 115, Serial 08836864, Binder, Germany). The residue in pear-shaped evaporating flask was then weighed determined for extracted filtrate (applied from Ades *et al*., 2012 and Samakradhamrongthai, 2011). The quantities were reported as mean and standard deviation of triplicate measurements. EE was calculated according to Eq. (5.2)

$$EE = [(\text{Total extract content (g)} - \text{surface extract content (g)})/\text{Total extract content (g)}] \times 100 \quad (5.2)$$

5.2.8 Microstructural characterization of optimized MAD encapsulated flavor powder

Morphology of optimized MAD encapsulated flavor powder

The optimized MAD flavor powder obtained from spray drying were examined using scanning electron microscope followed the method from 5.2.4

Glass transition temperature (T_g) of optimized MAD encapsulated flavor powder

The optimized MAD flavor powder was stored at 25% relative humidity in desiccator for 24 hr prior to T_g analysis. The samples were weighed (5±0.2 mg) and placed in an aluminum pan and then 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 the temperature ramping from 20°C to 120°C at the rate of 10°C per min. A sealed empty aluminum pan was used as reference. All measurements were performed in triplication (Chen, Zhong, Wen, McGillivray, & Quek, 2013).

X-Ray diffraction of optimized MAD encapsulated flavor powder

The formation of optimized MAD encapsulated powder from spray drying was verified using X-ray diffraction (XRD) compared with OSA starch and spray-dried OSA starch. The experiment was carried out by a Miniflex II Desktop X-ray Diffractometer equipped with a graphite crystal monochromator (Miniflex II, Rigaku Corp., 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 XRD peaks (Bhosale & Singhal, 2007).

5.2.9 Release profile of main aroma compounds from optimized MAD encapsulated flavor powder

The optimized MAD encapsulated flavor powder was sampling at 0, 1, 2, 3, 4, 5 toward 300 min. The extent of aroma released following the incubation in SSF was

measured released aroma using the static head space from the reaction medium and quantification by GC followed the method from 5.2.5.

5.2.10 Statistical analysis

All data were carried out in triplicate and reported as mean±standard deviation of mean (S.E.M.). The optimization of MAD microencapsulation 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 ($p < 0.05$).

5.3 Results and discussion

5.3.1 Morphology of MAD encapsulated flavor powder using Scanning Electron Microscopy (SEM)

The SEM micrographs of the MAD encapsulated flavor powder were conformed to many studies (Santana, de Oliveira, Pinedo, Kurozawa, & Park 2013; Kha, Nguyen, Roach, & Stathopoulos, 2014). The MAD encapsulated flavor powder showed various particle sizes in range of 16–35 μm as shown in Table 5.2. It is desirable to observe the morphology of particles. All sample of the MAD encapsulated flavor powder exhibited spherically regular shape and concaved surface which were typical characteristics of spray-dried microcapsules (Kha *et al*., 2014). There were absences of cavities which indicated that formation of a continuous film on the outer shell of microparticle and suggested higher encapsulation efficiency as reported by Frascareli, Silva, Tonon, & Hubinger (2012), who noticed the distribution curves for coffee oil microcapsules in a bimodal behavior. In addition, the occurrence of larger microcapsules at >30 μm can be qualified to cause agglomeration of collected powder. The decreasing feed solids concentration and increasing core to wall material ratio can affected encapsulate flavor particle size to be increased as reported by Beristain *et al.* (2001) and Frascareli *et al.*

(2012). These observations indicated the fact that the effect of particle size on encapsulation efficiency depends on the type of the core material. This observation is similar to that reported by Soottitantawat *et al.* (2005). They reported that larger powder size leads to higher stability and lower release of encapsulated material if the initial emulsion has a small size. This was specified that the MAD encapsulated flavor powder have lower permeability of volatile compounds, increasing the protection and retention of the aroma and flavor compounds (Carneiro, Tonon, Grosso, & Hubinger, 2013).

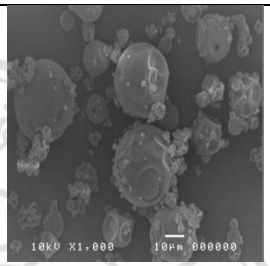
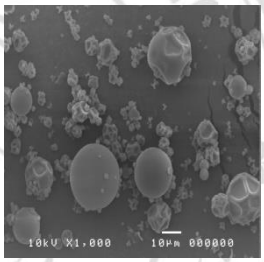
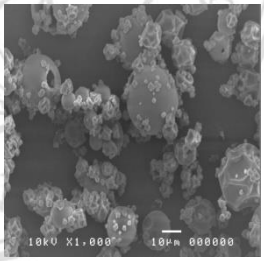
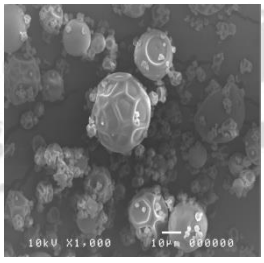
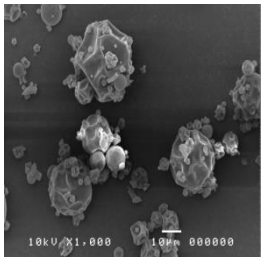
5.3.2 Kinetic study of the release of the main aroma compounds from MAD encapsulated flavor powder

The three main aroma compounds were analyzed and the results were presented in Fig. 5.1 as amount of each compound released in µg/ml. The rate constant of aroma release was evaluated using Avrami's equation (Weibull distribution function) as shown in Eq. (5.3).

$$R = 1\text{-}\exp[-kt]^{n} \qquad (5.3)$$

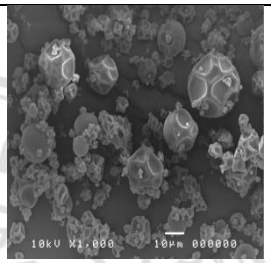
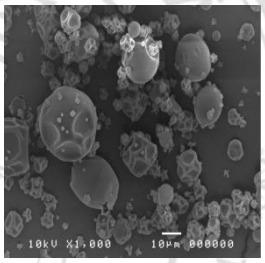
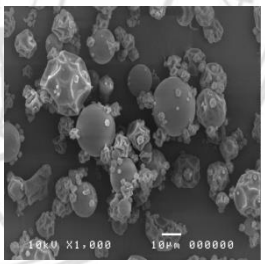
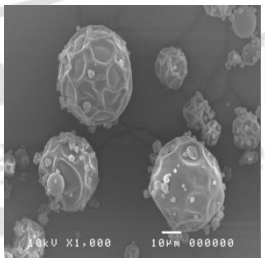
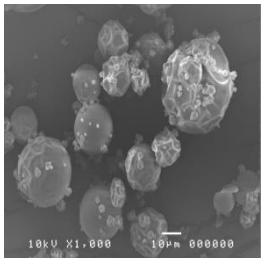
Where *R* is the release amount of aroma, *t* is the time that incubating in artificial saliva, *k* is the release rate of constant and *n* is a parameter representing the release mechanism. The Avrami's equation was chosen to employ in this experiment because of the fitted model that was applied to describe the shelf-life failure and was suitable to describe the release time-course of the encapsulated flavor powder (Yoshii *et al*, 2001; Soottitantawat *et al*, 2004; Szente & Sejtli, 2004). The encapsulation of MAD extract and OSA starch from treatment 9 was selected as an example because of the content of factors was in the middle level. This treatment was consisted of MAD extract 62.5 g and OSA starch 625.00 g per 1000 ml of water. The main aroma compounds were initially analyzed for released aroma content. The released aroma content of 2-methyl butanoic acid, linalool, and verbenone was analyzed from 0 min toward 300 min. The detected content was in range of 314.79–479.14 µg/ml, 193.48–575.96 µg/ml, and 115.08–317.96 µg/ml, respectively (Fig. 5.2).

Table 5.2 The SEM micrographs of spray dried MAD extract in OSA starch

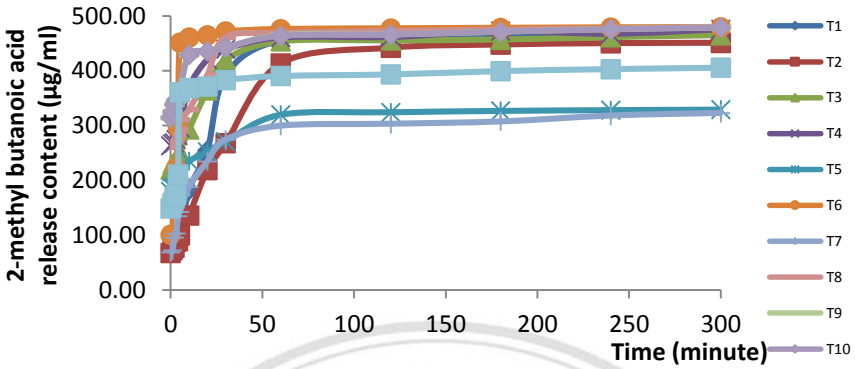
Treatment	MAD extract (%w/w, g of dry starch)	OSA starch (%w/v, g per 1000 ml of water)	SEM	Particle size (µm)
1	5.00 (12.50)	25.0 (250.00)		20.33±2.04e
2	15.00 (150.00)	100.0 (1000.00)		25.35±1.57c
3	15.00 (37.50)	25.0 (250.00)		22.11±2.54d
4	10.00 (100.0)	100.0 (1000.00)		27.43±1.40b
5	10.00 (9.47)	9.47 (94.70)		22.69±1.03d

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

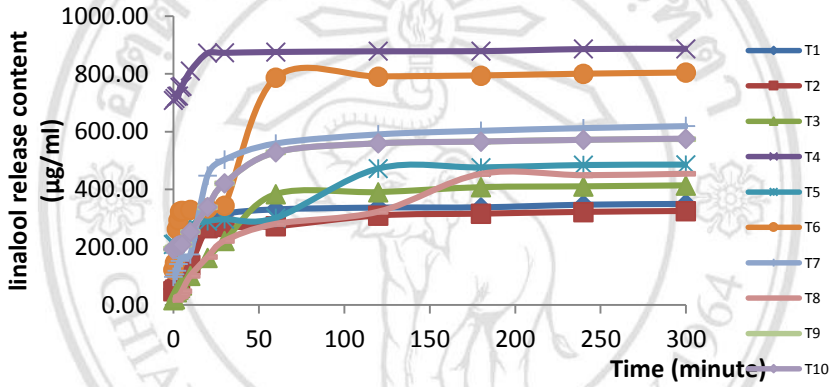
Table 5.2 (cont'd.) SEM micrographs of spray dried MAD extract in OSA starch

Treatment	MAD extract (%w/w, g of dry starch)	OSA starch (%w/v, g per 1000 ml of water)	SEM	Particle size (μm)
6	10.00 (115.53)	115.53 (1155.30)		16.74±1.52f
7	2.93 (18.31)	62.5 (625.00)		19.40±1.61e
8	17.07 (106.69)	62.5 (625.00)		25.24±2.42c
9	10.00 (62.50)	62.5 (625.00)		35.71±2.04a
10	10.00 (62.50)	62.5 (625.00)		35.14±2.22a

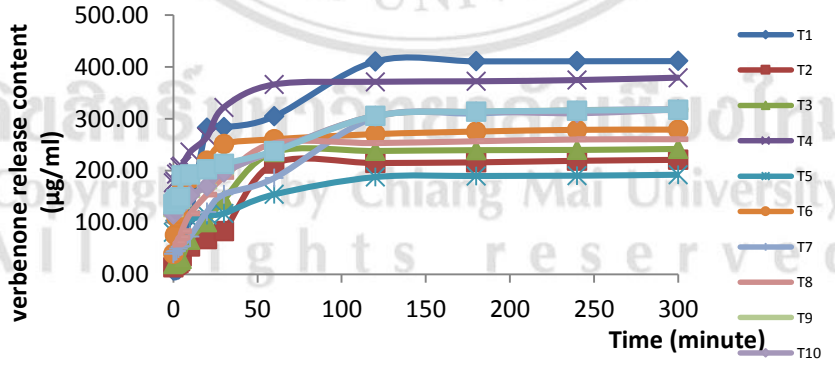
Note: The different letters in the same column mean significant difference ($p \leq 0.05$)



(a)



(b)



(c)

Fig. 5.1 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 (a) 2-methyl butanoic acid, (b) linalool, and (c) verbenone. The different treatments were marked as T1 – T10 with different colors and markers.

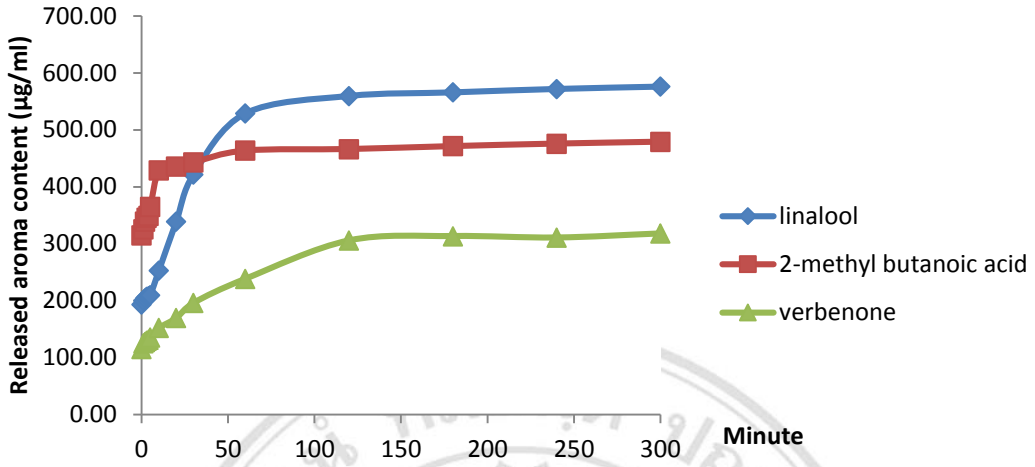


Fig. 5.2 Release of main aroma compounds during incubation in SSF (pH 7.0±0.2, 37 °C), Aroma release was presented as the amount from static head space.

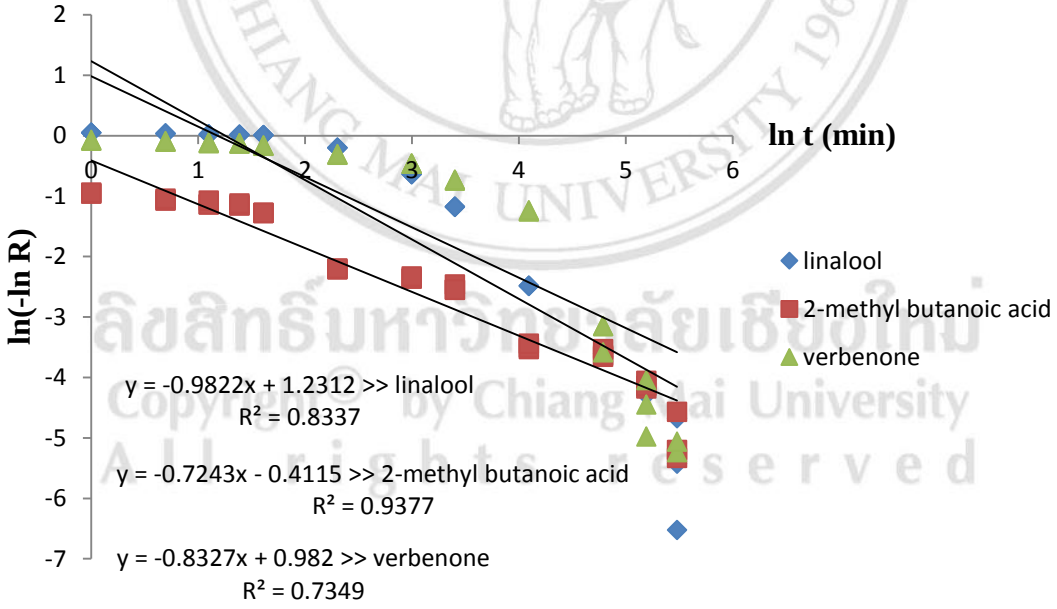


Fig. 5.3 Correlation of release time-course of main aroma compounds during incubation in SSF (pH 7.0±0.2, 37 °C) by Avrami's equation.

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 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 2-methyl butanoic acid, linalool, and verbenone were shown as *1.23* and *0.98*; *0.41* and *0.72*; *0.98* and *0.83*, respectively (Fig. 5.3).

The release rate constant of 2-methyl butanoic acid, linalool, and verbenone were in range of 0.78–2.06 min^{-1}, 0.33–2.01 min^{-1}, and 0.52–2.17 min^{-1}, respectively. The value of *n* was in range of 0.72–1.5 which suggested that release mechanism of encapsulated powder in SSF can be identified as first-order mechanism as indicated in Soottitantawat, Partanen, Neoh, & Yoshii (2015). The release time-course of aroma compounds using RSM showed that that the release rate constant of 2-methyl butanoic acid was not significant difference whereas the release rate constant of linalool and verbenone were significant difference (Table 5.3).

The release rate constant of linalool and verbenone were suggested that the MAD extract and the OSA starch affected the release rate constant (Table 5.4). The release rate constant linalool increased when the OSA starch increased while the MAD extract increased in term of exponential. Moreover, there were interaction between the MAD extract and the OSA starch toward decreasing of linalool release rate which suggested that linalool release rate decreased when both of the MAD extract and the OSA starch are decreased. The release rate constant of verbenone showed that there was interaction between the MAD extract and the OSA starch in the decreasing track which suggested that verbenone release rate constant was decreased when both of the MAD extract and the OSA starch were decreased. The contour plot from regression equation of responses revealed response surface of linalool release rate and verbenone release rate as shown in Fig. 5.4.

Table 5.3 Kinetic parameter of main volatile compounds from Avrami's equation

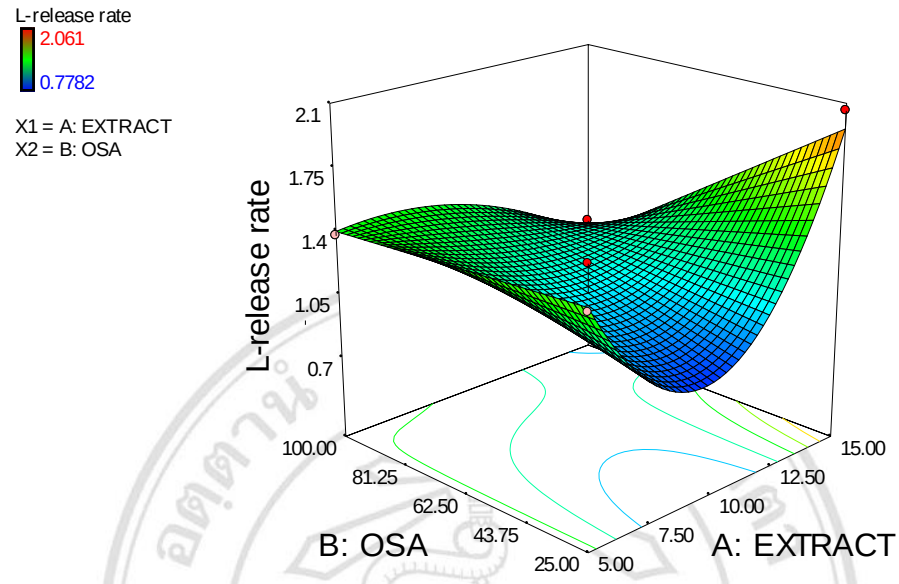
Factors		2-methyl butanoic acid			linalool			verbenone		
X_1	X_2	k (min^{-1})	n	R^2	k (min^{-1})	n	R^2	k (min^{-1})	n	R^2
5.00 (12.50)	25.0 (250.00)	1.4656	1.0163	0.9058	1.7086	1.1125	0.9345	1.6096	1.5954	0.8347
15.00 (150.00)	100.0 (1000.00)	1.3857	0.9388	0.9267	2.0146	1.2073	0.8303	2.0981	1.0862	0.9167
15.00 (37.50)	25.0 (250.00)	2.0610	1.0766	0.9127	0.5553	0.8970	0.8802	2.1745	1.2013	0.8667
10.00 (100.0)	100.0 (1000.00)	1.0311	0.7294	0.8993	0.2177	0.8503	0.8920	0.5165	0.8277	0.8909
10.00 (9.47)	9.47 (94.70)	0.7782	0.8055	0.8572	0.5030	0.9741	0.7544	0.7782	0.8055	0.7544
10.00 (115.53)	115.53 (1155.30)	1.4629	1.0627	0.9331	0.4775	1.3262	0.7985	0.9409	0.9992	0.8912
2.93 (18.31)	62.5 (625.00)	1.4890	0.9488	0.9527	0.9029	0.8043	0.9261	1.7757	0.9025	0.7677
17.07 (106.69)	62.5 (625.00)	2.0330	0.8988	0.9167	0.3250	0.9310	0.7553	1.5934	1.1198	0.8339
10.00 (62.50)	62.5 (625.00)	1.2312	0.9822	0.9377	0.4115	0.7243	0.8337	0.9820	0.8327	0.7349
10.00 (62.50)	62.5 (625.00)	0.8655	0.7570	0.9390	0.4203	0.7212	0.7327	0.7570	0.7570	0.7327
p-value		0.0576			0.0249			0.0069		

Note: X_1 = MAD extract (weight of dry starch), X_2 = OSA starch (g per 1000 ml of water)

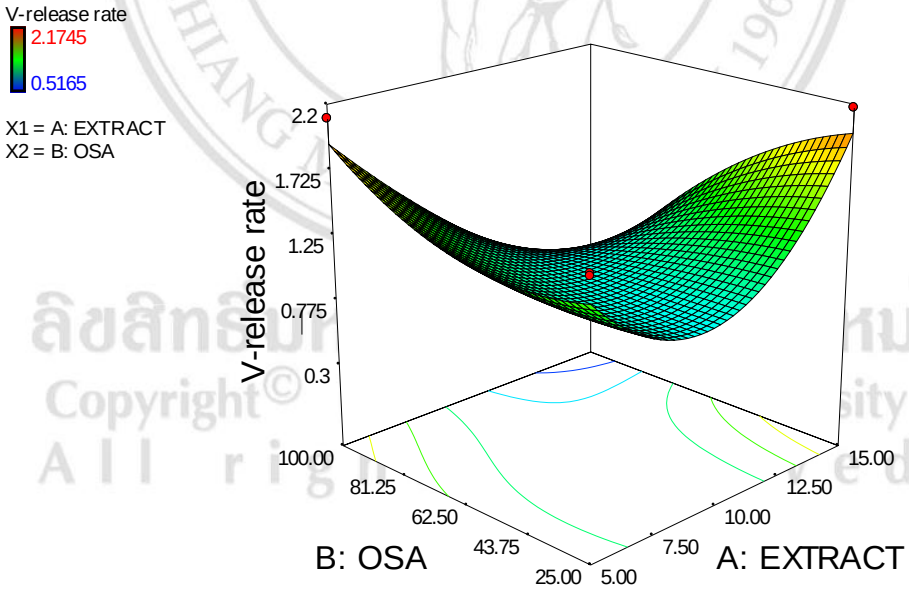
Table 5.4 Regression equation of significant responses from microencapsulated flavor powder using RSM

Aroma release rate constant	Regression equation (coded)	Adjusted R^2	*p-value*
linalool release rate (min^{-1})	$1.10+0.24X_2- 0.24X_1X_2 +0.35X_1^2-0.52\ X_1^2X_2$	0.7400	0.0249
verbenone release rate (min^{-1})	$0.98-0.54X_1X_2-0.44X_1^2\ X_2 -0.25\ X_1X_2^2$	0.8477	0.0069

Note: X_1 = MAD extract, X_2 = OSA starch



(a)



(b)

Fig. 5.4 The response surface demonstrated regression model between MAD extract and OSA starch; (a) the release rate constant of linalool and (b) the release rate constant of verbenone.

5.3.3 Physical and encapsulation properties of MAD encapsulated flavor powder

The results of physical and encapsulation properties from the MAD encapsulated powder illustrated that all designed treatments were significant difference. The yield recovery of the MAD encapsulated flavor powder from spray drying was in range of 32.60–65.42%. The highest yield recovery was from MAD extract 12.50 g of dry starch and OSA starch 250 g per 1000 ml of water whereas the lowest yield recovery was from MAD extract 150 g of dry starch and OSA starch 1000 g per 1000 ml of water. The moisture content and water activity obtained from encapsulates were in range of 2.00–4.20% and 0.214–0.312, respectively. The MAD extract 12.50 g of dry starch and OSA starch 250 g per 1000 ml of water provided the lowest moisture content at 2.00% whereas MAD extract 106.09 g of dry starch and OSA 625 g per 1000 of water provided the lowest water activity 0.214. The results of color value showed that lightness (L^*) was in range of 72.67–89.31, a^* was in range of 2.77–9.45, and b^* was in range of 13.87–22.80. The percentage of solubility in water of all treatment was higher than 90% and in range of 90.81–96.89%. The encapsulation properties of MAD extract encapsulated powder showed that the surface extract content was in range of 1.71–8.09%. The encapsulation efficiency was in range 11.94–73.94% (Table 5.5). The aroma release was determined by the percentages of aroma content in headspace within 5 min in SSF. The three main aromas from previous chapter were investigated. The results showed that the release of 2-methyl butanoic acid was in range of 95.69–451.66 µg/ml, linalool was in range of 48.15–756.81 µg/ml, and verbenone was in range of 29.51–208.38 µg/ml (Table 5.6).

Table 5.5 Physical and encapsulation properties of MAD encapsulated flavor powder

T[1]	MAD extract (g of dry starch)	OSA starch (g per 1000 ml water)	Yield recovery (%)	Moisture content (%)	Water activity	*L**	*a**	*b**	Solubility (%)	Surface content (%)	Encapsulation Efficiency (%)	Extract Recovery (%)
1	12.50	250.00	65.42±0.87a	2.00±0.42f	0.221±0.002f	82.16±0.54e	5.70±0.19e	20.43±0.30c	93.03±0.38d	2.33±0.39ef	66.66±3.67b	69.75±3.81c
2	150.0	1000.00	32.60±0.28f	3.08±0.84cd	0.312±0.002a	87.69±0.14b	3.39±0.08g	15.66±0.04f	91.97±0.40e	4.28±0.05b	46.59±2.51e	80.35±4.01b
3	37.50	250.00	56.83±0.64b	2.40±0.13def	0.217±0.001g	78.29±0.08g	7.48±0.10b	22.14±0.12b	96.89±0.06a	1.77±0.03fg	43.05±1.11e	31.10±0.64f
4	100.00	1000.00	37.33±1.13d	2.26±0.30ef	0.229±0.002e	84.69±0.28d	4.53±0.09f	18.11±0.43e	92.63±0.56de	1.92±0.19fg	73.94±1.91a	73.68±5.66bc
5	9.47	94.70	56.17±1.65b	4.20±0.38ab	0.282±0.002b	72.67±0.61h	9.45±0.45a	22.80±0.96a	94.13±0.08b	2.52±0.16e	57.08±2.10c	58.68±0.76e
6	115.53	1155.30	35.56±0.67e	2.83±0.27cde	0.247±0.002d	86.67±0.51c	2.91±0.16h	14.55±0.33g	90.81±0.48f	8.09±0.47a	11.94±1.14f	91.87±4.79a
7	18.31	625.00	55.55±0.01b	3.31±0.21c	0.217±0.001g	89.31±0.29a	2.77±0.09h	13.87±0.19h	93.23±0.26cd	3.07±0.13d	54.58±1.07cd	67.65±2.63cd
8	106.69	625.00	51.88±0.18c	2.51±0.41def	0.214±0.001h	80.40±0.02f	6.65±0.03c	21.63±0.08b	93.84±0.18bc	1.71±0.25g	72.30±3.93ab	61.57±1.77de
9	62.50	625.00	55.14±0.25b	4.31±0.52a	0.272±0.002c	82.20±0.32e	5.76±0.17e	20.18±0.31c	92.63±0.45de	3.90±0.70bc	47.30±7.15e	73.76±4.54bc
10	62.50	625.00	55.65±0.51b	3.50±0.24bc	0.271±0.003c	81.64±0.96e	5.76±0.14e	20.14±0.06c	92.97±0.63d	3.52±0.12cd	49.54±5.52de	70.28±6.22c
	p-value		<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

Table 5.6 Aroma release of main volatile compounds properties of spray-dried microcapsules

Treatment	MAD extract (g of dry starch)	OSA starch (g per 1000 ml of water)	Aroma content at 5th min (µg/ml)		
			2-methyl butanoic acid	linalool	verbenone
1	12.50	250.00	95.69±0.33i	93.50±0.28g	59.96±0.54e
2	150.0	1000.00	99.65±0.57h	109.71±0.43f	34.44±0.25g
3	37.50	250.00	251.80±1.01e	68.30±1.55h	29.51±0.42h
4	100.00	1000.00	296.21±0.66d	756.81±0.43a	208.38±0.26a
5	9.47	94.70	214.64±0.38f	265.82±0.29c	96.78±0.44c
6	115.53	1155.30	451.66±1.15a	326.72±0.43b	162.77±0.29b
7	18.31	625.00	142.63±0.69g	163.50±0.29e	54.42±0.68f
8	106.69	625.00	310.72±0.33c	48.15±0.43i	83.30±0.25d
9	62.50	625.00	362.50±0.33b	209.41±0.89d	135.62±0.54c
10	62.50	625.00	365.03±4.39b	209.50±0.99d	135.62±0.54c
	p-value		<0.001	<0.001	<0.001

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

5.3.4 Response surface of physical and encapsulation properties of MAD encapsulated flavor powder

The physical and encapsulation properties of the MAD encapsulated powder were submitted to generate a response surface to determine the optimized formulation of the OSA starch and the MAD extract to produce the MAD encapsulated flavor powder. The findings from response surface analysis demonstrated that there were 10 responses that fitted to create regression model which were yield recovery, water activity, color value (*L**, *a**, *b**), solubility, encapsulation efficiency, 2-methyl butanoic acid aroma release, linalool aroma release, and verbenone aroma release. The relationship of the MAD extract (X_1) and the OSA starch (X_2) was explained as show in Table 5.7.

The yield recovery affected by the increasing of X_2 until the percentage reached 70%, the yield recovery started to be decreased. The increasing of X_1 affected yield

recovery by increasing it until the percentage of X_1 reached 8%, the yield recovery then started to decrease as shown Fig. 5.5a. The increasing the OSA starch provided higher yield recovery because of the agglomeration of powder product as well as low retention of core material while lower the OSA starch reflected on low production yield. These actions suggested a delay in the formation of a semi-permeable layer by the internal components during drying (Sasone, Mencherini, Picerno, D'Amore, & Aquino, 2011).

The water activity was affected by interaction of X_1 and X_2. The increasing mixture increased water activity until X_2 reached 80% with X_1 at 12.5% as shown in Fig. 5.5b. The lightness (L^*) indicated that the increasing of X_2 increased the lightness whereas the decreasing of X_1 together increased the lightness as shown in Fig 5.5c. The color value a^* and b^* indicated that the increasing of X_2 decreased color value a^* and b^* whereas the increasing of X_1 decreased redness (a^*) and yellowness (b^*) as shown in Fig. 5.5d and Fig. 5.5e.

The solubility was affected from X_2 and the interaction of X_1 and X_2 as shown in Fig. 5.5f. The increasing of only X_2 affected the solubility to be decreased whereas the increasing of X_1 and X_2 altogether affected the solubility to be increased. As Murúa-Pagola, Beristain-Guevara, & Martínez-Bustos (2009) stated that high concentration of modified starch increased water activity in contrast decreased solubility as the fact the low water content powder lead to high value of water solubility. This is implied that starch hydrolysis products are generally used in combination of the OSA starch can reduce the oxygen permeability of the matrix in spray-dried powders, resulting in higher solubility values (Qi & Xu, 1999).

The increasing of X_1 affected encapsulation efficiency to be decreased until the percentage of X_2 reached 50%. After that the encapsulation efficiency established to be stabled whereas the interaction of X_1 and X_2 showed affected the encapsulation efficiency to be increased the encapsulation efficiency as show in Fig. 5.5g. Boutboul, Giampaoli, Feigenbaum, & Ducruet (2002) also stated that the aroma retention was increased with the increasing of starch material. The low viscosity of the OSA starch is

preferable along with high total solids concentration of the feed emulsion and protection of exceeded air inclusion in the microcapsules during spray drying.

The aroma release content of 2-methyl butanoic acid, linalool, and verbenone were affected by the MAD extract (X_1) and the OSA starch (X_2). The aroma release content at 5 min of 2-methyl butanoic acid showed that only X_1 affected aroma release to be increased as shown in Fig. 5.5h. The increasing X_1 and X_2 affected the aroma release content separately because of 2-methyl butanoic acid only formed complex with X_2 only between starch molecules during the homogenization (Kim & Maga, 1994). The increasing of X_2 and the increasing of X_1 altogether with X_2 affected linalool content to be increased as shown in Fig. 5.5i. The X_1, X_2 and their interaction were affected verbenone content as shown in Fig. 5.5j. The increasing all the factors affected the aroma release content of verbenone to be increased. The reason that the all three factors affected the aroma release contents because of linalool and verbenone formed complex better than 2-methyl butanoic acid. Linalool and verbenone were infusing into the OSA starch molecules and then form V-complex structure during the homogenization (Anantha & Milford, 1997; Jouquand, Ducruet, & Bail, 2006).

Table 5.7 Regression equation of significant responses from microencapsulated flavor powder using RSM

Attributes	Regression equation (coded)	Adjusted R^2	*p-value*
Yield recovery (%)	$53-7.28X_2+3.33X_1X_2-4.65X_2^2-5.80X_1^2X_2$	0.9600	0.0003
Water activity	$0.27-0.01X_2-0.02X_1X_2-0.03X_1^2+0.04X_1^2X_2-0.02X_1X_2^2$	0.9844	0.0002
L*	$80.91-2.43X_1+3.97X_2+2.08X_1^2$	0.8744	0.0012
a*	$5.95+1.37X_1-2.31X_2-0.64X_1^2+X_1^2X_2-0.65X_1X_2^2$	0.9837	0.0002
b*	$19.56+2.74X_1-2.56X_2-0.76X_1^2-1.70X_1X_2^2$	0.9243	0.0012
Solubility (%)	$92.95-1.25X_2+1.13X_1X_2^2$	0.5924	0.0179
Encapsulation efficiency (%)	$42.93-15.96X_2+12.74X_1X_2+11.72X_1^2+18.74\ X_1^2X_2$	0.7402	0.0248
2-methyl butanoic acid (µg/ml)	$318.75+73.80X_1+47.95X_2-75X_1^2$	0.5773	0.0435
linalool (µg/ml)	$166.25+168.08X_1X_2+73.62X_2^2+176.18X_1^2X_2+155.47X_1X_2^2$	0.9005	0.0024
verbenone (µg/ml)	$135.62+23.04X_1+30.83X_2+51.10X_1X_2-37.44X_1^2-6.98X_2^2$	0.8358	0.0216

Note: X_1 = MAD extract, X_2 = OSA starch

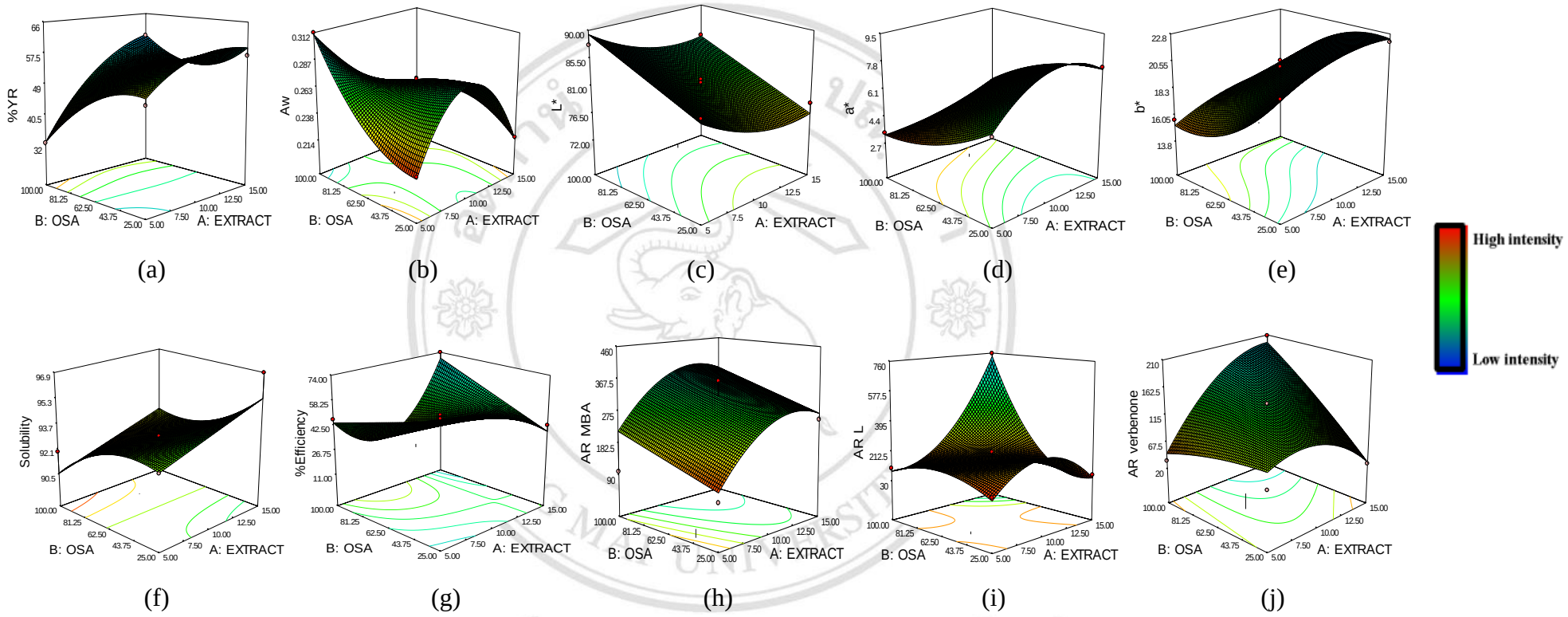


Fig. 5.5 The response surface demonstrated regression model between MAD extract and OSA starch; (a) yield recovery, (b) water activity, (c) color value *L**, (d) color value *a** (e) color value *b**, (f) solubility, (g) encapsulation efficiency, (h) 2-methyl butanoic acid aroma release content, (i) linalool aroma release content and (j) verbenone aroma release content.

5.3.5 Optimization and validation on MAD extract encapsulated powder

The predicted values with constrained of the highest and the lowest responses provided the highest yield recovery (41.36%), encapsulation efficiency (68.55%), 2-methyl butanoic acid release content (360.80 µg/ml), linalool release content (662.79 µg/ml), and verbenone release content (190.81 µg/ml) with the lowest water activity (0.230). The predicted response value indicated that the optimized formula for the MAD encapsulated flavor powder was consisted of the MAD extract 15% w/w of dry starch and the OSA starch 963.20 g per 1000 ml of water (Fig. 5.6). The optimized MAD encapsulated flavor powder was produced, validated and analyzed for all responses (Table 5.8). 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 0.02–10.04. This indicated that the results of validation were in perfect agreement between the predicted and measured values (Hu, 1999).

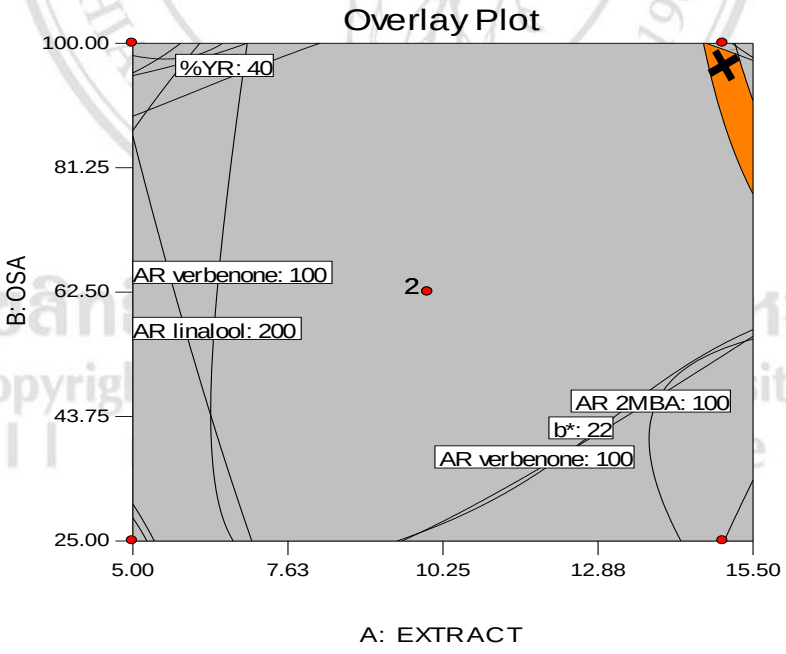


Fig. 5.6 The overlay plot of response surface demonstrated regression model between MAD extract and OSA starch.

Table 5.8 Comparison of prediction and validation value of MAD encapsulated powder with approximated error

Responses	Prediction value	Validation value	Approximated Error (%)
Yield recovery (%)	41.36	40.65±0.99	1.76
Moisture content (%)	3.04	3.19±0.06	4.93
Water activity	0.230	0.236±0.004	4.35
Color value			
*L**	84.13	85.39±0.95	1.50
*a**	4.98	4.48±0.06	10.04
*b**	17.86	17.64±0.55	1.23
Solubility (%)	92.74	90.96±0.65	1.92
Surface content (%)	4.50	4.57±0.32	1.56
Encapsulation Efficiency (%)	68.55	68.91±1.50	0.54
Extract recovery (%)	88.39	87.92±0.58	0.51
Aroma content at 5th min			
2-methyl butanoic acid (µg/ml)	360.80	360.74±0.69	0.02
linalool (µg/ml)	662.79	662.45±0.56	0.05
verbenone (µg/ml)	189.42	190.81±0.50	0.73

5.3.6 Microstructural characterization of optimized MAD encapsulated flavor powder

The external structure of the optimized MAD encapsulated flavor powder was observed using SEM. The images of encapsulated powder showed skin-forming morphology with a rounded external surface as shown in Fig. 5.7. The optimized MAD encapsulated powder showed spherically regular shape with shallowed dent of shrinkage which happened during early stage of drying and cooling. The particle size of optimized MAD encapsulated flavor powder was measured using ImageJ application and MAD encapsulated flavor powder particle size was 20.16±3.98 µm which conformed to Sahin-Nadeem, Torun, & Ozdemir (2011) and Saikia, Mahnot, & Mahanta (2015), who investigated on extract encapsulation using spray drying method. Glass transition temperature from optimized encapsulates flavor powder from spray drying 74.56°C. 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 70°C which indicated that the material was transformed to rubbery state when temperature of sample reached over 70°C and can be stored under temperature 70°C at 25% ambient relative humidity (Fig. 5.8).

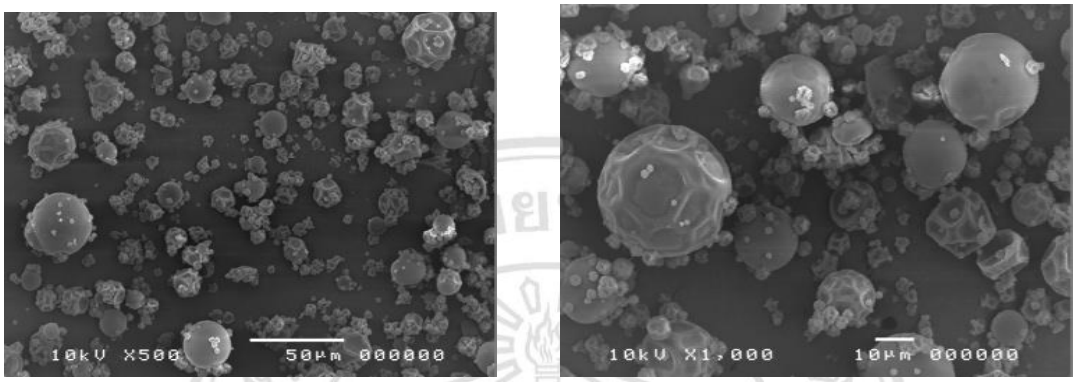


Fig. 5.7 The SEM micrographs of optimized MAD encapsulated flavor powder using spray drying.

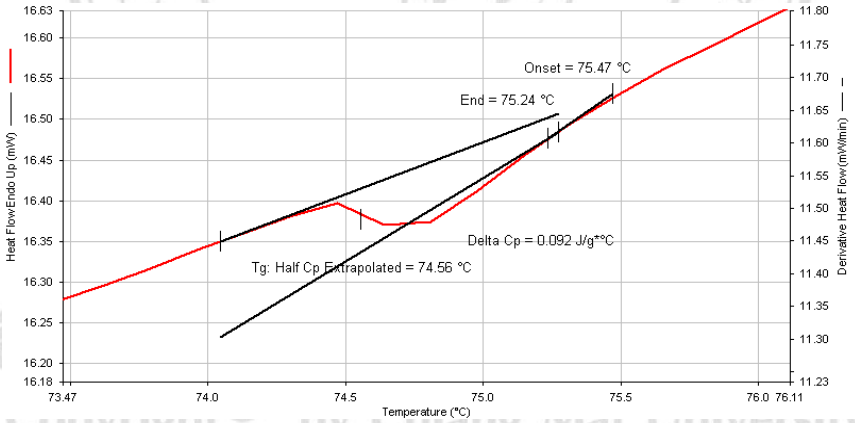


Fig. 5.8 The differential scanning calorimetry result of optimized MAD encapsulated flavor powder.

The changes of degree of crystallinity powder samples were analyzed using X-ray diffraction as shown in Fig. 5.9. The OSA starch and the optimized powder showed completely amorphous matrices, as specified from XRD patterns. 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 crystalline pattern of non-spray dried OSA starch showed C-type pattern (6˚, 9˚, 11˚, 15˚, 17˚, and 23˚) as in agreement with Wang and Wang (2002). The spray-dried OSA starch microcapsules also showed C-type pattern (15˚, 17˚, and 23˚) which suggested crystallinity of product was decreased as the amorphous increased. The encapsulated powder from spray drying created complexes of the OSA starch and the MAD extract resulting v-type polymorphs which provide Bragg angles of 2θ for V_7-type (13˚ and 18˚), V6h-type (20˚) also there were C-type (11˚, 15˚, and 23˚) crystalline pattern of OSA starch unchanged which indicated that OSA starch did not form complexes with MAD extract completely (Takeo, & Kuge, 1969; Buléon, Coloma, Plancot, & Ball, 1998; Le bail, Rondeau, & Buléon, 2005).

5.3.7 Kinetic release model of optimized MAD encapsulated flavor powder

The amounts of aroma release from the optimized MAD encapsulated flavor powder were different. The release profiles of 2-methyl butanoic acid, linalool, and verbenone were in range of 169.02–405.59 µg/ml, 448.34–690.56 µg/ml, and 85.09–223.60 µg/ml as shown in Fig. 5.10. Generally, high molecular weight flavor compounds were retained in encapsulated matrix more than low molecular flavor compounds. This behavior had been observed for spray dried with gum arabic from investigation of Rosenberg, Kopelman, & Talmon (1990) and Goubet, Le Quere, & Voilley (1998). The release rate constant of three main compounds followed Avrami's equation calculation suggested that linalool had the slowest release rate constant (1.26 min^{-1}), followed by verbenone (0.53 min^{-1}) and 2-methyl butanoic acid (0.24 min^{-1}) (Fig. 5.11 and Table 5.9). According to the results, 2-methyl butanoic acid was released from the complex in simulated saliva fastest, followed by verbenone and linalool. Therefore, the result was not agreed on many studies (Kim & Maga, 1994; Anantha & Milford, 1997; Jouquand *et al*., 2006) that suggested volatile compounds higher molecular weight comprised lower release rate. 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 is 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 complexes better that 2-methyl butanoic acid.

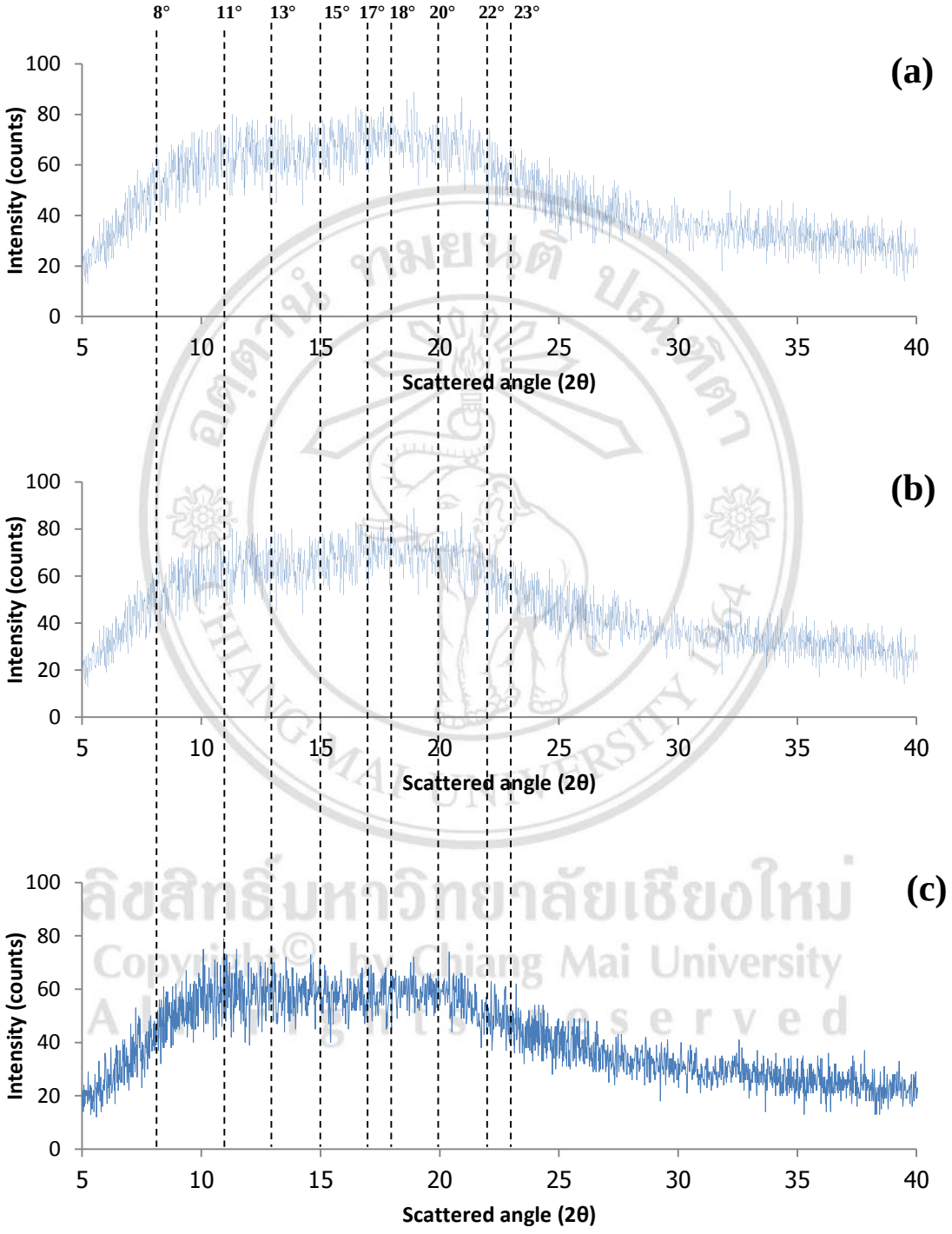


Fig. 5.9 X-ray diffraction scans of (a) non-processed OSA starch, (b) spray-dried OSA starch (non-extract), and (c) optimized microcapsules of MAD extract with OSA starch.

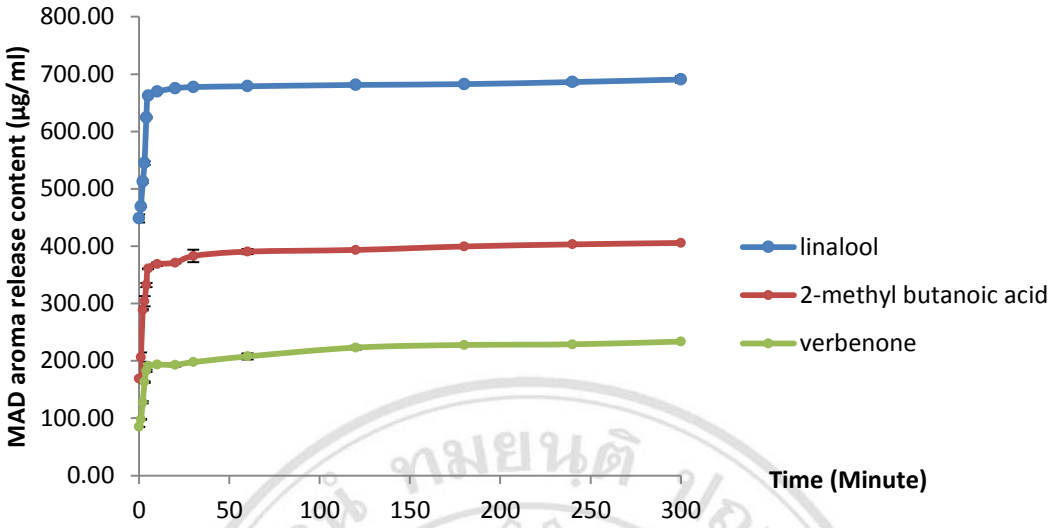


Fig. 5.10 The release of main aroma compounds from optimized MAD encapsulated powder during incubation in SSF (pH 7.0±0.2, 37 ˚C), Aroma release was presented as the amount from static head space.

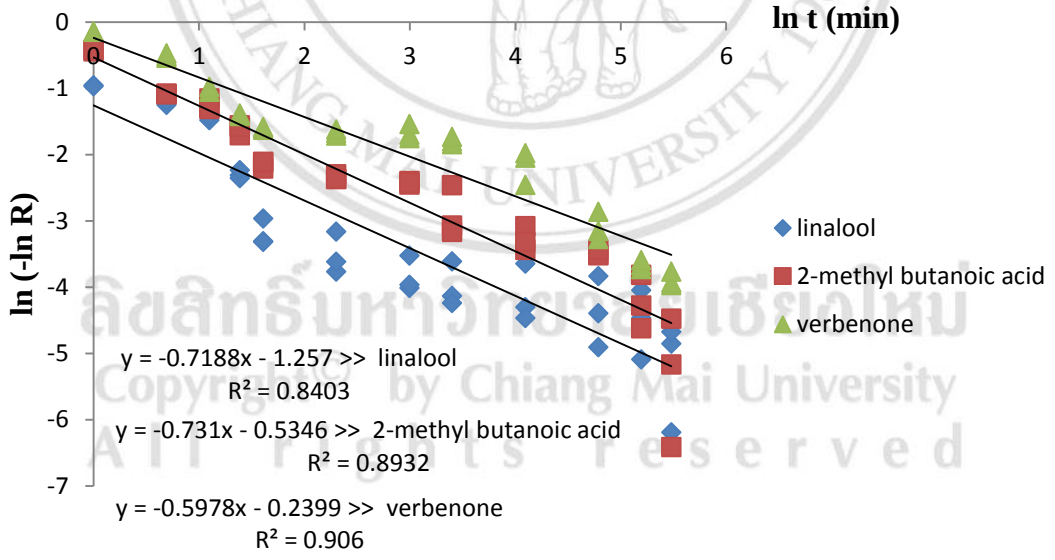


Fig. 5.11 Correlation of release time-course of main aroma compounds during incubation in SSF (pH 7.0±0.2, 37 °C) by Avrami's equation.

Table 5.9 The release rate constant and the kinetics parameters from Avrami's equation in SSF

Main aroma compounds	k (min^{-1})	n	R^2
2-methyl butanoic acid	0.24	0.7267	0.8512
linalool	1.26	0.7362	0.9005
verbenone	0.53	0.6003	0.9124

5.4 Conclusion

The optimized formula of MAD encapsulated flavor powder was consisted of the MAD extract and the OSA starch at 15% w/w of dry starch and 0.9632 per one part of water, respectively. The aroma release content of linalool was the highest, followed by 2-methyl butanoic acid and verbenone. The release rate of three main compounds in SSF followed Avrami's equation calculation suggested that linalool had slowest release rate constant, followed by verbenone and 2-methyl butanoic acid. This is 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 that 2-methyl butanoic acid. The findings from this experiment revealed that optimized MAD encapsulated flavor powder possessed controlled-release property in SSF. Those slower rates of aroma release rate found to be desirable characteristic of flavor powder that can be applied on many products that inquired to release aroma and flavor through enzymatic reaction in oral cavity.

5.5 References

Ades, H., Kesselman, E., Ungar, Y., & Shimoni, E. (2012). Complexation with starch for encapsulation and controlled-release of menthone and menthol. *Food Science and Technology,* 45, 277–288.

Anantha, N.R. & Milford, A.H. (1997). Cyclodextrin complexed flavors retention in extruded starch. *Journal of Food Science,* 62, 1057-1060.

AOAC. (2000). Official methods of analysis of AOAC international. *17th ed. Association of Official Analytical Chemists.* NO. 934.01.

Beristain, C.I., Garcia, H.S., & Vernon-Carter, E.J. (2001). Spray-dried encapsulation of cardamom (Elettaria cardamomum) essential oil with mesquite (Prosopis juli-ora) gum. *LWT-Food science and Technology,* 34, 398-401.

Bhosale, R., & Singhal, R. (2006). Process optimization for the synthesis of octenyl succinyl derivative of waxy corn and amaranth starches. *Carbohydrate Polymer* 66, 521-527.

Bunyapraphatsara, N. (1996). Herbs Plant folklore, Medicinal Plant Information Center, Mahidol University, Bangkok, p. 712.

Boutboul, A. Giampaoli, P., Feigenbaum, A., & Ducruet, V. (2002). Influence of the nature and treatment of starch on aroma retention. *Carbohydrate Polymers,* 47(1), 73-82.

Borrmann, D., Pierucci, A.P.T.R., Leite, S.G., & Leão, M.H.M.R. (2013). Microencapsulation of passion fruit (*Passiflora*) juice with n-octenylsuccinate-derivatised starch using spray-drying. *Food and Bioproducts Processing,* 91, 23-27.

Buléon, A., Gallant, D.J., Bouchet, B., Mouille, G., D'Hulst, C., Kossmann, J., & Ball, S. (1997). Starches from A to C: *Chlamydomonas reinhardtii* as a model microbiology system to investigate the biosynthesis of the plant amylopectin crystal. *Plant Physiology,* 115, 949-957.

Buléon, A., Coloma, P. Plancot, V., & Ball, S. (1998). Starch granules: structure and biosynthesis. *International Journal of Biological Macromolecules,* 23, 85-112.

Carneiro, H. C.F., Tonon, R.V., Grosso, C. R.F., & Hubinger, M. D. (2013). Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. *Journal of Food Engineering,* 115(4), 443-451.

Chen, Q., Zhong, F., Wen, J., McGillivray, D., & Quek, S.Y. (2013). Properties and stability of spray-dried and freeze-dried microcapsules co-encapsulated with fish oil, phytosterol esters, and limonene. *Drying technology,* 31, 707-716.

Dalgleish D.G. (2006). Food emulsions—their structures and structure forming properties. *Food Hydrocolloid.,* 20, 415-422.

Da Silva-Junior, A. A., De Matos, J. R., Formariz, T. P., Rossanezi, G., Scarpa, M. V., Do Egito, E. S. T., & De Olivera, A. G. (2009). Thermal behavior and stability of biodegradable spray-dried microparticles containing triamcinolone. *International Journal of Pharmaceutics*, 368, 45-55

Desai, K. G. H., & Park, H.J. (2005). Recent developments in microencapsulation of food ingredients. *Drying technology*, 23(7), 1361-1394.

Fernandes, R.V.B., Borges, S.V., & Botrel, D.A. (2014). Gum arabic/starch/maltodextrin/inulin as wall material on the microencapsulation of rosemary essential oil. *Carbohydrate Polymers*, 101, 524-532.

Ferreira, I., Rocha, S., & Coelho, M. (2007). Encapsulation of antioxidants by spray-drying. *Chemical Engineering Transactions,* 11(2), 713-717.

Flores-Martínez, H., Osorio-Revilla, G., & Gallardo-Velázquez, T. (2004). Optimal spray – drier encapsulation process of orange oil. *Drying 2004 – Proceedings of the 14th International Drying Symposium* (IDS 2004) São Paulo, Brazil, 22-25 August, (A), 621-627.

Frascareli, E.C., Silva, V.M., Tonon, R.V., & Hubinger, M.D., (2012). Effect of process conditions on the microencapsulation of coffee oil by spray drying. *Food and Bioproducts Processing,* 90, 413–424.

Hogan, S.A., McNamee, B.F., O'Riordan, E.D.,& O'Sullivan, M., (2001). Emulsification and microencapsulation properties of sodium caseinate/carbohydrate blends. *International Dairy Journal,* 11 (3), 137–144.

Hu, R. (1999). Food Product Design: A Computer-Aided Statistical Approach, Lancaster, Technomic Publishing Co., Inc. pp. 23-34.

Green, B.K., & Scheicher, L. (1955). Pressure Sensitive Record Materials. US Patent no. 2, 217, 507, Ncr C.

Goubet, I. Le Quere, L.J., & Voilley, J.A. (1998). Retention of aroma compounds by carbohydrate: Influence of their physiochemical characteristics and of their physical state-A review. *Journal of Agricultural and Food Chemistry,* 46, 1981-1990.

Jouquand, C. Ducruet, V., & Bail, L.P. (2006). Formation of amylose complexes with C6-aroma compounds in starch dispersion and its impact on retention. *Food Chemistry,* 96, 461-470.

Kha, T.C. Nguyen, M.H. Roach, P.D., & Stathopoulos, C.E. (2014). Microencapsualtion of Gac oil; Optimisation of spray drying conditions using response surface methodology. *Powder Technology,* 264, 298-309.

Kim, C.H., & Maga, J.A. (1994). Chain length and functional group impact on retention during extrusion, In Parliament, T.H., Morello, M.J. and McGorrin, R.J. (Eds), Thermally generated flavors: Mailard, Microwave and extrusion processes, p.105-153. CS Symposium Series 543, American Chemical Society. Washington, DC. USA.

Kumar, D., Kumar, S., Tapial, S, Kashyap, D., Kumar, A., & Prakash, O. (2012). A review of chemical and biological profile of genus *Michelia*. *Journal of Chinese Integrative Medicine*, 10(12), 1336-1341.

Le bail, P., Rondeau, C., & Buléon, A. (2005). Structural investigation of amylose complexes with small ligands: helical conformation, crystalline structure and thermostability. *Intermational Journal of Biological Macromolocules*, 35, 1-7.

Liu, X. D., Atarashi, T., Furuta, T., Yoshii, H., Aishima, S., Ohkawara, M., & Linko, P. 2001. Microencapsulation of emulsified hydrophobic flavors by spray drying. *Drying Technology*, 19(7), 1361-1374.

Murúa-Pagola, B., Beristain-Guevara, C.I., & Martínez-Bustos, F. (2009). Preparation of starch derivatives using reactive extrusion and evaluation of modified starches as shell materials for encapsulation of flavoring agents by spray drying. *Journal of Food Engineering*, 91, 380-386.

Naknean, P., & Meenune, M. (2010). Factors affecting retention and release of flavor compounds in food carbohydrates. *International Food Research Journal*, 17, 23-34.

Paibon, W., Yimnoi, C.A., Tembab, N., Boonlue, W., Jampachaisri, K., Nuengchamnong, N., Waranuch, N., & Ingkaniam, K. (2011). Comparison and evaluation of volatile oils from three different extraction methods for some Thai fragrant flowers. *International Journal of Cosmetic Science*, 33, 150-156.

Penbunditkul, P., Yoshii, H., Ruktanonchai, U., Charinpanitkul, T., Assabumrungrat, S., & Soottitantawat, A. (2012). The loss of OSA-modified starch emulsifier property during the high-pressure homogeniser and encapsulation of multi-flavour bergamot oil by spray drying. *International Journal of Food Science & Technology,* 47, 2325-2333. doi: 10.1111/j.1365-2621.2012.03106.x

Qi, Z.H, & Xu, A. (1999). Starch-based ingredients for flavor encapsulation. *Cereal Foods World*, 44(7), 460-465.

Reineccius, G.A. (1991). Carbohydrates for flavor encapsulation. *Food Technology,* 45, 144–147.

Rodriguez, S.D., Wilderjans, T.F., Sosa, N., & Bernik, D.L. (2013). Image texture analysis and gas sensor array studies applied to vanilla encapsulation by octenyl succinic anhydride starches. *Journal of Food Research,* 2(2), 36-48.

Rosenberg, M., Kopelman, I.J., & Talmon, Y. (1990). Factors affecting retention in spray drying microencapsulation in volatile materials. *Journal Agricultural and Food Chemistry,* 38, 1288-2627.

Sahin-Nadeem, H., Torun, M., &Ozdemir, F. (2011). Spray drying of the mountain tea (Sideritis stricta) water extract by using different hydrocolloid carriers. *LWT – Food Science and Technology,* 44, 1626-1635.

Saikia, S., Mahnot, N.K., & Mahanta. (2015). Optimisation of phenolic extraction from *Averrhoa carambola* pomace by response surface methodology and its microencapsulation by spray and freeze drying, *Food chemistry,* 171, 144-152.

Samakradhamrongthai, R., & Utama-ang, N. 2008. Development in instant mango tea. Internatioanl conference on tea production and tea products. 26 – 28 November, Mae Fah Luang University, Chiang Rai, Thailand, Poster presentation.

Samakradhamrongthai, R. (2011). Extraction of Champaca (*Michelia champaca* L.) essential oil and its application in instant Champaca-flavored tea powder. Master of science (Agro-industrial product development), Chiang mai University, Chiang Mai, Thailand.

Sanimah, S., Suri, R., Azizun, R. N., Hazniza, A., Radzali, M., Rusli, I., & Hassan, M. D. (2008). Volatile compounds of essential oil from different stages of *Michelia alba* (cempaka putih) flower development, 36(1), 109-119.

Santana, A.A., de Oliveira, R.A., Pinedo, A.A. Kurozawa, L.E., & Park, K.J. (2013). Microcapsulation of babasso cocnut milk. *Food Science and Technology, Campinas*, 33(4), 737-744.

Sasone, F., Mencherini, T., Picerno, P., D'Amore, M., & Aquino, R.P. (2011). Maltodextrin/pectin microparticles by spray drying as carrier for nutraceutical extracts. *Journal of Food Engineering*, 105, 468-476.

Soottitantawat, A., Yoshii, H., Furuta, T., Ohkawara, M., & Linko, P. (2003). Microencapsulation by Spray Drying: Influence of Emulsion Size on the Retention of Volatile Compounds. *Journal of Food Science*, 68, 2256-2262. doi: 10.1111/j.1365-2621.2003.tb05756.x.

Soottitantawat, A., Yoshii, H., Furuta, T., Ohgawara, M., Forssell, P., Partanen, R., Poutanen, K, & Linko, P. (2004). Effect of water activity on the release characteristics and oxidative stability of D-limonene encapsulated by spray drying. *Journal of Agricultural and Food Chemistry,* 52, 1269-1276.

Soottitantawat, A., Bigeard, F., Yoshii, H., Furuta, T., Ohkawara, M., & Linko, P., (2005). Influence of emulsion and powder size on the stability of encapsulated d-limonene by spray drying. *Innovative Food Science and Emerging Technology*, 6 (1), 107–114.

Soottiantawat, A., Partanen, R., Neoh, T.L., & Yoshii, H. (2015). Encapsulation of hydrophilic and hydrophobic flavors by spray drying. *Japan Journal of Food Engineering,* 16(1), 37-52.

Sosa, N., Zamora, M. C., Chirife, J., & Schebor, C. (2011). Spray-drying encapsulation of citral in sucrose or trehalose matrices: physicochemical and sensory characteristics. *International Journal of Food Science & Technology,* 46(10), 2096-2102.

Subcharoen, P. (1999). Herbal and Thai culture: Part II. Aromatic plants. Bangkok: The institute of Thai Medicine Printing; pp. 50-51.

Szente, L., & Szejtli, J. 2004. Cyclodextrins as food ingredients. *Trends in Food Science & Technology,* 15, 137-142.

Takeo, K., & Kuge, T. (1969). Complexes of starchy materials with organic compounds, III. X-ray studies on amylose and cyclodextrin complexes. *Agricultural and Biological Chemistry,* 33(8), 1174-1178.

Tari, T.A., & Singhal, R.S. (2002). Starch based spherical aggregates: reconfirmation of the role of amylose on the stability of a model flavouring compound, vanillin. *Carbohydrates Polymers,* 50, 279-282.

Wang, Y.J., & Wang, L.F. (2002). Characterization of acetylated wazy maize starches prepared under catalysis by different alkali and alkaline-earth hydroxides. *Starch/Stäke,* 54, 25-30.

Wang, J. J., Zeng, Z. W., Xiao, R. Z., Xie, T., Zhou, G. L., Zhan, X. R., & Wang, S. L. 2011. Recent advances of chitosan nanoparticles as drug carriers. *International Journal of Nanomedicine*, *6,* 765–774. doi:10.2147/IJN.S17296.

Watanabe, S., & Dawes, C. (1988). A comparison of the effects of tasting and chewing foods on the flow rate of whole saliva in man. *Archives of Oral Biology*, 33(10), 761-764.

Yamaguchi, M., Kanemori, T., Kanemaru, M., Takai, N., Mizuno, Y., & Yoshida, H. 2004. Performance evaluation of salivary amylase activity monitor. *Biosensors and Bioelectronics*, 20(3), 491-497.

Yoshii, H., Soottitantawat, A., Liu, X., Atarashi, T., Furuta, T., Aishima, S., Ohgawara, M., & Linko, P. 2001. Flavor release from spray-dried maltocextrin/gum arabic or soy matrices as a functional of storage relative humidity. *Innovative Food Science and Emerging Technologies*, 2, 55-61.