MICROENCAPSULATION OF HOLY BASIL OIL USING MODIFIED STARCH BY SPRAY DRYING METHOD

Miss Artima Lairattanakul

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การห่อหุ้มน้ำมันกะเพราระดับไมโครด้วยแป้งดัดแปรโดยใช้วิธีการอบแห้งแบบพ่นฝอย

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By	Miss Artima Lairattanakul
Field of Study	Chemical Engineering
Thesis Advisor	Associate Professor Apinan Soottitantawat, D.Eng.
Thesis Co Advisor	Uracha Ruktanonchai, Ph.D.

Accepted by the Faculty of Engineering, Chulalongkorn University in Partial Fulfillment of the Requirement for the Master of Engineering

.... Dean of the Faculty of Engineering
(Professor Supot Teachavorasinskun, D.Eng.)

THESIS COMMITTEE

.... Chairman
(Professor Anongnat Somwangthanaroj, Ph.D.)

.... Thesis Advisor
(Associate Professor Apinan Soottitantawat, D.Eng.)

.... Thesis Co-Advisor
(Uracha Ruktanonchai, Ph.D.)

.... Examiner
(Assistant Professor Rungthiwa Methaapanon, D.Eng.)

.... External Examiner
(Suvimol Surassmo, Ph.D.)

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กะเพราเป็นพืชสมุนไพรซึ่งสามารถพบได้ทั่วไปในประเทศไทยและเป็นที่รู้จักกันอย่างมากในการเป็นส่วนประกอบของผัดกะเพรา เนื่องด้วยกลิ่นที่เป็นลักษณะเฉพาะ นอกจากประโยชน์ในการนำไปประกอบอาหารแล้ว น้ำมันกะเพรายังถูกนำไปใช้ประโยชน์ในอุตสาหกรรมอีกหลากหลายด้าน เช่น เครื่องสำอาง น้ำหอม และทางการแพทย์ อย่างไรก็ตามปัญหาเกี่ยวกับการเสื่อมสภาพอันเนื่องมากจากแสง ความชื้น และอุณหภูมิสูงในระหว่างขั้นตอนการจัดเก็บเป็นหนึ่งในปัญหาสำคัญที่ทำให้ประสิทธิภาพของน้ำมันกะเพราลดลง ในงานวิจัยครั้งนี้ได้เสนอเทคนิคการกักเก็บน้ำมันกะเพราในรูปของผงอนุภาคซึ่งได้มากจากกระบวนการอบแห้งแบบพ่นฝอย โดยใช้แป้งดัดแปรในการห่อหุ้ม น้ำมันกะเพราประกอบด้วยสารสำคัญ 3 ตัวได้แก่ ยูจีนอล เมทิลยูจีนอล และบีตาคาริโอฟิลีน ซึ่งเป็นสารสำคัญที่ส่งผลต่อคุณสมบัติของน้ำมันกะเพรา เป้าหมายในงานวิจัยครั้งนี้ มุ่งหวังเพื่อศึกษาสภาวะที่เหมาะสมที่สุดในการห่อหุ้มน้ำมันกะเพราซึ่งสามารถเพิ่มเสถียรภาพและกักเก็บสารสำคัญทั้งสามในน้ำมันได้ อัตราส่วนโดยมวลของน้ำมันต่อสารห่อหุ้ม และอุณหภูมิอากาศขาเข้าของเครื่องอบแห้งแบบพ่นฝอยถูกเลือกเป็นปัจจัยหลักในการศึกษา และวิเคราะห์ผลจากคุณสมบัติของผงแห้งและปริมาณสารสำคัญที่ถูกกักเก็บ ผลการศึกษาพบว่าอัตราส่วนโดยมวลของน้ำมันต่อสารห่อหุ้มเท่ากับ 1 ต่อ 4 เป็นสภาวะที่เหมาะสมที่สุดในการห่อหุ้มน้ำมันกะเพราด้วยกระบวนการอบแห้งแบบพ่นฝอย

สาขาวิชา	วิศวกรรมเคมี	ลายมือชื่อนิสิต	....
ปีการศึกษา	2566	ลายมือชื่อ อ.ที่ปรึกษาหลัก	....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม	....

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Artima Lairattanakul : MICROENCAPSULATION OF HOLY BASIL OIL USING MODIFIED STARCH BY SPRAY DRYING METHOD. Advisor: Assoc. Prof. APINAN SOOTTITANTAWAT, D.Eng. Co-advisor: Uracha Ruktanonchai, Ph.D.

Holy basil is a local herb that can planted all over Thailand. It is the significant ingredient in Thai famous dish named "Pad ga prao" because of its unique scent which is released when cooked. Apart from the cuisine, its essential oil has been used in various industries including cosmetics, perfumery, and pharmaceuticals. However, their degradation under light, moisture, and high temperature during storage is the main problem of their utility. In this research, the encapsulation process is selected to prevent the problem. The modified starch was used as wall materials. Holy basil oil (HBO) has three main components: Eugenol, Methyl-eugenol, and beta-caryophyllene, which significantly affect its benefits. This research aims to study the most suitable condition that can increase the stability of the oil and retain three main components in HBO by spray drying method. The mass ratio of oil to modified starch and air inlet temperature of the spray dryer were studied and the effect with the encapsulated powder was evaluated by the properties of encapsulated power and retention of the components. From the result, the conclusion led to the mass ratio of HBO to wall materials equal to 1 to 4 is the most suitable condition to encapsulate the HBO by spray drying method.

Field of Study:	Chemical Engineering	Student's Signature ....
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LIST OF ABBREVIATIONS

EOs	Essential oils
HBO	Holy basil oil
OSA	Octenyl succinic anhydride
EE	Encapsulation efficiency percentage
GC	Gas Chromatograph
GC-MS	Gas Chromatograph-Mass spectroscopy
DI water	Deionized water
Encapsulated powder	The microcapsule in powder form which contain the Holy basil oil that encapsulated by wall material
Ratio	The ratio of Holy basil oil to wall material (w/w)

CHAPTER I

INTRODUCTION

1.1 Motivation

Holy basil is a local herb which can plants all over Thailand. It is the significantly ingredient in Thai famous dish naming “Pad ga prao” because of its unique scent which is released when cooked. Apart from the cuisine, its essential oil has been used in various industries including cosmetics, perfumery and pharmaceutical. It has many pharmacological activities for example antimicrobial, immunomodulatory, antistress, anti-inflammatory, antidiabetic and antiulcer [1-3]. From these advantages, they are received attention to use in many fields, but the restriction of sensibility, stability and the solubility are the main problems which are limiting their usage [4, 5]. Therefore, the preservation technique is required to solve these problems.

Spray drying method is the encapsulation technique that transform the emulsion into the encapsulated powder, it can use with high boiling point temperature substances and has no effect with the deduction of substance during the process [4]. It is commonly used because of the continuous to enlarge from the laboratory scale into the industrial scale [5, 6]. The factor of encapsulation efficiency in spray drying method consist of 2 factors, emulsion preparation and spray drying condition. The spray dryer has many parameters which can adjust including air inlet temperature, gas flow rate and the size of droplet from the atomizer [6].

Emulsion preparation is the method that encapsulated the substances in the wall material to protect them from the environment resulting in long shelf life and improving some of physical properties. Moreover, it can also control the release rate of core substances which is resulting in long time activated of the substances [5, 7]. From these details, the suitable wall materials and ratio of core substances to wall materials are required to get the most appropriate condition to prepare the emulsion.

Wall materials in carbohydrate groups was used to encapsulate the volatile compound and resulting in great encapsulation efficiency and stability; for instance, the using of modified starch (trademark name HI-CAP®100) to encapsulate the linalool [8], beta-cyclodextrin to encapsulate the eugenol [9] and gum arabic and maltodextrin to encapsulate the beta-caryophyllene [10]. Even though, the encapsulation efficiency and stability of the encapsulated powder from spray drying method were studied and confirmed, but the similarity of profile of the significantly substances before and after spray dying method that correlated with the unique scent of the essential oil were not investigated. In this experiment, HI-CAP®100 and white Holy basil oil was selected to be the wall material and essential oil. The condition of emulsion preparation and spray drying method condition were determined to find the most efficient condition to encapsulate the Holy basil oil which gave the high oil retention and high encapsulation efficiency. Moreover, the most resemble profile compared to the initial Holy basil oil before the spray drying method was also evaluated.

1.2 Research objectives

1.2.1 To determine the effect of mass ratio of Holy basil oil to wall material on the encapsulated powder.

1.2.2 To investigate the effect of spray drying condition on the encapsulated powder.

1.3 Research scopes

1.3.1. Determine the profile of components of white Holy basil oil.

1.3.2. Study the effect of mass ratio between Holy basil oil and modified starch on the encapsulated powder by varying the ratio at 1 to 2, 1 to 3 and 1 to 4.

1.3.3. Study the effect of air inlet temperature of spray dryer on the encapsulated powder by varying the air inlet temperature of spray dryer at 120, 140, 160 and 180 degree celsius.

1.3.4. Characterization of physical and chemical properties of encapsulated powder.

1.3.4.1. Physical characteristic of encapsulated powder

- Emulsion and redispersed emulsion size

- Moisture content

1.3.4.2. Chemical characteristic of encapsulated powder by Gas Chromatograph

- Component retention of encapsulated powder

The percentage of total content of marker component from encapsulated powder compared with the content of marker component in initial feed emulsion

- Surface oil percentage

The percentage of content of marker component which easily to wash from encapsulated powder compared with the total content of marker component from encapsulated powder

- Encapsulation efficiency of encapsulated powder (%EE)

The percentage of marker component which is entrapped in encapsulated powder compared to the content of marker component in initial feed emulsion

- Similarity of profile

The comparison of similarity percentage of components between the initial Holy basil oil and extracted from encapsulated powder after spray drying method

1.4 Expected benefits

1.4.1 The relation between mass ratio of oil to wall materials in emulsion preparation step to the properties of encapsulated powder.

1.4.2 The relation between air inlet temperature of spray dryer to the properties of encapsulated powder.

1.4.3 The information of the encapsulation condition which gave the most resemble profile compared to initial Holy basil oil

CHAPTER II

THEORY & LITERATURE REVIEW

2.1 Holy basil oil

It is commonly known as Tulsi or Holy basil [1, 11]. There are two common types of Holy basil (Ocimum sanctum/tenuiflorum) in Thailand which have varieties namely, white and red [11]. It has used in Thai culinary dishes for a long time and has been the main ingredient of Thai famous menu naming “Pad ga prong”. It is one of herbals that can extracted essential oil from each part of the plants by steam distillation process. The essential oil from different parts of Holy basil leads to the different compositions in the essential oil. In the oil extracted from leaves, the main components are eugenol and methyl eugenol which is volatile compounds while the oil extracted from seed mainly consists of linoleic, linolenic and oleic which is fixed oil. Moreover, the different of cultivation area was led to the variation of the chemical constituents percentage which is also affected its properties [1]. It has been used in various industries including food, medical and cosmetics. It has many pharmacological activities for example antimicrobial, immunomodulatory, anti-stress, anti-inflammatory, antidiabetic and antiulcer [1-3].

Figure 1 The picture of Holy basil trees

(a) Red Holy basil and (b) White Holy basil [11]

In 2018, Tangpao T., et al. [11] studied about the aromatic identities of 5 common Thai basils consist of Lemon basil, red Holy basil, Thai basil, Tree basil and white Holy basil. Each type of basil oil was planted at Chiang Mail university, extracted from fresh leaves by hydro-distillation, then analyzed by Gas Chromatograph to receive the qualitative and quantitative information of volatile components. Sixty-seven compounds were investigated and the result was reported in microgram per milliliter. In the addition, The Principal Component Analysis (PCA) was used to identify the relation of volatile components to odor of the oil. The results illustrated that the 3 major components of white Holy basil oil are methyl eugenol (98.44 µg/mL oil), α-cubebene (9.94 µg/mL oil) and α-copaene (4.74 µg/mL oil), while linalool and eugenol were also detected in 1.09 and 1.5 microgram per milliliter oil. From PCA, the white Holy basil oil has the herb and spice odor which is affected from 3 components; methyl eugenol, β-caryophyllene and α-cubebene [11].

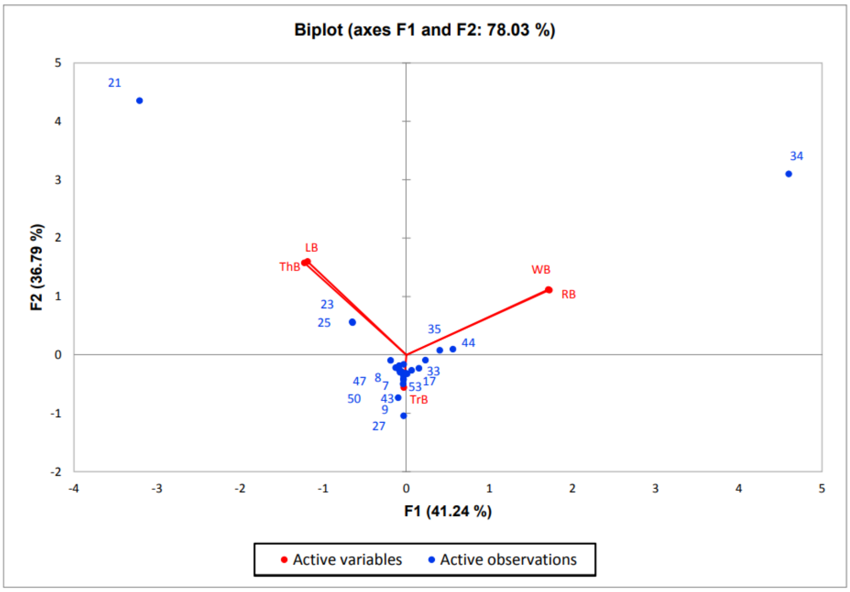


Figure 2 Principle components analysis (PCA)

biplot illustrated the relationship among the chemical components and odor WB = white Holy basil oil, 34 = Methyl eugenol, 35 = Beta-caryophyllene, 44 = α-cubebene [11].

The components of white Holy basil oil were investigated again in 2021 when Tangpao T., et al. [7] studied about the Encapsulation of Basil Essential Oil by Paste Method. The fresh leaves were collected from the local contract farmer and extracted by hydro-distillation. The white Holy basil oil contained 23 compounds consisting of methyl eugenol, trans-caryophyllene, estragole, eugenol and linalool in 372.57, 99.38, 84.17, 83.04 and 7.41 microgram per milliliter oil, orderly [7].

From the information mentioned above, the white Holy basil oil showed the different composition of components when the oil was extracted from different sources, but they also have the methyl eugenol as the significant component. These information leads to the conclusion that the components from the oil have a variation depending on the surrounding of cultivation such as climate and temperature, so the analyzation of the oil components is required to get the details about its components and determine the significant components to analyze in the experiment.

2.1.1 Eugenol

Eugenol is a phenolic compound which has many benefits in medical treatment such as asthma, anti-inflammatory, anti-oxidation activity and anti-bacterial activity. It has great content in clove oil, tulsi leaves (Ocimum sanctum), bay leaves (Cinnamomum tamala) and clove buds (Syzygium aromaticum) [12]. Moreover, it is also used in fragrant, favoring agent in a variety of food and cosmetic products [9, 13].

In 2019, Talon E., et al [14]., studied about the encapsulation of eugenol by spray-drying using maltodextrin that mixed with whey protein (WP) or soy lecithin (LE) as the wall material. The results showed that formulations of maltodextrin with WP or LE at ratio of maltodextrin to WP or LE equal to 1:42 (w/w) showed high encapsulation efficiencies approximately 95–98% which led to great antibacterial effect [14].

Table 1 Physical properties of eugenol

Name	4-allyl-2-methoxyphenol
Molecular formular	$C_{10}H_{12}O_2$
Molecular weight	164.20 g/mol
Density (25°C)	1.067 g/mL
Boiling point (1 atm)	254 °C
Water Solubility	slightly soluble

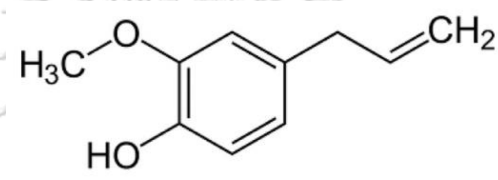


Figure 3 Chemical structure of eugenol [9].

2.1.2 Methyl eugenol

Methyl eugenol is a substance which is found in various plants. It can be used in medical treatment because of its medical properties such as anti-inflammatory, anti-allergic, tissue repair and wound healing [15]. In the addition, It is one of a significantly substance which is affected to the unique scent (spice-like aroma) of white Holy basil oil [11].

Table 2 Physical properties of methyl eugenol

Name	4-allyl-1, 2-dimethoxybenzene-carboxylate
Molecular formular	$C_{11}H_{14}O_2$
Molecular weight	178.23 g/mol
Density (25°C)	1.036 g/mL

Boiling point (1 atm)	254.7°C
Water Solubility	insoluble

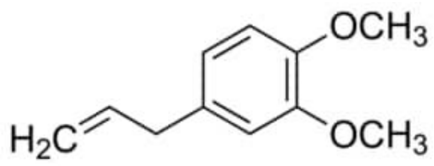


Figure 4 Chemical structure of methyl eugenol. (Ref. Sigma-Aldrich)

2.1.3 Beta-caryophyllene

Beta-caryophyllene is a terpenic compounds which has many medical properties such as anticancer, antifungal, and immunomodulatory activities [16]. Moreover, it is one of a significantly substance which is affected to the unique scent (spice-like aroma) of white Holy basil oil [11].

In 2014, Suzana et al. [10] studied about the microencapsulation of essential oil from Fruits of *Pterodon emarginatus* which has Beta-caryophyllene as the remarkable components and using Gum Arabic and Maltodextrin as wall materials by spray drying method. Two variables are investigated consist of emulsion composition and spray dryer condition. The result was report in encapsulation efficiency, stability and the morphology of the encapsulated powder. The result shows that the emulsion composition between essential oil and Gum Arabic to Maltodextrin equal to 1 to 3 to 3.6 and spray dryer condition at air inlet temperature equal to 160°C, Spray nozzle equal to 1.2 millimeter and flow rate at 4 milliliter per minute gave the most efficient protection for essential oil. The encapsulation efficiency was equal to 98.63%. The stability of encapsulate powder after 45 days displayed the great content of beta-caryophyllene and the morphology of encapsulated powder from scanning electron microscope (SEM) illustrated the smooth and spherical particles. [10]

Table 3 Physical properties of beta-caryophyllene

Name	2-Methylene-6,10,10-trimethyl bicyclo[7.2.0]undec-5-ene
Molecular formular	$C_{15}H_{24}$
Molecular weight	204.35 g/mol
Density (25°C)	0.901 g/mL
Boiling point (1 atm)	268°C
Water Solubility	insoluble

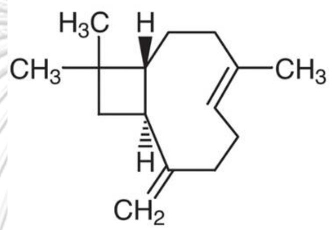


Figure 5 Chemical structure of Beta-caryophyllene (Ref. TCI, Japan)

2.2 Encapsulation techniques

Encapsulation is the technique which is using to cover the substances that desire to protect. It can use to overcome the stability causing by the sensitivity to oxygen, light, heat, moisture and solubility properties of essentials oil. In the addition, this process leads to the easier handling of the active compound, a better protection during storage and transportation and a better control in the release of products [5, 6, 14, 17]. Moreover, this technique can be using in masking purposes to cover the undesirable flavor, color and taste of some substances such as fish oil [14, 17, 18]. Encapsulation is divided into 2 main categories; cooling of melt and rapid evaporation such as freeze drying and spray drying. Spray drying method is received attention to used more than cooling of melt due to the advantages of using with high boiling point temperature substances and no effect of the loss of substance from the heat of this process [4]. The key point of encapsulation technique is the properties of the initial emulsion which is affected by the substances and wall materials. In the addition, the substance to wall materials ratio and release kinetics into a medium are also considered [14].

The emulsion consists of at least 2 immiscible liquids, generally oil and water. One of the liquids is dispersed in the other as small spherical droplets. The system of the emulsion divided into 2 main types; oil-in-water (O/W) emulsion or water-in-oil (W/O) emulsion and oil-in-water-in-oil (O/W/O) or water-in-oil-in-water (W/O/W).

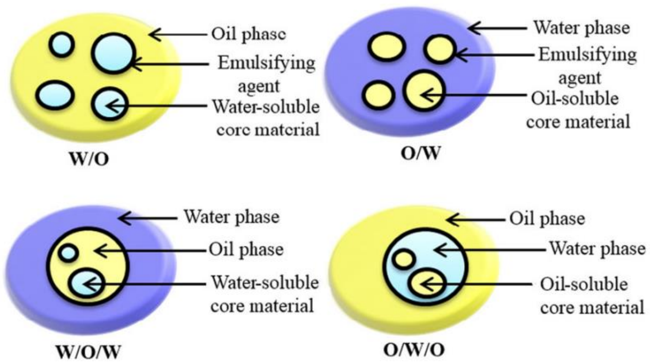


Figure 6 The Illustration of 4 emulsion system

(a) oil-in-water (O/W), (b) water-in-oil (W/O), (c) oil-in-water-in-oil (O/W/O) and (d) water-in-oil-in-water (W/O/W) [19]

The effect of emulsion preparation step in the topic of mean size of emulsion on the flavor retention of volatile compound was studied by Soottitantawat A., et al. in 2003 [20]. The conclusion led to the point that the increasing of emulsion droplet size decreasing the retention of flavors and the usage of atomizer led the distribution curve to change into the smaller size of emulsion.

2.2.1 Encapsulation of the essential oil by spray drying

The essential oil was encapsulated by spray drying method and resulted in high encapsulation efficiency and stability when using the modified starch as the wall materials or carrier matrix. Most of the substances in the essential oil were the active compounds which are sensitive to environment condition such as d-limonene [21], eugenol [12, 14], citral [22], linalool [8] and beta-caryophyllene [10] which has many pharmacological properties.

In 2005, Soottitantawat A., et al. [21] studied about the encapsulation of d-limonene by mixing the carbohydrate group wall materials including gum arabic, maltodextrin and modified starch (HI-CAP 100) in different proportion, then studied about the influence of emulsion and powder size on the stability of encapsulated powder. The results showed that when the powder and emulsion size were increased resulting in the reducing of the release and the oxidation rate of encapsulated powder. In the addition, the highest stability was observed when the modified starch was used as the wall material.

In 2009, Kaewpanha M., et al. [22], studied about the capsulation of lemongrass oil with modified starch (CAPSUL) and gum arabic mixed maltodextrin and focus on the leading substance names citral. The results show that when the modified starch was used as the wall materials, the retention of citral was higher than gum arabic mixed maltodextrin. In the addition, the increasing of oil to wall materials ratio resulting in high surface oil which is not desire from the encapsulated powder due to the motive of the oxidation circumstances of the oil that affected the stability of encapsulated powder [22].

In 2015, Rajabi H., et al [23] studied about the encapsulation of saffron bioactive components with maltodextrin, gum Arabic and gelatin and got the result that when using the mixture of maltodextrin, gum arabic and gelatin as the wall materials at 40% total solids leading to the highest retention of saffron bioactive components [23].

In 2020, Mahdi A. A., et al. [24] studied about the capsulation of fingered citron extract with gum arabic, modified starch, whey protein, and maltodextrin. The results show that when using gum arabic/maltodextrin/modified starch (GMS) in equally ratio as the wall materials resulting in the encapsulation efficiency equal to 87.20% and also improved the thermal stability which indicated a good storage stability [24]. The suitability of spray drying method to encapsulate the active compound was also confirmed in 2021, Rehman A., et al. studied about the co-entrapped of borage seed oil (BSO), resveratrol (RES) and curcumin (CUR) using the OSA-modified starch (HI-CAP®100) and purity gum 200 as the wall materials. The result showed the high encapsulation efficiency approximately 93.05%, great oxidative stability and lower water activity and lower moisture contents [25].

From all the reference that mention in 2.1. to 2.2 leads to the point that when the essential oil were encapsulated with the wall material in carbohydrate group by spray drying method, for example modified starch [8, 22, 24, 25], β-cyclodextrin [8] and gum arabic and maltodextrin [9, 23, 24] indicating the high encapsulation efficiency and stability, so the wall material in carbohydrate group and spray drying method should be considered to be the suitable way of protect the Holy basil oil. Furthermore, the encapsulation efficiency and content of total oil and surface oil [22] should be investigated for the stability of the encapsulated powder.

2.2.2 The profile of Essential oil in encapsulated powder

From the research in 2011 of Penbunditkul et al. [8], The HI-CAP®100 was used to encapsulation the volatile compounds by spray drying method using the bergamot oil as the representative and focus on 3 components consists of d-limonene, linalool and linalyl acetate. The result about the effect of air inlet temperature of spray dryer on flavor retention of Bergamot oil was shown in **Table 1**. The result showed in percentage which is compared with the initial oil before the spray drying method. After the process, the ratio of component was changed because of the unequally reduced of the substances. Therefore, the ratio of the marker components in essential oil after the spray drying should be investigated to get the most resemble profile of composition which is affected to the properties and unique scent of each essential oil.

Table 4 Effect of air inlet temperature of spray dryer on flavor retention of Bergamot oil [8]

Air inlet temperature (°C)	Flavor retention (%)		
	D-limonene	Linalool*	Linalyl acetate
120	92 ± 2.92	111 ± 1.80	93 ± 3.96
140	95 ± 2.71	112 ± 4.07	93 ± 4.55
160	99 ± 1.75	120 ± 4.07	98 ± 3.82

*More than 100 because the transformation of Linalyl acetate to Linalool

2.2.3 Encapsulation of Holy basil oil

Holy basil oil was encapsulated by many wall material or carrier matrix to protect its ability to use by preventing the loss of significantly substances and improve its stability. Most of the experiments focus on the highest quantity substances in the Holy basil oil; eugenol or Methyl eugenol depending on the varieties white or red Holy basil [11].

For the review of Holy basil oil encapsulation which is focus on the content of Holy basil oil. In 2016, in the encapsulated Holy Basil Essential Oil in Gelatin by Ngamakeue N., et al, [2] studied about the Encapsulation of Holy Basil Essential Oil

(HBEO) in Gelatin and coated with different concentration of Palmitic Acid in Carboxymethyl Cellulose. The HBEO was purchased from Thai-China Flavours and Fragrances Industry, Thailand (TCFF). It is extracted from leaves and flowers by Steam-distillation process. The content of HBEO was determined by UV–Vis spectrophotometer and calculated by **Equation 1**. The result showed that at the increasing of the palmitic acid concentrations resulted in the decreasing of HBEO contents in the microencapsulated. The optimal concentration of palmitic acid in the coating emulsion was 2% (w/v), which provide the effective protection in the physical appearance, color, surface HBEO content, and antioxidant activity of the microcapsules after 3 months of storage [2].

$$\text{Microencapsulated HBEO content (\%)} = \frac{W_E}{W} \times 100 \tag{2}$$

When W_E is the microencapsulated HBEO weight (mg) and W is the microcapsule weight (mg).

In 2021, Tangpao T., et al [7] studied about the encapsulation of Holy basil oil by Paste Method. The result showed that when the white Holy basil (WHO) oil was encapsulated by the combination of the maltodextrin and gum arabic at the ratio of 25 to 75 gave the highest loading efficiency of the oil equal to 9.40%. Moreover, this research was investigated the relation of Oriental Fruit Fly attractive efficiency and the release of the Basil Essential Oil and got the result that WBO-encapsulated products can improve the fruit fly attractive efficiency by maintaining the release rate of basil essential oil [7].

2.3 Spray drying

The spray drying is one of encapsulation technique which transformed the emulsion consisting of substances that encapsulated by wall materials into powder in the short time. It is one of the most popular technique because of reasonably priced, simple, flexible and the most feasible from the industrial point of view [6, 14, 18, 19]. It is commonly used for the encapsulations of oils such as Anchovy oil, Fish oil and Tuna oil [6, 19]. The brief details of the spray drying method is illustrated in **Figure 7**; First, the emulsion is fed through the atomizer and became fine droplets. In the drying chamber, the droplets are fed in the same time with high temperature gas in co-current or counter current way and immediately transformed the emulsion fine droplets into encapsulated powder.

To receive the great properties of encapsulated powder including the low content of surface oil, low moisture content and great stability, the optimal condition is required. From **Figure 7,** The spray drying method has many variable factors such as air inlet temperature, gas flow rate, feed flow rate and the atomizer type which can be adjust to find the most suitable condition for each experiment [6]. These factors directly affect to drying rate which related to the crust formation of droplet which acts as the selective membrane that influence the flavor retention and oil content of the encapsulated powder, so the optimal condition of drying rate is expected to get the most suitable condition for the encapsulated powder that gave the highest flavor retention and lowest surface oil content. In the addition, the stability of encapsulated powder was depending on types of substances, content of surface oil and moisture content of the powder [8], so the condition of the surrounding that used to keep the encapsulated powder is also the significantly factor.

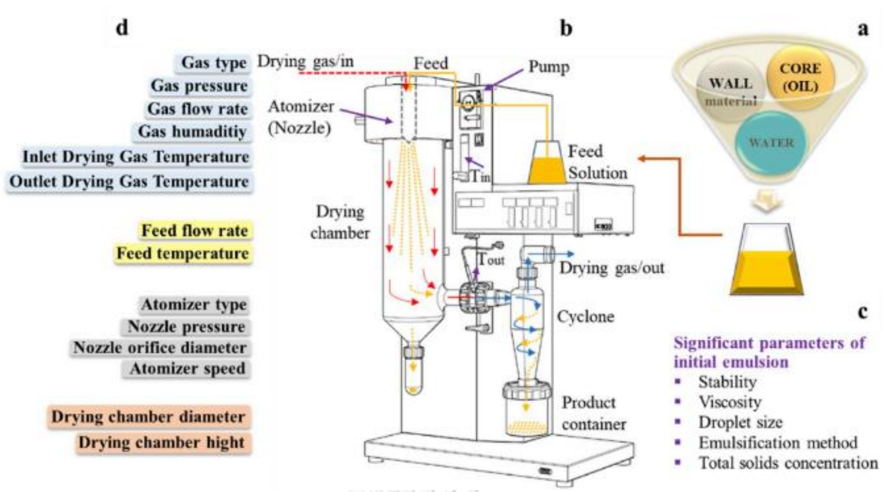


Figure 7 A schematic illustration of the spray drying procedure for encapsulation of functional oils

(a) emulsification step and (b) drying step; (c) supplemented with the significant parameters of initial emulsion and (d) the list of measurable parameters of the spray drying method related to the drying step; Abbreviation: T, temperature [6].

2.4 Wall materials

Wall materials can be divided into 3 groups consists of carbohydrates, proteins and lipids. The carbohydrates and proteins groups are commonly used in spray drying and lipids group are used in liposome delivery system. To improve the emulsifying properties and film-forming capabilities the carbohydrates and proteins groups are mixed together in individual usage. It is the primary factor of encapsulation efficiency and stability of the encapsulated powder because it protected substance throughout the processing and storage [5, 6, 25].

2.4.1 HI-CAP®100

Modified-starch is the starch that was modified by enzyme or mixing with other chemical substances to obtain the required properties to use in the various industry such as food and medication [26]. It has a emulsifying function because of amphiphilic starch characteristic [27]. It can encapsulated the substances for 80% and improve the feature to protect the substance at high temperature [28].

HI-CAP®100 is trademark of OSA-modified starch that was provided by Ingredion (Thailand) Co., Ltd. Its linear structure causing the dense arrangement of hydrophilic line at the oil droplets' surface that prevent the gathering of oil droplets resulting in small oil droplet that correlated with the size of feed emulsion [22] .It is derived from waxy maize and has properties that suited for the encapsulation of flavors at high oil loading because of great emulsifying properties. Moreover, it also has wide application in the food industry mainly as emulsifier, encapsulating agent and fat replacer. The great properties for encapsulation the volatile compounds were confirmed by the experiment in 2005 by Soottitantawat A., et al. [21]. The HI-CAP®100 was used to encapsulation the d-limonene by spray drying method compared with other wall materials in carbohydrate groups consisted of gum arabic and maltodextrin. The result showed that HI-CAP®100 showed a higher stability of encapsulated d-limonene than the other. Moreover, in 2011 of Penbunditkul et al. [8], The HI-CAP®100 was used to encapsulation the volatile compounds by spray drying method using the bergamot oil as the representative and focus on 3 components consists of d-limonene, linalool and linalyl acetate. The result showed that in suitable condition of spray drying, the HI-CAP®100 gave the high retention of d-limonene, Linalool and Linalyl acetate equaled to 75.14%, 87.34% and 62.72%, respectively. The mechanism of starch modification to OSA-modified starch showed in **Figure 8** [29]. In the addition, the experiment in 2021 was also confirmed these properties of the HI-CAP®100, it was used to co-entrapped the borage seed oil (BSO), resveratrol (RES) and curcumin (CUR) by spray drying. The result showed the high encapsulation efficiency approximately 93.05% and great oxidative stability with lower water activity and moisture contents [25].

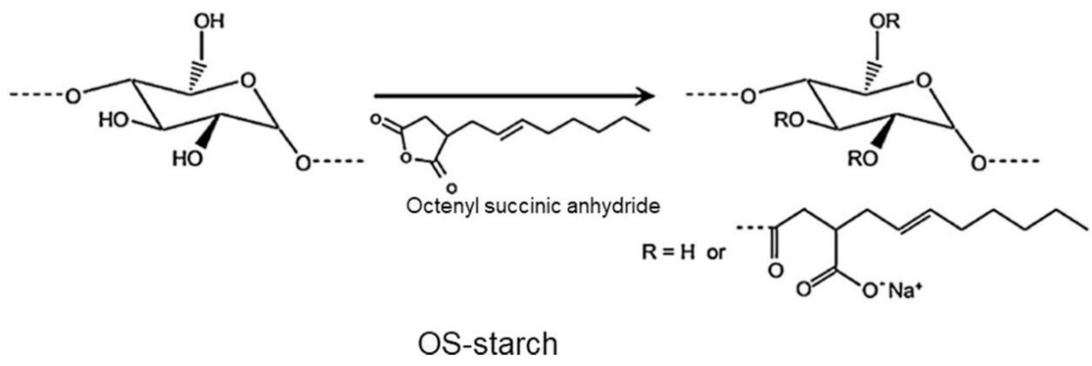


Figure 8 The mechanism of starch modification to OSA-modified starch [27].

CHAPTER III

EXPERIMENTAL

3.1 Materials

Materials	Company
White Holy basil oil	Thai - China Flavours and Fragrances Industry Co., Ltd. (Thailand).
HI-CAP®100	Ingredion (Thailand) Co., Ltd.
Hexane (Analytical grade)	Fisher chemical (Belgium)
Deionized water	Ultrafiltration module UV-modules, ELGA PURELAB Ultra Genetic
Ethanol (Analytical grade)	Sigma-Aldrich, Singapore
Eugenol	RCI Labscan, Thailand
Methyl eugenol	Aldrich, Singapore
Beta-caryophyllene	Tokyo Chemical Industry, Japan

3.2 Apparatus

3.2.1 Spray dryer

Company: BUCHI (Thailand) Ltd.

Model: Mini spray dryer B-290

Application: Transformed the emulsion consisted of substance and wall materials into the powder.

Figure 9 Mini spray dryer B-290, BUCHI (Thailand) Ltd.

3.2.2 High speed homogenizer

Company: IKA® Works (Thailand) Co. Ltd.

Model: S25N-25G

Application: Preparing the emulsion

Figure 10 High speed homogenizer Model S25N-25G

3.2.3 Laser scattering particle size distribution analyzer

Company: HORIBA, Ltd.

Model: Partica LA-950V2

Dynamic range: 0.01 - 3000 μm

Application: Analyze the dispersed particle size

Figure 11 Laser Scattering Particle Size Distribution Analyzer (HORIBA, Ltd.)

3.2.4 Ultrasonic bath

Company: CREST ULTRASONICS (THAILAND) LTD.

Model: 1875D

Application: Extracted the components from the encapsulated powder

Figure 12 Ultrasonic bath, CREST ULTRASONICS (THAILAND) LTD.

3.2.5 Gas Chromatograph

Company: Agilent Technologies (Thailand) Co.,Ltd.

Model: Agilent 7890A

Application: Analyzed the content of components

Figure 13 Gas Chromatograph (Agilent Technologies (Thailand) Co.,Ltd.

3.2.6 Gas Chromatograph – Mass spectroscopy (GC-MS)

Company: Agilent Technologies (Thailand) Co.,Ltd.

Model: Agilent 7890A – 5975C

Application: Specify the components in the substance

Figure 14 Gas Chromatograph – Mass spectroscopy (Agilent Technologies (Thailand) Co.,Ltd.

3.2.7 Oven

Company: Memmert GmbH

Model: 30 - 1060

Application: Heat substances in the experiments

Figure 15 Oven (Memmert GmbH)

3.2 Methodology

3.2.1 Preparation of dissolved wall material and emulsion

All emulsions were oil in water emulsions. First, the wall material was dissolved in deionized water to prepare the wall material solution. The solution was stirred overnight to create the saturated solution [8], then the essential oil was added. The emulsions were produced using a high-speed homogenizer at the rotation speed of 8,000 rpm for 3 minutes.

Table 5 Composition of essential oil and wall material in emulsion

	Solid content (%w/w)	Ratio of oil to wall materials (w/w)	Wall material solution (g)	Essential oil (g)
1	40	1:2	120	24

2	40	1:3	120	16
3	40	1:4	120	12

3.2.2 Preparation of encapsulated Holy basil oil powder

The emulsion consists of Holy basil oil that encapsulated in wall material was transform into powder by spray dryer at the specific condition. The encapsulated powder was kept in the freezer until characterization process.

Table 6 Spray drying method condition

Feed rate	Approximately 11 mL/min (40% pump)
Air inlet temperature	120, 140, 160, 180 °C
Atomizer	Two fluid nozzle diameter = 7 mm

3.2.3 Physical characteristic of encapsulated powder

3.2.3.1 The weight of encapsulated Holy basil powder (neglected moisture content)

The weight of encapsulated Holy basil powder was determined by weighed the vacant plastic bottles. Then, the encapsulated powder from spray drying was filled. After that, the weights of the contained bottles were measured, and the % moisture content was used to neglect the water from the encapsulated powder. The weight of encapsulated Holy basil powder was calculated by the following equation (1):

$$W_{NH} = \frac{100 - \%\text{moisture content}}{100} \times W_{powder} \quad (1)$$

where W_{NH} is the weight of encapsulated Holy basil powder which is neglected the moisture content and W_{powder} indicate the weights of encapsulated Holy basil powder from spray drying method.

3.2.3.2 Yield percentage of encapsulated Holy basil powder

%Yield of encapsulated Holy basil powder was calculated by the following Equation (2):

$$\%Yield = \frac{W_{NH}}{W} \times 100 \qquad (2)$$

where W_{NH} is the weight of encapsulated Holy basil powder which is neglected the moisture content and W is the total weight of Holy basil oil and modified starch solution in emulsion preparation step.

3.2.3.3 Size of particle by Laser diffraction analysis

Laser diffraction analysis was performed by HORIBA model LA-950V2. Water was used as the dispersion medium to measure the emulsion and redispersed emulsion size and the ethanol was used as the dispersion medium to measure the encapsulated powder size. The refractive index of Holy basil oil, water and ethanol were set at 1.515, 1.333 and 1.361, respectively.

3.2.4 Chemical characteristic of encapsulated powder

3.2.4.1 GC and GC-MS analysis

The Gas Chromatograph (GC) analysis was performed using Agilent GC 7890A and the Gas Chromatograph – Mass spectroscopy (GC-MS) analysis was performed using

Agilent GC 7890A with 5975C mass spectrometer. The flame ionization detector (FID) was used and equipped with DB-5HT column (30 m x 0.25 i.d.; film thickness 0.10 μm). The injection volume is 1 μL in a split mode (1:10). Helium was used as carrier gas at 1 mL/min flow rate. The oven temperature was set at 40 °C for 2 minutes, and increased by 10 °C/min until a final temperature 250 °C was reached and held for 5 min.

3.2.4.2 The determination of maker components before and after spray drying

The marker components consist of eugenol, methyl eugenol and beta-caryophyllene are measured by Gas Chromatograph (GC) and calculated by the external standard calibration curve of standard solution. To create the calibration curve, the standard solution at 1,500 1,000 600 400 300 100 50 20 and 10 ppm were prepared in hexane then measure triplicate by GC. The results were shown in the average value.

The extraction step was divided into 2 parts:

Part 1 Extraction of eugenol and methyl eugenol

Encapsulated powder was weighed 0.1 g, dissolved by 2 mL of DI water until become the emulsion. Then, 4 mL of hexane was added and sonicated by Ultrasonic bath with iced water for 1 hours. The hexane phase was used to analyze the content of eugenol and methyl eugenol.

Part 2 Extraction of beta-caryophyllene

Encapsulated powder was weighed 0.1 g, dissolved by 2 mL of DI water until become the emulsion. Then, 3 mL of ethanol was added and followed by 4 mL of hexane, then sonicated by Ultrasonic bath with iced water for 1 hours. The hexane phase was used to analyze the content of beta-caryophyllene.

The content of maker components in the emulsion before spray drying was calculated by the following Equation (3):

$$A_{IE} = \frac{PPM \times V_i}{V_f} \times \frac{W_{HO}}{W_{DS}} \times 10^{-3} \quad (3)$$

where A_{IE} is the content of marker components per 1 g of HI-CAP®100; PPM is the concentration of marker components in the Holy basil oil solution; V_i is the volume of the initial solution; V_f is the volume of the initial solution which is used to prepare the solution for GC analysis; W_{HO} is the total weight of Holy basil oil in emulsion preparation step and W_{DS} is the weight of HI-CAP®100 which is used in emulsion preparation step.

The content of maker components in encapsulated powder after spray drying was calculated by the following Equation (4):

$$A_{AP} = \frac{1}{W_{DS}} \times \frac{PPM \times V_i}{V_f} \times V_E \times 10^{-3} \quad (4)$$

where A_{AP} is the content of marker components per 1 g of HI-CAP®100; W_{DS} is the weight of HI-CAP®100 which is calculated from the encapsulated Holy basil powder using in extracted step; PPM is the concentration of marker components; V_i is the volume of the initial solution; V_f is the volume of the initial solution which is used to prepare the solution for GC analysis and V_E is the volume of hexane which is used in extract step.

3.2.4.3 Surface oil of encapsulated powder

Encapsulated powder was weighed 1 g and vortexed with 5 mL of hexane for 30 second. The hexane phase was used to analyze the content of components.

The content of maker components at the surface of encapsulated powder after spray drying was reported in percentage and calculated by the following Equation (5):

$$\%\text{Surface oil} = \frac{A_S}{A_{AP}} \times 100 \quad (5)$$

where the %surface oil is the content of maker components at the surface of encapsulated powder compared with all extracted; A_S is the content of marker components at the surface of encapsulated powder per 1 g of HI-CAP®100; A_{AP} is the content of marker components per 1 g of HI-CAP®100 calculated in Equation 4.

3.2.4.4 Retention percentage

The retention percentage of encapsulated powder was calculated by the following Equation (6):

$$\%\text{Retention} = \frac{A_{AP}}{A_{IE}} \times 100 \quad (6)$$

where A_{AP} is the content of marker components which is extracted from encapsulated powder (mg/g of dry solid) and A_{IE} is the content of marker components in emulsion preparation step (mg/g of dry solid)

3.2.4.5 Encapsulation efficiency

The encapsulation efficiency (%EE) of encapsulated powder was calculated by the following Equation (7):

$$\%\text{EE} = \frac{(A_{AP} - A_S)}{A_{IE}} \times 100 \quad (7)$$

where A_{AP} is the content of marker components which is extracted from encapsulated powder (mg/g of dry solid); A_{IE} is the content of marker components in emulsion preparation step (mg/g of dry solid) and A_S is the content of marker components on the surface of encapsulated powder (mg/g of dry solid)

3.2.5 Similarity of profile

The similarity percentage of each component was calculated by the following Equation, then created hierarchical clustering heatmaps by MetaboAnalyst 5.0 to determine the similarity of components profile from initial Holy basil oil compared with the encapsulated powder.

$$\%\text{Containing} = \frac{\text{A}}{\text{All}} \times 100 \qquad (7)$$

where A is the content of marker components (mg/g of dry solid) and All is the sum of the content of marker components (mg/g of dry solid)

The containing percentage from initial Holy basil oil and from extracted of encapsulated powder were compared and reported in similarity percentage.

$$\%\text{Similarity} = \frac{\text{E}}{\text{O}} \times 100 \qquad (8)$$

where O is the containing percentage from initial Holy basil oil and E is the containing percentage from extracted of encapsulated powder

3.2.6 The storage stability of encapsulated powder

The encapsulated powder of all ratios at air inlet temperature of spray dryer equal to 160°C were sealed in foil bag and kept at 40 °C in oven for 1, 2 and 3 months. The content of components that retained in the encapsulated powder was analyzed by the same method as topic 3.2.2.

3.2.7 Statistical analysis

The statistical analysis was analyzed by IBM SPSS Statistics. Post Hoc Multiple Comparisons was set to Tukey as equal variances assume. The data presented in Table and Figure are at 95% confidence levels of the fitting values. The same group of data were shown by the same English alphabet.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 The study of components in Holy basil oil

4.1.1 Analyze the components in Holy basil oil

White Holy basil oil from TCFF was prepared to analyze by mixed the Holy basil oil with hexane in the range concentration of 500 to 600 ppm. The analysis was performed by GC-MS which determine the components comparing with wiley7n library. Thirty components were found as shown in appendix. **Figure 16** showed the four dominant peak of Holy basil oil.

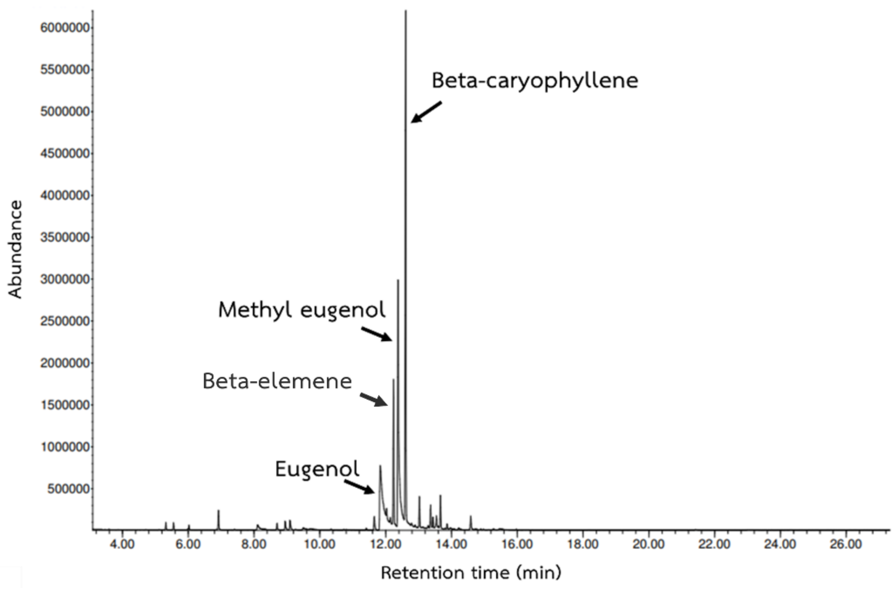


Figure 16 Chromatogram of white Holy basil oil

The significant peaks were characterized to be eugenol, methyl eugenol, beta-elemene and beta-caryophyllene, orderly. Although, the beta-elemene showed the higher peak more than eugenol, but when the relative content was determined the beta-elemene has a content around 9 percent which is two times lower than eugenol.

So, only three components were interested in this experiment. This result was correlated with the result from Tangpao T., et al. [7, 11]. Eugenol, methyl eugenol and beta-caryophyllene were illustrated to be the predominant components in white Holy basil oil. Apart from their dominant in quantitative result, methyl eugenol and beta-caryophyllene are the components that effect the herb and spice odor which is the unique scent of Holy basil oil. So, 3 components consisting of eugenol, methyl eugenol and beta-caryophyllene were selected to be the marker components in this experiment.

Table 7 Outstanding components in Holy basil oil

Order	Component	Relative content (%)	mg/g oil
1	eugenol	18.01	206.73
2	methyl eugenol	25.29	297.55
3	beta-caryophyllene	30.61	357.65

4.1.2 The extraction of marker components for analysis

After the Holy basil oil was encapsulated in the encapsulated power. The investigation of the content of components after encapsulation process was required to determine the efficiency of the encapsulation condition. At the first place, hexane was choosing to be the suitable solvent to extract three marker components because of the compatibility in polarity properties with them, but when the experiment occurred the problem of the beta-caryophyllene extraction was appeared. Only two components can be completely extracted from the encapsulated powder including eugenol and methyl eugenol meanwhile the quantity of beta-caryophyllene was less than it should be referring from **Table 7.** It has the lowest content in the extract that is inconsistent with the relative content in initial Holy basil oil. From this problem, the addition of another extraction solvent was determined.

In the preliminary extraction method; firstly, the encapsulated powder was dispersed in the water to get the emulsion. So, water and hexane were specified to be the solvent. Since, these two substances have an opposite in polarity properties, the ethanol which has the polarity value between these two substances was consider to use in the experiment. The suitable volume of ethanol was calculated from Liquid-Liquid extraction (LLE) of ethanol, water and hexane [30].

Table 8 The polarity properties of substances

Components	Solubility parameter
hexane	7.3
ethanol	11.2
water	21.0

After the emulsion was prepared by the detail as shown in **Table 9** and extraction solvent was added in the emulsion following in **Table 10.** The extraction was performed by the ultrasonic bath. It was filled with iced water to reduce the heat affect in extraction process. The extraction time was used at 1 hour, to reassure that all components were extracted from the encapsulated powder. The result showed that after the ethanol was involved, the beta-caryophyllene can extract from the encapsulated powder, but the low content of the others after the ethanol was involved can be observed as showed in **Table 11**. So, the extraction method is divided into two parts; First, hexane was used to extract the eugenol and methyl eugenol and second, ethanol and hexane was used to extract the beta-caryophyllene.

Table 9 Weight of encapsulated powder in extracted method experiment

	Weight of encapsulated powder (g)	
	Extracted eugenol and methyl eugenol (g)	**Extracted beta–caryophyllene (g)**

n1	0.1232	0.1004
n2	0.1239	0.1251
n3	0.1096	0.1043

Table 10 Brief details of extraction method experiment

			Extracted eugenol and methyl eugenol		Extracted beta - caryophyllene	
		Water (mL)	Hexane (mL)	Ethanol (mL)	Hexane (mL)	Ethanol (mL)
Volume of solvent (mL)	n1	2	4	-	4	3
	n2	2	4	-	4	3
	n3	2	4		4	3
Extraction method	Ultrasonic baht with iced water					
Time (h)	1 hour					

Table 11 The quantity of the components when using the different solvent for extraction method

Component	Relative content (%)		mg/ g dry solid	
	Hexane	Hexane + EtOH	Hexane	Hexane + EtOH
eugenol	24.66	2.86	49.81 ± 2.60	5.90 ± 0.50
methyl eugenol	40.04	22.40	54.89 ± 2.72	31.74 ± 1.84
β-caryophyllene	9.36	38.89	10.35 ± 1.94	47.54 ± 4.37

4.2 Effect of mass ratio of Holy basil oil and wall materials

4.2.1 Effect of mass ratio on the emulsion size

Since, the emulsion size was reported to affect the component retention and surface oil content of the encapsulated powder [21]. So, the studied of mass ratio of

Holy basil oil and wall materials is required. In this experiment, the three different mass ratios of Holy basil concluded 1 to 2, 1 to 3 and 1 to 4 were studied. The wall material was prepared at 40% (w/w) with water, then stirred overnight to avoid the non-saturated of wall materials solution which cause the disadvantage to the component retention and surface oil of encapsulated powder [8]. The particle size was measured by laser scattering particle size analyzer.

Table 12 The feed emulsion size (micrometer)

Ratio of oil to wall materials (w/w)	1:2	1:3	1:4
Emulsion size (μm)	0.592 ± 0.008^{a}	0.485 ± 0.016^{b}	0.459 ± 0.008^{b}

Table 12 showed the effect of Holy basil oil content to the emulsion size. The different of oil quantity resulting in different of emulsion size. When the quantity of oil increased, the emulsion size was increased in the same trend. This can be explained by the decreasing in emulsify properties of the wall materials as the content of oil increased [22, 31]. The size from all mass ratio gave the nearby emulsion size around 0.6 micrometer which related with the suitable emulsion size as reported by P. Penbunditkul, et al and A. M. Bakry, et al [8, 19]. All size was showed in well distributions curve as represent in **Figure 17**.

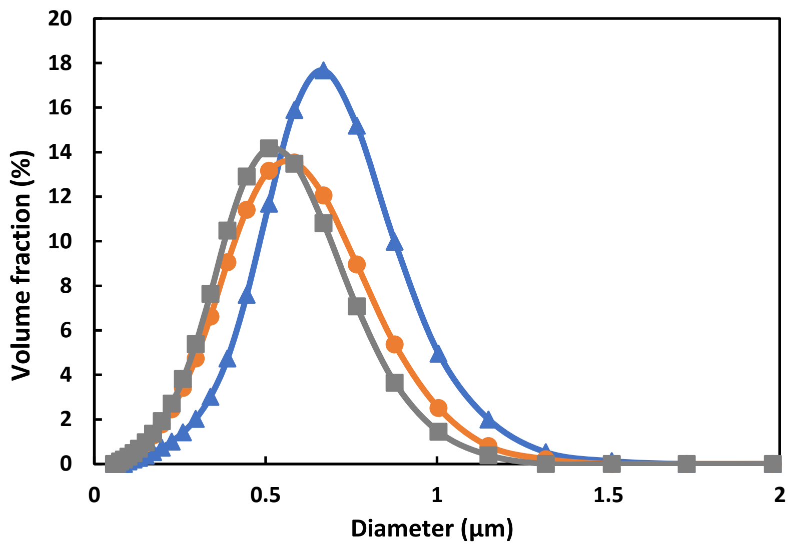


Figure 17 The distribution curve of emulsion size

(The symbol related to ratio of Holy basil oil to wall materials (w/w);

△ 1 to 2, o 1 to 3 and ☐ 1 to 4)

4.2.2 Effect of mass ratio on the operating parameters of spray dryer

The emulsion was spray dried at specific condition to study the effect of Holy basil oil content in feed emulsion. In all operation, three parameters of spray dryer were fixed consist of drying air flow rate at 40 kilogram per hour, air inlet temperature at 160°C and feed emulsion rate at 40% pump which approximately equal to 14.9 gram per minutes. The others parameters were showed in **Table 13**. From the result, the outlet temperature of spray dryer from all mass ratio showed the insignificant value in statistical analysis which mean the Holy basil oil content were not affected the outlet temperature of spray dryer.

Table 13 Effect of mass ratio on operating parameters of spray dryer

Mass ratio of Holy basil oil to wall materials	Air inlet temperature of spray dryer (°C)	Outlet temperature of spray dryer (°C)
1:2	160	86.67 ± 1.53[a]

1:3	160	85.33 ± 1.15[a]
1:4	160	85.00 ± 1.73[a]

4.2.3 Effect of mass ratio on encapsulated powder

4.2.3.1 Effect of mass ratio on moisture content of encapsulated powder

The encapsulated was prepared at spray dryer condition as previous described with the three different Holy basil oil content. The weight of encapsulated powder before and after heated by oven were compared and reported in percentage. **Table 14** showed the insignificant in statistical analysis of moisture content for all mass ratio which represent the no effect of mass ratio to moisture content of encapsulated powder. This result is correlated with the previous report, that air inlet temperature of the spray dryer has the dominant effect to the moisture content of encapsulated powder [22].

Table 14 Effect of mass ratio on moisture content of encapsulated powder

Mass ratio of Holy basil oil to wall materials	Air inlet temperature of spray dryer (°C)	Moisture content (%w/w)
1:2	160	1.83 ± 0.47[a]
1:3	160	1.21 ± 0.27[a]
1:4	160	1.85 ± 0.19[a]

4.2.3.2 Effect of mass ratio on redispersed emulsion and encapsulated powder size

Due to the effect of redispersed emulsion size to the diffusion rate of components after the spray drying process which effect the retention of the components and the effect of oil droplet size with is one of the factor to consider about the application, size of redispersed emulsion is determined. The redispersed emulsion was prepared from the mixing of encapsulated powder with water and vortex until the emulsion was observed. The particle size was measured by laser scattering particle size analyzer. **Table 15** showed the decreasing of redispersed emulsion and encapsulated powder size as the content of oil decreased. The size of redispersed emulsion was about the half of the emulsion size. This situation can be explained by the reducing of oil droplet size due to the atomization process when the emulsion was fed into the spray dryer [22, 32]. In the addition, the redispersed emulsion size showed the well distribution and narrow range of distribution curve comparing with feed emulsion as shown in **Figure 18.**

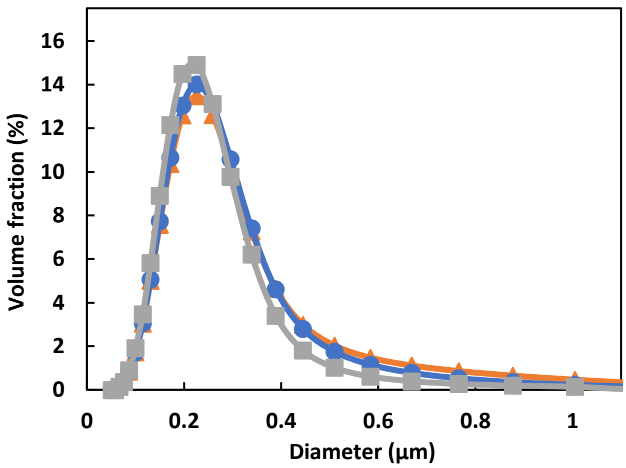


Figure 18 The distribution curve of redispersed emulsion size (The symbol related to ratio of Holy basil oil to wall materials (w/w); △ 1 to 2, o 1 to 3 and □ 1 to 4)

4.2.3.3 Effect of mass ratio on retention of encapsulated powder

After the spray drying method was completed, the encapsulated powder was extracted to determine the remaining of the component and analyzed by GC. It was compared with the content of the component in feed emulsion which represent the efficiency of the encapsulation process.

From **Table 16**, the mass ratio 1 to 4 which has the highest wall material represented the highest retention percentage compared to the others. In contrast, when the quantity of oil increased, the lower of retention percentage were observed. This is correlated to the previous reports [8, 22] and can be explained by the increasing of the rate of formation of semipermeable membrane due to the increasing of wall material resulting in the lower component loss during spray drying method according to the selective diffusion theory. When the type of maker components was determined, the retention percentage of eugenol was higher than other marker components. In this point, the chemical functional group of components took placed because of great ability to adsorbed to the starch more than the other of alcohol functional group. In the addition, molecular weight, polarity and volatility of components were also the variable of adsorption [8, 33, 34]. In the addition, the emulsion size and redispersed size showed the same trend in the increasing of retention percentage when size of particle are decreased as showed in **Figure 19** [20].

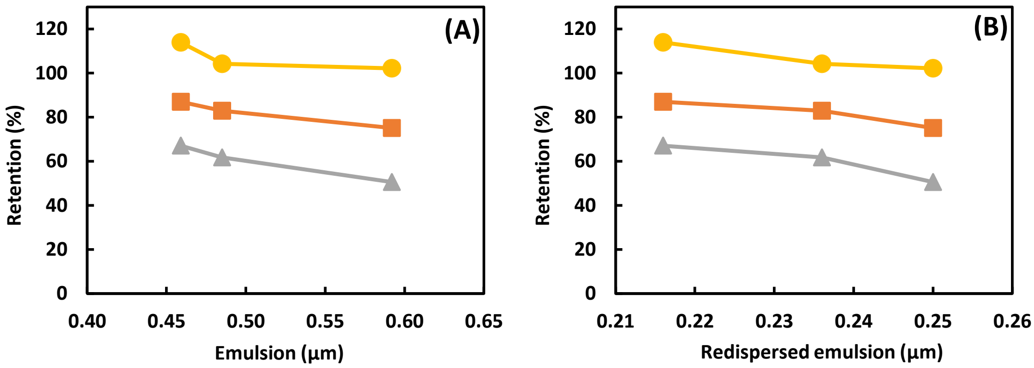


Figure 19 Trend of physical size to retention percentage of encapsulated powder (A) Emulsion size, (B) Redispersed emulsion size (The symbol related to each marker components; ○ eugenol, □ methyl eugenol and △ beta-caryophyllene)

Table 15 Effect of the mass ratio of Holy basil oil to wall materials on physical properties of encapsulated powder.

Mass ratio of Holy basil oil to wall materials	Emulsion size (μm)	Redispersed emulsion size (μm)	Encapsulated powder size (μm)
1:2	0.592[a]	0.250[d]	12.304 ± 0.239[f]
1:3	0.485[b]	0.236[c]	12.714 ± 0.404[e,f]
1:4	0.459[b]	0.216[c]	10.085 ± 0.365[e]

*The statistical analysis was compared in same group of physical size

Table 16 Effect of the mass ratio of Holy basil oil to wall materials on chemical properties of encapsulated powder.

Mass ratio of Holy basil oil to wall materials	Retention of components (%)			Surface oil content (%)			Encapsulation efficiency (%)		
	Eugenol	Methyl eugenol	Beta-caryophyllene	Eugenol	Methyl eugenol	Beta-caryophyllene	Eugenol	Methyl eugenol	Beta-caryophyllene
1:2	102.15 ± 3.80[a]	75.10 ± 0.85[a]	50.57 ± 5.08[a]	7.52 ± 1.53[a]	8.46 ± 1.63[a]	9.21 ± 1.44[a]	94.42 ± 1.95[a]	68.74 ± 0.47[a]	45.94 ± 5.11[a]
1:3	104.29 ± 7.69[a]	82.96 ± 6.62[a,b]	61.79 ± 2.27[a,b]	0.83 ± 0.3[b]	2.07 ± 0.50[b]	3.15 ± 0.47[b]	103.43 ± 7.75[a,b]	81.26 ± 6.64[b]	59.84 ± 2.10[b]
1:4	113.93 ± 5.07[a]	86.97 ± 3.40[b]	67.05 ± 5.80[b]	0.64 ± 0.07[b]	2.26 ± 0.55[b]	4.08 ± 0.70[b]	113.20 ± 5.11[b]	84.99 ± 2.97[b]	64.33 ± 5.84[b]

*The statistical analysis was compared in same group of components in each topic

4.2.3.4 Effect of mass ratio on content of marker components

In this experiment, the three components consist of eugenol, methyl eugenol and beta-caryophyllene which is dominant in quantity in Holy basil oil were selected to be the marker components in this experiment. From three different of mass ratio, the increasing of Holy basil oil content was represented by ratio 1 to 4, 1 to 3 and 1 to 2, orderly. From **Table 17,** the highest content of marker components was showed when mass ratio equal to 1 to 2 and the lowest content was showed when mass ratio equal to 1 to 4 which is correlated with the quantity of oil in each mass ratio.

Table 17 Effect of mass ratio on content of marker components

Mass ratio of Holy basil oil to wall materials	Air inlet temperature of spray dryer (°C)	Content of marker components (mg/ g dry solid)		
		Eugenol	Methyl eugenol	Beta-caryophyllene
1:2	160	114.25 ± 4.28[a]	124.07 ± 1.43[a]	83.63 ± 8.37[a]
1:3	160	77.28 ± 5.86[b]	85.03 ± 6.93[b]	88.30 ± 2.99[a]
1:4	160	49.86 ± 2.20[c]	55.68 ± 2.23[c]	52.81 ± 4.59[b]

*The statistical analysis was compared in same group of components

4.2.3.5 Effect of mass ratio on surface oil and encapsulation efficiency

Apart from the determine of retention which represent the remaining components in encapsulated powder, the surface oil was one of the important factors which represent the great stability of the encapsulated powder because of the motive of the oxidation circumstances of the oil that affected the stability of encapsulated powder [22]. When these two factors were considered simultaneously, the result led to represent in the encapsulation efficiency which is represented the component which is entrapped in the encapsulated powder.

Table 16 showed the reduction of surface oil when the oil content is decreased. In contrast, the encapsulation efficiency is increased in co-current way. This result can be explained by the increasing of emulsifying of wall material when the lower of Holy basil oil content was occurred which enhanced the ability to encapsulated the marker components of wall material [22, 31].

4.2.3.6 Effect of mass ratio on profile similarity

Apart from the content of component which is determined from the ability to entrapped the components by wall material. For essential oil, the unique scent is the important thing to represent the characteristic of each plant, especially when using them in aroma industrial such as cuisine and perfume [1, 2] . The unique scent of each plant come from the specific proportion of the volatile compounds in the essential oil, so the determination of the proportion of the compounds is required to get the condition which represent the most resemble scent comparing to the initial oil.

The comparison was showed in similarity percentage. Firstly, the proportion percentage of each component was calculated by compared with the summary of three marker components. Then, the similarity percentage was calculated from the comparison of sample and Holy basil oil. The most resemble profile was expected to have the 100 percentage of similarity. **Figure 20** showed the similarity percentage of different mass ratio compared with Holy basil oil. The result illustrated that the similarity percentage of each substance was different. For eugenol, the Holy basil oil showed the low similarity percentage compared to the result form encapsulated powder which mean the content of eugenol in encapsulated powder is higher than Holy basil oil. For methyl eugenol. the similarity percentage is insignificantly as label by the same alphabet. For beta-caryophyllene, the Holy basil oil showed the high similarity percentage compared to the result form encapsulated powder which mean the content of beta-caryophyllene in encapsulated powder is lower than Holy basil oil. The result of eugenol and beta-caryophyllene were correlated with the explanation in retention part, that eugenol can be entrapped in encapsulated powder more than beta-caryophyllene because of its functional group.

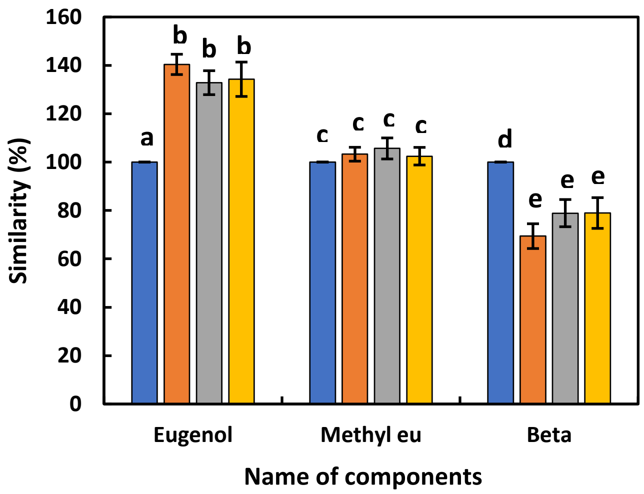


Figure 20 Effect of mass ratio on similarity percentage

The x axis; eugenol, methyl eugenol and beta-caryophyllene

(The color related to mass ratio of Holy basil oil to wall material; ■ Holy basil oil, ■ 1 to 2, ■ 1 to 3, ■ 1 to 4)

4.3 The effect of air inlet temperature of spray dryer to encapsulated powder

4.3.1 Effect of spray drying condition on the operating parameter of spray dryer

The emulsion with three different of oil content was spray dried at specific condition to study the effect of air inlet temperature of spray dryer. The air inlet temperature was studied at 120, 140, 160 and 180°C. In all operation, two parameters of spray dryer were fixed consist of drying air flow rate at 40 kilogram per hour and feed emulsion rate at 40% pump which approximately equal to 14.9 gram per minutes. The others parameters were showed in **Table 18**. From the result, the effect of oil content to outlet temperature were not observed as represent by the indifferent value when the same inlet temperature was performed. The variation of outlet temperature of spray dryer was represented when the different inlet temperature was operated. As the inlet temperature increased, the outlet temperature was increased which represented the influence of air inlet temperature to the outlet temperature of spray dryer.

Table 18 Effect of spray drying condition on the operating parameter of spray dryer

Air inlet temperature of spray dryer (°C)	Mass ratio of Holy basil oil to wall materials	Air outlet temperature of spray dryer (°C)
120	1:2	66.33 ± 2.08^{a}
	1:3	65.67 ± 3.51^{a}
	1:4	68.00 ± 1.00^{a,b}
140	1:2	75.67 ± 0.58^{c}
	1:3	75.67 ± 1.53^{c}
	1:4	73.67 ± 2.08^{b,c}
160	1:2	86.67 ± 1.53^{d,e}
	1:3	85.33 ± 1.15^{d}
	1:4	85.00 ± 1.73^{d}
180	1:2	99.00 ± 5.57^{f}
	1:3	98.00 ± 1.00^{f}
	1:4	93.33 ± 1.15^{e,f}

4.3.2 Effect of air inlet temperature of spray dryer on encapsulated powder

4.3.2.1 Effect of air inlet temperature on moisture content

The moisture content of encapsulated powder was one of disadvantage properties that affect the stability of encapsulated powder due to the oxidation circumstances [22]. So, the moisture content of encapsulated powder was measured to consider this topic. The encapsulated powder was heated by oven, and calculated the percentage by compared the weight of encapsulated powder before and after heated process. From **Figure 21**, the moisture content showed the reducing trend towards the increasing of air inlet temperature of spray dryer from 120 to 180 °C. The same trend in all mass ratio were observed, which represent the dominant effect of air inlet temperature to moisture content of encapsulated powder because of the high different in temperature between air and encapsulated powder which is leading to high evaporation rate of water [22]. The effect of moisture content is also observed with the yield percentage of the encapsulated powder from the spray dryer as shown in **Table 19**. The yield percentage has a range of 55 to 70 which has increased when the air inlet temperature of spray dryer was increased. As shown in the **Figure 7**, the spray dryer has a drying chamber and cyclone which is the pathway that encapsulated powder can be contact and stick at their inner surface. When the air inlet temperature of spray dryer was increased, the moisture content was decreased which make less of encapsulated powder was sticked and easily pass through to the product container.

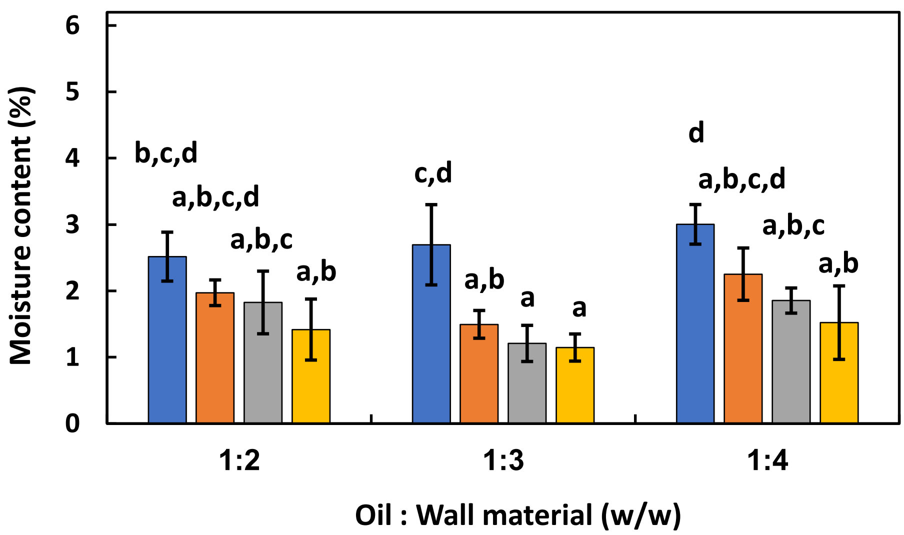


Figure 21 Effect of air inlet temperature on moisture content

(The color related to air inlet temperature of spray dryer;
■ 120°C, ■ 140°C, ■ 160°C, ■ 180°C)

Table 19 Effect of inlet temperature of spray dryer on moisture content and yield of encapsulated powder.

Inlet temperature of spray dryer (°C)	Mass ratio of Holy basil oil to wall materials	Moisture content (%)	Yield (%)
120	1:2	$2.52 \pm 0.37^{b,c,d}$	55.89 ± 0.55^{a}
	1:3	$2.70 \pm 0.60^{c,d}$	61.43 ± 2.08^{b}
	1:4	3.00 ± 0.30^{d}	54.39 ± 2.36^{a}
140	1:2	$1.97 \pm 0.19^{a,b,c,d}$	61.43 ± 2.08^{b}
	1:3	$1.50 \pm 0.21^{a,b}$	$64.30 \pm 1.90^{b,c,d}$
	1:4	$2.25 \pm 0.39^{a,b,c,d}$	56.20 ± 3.12^{a}
160	1:2	$1.83 \pm 0.47^{a,b,c}$	$64.85 \pm 0.59^{b,c,d}$
	1:3	1.21 ± 0.27^{a}	$66.97 \pm 0.84^{c,d}$
	1:4	$1.85 \pm 0.19^{a,b,c}$	$62.43 \pm 1.24^{b,c}$
180	1:2	$1.42 \pm 0.46^{a,b}$	68.62 ± 0.26^{d}
	1:3	1.15 ± 0.20^{a}	68.51 ± 0.36^{d}
	1:4	$1.52 \pm 0.55^{a,b}$	$65.69 \pm 1.02^{b,c,d}$

*The statistical analysis was compared in same group

4.3.2.2 Effect of air inlet temperature on redispersed emulsion and encapsulated powder size

Figure 22 showed the effect of four different air inlet temperature on redispersed emulsion size and encapsulated powder size at different oil content. The redispersed size of mass ratio 1 to 2 and 1 to 3 tend to increase with the air inlet temperature, while mass ratio 1 to 4 is static which represent the great ability to encapsulate the Holy basil oil in this condition. For the encapsulated powder size, the range of powder were around 10 to 15 micrometer and the mass ratio of Holy basil oil to wall material at 1 to 4 demonstrates the lowest encapsulated powder size. The encapsulated powder size tends to increased slightly when the air inlet temperature increased. This can be explained by the rapidly dry of the encapsulated powder surface when the high temperature was performed which caused the prevention of water evaporation from the encapsulated powder and resulting in large particle size [22].

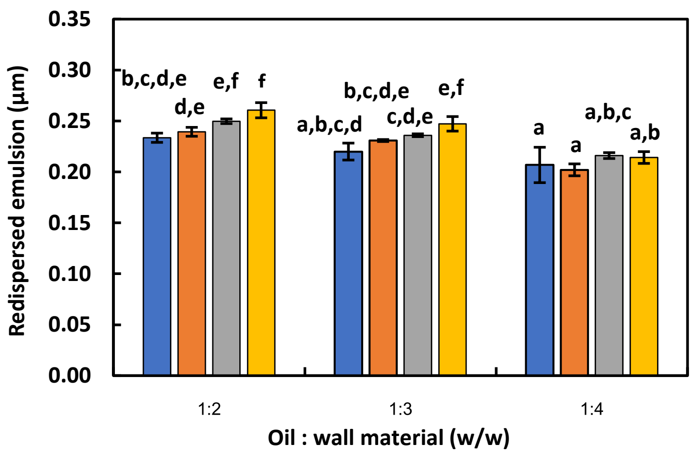


Figure 22 Effect of air inlet temperature on redispersed emulsion size (The color related to air inlet temperature of spray dryer; 120°C, 140°C, 160°C, 180°C)

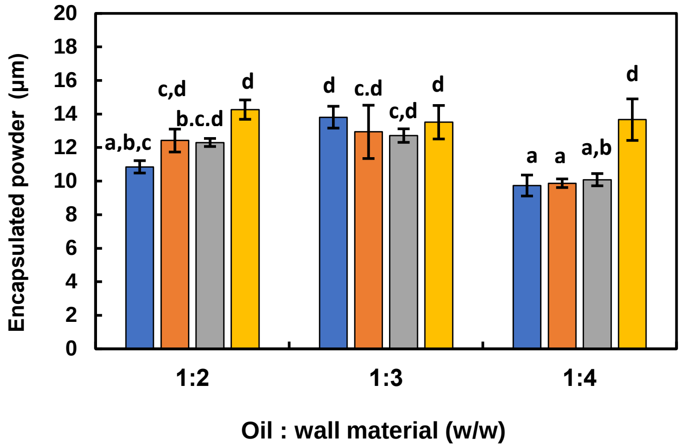
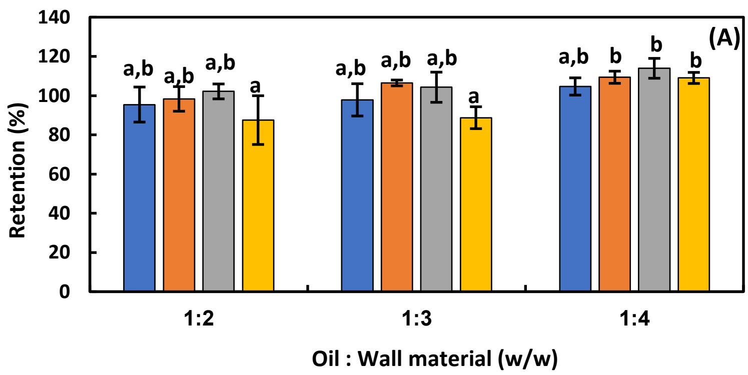


Figure 23 Effect of air inlet temperature on encapsulated powder size (The color related to air inlet temperature of spray dryer; 120°C, 140°C, 160°C, 180°C)

4.3.2.3 Effect of air inlet temperature on retention

The spray drying method used the high air inlet temperature to process, so the consideration of retention percentage which is indicated the content of components in encapsulated powder after spray drying was required. It was calculated by compared the content of marker components which extracted from encapsulated powder with the component in initial feed emulsion.



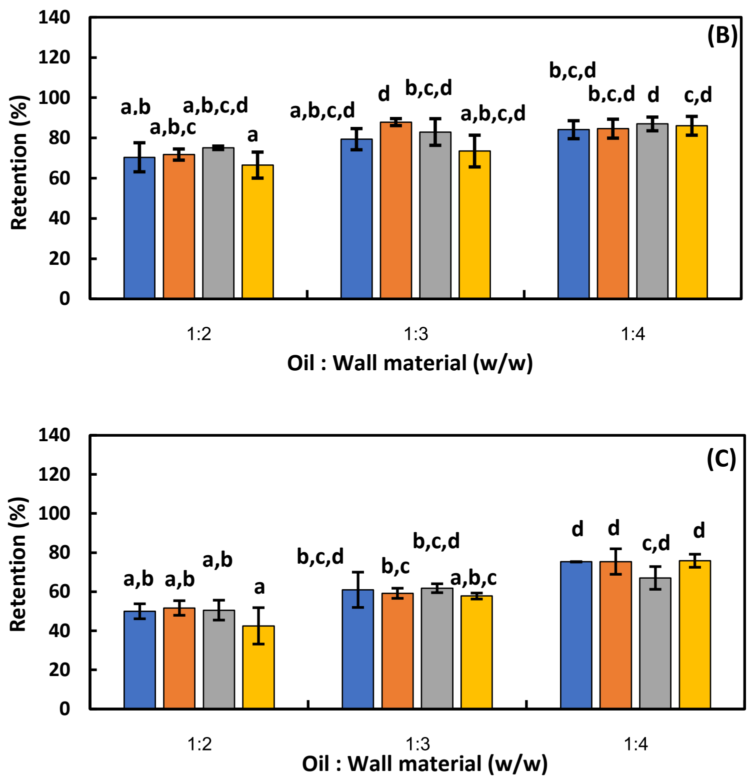


Figure 24 Effect of air inlet temperature on retention

(A) Eugenol, (B) Methyl eugenol, (C) Beta-caryophyllene

(The color related to air inlet temperature of spray dryer;

120°C, 140°C, 160°C, 180°C)

From **Figure 24**, the same mass ratio represents the same value of retention percentage, although the inlet temperature of spray dryer was different which represent the great ability to protect the component from heat of the wall material. The mass ratio of Holy basil oil to wall material at 1 to 4 represent the highest retention percentage of all components which correlated to previous part that the mass ratio 1 to 4 was also gave the lowest of emulsion size aside from the high retention percentage because of the great emulsifier properties of wall materials. As for the effect of type of component in retention percentage, the result followed the same described in the previous section.

4.3.2.4 Effect of air inlet temperature on content of components

From the probability of disadvantage effect from losing of components from heat which is performed by the air inlet temperature of spray dryer, the content of components after the spray drying method was investigated. The result showed the mg of marker component per 1 g of dry wall material.

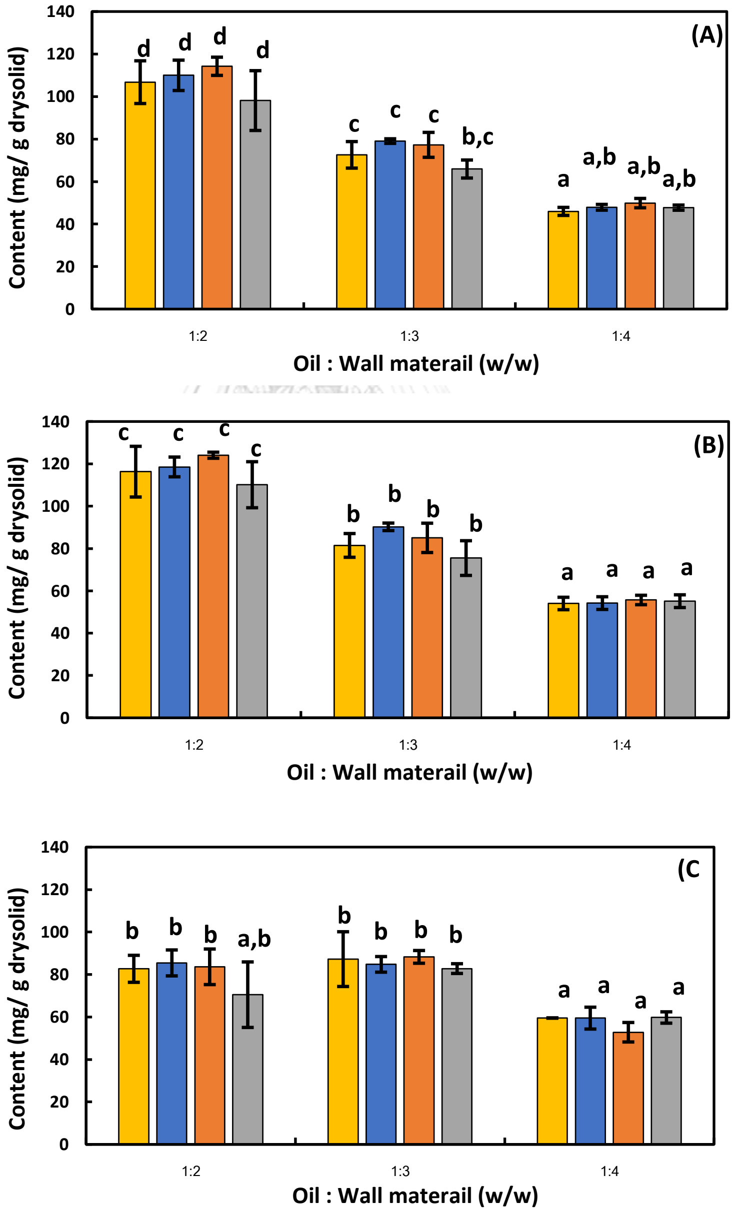


Figure 25 Effect of air inlet temperature on content of components

(A) Eugenol, (B) Methyl eugenol, (C) Beta-caryophyllene

(The color related to air inlet temperature of spray dryer;

120°C, 140°C, 160°C, 180°C)

Figure 25 showed the different in content of components in different mass ratio of Holy basil oil which is correlated with the quantity of oil in the emulsion preparation step. The same value of content of the components were observed at different air inlet temperature of spray dryer which represent the efficiency protection of all components from the wall material. So, the air inlet temperature was determined to have no effect with the content of components.

4.3.2.5 Effect of air inlet temperature on surface oil and encapsulation efficiency

When the quantity of oil increased as labeled by 1 to 4, 1 to 3 and 1 to 2, orderly. From **Figure 26**, surface oil at mass ratio 1 to 2 was increased with the inlet temperature of spray dryer meanwhile the other mass ratios are static. This result can be explained by the low emulsifying of wall material when the oil content was increased and unsuitable drying rate of spray dryer which affect the limitation of storage of the wall materials. The water solubility was also affecting the surface oil. The component that has high water solubility is tend to have the high surface oil because it easily diffused with the water through the crust of the encapsulated powder [8, 22]. From the components' properties, the three marker components are all insoluble in water so the nearest value of the components in surface oil content for same mass ratio were observed. Since the retention was statistically equally in same mass ratio as discussed previously, so the content of surface oil was the determinant to the encapsulation efficiency. **Figure 27**, the lowest encapsulation efficiency was presented by mass ratio 1 to 2 which showed the highest surface oil content and the highest encapsulation efficiency was presented by mass ratio 1 to 4 which showed the lowest surface oil content.

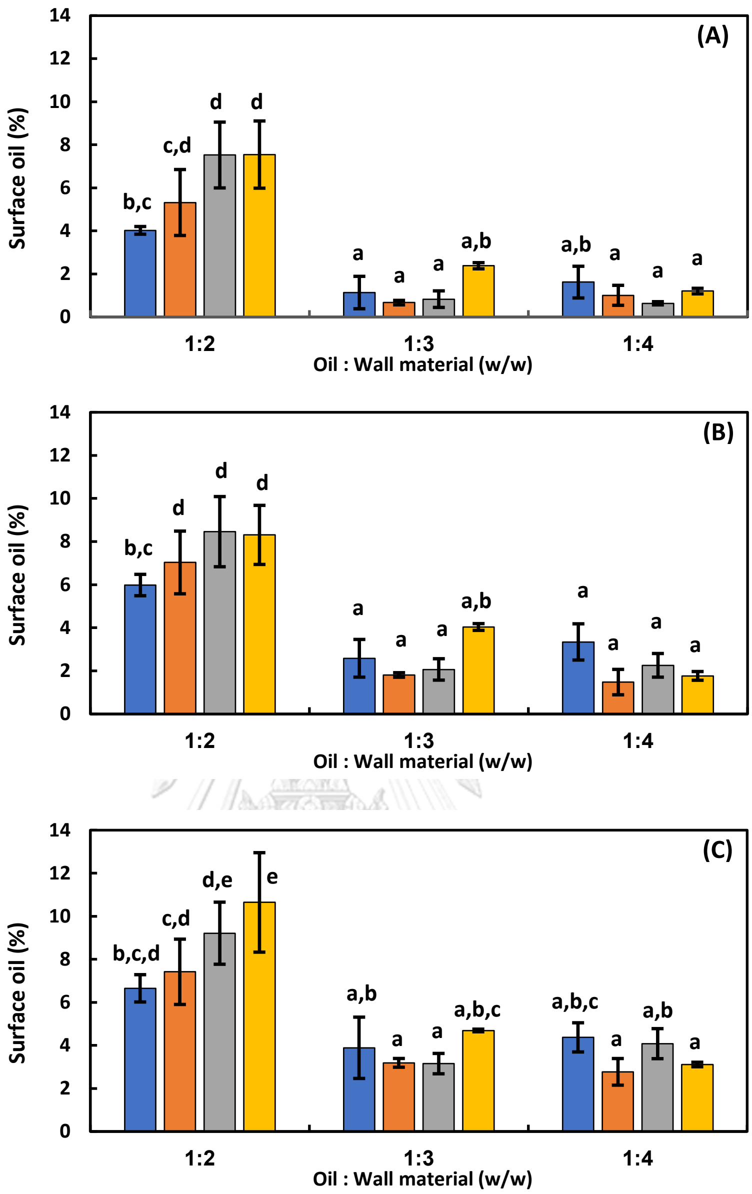


Figure 26 Effect of air inlet temperature on surface oil content

(A) Eugenol, (B) Methyl eugenol, (C) Beta-caryophyllene

(The color related to air inlet temperature of spray dryer;

120°C, 140°C, 160°C, 180°

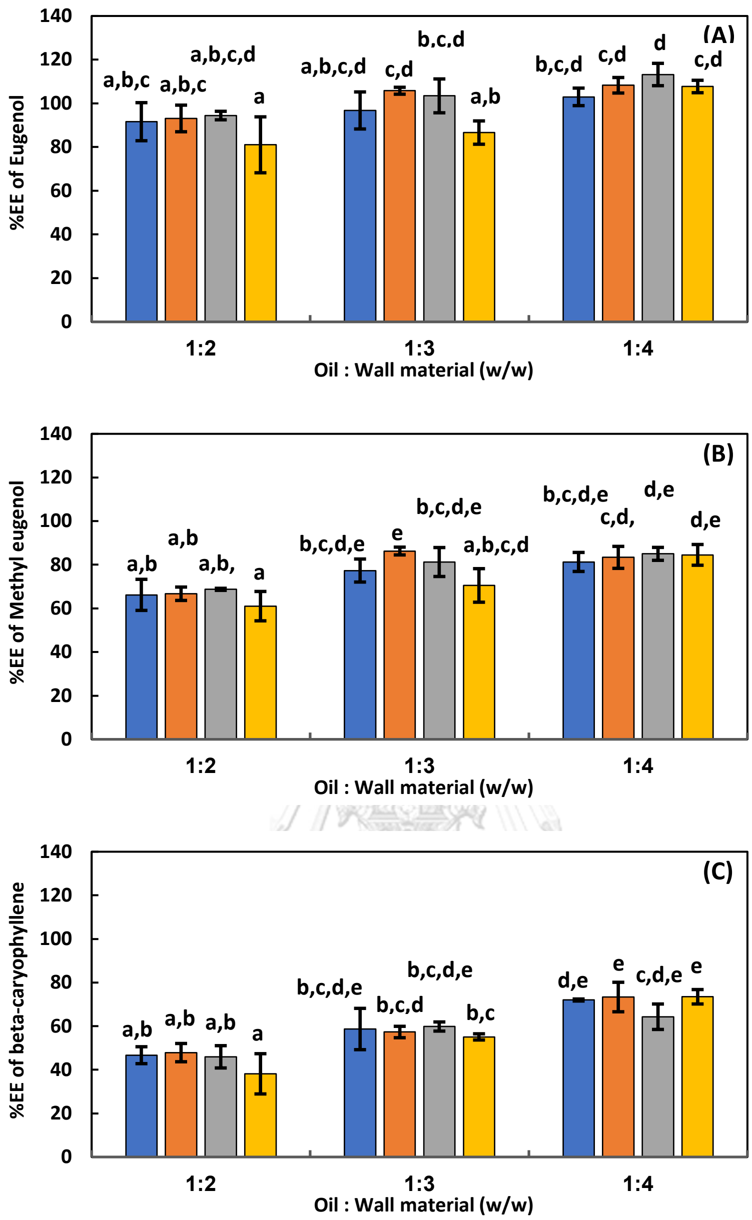


Figure 27 Effect of air inlet temperature on encapsulation efficiency

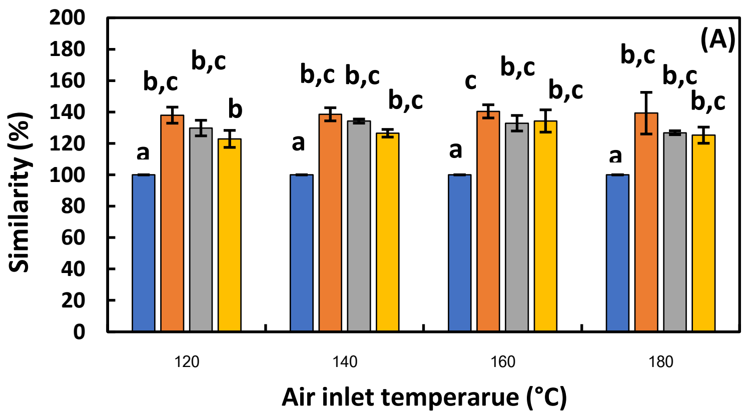
(A) Eugenol, (B) Methyl eugenol, (C) Beta-caryophyllene

(The color related to air inlet temperature of spray dryer;

120°C, 140°C, 160°C, 180°C)

4.2.3.6 Effect of air inlet temperature on profile similarity

The similarity percentage was calculated as described above. Then, analyzed by MetaboAnalyst to report the hierarchical clustering heatmaps with dendrogram which can illustrate the comparison of Holy basil oil to the sample from different treatment condition by spray drying method. The result of similarity percentage from encapsulated powder was showed in **Figure 28**, the percentage of each mass ratio represented the insignificant value even if the different air inlet temperature of spray dryer was performed. From eugenol and beta-caryophyllene, the different of percentage comparing to the Holy basil oil were observed due to the content of the components after the spray drying method as previously described. The hierarchical clustering heatmaps was showed in **Figure 29**, intensity of the color from blue to red illustrated the content of maker component from lowest to highest. The dendrogram which showed above the heat map represent the group of each data, the data in the same group were determined to resemble to each other. The most resemble profile compared to Holy basil oil was observed at mass ratio of Holy basil oil to wall material at 1 to 4 when inlet temperature of spray dryer was set at 140°C. The latter were showed by mass ratio 1 to 4 at inlet temperature 120°C and 180°C, respectively.



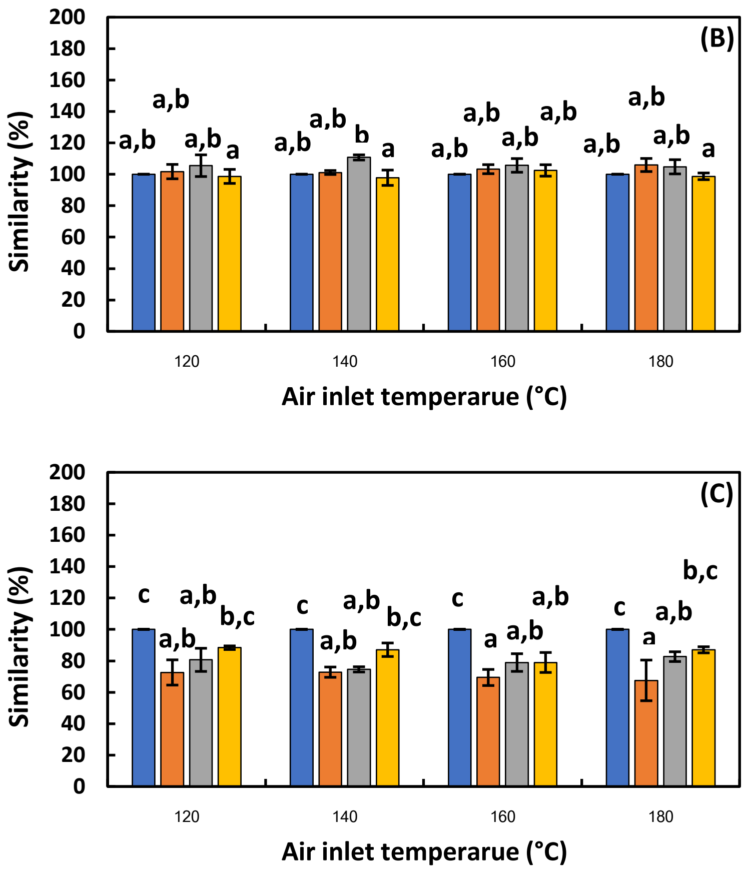


Figure 28 Effect of air inlet temperature on similarity percentage

(A) Eugenol, (B) Methyl eugenol, (C) Beta-caryophyllene

(The color related to mass ratio of Holy basil oil to wall material; Holy basil oil, 1 to 2, 1 to 3, 1 to 4)

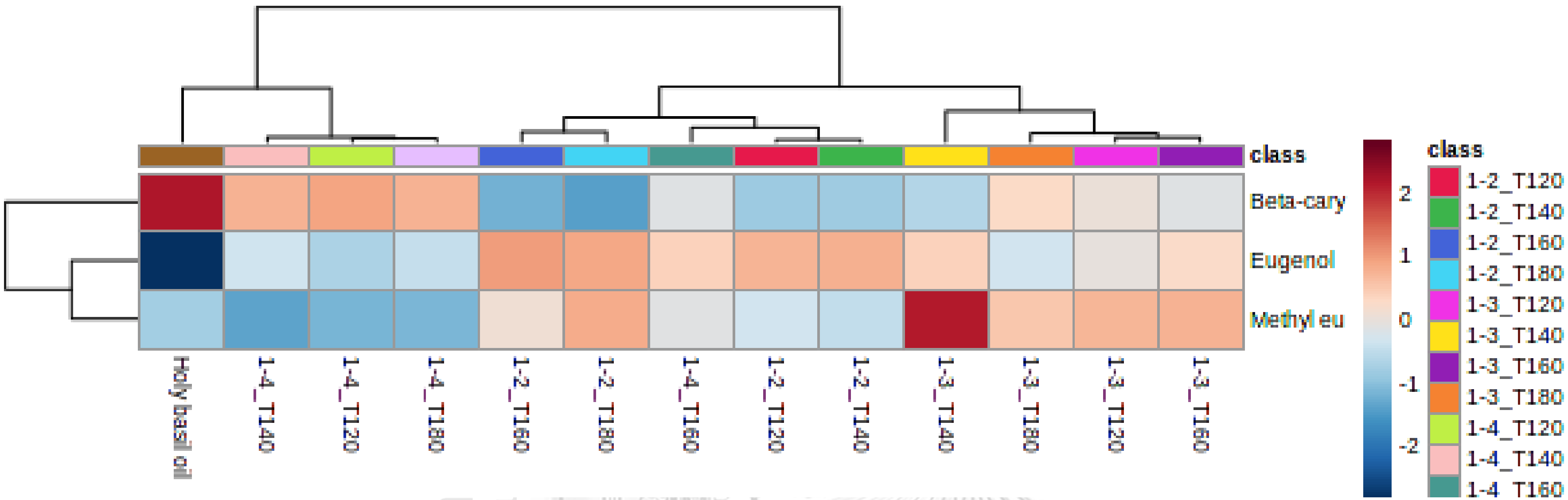


Figure 29 Heat map of the components profile

4.4 The storage stability of encapsulated powder

4.4.1 Effect of time storage to the content of components

The mass ratio at 1 to 2, 1 to 3 and 1 to 4 that performed by spray dryer at air inlet temperature 160°C were kept at 40°C in the oven for 1, 2 and 3 months. When the time was reached, the remaining of component was analyzed by GC to determine the effect of storage with the content of marker components in encapsulated powder. From **Figure 30**, when the content of marker components from different time storage was compared, mass ratio of Holy basil oil to wall material equal to 1 to 4 show no different in value which represented the great ability of modified starch to protect the Holy basil oil during storage [31].

4.4.2 Effect of storage time on profile similarity

From the utility to use in aroma field. The profile of encapsulated powder after storage which related with the unique scent of Holy basil oil was also investigate. The similarity percentage of Holy basil oil was fixed at 100 as the reference. The result showed in **Figure 31,** all mass ratio represented the resemble of profile after the storage as compared by the encapsulated powder before the storage. The high similarity percentage of eugenol and low similarity percentage of beta-caryophyllene were observed which can described by the content of the components after spray drying method as described previously.

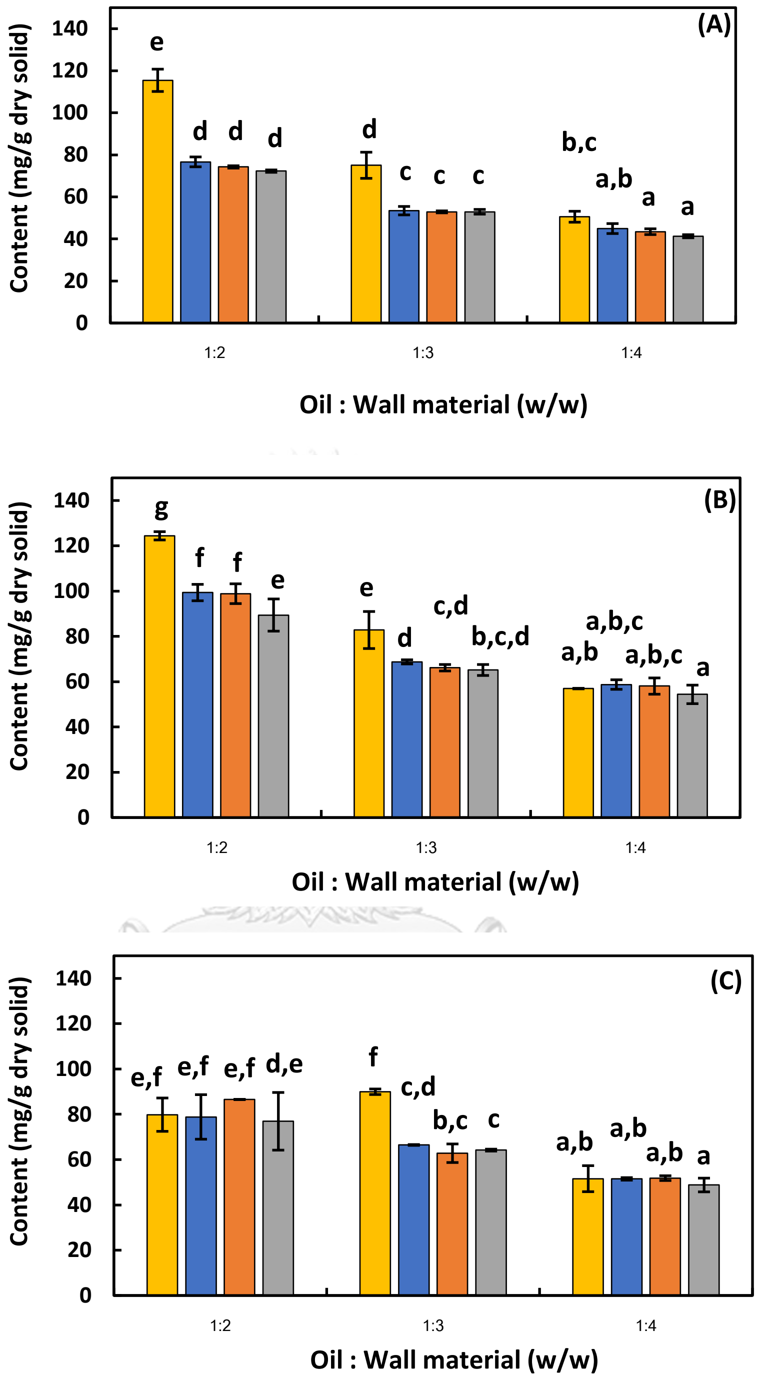


Figure 30 Content of components after storage at 40 ° C

(A) Eugenol, (B) Methyl eugenol, (C) Beta-caryophyllene

(The color related to time duration of storage;

0 month, 1 month, 2 month, 3 month)

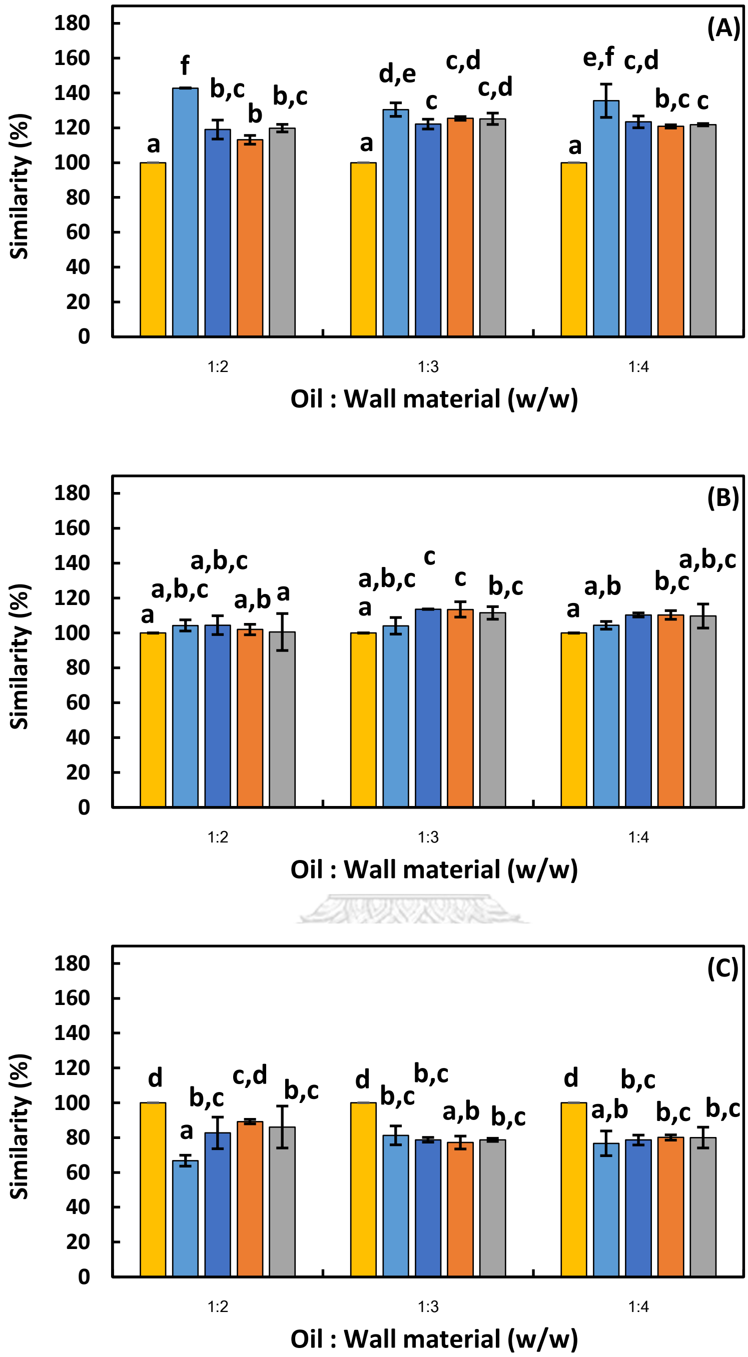


Figure 31 Effect of storage time on profile similarity

(A) Eugenol, (B) Methyl eugenol, (C) Beta-caryophyllene

(The color related to time duration of storage;

Holy basil oil, 0 month, 1 month, 2 month, 3 month)

CHAPTER V

SUMMARY

To the best of the authors’ knowledge, there was the few experiments about the encapsulation of Holy basil oil. In this experiment, three marker components consist of eugenol, methyl eugenol and beta-caryophyllene were studied. The encapsulated powder was successfully prepared by spray drying method using the modified starch which is the wall material in carbohydrate group. Three formulation of initial feed emulsion were performed at mass ratio of Holy basil oil to wall material equal to 1 to 2, 1 to 3 and 1 to 4. The condition of spray dryer was set at 4 different air inlet temperatures; 120, 140, 160 and 180 °C

From the result, the mass ratio of Holy basil oil to wall material showed the affects with the emulsion size and redispersed emulsion size. Moreover, the effect of particle size and retention percentage was observed. The low content of oil showed the small size of droplet and high retention percentage. The air inlet temperature of spray dryer has affected with the moisture content and surface oil of encapsulated powder. When the high air inlet temperature was used the moisture content was decreased and surface oil was increased in mass ratio 1 to 2. The high encapsulation efficiency is detected at mass ratio 1 to 4 at all air inlet temperature of spray dryer, moreover the most similarity of profile and high remaining of component after storage were also observed. From all of above, the mass ratio equal to 1 to 4 was recommend to encapsulate the Holy basil oil.

REFERENCES

1. Mahajan, N., et al., *A phytopharmacological overview on Ocimum species with special emphasis on Ocimum sanctum.* Biomedicine & Preventive Nutrition, 2013. 3(2): p. 185-192.
2. Ngamakeue, N. and P. Chitprasert, *Encapsulation of Holy Basil Essential Oil in Gelatin: Effects of Palmitic Acid in Carboxymethyl Cellulose Emulsion Coating on Antioxidant and Antimicrobial Activities.* Food and Bioprocess Technology, 2016. 9(10): p. 1735-1745.
3. Singh, D. and P.K. Chaudhuri, *A review on phytochemical and pharmacological properties of Holy basil (Ocimum sanctum L.).* Industrial Crops and Products, 2018. 118: p. 367-382.
4. Kanaujia, P., et al., *Amorphous formulations for dissolution and bioavailability enhancement of poorly soluble APIs.* Powder Technology, 2015. 285: p. 2-15.
5. Veiga, R.D.S.D., et al., *Essential oils microencapsulated obtained by spray drying: a review.* Journal of Essential Oil Research, 2019. 31(6): p. 457-473.
6. Geranpour, M., E. Assadpour, and S.M. Jafari, *Recent advances in the spray drying encapsulation of essential fatty acids and functional oils.* Trends in Food Science & Technology, 2020. 102: p. 71-90.
7. Tangpao, T., et al., *Encapsulation of Basil Essential Oil by Paste Method and Combined Application with Mechanical Trap for Oriental Fruit Fly Control.* Insects, 2021. 12(7).
8. Penbunditkul, et al., *Loss mechanism of multi-flavor compounds in microencapsulation process by spray drying.* 2011.
9. Joardar, A., et al., *Host-guest complexation of eugenol in cyclodextrins for enhancing bioavailability.* Journal of Molecular Liquids, 2020. 319: p. 114336.
10. Alves, S.F., et al., *Microencapsulation of Essential Oil from Fruits ofPterodon emarginatusUsing Gum Arabic and Maltodextrin as Wall Materials: Composition and Stability.* Drying Technology, 2013. 32(1): p. 96-105.
11. Tangpao, T., H.H. Chung, and S.R. Sommano, *Aromatic Profiles of Essential Oils from Five Commonly Used Thai Basils.* Foods, 2018. 7(11).

12. Ghosh, S., et al., *Food application of an encapsulated phytochemically rich SC-CO2 extract of a polyherbal mix of tulsi, bay and cardamom: Shelf-life and frying stability of soybean oil.* Journal of Food Engineering, 2016. 171: p. 194-199.

13. Nuchuchua, O., et al., *Physicochemical investigation and molecular modeling of cyclodextrin complexation mechanism with eugenol.* Food Research International, 2009. 42(8): p. 1178-1185.

14. Talon, E., et al., *Encapsulation of eugenol by spray-drying using whey protein isolate or lecithin: Release kinetics, antioxidant and antimicrobial properties.* Food Chem, 2019. 295: p. 588-598.

15. Wang, M., et al., *Methyl eugenol attenuates liver ischemia reperfusion injury via activating PI3K/Akt signaling.* Int Immunopharmacol, 2021. 99: p. 108023.

16. Lee, H.Y. and M.J. Ko, *Thermal decomposition and oxidation of beta-caryophyllene in black pepper during subcritical water extraction.* Food Sci Biotechnol, 2021. 30(12): p. 1527-1533.

17. Timilsena, Y.P., M.A. Haque, and B. Adhikari, *Encapsulation in the Food Industry: A Brief Historical Overview to Recent Developments.* Food and Nutrition Sciences, 2020. 11(06): p. 481-508.

18. Mohammed, N.K., et al., *Spray Drying for the Encapsulation of Oils-A Review.* Molecules, 2020. 25(17).

19. Bakry, A.M., et al., *Microencapsulation of Oils: A Comprehensive Review of Benefits, Techniques, and Applications.* Compr Rev Food Sci Food Saf, 2016. 15(1): p. 143-182.

20. Soottitantawat, A., et al., *Microencapsulation by Spray Drying :Influence of Emulsion Size on the Retention of Volatile Compounds.* 2003.

21. Soottitantawat, et al., *Influence of emulsion and powder size on the stability of encapsulated d-limonene by spray drying* 2005.

22. Kaewpanha, M., Apinan Soottitantawat, and T. Charinpanitkul, *Encapsulation of lemongrass oil by spray drying.* 2009.

23. Rajabi, H., et al., *Retention of saffron bioactive components by spray drying encapsulation using maltodextrin, gum Arabic and gelatin as wall materials.* Food Hydrocolloids, 2015. 51: p. 327-337.

24. Mahdi, A.A., et al., *Microencapsulation of fingered citron extract with gum arabic, modified starch, whey protein, and maltodextrin using spray drying.* Int J Biol Macromol, 2020. 152: p. 1125-1134.

25. Rehman, A., et al., *Spray dried nanoemulsions loaded with curcumin, resveratrol, and borage seed oil: The role of two different modified starches as encapsulating materials.* Int J Biol Macromol, 2021. 186: p. 820-828.

26. Wang, Y. and G. Zhang, *The preparation of modified nano-starch and its application in food industry.* Food Research International, 2021. 140: p. 110009.

27. Anggriawan, R., A. Maksum, and N. Nurhayati, *Production and Application of Octenyl Succinic-Modified Starch as Fat Replacer: A Review of Established and Recent Research*. 2020, Preprints.org.

28. Zanoni, F., et al., *Microencapsulation by spray-drying of polyphenols extracted from red chicory and red cabbage: Effects on stability and color properties.* Food Chemistry, 2020. 307: p. 125535.

29. Altuna, L., M.L. Herrera, and M.L. Foresti, *Synthesis and characterization of octenyl succinic anhydride modified starches for food applications. A review of recent literature.* Food Hydrocolloids, 2018. 80: p. 97-110.

30. Huang, C.-y., et al., *Measurements and Correlations of Liquid-liquid-Equilibria of the Mixtures Consisting of Ethanol, Water, Pentane, Hexane, and Cyclohexane~!*2009-08-31~*!*2009-11-02~*!*2010-06-24~*!* The Open Thermodynamics Journal, 2010. 4: p. 102-118.

31. Shu, B., et al., *Study on microencapsulation of lycopene by spray-drying.* Journal of Food Engineering, 2006. 76(4): p. 664-669.

32. Taboada, M.L., et al., *Breakup and Coalescence of Oil Droplets in Protein-Stabilized Emulsions During the Atomization and the Drying Step of a Spray Drying Process.* Food and Bioprocess Technology, 2021. 14(5): p. 854-865.

33. Goubet, I., J.L. Le Quere, and A.J. Voilley, *Retention of Aroma Compounds by Carbohydrates: Influence of Their Physicochemical Characteristics and of Their Physical State. A Review.* Journal of Agricultural and Food Chemistry, 1998. 46(5): p. 1981-1990.

34. M. Le Thanh, P.T., M.A. Thibaut, A. Voilley, *Interactions between volatile and non-volatile compounds in the presence of water.* food chemistry, 1992. 43(2): p. 129-135.

Appendix

Results

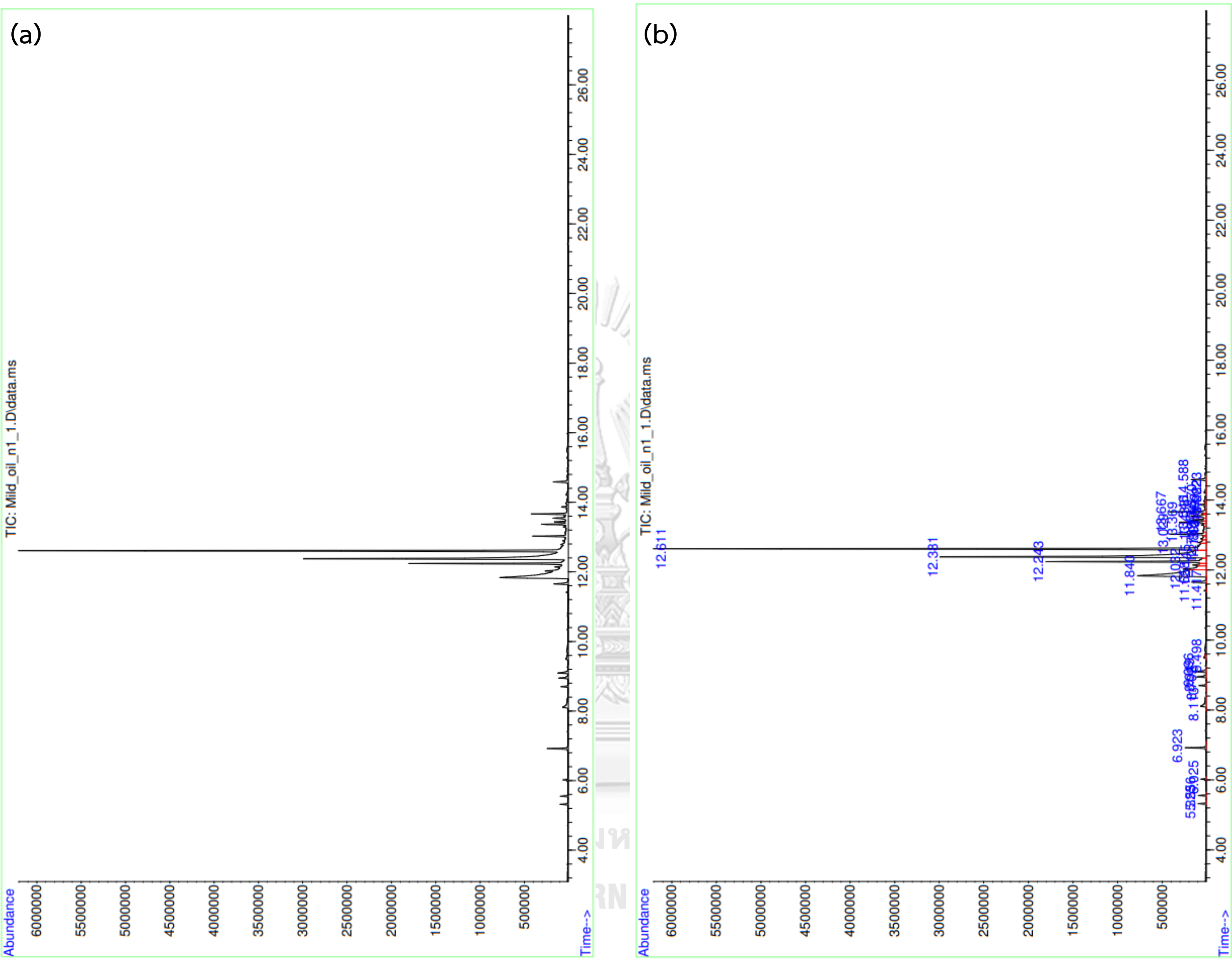


Figure 32 Chromatogram of Holy basil oil from GC

(a) No retention time, (b) With retention time (min)

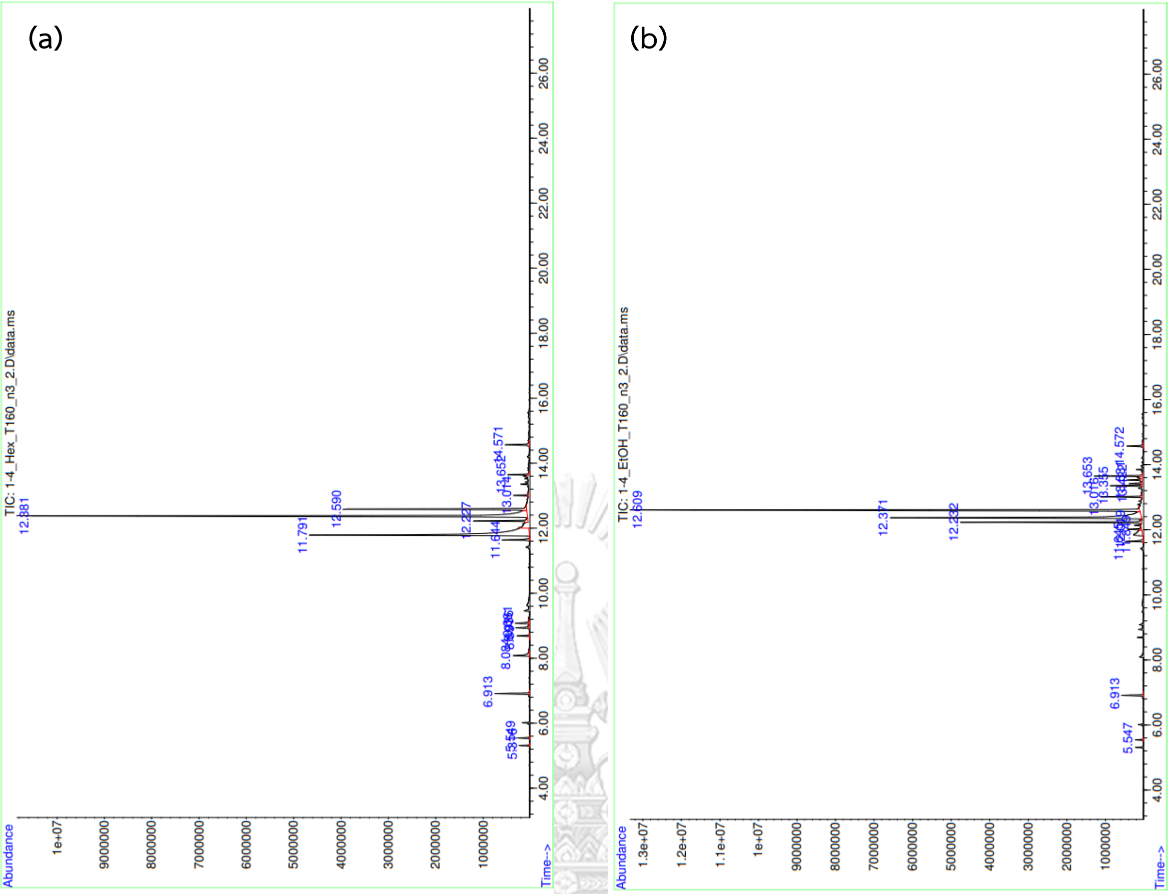


Figure 33 Chromatogram of extracted from GC

(a) Extracted with Hexane, (b) Extracted with Hexane and Ethanol; ratio of Holy basil oil to HI-CAP100 equal to 1 to 4 and air inlet temperature of spray dryer is 160°C

Table 20 The chemical compositions of the white Holy basil oil from TCFF analyzed by GC-MS

From the results, the Thirty volatile compounds were detected.

Order	Components	Area%
1	α-pinene	0.33
2	camphene	0.35
3	β-pinene	0.22
4	1,8-cineole	1.04

5	α-terpinolene	0.91
6	camphor	0.36
7	isoborneol	0.61
8	borneol	0.72
*9	α-terpineol	0.15
10	cyclohexasiloxane	0.08
11	camphene	0.91
12	eugenol	18.01
15	β-elemene	8.70
16	methyl eugenol	25.97
17	β-caryophyllene	30.61
*18	methyl eugenol	0.97
20	4,7,10-cycloundecatriene	1.93
21	naphthalene	0.46
22	β-cubebene	1.43
23	β-selinene	0.92
24	α-selinene	1.14
*25	cycloheptasiloxane	0.27
26	naphthalene	2.01
27	δ-cadinene	0.41
28	Tricyclo[5.4.0.02,8]undec-9-ene	0.29
*29	trans-2-Isopropylbicylclo[4.3.0]no n-3-ene-8-one	0.25
30	caryophyllene oxide	0.93

*%Similarity compared with library less than 85%

<u>External standard calibration curve</u>

The standard of 4 marker components were prepared individually in hexane to determine the retention time of each component by Gas Chromatography (GC). The results showed in **Table 21**.

Table 21 Estimate retention time of each component

Components	Retention time (minutes)
Eugenol	11.708
Methyl eugenol	12.282
Beta-caryophyllene	12.457

<u>Example of calculation</u>

<u>Condition</u> Mass ratio of Holy basil oil to wall material = 1 to 4 (w/w)

Spray dryer condition = Inlet temperature 160°C

1) Yield percentage

From equation $\%Yield = \frac{W_{NH}}{W} \times 100$

where W_{NH} is the weight of encapsulated Holy basil powder which is neglected the moisture content and W is the total weight of Holy basil oil and modified starch solution in emulsion preparation step.

<u>Step 1</u> Calculation of W_{NH}

$$W_{NH} = \frac{100-\%\text{moisture content}}{100} \times W_{powder}$$

where W_{powder} indicate the weights of encapsulated Holy basil powder from spray drying method.

$$W_{NH} = \frac{100-2.06}{100} \times 37.56 \quad = 36.79 \text{ g}$$

Step 2 Calculation of W

Wall material solution percentage

$$= \frac{\textbf{Weight of wall material}}{\textbf{Weight of wall material+DI water}} \times 100$$

$$= \frac{\textbf{640.15}}{\textbf{640.15+960.04}} \times 100 \qquad = 40.00\ \%\ (w/w)$$

Weight of wall material in emulsion

= Wall material solution percentage x Weight of wall material solution

$$= \frac{\textbf{40}}{\textbf{100}} \times 120.06 \qquad = 48.03\ g$$

So, W = Weight of wall material in emulsion + Weight of Holy basil oil

= 48.03 + 12.03 = 60.60 g

Step 3 Calculation of Yield percentage

$$\%Yield = \frac{\mathbf{W_{NH}}}{\mathbf{W}} \times 100$$

$$\%Yield = \frac{\textbf{36.79}}{\textbf{60.60}} \times 100 \qquad = 61.25\%$$

2) Retention percentage

The volume of extraction when ethanol and hexane was calculated by LLE of water, ethanol and hexane to determine the volume of extraction phase. The detail which is used to calculate mole of each component from volume in extraction methos was shown in Table.

Table 22 Details of each component in extraction step.

Substance	Density (g/cm^3)	Molecular weight	Mole
Water	995.41 x 10^{-3}	18.02	0.111
Ethanol	779.8 x 10^{-3}	46.07	0.051
Hexane	650.7 x 10^{-3}	86.18	0.030

From the table, the total mole fraction equal to 0.192 mol. Mol of each component was divided by total mole to get the mole fraction of each component in the mixing substance.

Mole fraction

Water = $\frac{0.111}{0.192}$ = 0.58, Ethanol = $\frac{0.051}{0.192}$ = 0.26, Hexane = $\frac{0.030}{0.192}$ = 0.16

The mole fraction of each component was used to find the mixing point in **Figure 28**.

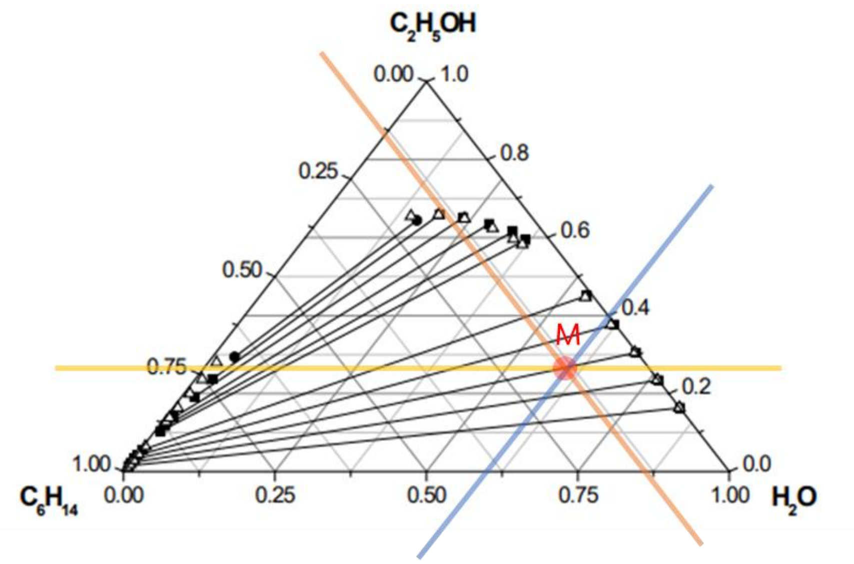


Figure 34 LLE data of ethanol - water - hexane ternary mixture with mixing point in this experiment.

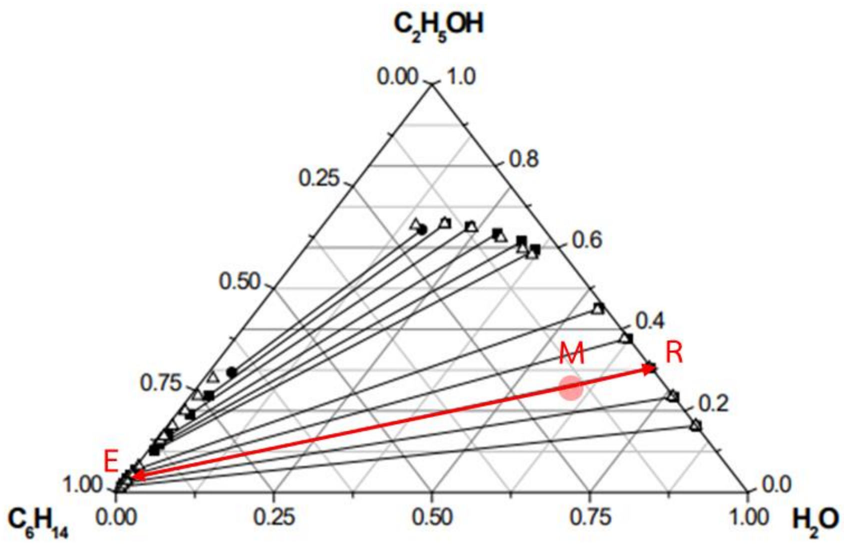


Figure 35 ER line from LLE and mixing point

The lever rule was used to calculated the content of mole in extract part which is illustrated by E.

$$E \times \overline{EM} = R \times \overline{RM}$$

$$E \times 4.9 = R \times 0.8$$

$$R = 6.125E$$

From total mole = 0.192

$$E + R = 0.192$$

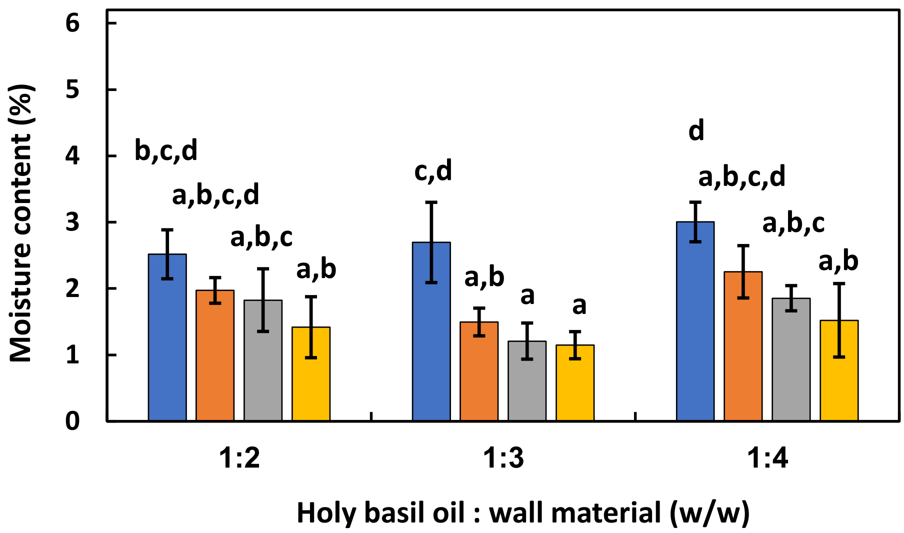
$$E + 6.125E = 0.192$$

$$E = 0.03$$

The mole of hexane equal to 0.03, so the extraction phase is assumed equal to the volume of hexane which added in the extraction step of hexane and ethanol.

Example of statistical analysis interpret

This graph showed the result of effect of air inlet temperature on moisture content. The color related to air inlet temperature of spray dryer; 120°C, 140°C, 160°C and 180°C.



The data were compared by one way ANOVA analysis at 95% confidence levels of the fitting values and represent the same group of data by label the same alphabet. For example, in all mass ratio of Holy basil oil to wall material when the inlet temperature was set at 140, 160 and 180 °C, the alphabet a was represent in all group which mean that all of the data has no significantly difference in statistical analysis. For data which is labeled by more than 1 alphabet, that data was determined to has no significantly difference in statistical analysis with all groups that labeled with the same alphabet. For example, when the mass ratio of Holy basil oil to wall material at 1 to 2 at air inlet temperature 180°C was determined, this data has no significantly difference in statistical analysis with group of a as showed previously, and group of alphabet b consist of all air inlet temperature of mass ratio 1 to 2, mass ratio 1 to 3 at air inlet temperature 140°C and mass ratio 1 to 4 at air inlet temperature 140, 160 and 180°C.

VITA

NAME	artima lairattanakul
DATE OF BIRTH	2 August 1998
PLACE OF BIRTH	Bangkok
INSTITUTIONS ATTENDED	Chulalongkorn university
HOME ADDRESS	805/44 Soi Tessabanbangpoo 59/2 Tambon Taibanmai Amphor Muang Samutprakarn 10280