Microencapsulation of Natural Vanilla (*Vanilla planifolia*) Extract in Beta-cyclodextrin by Using Kneading Method

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Ms. Ampapan Jongyin

ID. 5436811

A special project submitted to the faculty of Biotechnology, Assumption University in part of fulfillment of the requirement for the degree of Bachelor of Science in Biotechnology

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By	:	**Ms. Ampapan Jongyin**
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Level of study	:	**Bachelor of Science**
Department	:	**Food technology**
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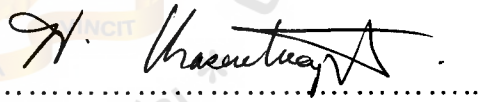

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(Advisor)

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Abstract

This researchaimed to encapsulate vanilla extract by using inclusion complex of β-cyclodextrin and also investigate the qualities of the encapsulated powder in terms of vanillin content, moisture content, and stability under accelerated condition. Natural vanilla extract was made from solvent extraction of vanilla (*Vanilla planifolia*) pods from Royal Project, Khun Wang Center, Chaing Mai, Thailand. Vanilla pods were cut and soaked in ethanol for 72 h at room temperature with continuous shaking at 150 rpm. The two studied factors for extraction condition were ethanol concentration (35, 45 and 55%) and ratio between vanilla pods and ethanol (1:4, 1:5 and 1:6).Both ethanol content and ratio of vanilla to ethanol had positive effects on vanillin content.The extraction with 55% ethanol and the ratio of vanilla pods to ethanol as 1:4 provided the highest vanillin content of 341.23 mg/100 mL of vanilla crude extract. Natural vanilla extract was then encapsulated in β-cyclodextrin cavity using kneading method. In this method, vanilla extract (3, 6 and 9%) was added into hydrated β-cyclodextrin paste and then kneaded for 5, 10 and 15 min. The obtained paste was then dried in cabinet dryer at 40°C for 18 h. The vanillin content of encapsulated natural vanilla extract was determined using HPLC. The amount of vanilla extract and kneading time gave significant (p<0.05)effect on the encapsulation efficiency. The greatest encapsulation efficiency found was 94.50% when 9% vanilla extract and 10 minutes of kneading time were used. Thestability of encapsulated natural vanilla powder was also investigated at various temperatures (35, 45 and 55°C) and water activities (0.53, 0.64 and 0.75). The interaction of temperature and water activity gave significant effect on the second order kinetic reaction of the encapsulated vanilla powder (p<0.05).Themost suitable condition of storage was 35°C with a_w of 0.64, providing the kinetic constant (k) of 0.0024and R^2 of 0.92 with $t_{half\text{-}life}$ of 4.54weeks.

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Introduction

Vanilla (*Vanilla planifolia)* is a tropical, flowering, climbing plant in a family of *Orchidaceae* (Menon and Nayeem, 2013). The part of use for vanilla is usually vanilla beans or vanilla pods which are mainly used for producing flavoring agents. Vanilla beans are harvested green with no flavor. However, the subsequent curing process after harvesting helps to develop vanilla flavor (Menon and Nayeem, 2013). Curing process is the process that allows flavor precursors to contact with enzymes to create flavor compounds (Havkin-Frenkel et al., 2004). Among hundreds flavor compounds, vanillin (4-hydroxy-3-methoxy benzaldehyde) is the main chemical constituent responsible for the vanilla flavor. Vanillin composes about 85% of total volatile compounds (Huesgen, 2011). In general, vanilla pod is graded based on the length, appearance (color, sheen, presence of any splits, presence of blemishes), and moisture content of the pods (Havkin-Frenkel et al., 2011). Whole, dark, plump and oily pods that are visually attractive, with no blemishes, and that have higher moisture content are graded most highly. Such pods are particularly prized by chefs for their appearance and can be featured in gourmet dishes. Beans that show localized signs of disease or other physical defects are cut to remove the blemishes; the shorter fragments left are called "cuts" and are assigned lower grades, as are pods with lower moisture contents. Lower-grade vanilla pods are used in the production of vanilla flavoring extract and in the fragrance industry. The flavor is extracted by using ethanol as a solvent via several methods. The vanilla extract is referred as a brown solution containing a defined level of extractable matter of vanilla beans in 35% alcohol (Hocking, 1997). Vanilla flavor is available in the markets in many forms including natural vanilla extract, vanilla flavoring, and vanilla-vanillin extract and flavoring (Havkin-Frenkel et al., 2011). Natural vanilla extract is considered as a high quality flavoring agent so its price is also high. As liquid form, the natural vanilla extract solution had raised problems on process handling and storage of the solution. Also vanillin content can be interfered by enzymatic oxidation (Anklam et al., 1996), thermal oxidation (Mourtzinos et al., 2009), and light-induced oxidation (Kumar et al., 2012). To

avoid these problems, conversion of natural vanilla extract to powder by using microencapsulation technique is recommended.

Microencapsulation is the technique used for entrapping a sensitive material within another material or matrix in order to increase its stability and prevent the undesirable interaction with the environment. The encapsulation methods are classified into 2 groups as chemical and physical methods based on the properties of the core materials as well as encapsulation methods. The encapsulants are also varied wildly as carbohydrate base, gums base, and protein base (Madene et al., 2006). Cyclodextrin is one of the most well-known encapsulants used in food manufacturing. It is a modified starch in a family of cyclic oligosaccharides. It forms a cage-like structure which can entrap a material inside. This encapsulation technique is so called the molecular inclusion technique which is one of the chemical encapsulation methods. There are three major cyclodextrins which are α-cyclodextrin, β-cyclodextrin, and γ-cyclodextrin. Among these three cyclodextrins, β-cyclodextrin is the most useful one with a low price and easily accessed (Martin Del Valle, 2003). β-cyclodextrin has an inner hydrophobic cavity which allows it to trap a variety of aromatic molecules inside. Meanwhile, an external surface has a hydrophilic property to allow it to be used in several food systems (Madene et al., 2006). Kayaci and Uyar (2012) had studied the encapsulation of vanillin/cyclodextrin inclusion complex in polyvinyl alcohol (PVA) nanowebs. The result showed that without cyclodextrin-inclusion complex, the vanillin could not be preserved throughout the process. Also there was a study on the encapsulation of cinnamon oil in β-cyclodextrin which showed that β-cyclodextrin could entrap more than half of the total flavor compounds presented in the original oil (Petrovic et al., 2010). Therefore, this research was aimed to optimize the microencapsulation condition of Thai natural vanilla extract by using β-cyclodextrin. Moreover, the stability of encapsulated power during the storage was also investigated.

Objectives of the study

1) To optimize extraction condition of vanilla pods using solvent extraction.
2) To optimize microencapsulation condition of Thai natural vanilla extract using β-cyclodextrin.
3) To investigate the storage stability of microencapsulated Thai natural vanilla powder.

Literature review

1. Vanilla

1.1 What is vanilla?

Flat plane-leaved vanilla is referred to a climbing orchid which scientific name is *Vanilla planifolia.* It is a flowering plant that is belonged to the family *Orchidaceae*. It is a thick, leafy, evergreen vine orchid. Its flower has a trumpet shape with cream to yellow color. However, not like other orchid, the commercial part of vanilla is a fruit or so called a pod (Menon and Nayeem, 2013). Vanilla is considered as the world's third most expensive spice (Parthasarathy et al., 2008). Vanilla is cultivated in tropical and subtropical climates with the preferred temperature of 21-32°C. It produces flower after 3 to 4 years of cultivation and after that flowers annually. The vanilla pod is allowed to develop for about 8 to 10 months before harvested. The mature pods are harvested green with no flavor (Figure 1). Then the pods are subjected to a curing process for 3 to 6 months in order to develop prized vanilla flavor (Havkin-Frenkel, 2004). Curing process involves four major stages including killing, sweating, drying, and conditioning. Curing process allows the contact between flavor precursors and enzymes to produce flavor compounds such as vanillin, vanillic acid, anisaldehyde, hydroxyl benzoic acid, and etc. (Menon and Nayeem, 2013). The cured vanilla pods are dried, shriveled, and black in color (Figure 2).

Figure 1: Fresh vanilla pods (Menon and Nayeem, 2013)

Figure 2: Cured vanilla pods (Johnson, 2015)

1.2 The history of vanilla

Vanilla is first cultivated by the Totonac people of ancient Maxico. Maxico was continued to be the main producer of vanilla until mid-19th century (Menon and Nayeem, 2013). After mid-19th century, the new technique of vanilla cultivation was introduced by French vanilla grower and the cultivation of vanilla has been spread out to other areas of the world (Parthasarathy et al., 2008). According to UN Food and Agriculture Organization (2005), the three largest vanilla grower areas are Madagascar, India, and China in which Madagascar is the largest grower producing approximately 3 tons of a total world production of 7.3 tons.

1.3 Vanilla extraction

According to US Food and Drug Administration (2002), vanilla extract is the solution in aqueous ethyl alcohol of extractable matters of vanilla beans. The ethyl alcohol content is not less than 35 percent by volume and the content of vanilla constituent is not less than one unit per gallon. However, the vanilla constituent may be extracted from vanilla beans by several extraction procedures. Generally, cured vanilla pod is soaked in ethanol for several hours to days to obtain natural vanilla extract. The extraction yield depends mainly on the pod quality and also pretreatment and extraction process. Sujalmi *et. al.* (2005) had studied on the effect of pre-treatment conditions and extraction procedure on the yield of vanillin content in vanilla extract. They had found that the pre-extraction treatment for cured vanilla sliced about 2.5 cm gave higher vanillin content comparing to the whole pods one as slicing gave more contact surface between the sample and the solvent. Also the vanillin content resulted from the Soxhlet extraction was higher than that from the percolation extraction. The reason was that there was continually solvent purity process in the soxhlet extraction. Another study, Waliszewski *et al.* (2007) had found that hydration and enzymatic pretreatment of vanilla pods could enrich the vanillin content in the extract. By which prehydration of vanilla pods with water or 5% ethanol can improve the subsequence hydrolyzing activity of three enzymes preparations (Crystalzyme PML-MX, Zymafilt L-300, and Novozym 342). Further, the enzymes will perform cellulolytic activity which enhances the amount vanillin obtained from extraction process. In addition to pre-treatments and extraction procedures, there was a study about the extraction aid with Ultrasound-assisted. The result from the study showed that the vanillin extraction process from cured vanilla pods at 30°C with 40% ethanol for 1 hour yielded about the same amount of vanillin as water bath extraction at 56°C for 15 hours (Rasoamandrary *et al.*, 2013). Apart from extraction techniques, solvent used for extraction is also considered. Galetto and Hoffman (1978) found that the use of pentane as solvent for vanilla extract resulted in high amount of ethyl ethers which contribute to the overall character of vanilla. On the other hand, commercial vanilla extract that did not use pentane as a solvent, ethers were found in much lesser amount.

1.4 Chemical constituent in vanilla extract

Vanilla extract is generally composes of sugar, non-sugar carbohydrates, amino acids and proteins, phenolic compounds, and minerals (Havkin-Frenkel and Belanger, 2011). Chemical analyses of vanilla extract reveal that there over hundred volatile compounds responsible for vanilla flavor. The five most important volatile compounds include vanillin, p-hydroxybenzaldehyde, vanillic acid, p-hydroxybenzyl methyl ether, and acetic acid. Among these compounds, aldehyde vanillin is the most abundant (Anklam *et al.,* 1996). Vanillin is constituted 85 percent of the total volatiles in vanilla extract (Menon and Nayeem, 2013).

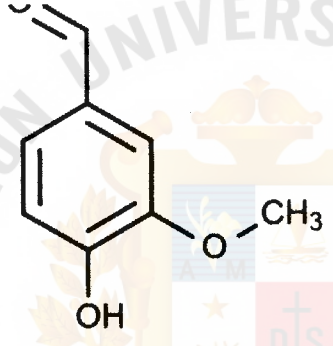

Figure 3: Chemical structure of vanillin (Helmenstine)

Vanillin or 4-hydroxy-3-methoxy benzaldehyde is classified as a phenolic aldehyde with the molecular formula as $C_8H_8O_3$. Its functional groups include aldehyde, ether, and phenol (Kumar *et al.*, 2012). In term of solubility, vanillin is very soluble in organic solvents such as acetone, hot benzene, petroleum ether, and alcohol (Towt, 1951). It is primarily responsible for the flavor strength and sweet odour of vanilla extract. Vanillin could be found naturally in cured vanilla beans (2-2.5%) under optimum conditions (Havkin-Frenkel and Belanger, 2011). Vanillin in vanilla is produced from a glycoside precursor compound called glucovanillin via an enzymatic catalysis. Vanilla pods accumulate glucovanilla in the placental tissue surrounding the seeds in the inner core of the pods during growth. The glucovanilla is converted to vanillin by β-glucosidase enzyme during curing process (Havkin-Frenkel *et al.,* 2004). Apart from extracting from vanilla pods, vanillin can also be synthesized via chemical processes. The

compounds that could be used in synthesis of vanillin include eugenol, isoeugenol, guaiacol, ferulic acid, and lignin (Towt, 1951). From recent study shows that less than 1% of vanillin produced each year comes from vanilla while the remaining is synthesized vanillin from other compounds (Parthasarathy *et al.*, 2008).

1.5 Vanillin stability

As vanillin is the most important compound in vanilla extract, the decomposition of vanillin is significant for the quality of vanilla extract. Vanillin contains both aldehyde and phenol functional groups, so it can undergo several types of reaction (Mourtzinos *et al.*, 2009). There is a review on vanillin suggested that vanillin is sensitive to sunlight as vanillin absorbs UV light at wavelengths of 308 and 278 nm and photo-transformation is possible (Kumar *et al.*, 2012). Moreover, Anklam *et al.* (1996) found that vanillin could be oxidized to vanillic acid in fresh and pasteurized milk. This was showed to be pH-dependent oxidation and thermolabile xanthine oxidase enzyme was showed to be partly responsible for the oxidation. In addition, the researchers also found that the oxidation from vanillin to vanillic acid was much faster in fresh milk sample spiked with lower concentration of vanillin. Apart from these, there was also a study on thermal oxidation of vanillin by the detection of vanillic acid using GC-MS. The result showed that vanillin could undergo thermal oxidation into vanillic acid; and the oxidation rate was governed by both temperature and heating time (Mourtzinos *et al.*, 2009).

1.6 Vanilla extract quality determination

Quality of vanilla extract could be determined by various factors (Merory, 1960) (Table 1). Among the factors, the amount of vanillin content in the extract is a major criterion. As vanillin is the most influenced compound for the vanilla flavor, high vanillin content indicates a high quality vanilla extract and also indicates the effectiveness of the extraction process.

Table 1: Quality parameters for vanilla extract.

Quality parameter	Minimum	Maximum	Average
Vanillin (g/100 ml)	0.11	0.35	0.19
Ash (g/100 ml)	0.220	0.432	0.319
Soluble ash (g/100ml)	0.179	0.357	0.265
Lead number (Winton)	0.40	0.74	0.54
Alkalinity of total ash (N/10 acid/ 100 ml)	30.00	54.00	30.00
Alkalinity of soluble ash (N/10 acid/ 100 ml)	22.00	40.00	42.00
Total acidity (N/10 acid/ 100 ml)	30.00	52.00	30.00
Acidity other than vanillin (N/10 acid/ 100 ml)	14.00	42.00	

Source: Merory (1960).

In order to measure the vanillin content, there are several techniques from simply measuring from absorbance to a more complicated technique as high performance liquid chromatography or HPLC technique. The selection of the isolation technique depends on how accurate the result is needed. For absorbance measuring technique, the absorbance of the vanilla extract solution is measured at 270, 348, and 380 nm under UV-visible spectrophotometer. The peak of absorbance of vanillin is measured at 348 nm while the absorbance's at 270 and 380 nm are used to correct the result for a more accurate value (Rasoamandrary *et al.,* 2013). However, the more complex technique as high performance liquid chromatography or HPLC is more reliable as the amount of vanillin is directly measured. Sujalmi *et al.* (2005) had compared the use of HPLC and ISO spectrophotometric assay in determination of the amount of vanillin content in vanilla. For HPLC, the vanillin was separated on a reverse phase C18 column using methanol-acidified water with the flow rate at approximately 4 mL/min and was detected at 280 nm. While the standard vanillin solutions for HPLC were prepared in 99.9% ethanol. The result revealed that the spectrophotometric assay consistently yielded higher values than the HPLC technique. The researchers had suggested that it was a result of impurity of the extracted solution. As other substances contained in the solution were also absorbed at the same wavelength as vanillin. Although HPLC technique also used the UV detector to

detect the vanillin, but the sample went through C18 column which separated the impurities out before detected by the UV detector.

1.7 Application of vanilla extract

1.7.1 Use in food

The use of vanilla in food has been recorded since pre-Columbian that it was used to flavor special drink prepared from water, cocoa beans, and spices (Parthasarathy *et al.*, 2008). Vanilla is usually used in sweet and creamy foods while its usage in salty foods is unusual. Recently, there is a research showed that vanilla is considered one of the most widely used flavors in foods including confectionary, beverages, ice cream, and bakery (Jadhav *et al.*, 2009). The popularity of vanilla flavor could be confirmed by the sample of beverages containing vanilla (Havkin-Frenkel and Belanger, 2011) (Table 2).

Table 2: Samples of beverages containing vanilla

Product	Brand	Mfg. Company
Vanilla Frappucino	Starbucks	Pepsi
Cream Liqueur	Starbucks	Pepsi
Super Protein Vanilla Al'mondo	Odwalla	Caca Cola
Vanilla Shake	Slim Fast	Unilever
Homemade Creamy Vanilla Shake	Ensure	Abbott
Nutripals Vanilla Drink	PediaSure	Abbott
Vanilla Drink Powder	Nesquik	Nestle
Vanilla Twist Vodka	Smirnoff	Diageo
Orange Dream Lcee	Lcee	J&J Snack Foods Corp.
Tazo Chai Tea Latte	Tazo	Starbucks
Classic Vanilla Soy Milk	Soy Dream	Hain Celestial Group
Vanilla Rice Drink	WestSoy	Hain Celestial Group
Orange & Cream Soda	Jones Soda Co.	Jones Soda Co.
Cream Soda	Mug Root beer	Pepsi
Vanilla Cream Root Beer	Barq's	Coca Cola
Diet Coke Black Cherry Vanilla	Caca Cola	Coca Cola
Cherry Vanilla	Dr Pepper	Cadbury Schweppes

Source: Havkin-Frenkel and Belanger (2011)

1.7.2 Use in fragrance and perfume

Vanilla has long been used as a composition in fragrance due to its beautiful, extremely long lasting, and diffusive vanilla flavor. Approximately 50 percent of 10,000 fragrances produced make use of vanilla in the formula. One of the most famous fragrances that use vanilla as an ingredient is a beautiful oriental blend called Shalimar. However, for the fragrances that do not use vanilla is due to the instability and color of vanilla (Havkin-Frenkel and Belanger, 2011).

1.7.3 Medicinal and pharmaceutical use of vanilla

Vanillin, a major aroma compound in vanilla extract, has been reported on the antimicrobial and antioxidant properties. It is active against both Gram-positive and Gram-negative bacteria and also effective against yeasts and moulds (Parthasarathy *et al.*, 2008). Traditionally, vanilla plant has been used to treat against several diseases including dysmenorrhea, fever, hysteria, tooth ache, and ulcers (Menon and Nayeem, 2013). However the use of vanilla to treat against diseases has faded away as more effective medicines have been discovered. Recently, the use of vanilla in pharmaceutical industries is usually as flavoring agent for medicine (Jadhav *et al.*, 2009).

2. Microencapsulation Technology

2.1 Definition

Encapsulation is the method of incorporate one material or a mixture of materials inside another material and form small capsules/particles. The coated material is so called core or active material while the coating material is called wall, shell material, or carrier agent. The core material can be a single compound or a mixture as well as the carrier that can be single or multilayers. The capsules or particles that are obtained from the encapsulation technique have to be able to protect the active material against external environment and also be able to release their contents at controlled rate. The size of microcapsule formed can vary from a few millimeters to <1 μm (Madene *et al.*, 2006).

Microencapsulation technology has developed since 1950s in the research into pressure-sensitive coating for the manufacture of carbonless copying paper (Green and Scheicher, 1955). Recently, this technology is found to have many applications in cosmetics, food, and drug industries as a flavor carrier and a shelter to protect active compounds against reactions, oxidation, evaporation, or migration (Rocha *et al.,* 2011).

In food industry, microencapsulation has been applied to many products such as vitamins, oil, flavor, and aroma compounds. Recently, microencapsulated aroma compounds have been increased interest as microencapsulation could limit the degradation or loss of the aroma compounds during processing and storage (Madene *et al.,* 2006).

2.2 Purpose of microencapsulation

As the knowledge about microencapsulation technology has increased, the use of this technology is also increased into wilder purposes. The primary purpose of microencapsulation is to protect the active ingredients from the external environment such as light, moisture, heat, or other extreme conditions. This is to enhance stability and prevent the loss of the active compounds. There is a research on the microencapsulation of flavors in carnauba wax by using vanilla as an example of flavors. The study showed that vanillin inside the wax started to evaporate at 200°C while matrix degradation started at 250°C. This result indicated the increase in stability of vanillin inside the matrix (Milanovic *et al.,* 2010). Microencapsulation in foods also utilized to mask undesired odors and tastes (Gibbs et al, 1999). As the compound is entrapped inside a wall material, lower perception of the compound could be obtained in food products. Choi *et al.* have studied the effect of microencapsulated chitooligosaccharide on physical and sensory properties of milk. They found that microencapsulation technology cold mask bitter flavor and color of chitooligosaccharide when added to milk. Another purpose of microencapsulation is to improve solubility of compounds. Rachmawati *et al.,* (2013) reported that curcumin-β-cyclodextrin nanoparticles inclusion complex prepared by various methods were resulted in improved curcumin solubility. Moreover,

microencapsulation could be applied to promote easier handling of a product by changing liquid product into powder form.

2.3 Encapsulation wall materials

There are numerous type of coating material that can be used for microencapsulation including proteins, carbohydrates, lipids, gums, and cellulose. Each type of material has its own characteristic which could be suitable for a certain core material. The coating material selection depends on several factors including the expected final product characteristics, nature of core material, the encapsulation technique implemented, economic purpose, and also the regulation in each country. Carbohydrate materials are commonly used with spray-dried encapsulation. The advantages of carbohydrate materials are that they have low viscosity at high solid content and good solubility that is preferred in many food applications. The materials in this group are widely used in the food industry to retain and protect the volatile compounds. The examples of the carbohydrate materials are starches, β-cyclodextrins, maltodextrins, corn syrup, and acacia gum. For protein materials, they are not wildly used in the flavor encapsulation due to its amphiphilic properties, self-associated property, and interactions with variety of compounds. The materials in this group include sodium caseinate, whey protein, and isolated soy protein (Madene *et al.*, 2006). Another type of wall material is gum. Normally, gums are tasteless; however, they have pronounced effect on the flavor of foods. For instant, hydrocolloids decrease sweetness of foods (Goodshall, 1997).

2.4 Microencapsulation techniques

Microencapsulation can be achieved via various techniques. The selection of the techniques depends on many factors including nature of the active compound and coating material, amount of compound to be encapsulated, expected product objectives and requirement, and availability of machines. The process of encapsulation consists of two steps which are emulsification of core material and drying of emulsion. The first one, emulsification of core material, is the process that blends a core material with a wall

material. While the second one, drying of emulsion, is the process in which emulsion of core and wall materials is dried to obtain microparticles (Madene *et al.*, 2006). Microencapsulation technique is generally classified into chemical technique and physical technique. The characteristics and applications of each encapsulation process are defined in Table 3.

Table 3: Characteristics and applications of different encapsulation methods

Type	Encapsulation method	Particle size (μ m)	Load (%)	Encapsulated form	Application area
Chemical techniques	Simple coacervation	20-200	<60	Paste/powder/capsule	Chewing gum, toothpaste, baked foods
	Complex coacervation	5-200	70-90		
	Molecular inclusion	5-50	5-10	Powder	Confectionary, instant drinks, extruded snack
Mechanical techniques	Spray-drying	<40	<40	Powder	Confectionary, milk powder, instant deserts, food flavors, instant beverages
	Spray-chilling	10-20	10-20	Powder	Prepared dishes, ices
	Extrusion	6-20	6-20	Powder/granule	Instant beverages, confectionary, teas
	Fluidised bed	60-90	60-90	Powder/granule	Prepared dishes, confectionary

Source: Madene *et al.* (2006)

2.4.1 Molecular inclusion

Molecular inclusion is the process that creates interaction between compounds in which a smaller guest molecule fits into and surrounds by the lattice of the other (Godshall, 1997). In this technique, cyclodextrins are used as they contain lipophilic central cavity and hydrophilic surface. During complex formation, water molecules inside the central cavity are replaced by lower polar molecules. Cyclodextrin can hold an active compound up to the temperature of 200°C. However, the release of the compound is allowed by moist and temperature condition in the mouth (Gibbs *et al.,* 1999). The complexing method of cyclodextrins and an active compound could be various. However, the most used methods are as followed;

1) Stirring or shaking a cyclodextrin with an active compound in aqueous solution and filtering out the precipitate complex
2) Blending solid cyclodextrin with an active compound by high speed mixer and bubbling the active compound, as vapours, through a solution of cyclodextrin.
3) Kneading an active compound with the cyclodextrin-water paste (Madene *et al.,* 2006).

3. Cyclodextrins

3.1 What is cyclodextrins?

Cyclodextrins, also called cycloamyloses or cyclomaltoses, are cyclic oligosaccharides consisting of at least six glucopyranose units liked by α-(1,4) glycosidic bonds. They are produced from intramolecular transglycosylation reaction of degraded starch by cyclodextrin glucanotransferase enzyme (Martin Del Valle, 2003). There are several types of cyclodextrins, however, the most common used cyclodextrins are α-cyclodextrin, β-cyclodextrin, and γ-cyclodextrin. The difference of theses cyclodextrins is that they consist of different numbers of glucopyranose units making different size of molecules. α-, β-, and γ-cyclodextrins consist of six, seven, and eight glucopyranose units, respectively (Dardeer, 2014). The most interesting property of cyclodextrins is the

ability to form an inclusion complex that gives out many applications in pharmaceutical, food, and cosmetic industries (Martin Del Valle, 2003).

3.2 Structure of cyclodextrins

Cyclodextrins are cyclic oligosaccharide compose of α-(1-4) linking glucopyranose units. They possess a cage-like structure with consist of lipophilic inner cavity and hydrophilic external surface (Martin Del Valle, 2003). The size of the cavity depends on the number of glucose units. The more the glucose units, the larger the cavity size. As cyclodextrins form a truncated cone structure instead of perfect cylinder, they have two hydroxyl groups. The primary hydroxyl group (C_6) is placed in the narrow edge of the cone while the secondary hydroxyl groups (C_2 and C_3) are located in the wider edge of the cone. The central cavity of the cone is created by the carbon chain and peptide linkage of the glucose residuals which causes a lipophilic property (Dardeer, 2014).

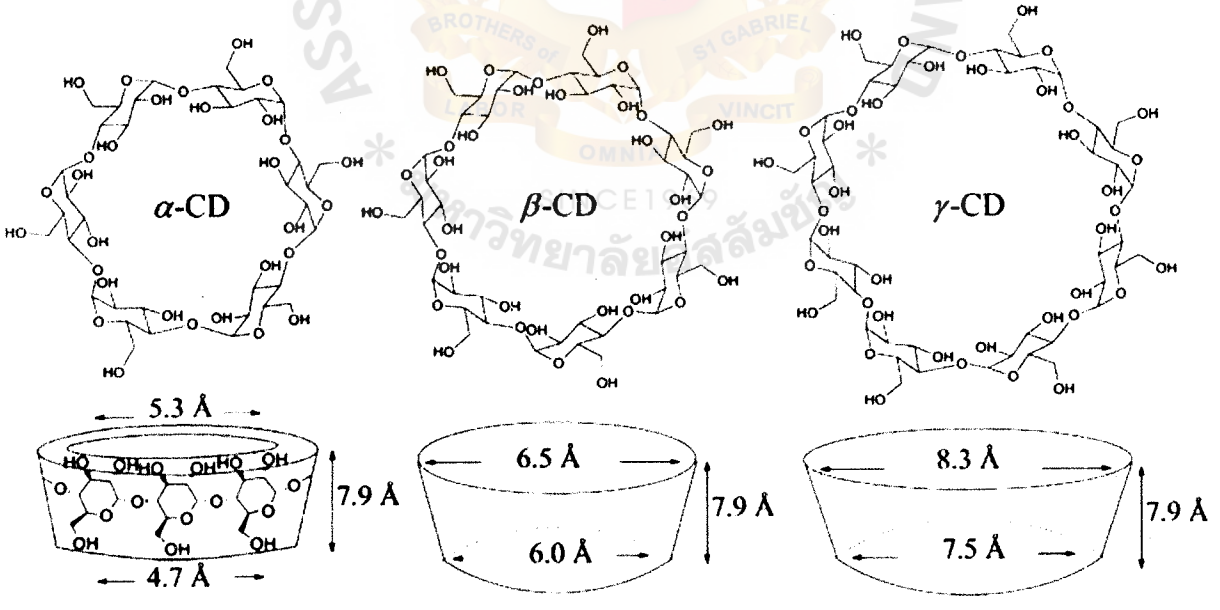

Figure 4: Chemical structures of different types of cyclodextrins (Freitag and Galoppini, 2010)

3.3 Types of cyclodextrins

Cyclodextrins are cyclic carbohydrates containing different numbers of glucopyranose units. However, there is no existing of cyclodextrins that consist of less than six glucopyranose units. There are many types of cyclodextrins have been reported including δ, η, θ, €, and £-cyclodextrins. Among theses, α, β, and γ-cyclodextrins which consist of six, seven, and eight glucopyranose units, respectively, are the most common types. Some important properties of the three cyclodextrins are shown in Table 2.4. Apart from different in cavity sizes, differ in glucopyranose units also affects the solubility of the particles. Among the three cyclodextrins, γ-cyclodextrin is the most soluble one according to the fact that its glucopyranose units are not in the same plane and its structure is more elastic. While 4 of 6 hydrogens of α-cyclodextrin could form hydrogen bond making the solubility of α-cyclodextrin higher than β-cyclodextrin (Dardeer, 2014).

Table 4: Some important properties of cyclodextrins

Characteristic	**α - cyclodextrin**	**β - cyclodextrin**	**γ -cyclodextrin**
No. of glucose units	6	7	8
Molecular weight (Da)	972	1135	1297
No. of water molecules in cavity	6	11	17
Water solubility at 25°C (%w/v)	14.5	1.85	23.2
Half-life in 1 M HCl at 60°C (h)	6.2	5.4	3.0
Melting onset (°C)	≈275	≈280	≈275
Diameter of central cavity (nm)	0.5-0.6	0.6-0.8	0.8-1.0
Diameter of outer periphery (nm)	1.4-1.5	1.5-1.6	1.7-1.8
Height of the torus (nm)	0.8	0.8	0.8

Source: Marques, 2010

3.4 Cyclodextrins in inclusion complex

The most notable property of cyclodextrins is their ability to form an inclusion complex with a very wide range of compounds (solid, liquid, and gaseous). Cyclodextrins complexes are formed through molecular inclusion method. In this complex, a guest molecule is held inside the cavity of cyclodextrins molecule (Martin Del Valle, 2003). The formation is due to the liking of hydrophobic molecules in core material within the hydrophobic cavity of the cyclodextrins. The main driving force for the molecular inclusion is the involve of enthalpy-rich water molecules from the cavity and the cavity is replaced by the hydrophilic core material molecules. However, the linking of the core material and the cavity is not permanent; no covalent bond is broken or formed during the inclusion. The linking strength is depending on the local connection between surface atoms and the way the material fit to the cavity (Dardeer, 2014).

3.5 Application of cyclodextrins

3.5.1 Application in foods and flavors

Cyclodextrins are used in foods formulations for many purposes. They could form inclusion complexes with variety of molecules including fats, flavors, and colors (Martin Del Valle, 2003). There are many reviews describing the use of cyclodextrins in food and flavor applications. Cyclodextrins have been recommended for application in food processing and also use as food additive for various purposes. The aims of using cyclodextrins in food and flavor applications could be as the following (Astray *et al.*, 2009):

- Encapsulation of flavor compounds to prevent aroma degradation and loss during process
- Protect food components against oxidative degradation, heat induced change, and light induced degradation

- Taste modification and elimination of unwanted flavors.
- Remove cholesterol from animal products
- Food preservation

3.5.2 Application in pharmaceutical

In pharmaceutical formulations, cyclodextrins are normally used as solubilizer to improve solubility of drugs. However, sometimes, they are also used as stabilizer or to reduce local drug irritation (Loftsson and Dominoque, 2007). One of the unique properties of cyclodextrins is their ability to enhance drug delivery through biological membranes. It is generally recognized that cyclodextrins act as a true carrier by keeping lipophilic drug molecules inside and delivering them at the surface of biological membranes (Martin Del Valle, 2003).

3.5.3 Application in cosmetics

The ingredients to be encapsulated in cosmetic product formulation could be colorant, fragrance, or active ingredients. There are numerous reports on the use of cyclodextrins in cosmetic products. Some purposes of use cyclodextrins in cosmetic produces are as the following (Buschmann and Schollmeyer, 2002):

- Protection of the active molecules
- Improve solubility of the active molecules
- Elimination of unwanted odors
- Improvement of handling

Materials and Methods

1. Solvent extraction of vanilla

1.1 Vanilla pod preparation

Cured vanilla pods of *V. planifolia*, Royal Project, Khun Wang Center, ChaingMai, were cut into small pieces. The cut pods were then stored in dried condition at room temperature for further extraction.

1.2 Solvent extraction

A factorial experimental and randomized block designs with 3 replications used to study the effects of two factors on vanillin content of vanilla extract. The two investigated factors (independent variables) were vanilla-ethanol ratio (1:4, 1:5, and 1:6) and ethanol concentrations (35, 45, and 55%). The cut vanilla pods were subjected to a total of nine treatments with the combination of the two factors as in Table 5.

Table 5: Treatment combination of extraction condition of vanilla pods

Treatment	Ethanol concentration (%v/v)	Vanilla:ethanol ratio
1	35	1:4
2	35	1:5
3	35	1:6
4	45	1:4
5	45	1:5
6	45	1:6
7	55	1:4
8	55	1:5
9	55	1:6

The cut vanilla pods were weight according to the vanilla:ethanol and then put into 125 ml Erlenmeyer flasks. Ethanol/water with different concentrations was added according to the ratios. Vanilla pods of all treatments were soaked in ethanol/water for 72 hours at room temperature with continuous shaking at 150 rpm. The extracts were filtered through No.1 filter. The collected supernatants were then kept in dark containers at 4°C μm (adapted from Rasoamandrary *et al.*, 2013).

2. Microencapsulation of vanilla extract in β-cyclodextrin by using kneading method

The optimum encapsulation conditions were determined using A factorial experimental and randomized block designs with 3 replications. Vanilla extract was subjected to a total of nine treatments with the combination of amount of vanilla extract (3, 6 and 9%) used and kneading time (5, 10 and 15 minutes) as shown in Table 6.

Table 6: Treatment combination for microencapsulation of natural vanilla extract

Treatment	Amount of vanilla extract (%)	Kneading time (min)
1	3	5
2	3	10
3	3	15
4	6	5
5	6	10
6	6	15
7	9	5
8	9	10
9	9	15

Forty grams of β-cyclodextrin powder were hydrated with 30 mL of water. Then, the mixture was kneaded by a hand mixer at high speed to let water occupy β-cyclodextrin cavity. After that, appropriate amount of vanilla extract was added. The mixture was kneaded continuously for different length of times. After kneading, the mixture was dried in a tray dryer at 40°C for 18 hours. The dried powder was blended and sieved. The microencapsulated vanilla powder was kept in a dark and dried container.

3. Analysis of vanillin content in vanilla extract

3.1 Sample preparation

All vanilla samples were diluted at 1:1 ratio with deionized water. Then, the samples were filtered through 0.22 μm syringe filter and centrifuged at 10000 rpm for 20 minutes. The collected supernatants were kept in dark container at 4°C.

3.2 Standard preparation

Vanillin standards (Sigma, Singapore) were prepared at concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml by using methanol as a solvent. All standard solutions were filtered through 0.22 μm syringe filter and kept at 4°C prior to analysis.

3.3 HPLC condition

The vanillin content was analyzed using high performance liquid chromatography (HPLC) method. The column used was C18 column (250 x 4.6 mm, 5μm, Waters) with a multiphasic gradient at a flow rate of 1 mL/min and injection volume of 10 μL. Solvent A was 20 mM sodium acetate, pH6 and solvent B was methanol. The proportions of solvents were as followed;

0 min	Solvent A 100%	Solvent B 0%
7 min	Solvent A 50%	Solvent B 50%

10 min	Solvent A 55%	Solvent B 45%
20 min	Solvent A 100%	Solvent B 0%

The result was detected by UV visible spectrophotometer at absorbance of 260 nm (adapted from Yoon *et al.*, 2005).

4. Encapsulation efficiency (EE)

The value of vanillin content was used to determine the encapsulation efficiency (EE) using the following equation;

$$EE(\%) = \frac{Vanillin\ content\ in\ powder \times weight\ of\ powder}{Vanillin\ content\ in\ vanilla\ extract \times volume\ of\ vanilla\ extract\ used} \times 100$$

5. Yield of vanilla powder

The production yield of vanilla powder was calculated according to the following equation;

$$Yield\ (\%) = \frac{Weight\ of\ powder}{Total\ weight\ of\ all\ ingredients} \times 100$$

6. Moisture content analysis

One gram of vanilla powder was placed in a dried moisture can and dried at 105°C for 8 hours. The samples were then cooled down in a desicator and weighed (AOAC, 1990). The moisture content is expressed as a percentage of the initial sample using the following equation;

$$MC(\%) = \frac{initial\ weight\ -\ final\ weight}{initial\ weight} \times 100$$

7. Stability of vanilla powder

Stability of vanilla powder was determined by A factorial experimental and randomized block designs with 2 replications based on two investigated factors, temperature and water activity. Vanilla powder was subjected to a total of nine treatments as in Table 7.

Table 7: Treatment combination of shelf life study of encapsulated vanilla powder

Treatment	Temperature (°C)	a_w
1	37	0.53
2	37	0.64
3	37	0.75
4	45	0.53
5	45	0.64
6	45	0.75
7	55	0.53
8	55	0.64
9	55	0.75

Vanilla powders were kept at controlled conditions for 5 weeks. The samples were taken every week for moisture content analysis and vanillin content analysis. Then the half-life of the powder was determined.

Results and Discussion

1. Solvent extraction of vanilla

The vanillin content in vanilla extracts made by solvent extraction varied with different alcohol content and vanilla:ethanol ratio. It was recognized that alcohol content and ratio of vanilla:ethanol had significant ($p<0.05$) positive effect on the vanillin content. Therefore, increasing in alcohol content and the amount of vanilla pods resulted in higher vanillin content. While the interaction effect of alcohol and the ratio has no significant ($p\geq0.05$) effect on vanillin content. From the result in Table 8, the highest vanillin contents were found in treatment 7 (341.23 mg/100ml) and treatment 4 (322.81 mg/100ml). These two treatment were made up from the ratio of 1:4 as vanilla:ethanol and alcohol content of 55% and 45% for treatments 7 and 4, respectively. Meanwhile, the lowest vanillin contents were found in treatment 3 (221.18 mg/100ml), treatment 6 (220.92 mg/100ml), and treatment 2 (201.84 mg/100ml).

The positive effect of alcohol concentration on vanillin content might be because vanillin was more soluble in ethanol than water. So the higher amount of ethanol in the solution, the higher amount of vanillin could be extracted. Moreover, the positive effect of the vanilla:ethanol ratio might come from the higher amount of extractable matters when the ratio of vanilla pods was increased.

Table 8: Vanillin content in natural vanilla extract from different conditions

Alcohol concentration (%)	Vanilla:Ethanol ratio	Vanillin content (mg/100 ml)
35	1:4	301.10±3.04[b*]
	1:5	201.84±5.60[d]
	1:6	221.18±1.18[d]
45	1:4	322.81±25.96[ab]
	1:5	247.50±15.94[c]
	1:6	220.92±7.57[d]
55	1:4	341.23±13.26[a]
	1:5	268.91±5.11[c]
	1:6	253.95±21.61[c]

* The same letter indicates no significant difference at 95% confidential level.

2. Microencapsulation of vanilla extract in β-cyclodextrin using kneading method

2.1 Production yield

The production yield of encapsulated vanilla powder prepared by kneading method varied with different amount of vanilla extract used and kneading time. From Table 9, the highest production yield was obtained from treatment 3 (88.3%) in which 3% of vanilla extract was used with 15 minutes of kneading time. It was recognized that amount of vanilla extract had significant ($p<0.05$) negative effect on the production yield. So as amount of vanilla extract increased, the lower production yield could be obtained. On the other hand, kneading time and interaction effect between amount of vanilla extract and kneading time showed no significant effect ($p\geq0.05$) on the production yield.

The negative effect of the amount of vanilla extract on the production yield might be the result of the loss of volatile compounds such as ethanol during drying. An increasing in the amount of vanilla extract also increased the amount of volatile

compounds, mainly ethanol. So, more weight loss was found in the treatments with higher percentage of vanilla extract in the formulation.

2.2 Encapsulation efficiency

The encapsulation efficiency of vanilla extract in β-cyclodextrin complex was determined based on the amount of retained vanillin in the powder. From Table 9, it could be seen that the highest encapsulation efficiency was found in treatment 8 (94.5%) and treatment 5 (80.97%). The two treatments were made from 9% and 6% of vanilla extract with 10 minutes of kneading time. Moreover, ANOVA test showed that the two factors, amount of vanilla extract and kneading time, had significant effect ($p<0.05$) on the encapsulation efficiency, while an interaction effect between the two factors had no significant effect ($p\geq0.05$) on the encapsulation efficiency.

For amount of vanilla extract, an increasing in the amount of vanilla extract tended to increase the encapsulation efficiency. Lower amount of vanilla extract, the contents might be degraded during preparation of the powder. Also, 6% and 9% of vanilla extract might fit better to the size of the cavity of β-cyclodextrin. This was supported by a study of Madene *et al.* (2006) which concluded that the most suitable load for the inclusion complex with cyclodextrins was 5-10%.

For kneading time, the highest encapsulation efficiency was found at 10 minutes of kneading time. This might be occur because the lower kneading time (5 minutes) might not be sufficient to force vanilla extract into the complex so vanillin outside the complex was degraded during the preparation process. On the other hand, prolonged kneading time (15 minutes) may sharply decrease the retention of vanilla extract as to approach the equilibrium. The result might be supported by the study of Furuta et al. (1994) who studied on the encapsulation of limonene in β-cyclodextrin/maltodextrin complex by kneading method. The limonene retention profile showed that after reaching the maximum retention (4 minutes), the limonene retention was sharply decreased to reach the equilibrium. So the same phenomena may occur for the encapsulation vanilla in β-cyclodextrin complex.

Table 9: Some physical and chemical properties of encapsulated vanilla powder made from different conditions

Amount of vanilla extract (%)	Kneading time (min)	Yield (%)	Moisture content (%)	Vanillin EE (%)
3	5	79.23±9.38^{abc*}	6.61±1.04^{a}	7.96±7.42^{d}
	10	83.21±6.67^{ab}	6.93±1.00^{a}	22.72±5.64^{d}
	15	88.30±2.81^{a}	6.09±0.33^{a}	3.91±4.40^{e}
6	5	68.77±2.06^{c}	7.05±1.56^{a}	66.78±11.75^{bc}
	10	81.96±2.32^{ab}	6.58±1.13^{a}	80.97±9.88^{ab}
	15	75.87±10.83^{bc}	6.87±1.46^{a}	64.60±14.70^{c}
9	5	73.88±6.99^{bc}	7.49±1.96^{a}	75.09±6.63^{bc}
	10	74.05±2.29^{bc}	6.44±0.23^{a}	94.50±5.95^{a}
	15	78.64±3.73^{abc}	6.75±0.27^{a}	66.52±14.64^{bc}

* The same letter indicates no significant difference at 95% confidential level.

2.3 Stability of vanilla powder

Storage quality of encapsulated vanilla powder in term of moisture content and vanillin retention was varied according to different temperatures and water activities. For moisture content of the powder (Figure 5), all treatments showed the same trend in which moisture contents were gradually raised during the first two weeks. Then, moisture contents were sharply increased during the third to the fifth weeks. Temperature gave significant different on the moisture absorption of the powder (p<0.05). As showed in Figure 5, at 55°C, for all a_w, moisture contents of the powder were increased at slower rate than those of 35°C and 45°C. Also the final moisture contents at the fifth week of the powder at 55°C for a_w 0.53 (13.45%) and 0.64 (13.45%) were significantly lower than other conditions (p<0.05) (Table 10). The reason that 55 °C gave lower rate of moisture absorption might be that this temperature was high enough to evaporate water out from

the powder. So, lower moisture content than at other temperatures was resulted for prolonged storage. Considering on a_w, it gave no significant different on the moisture absorption of the powder ($p>0.05$) as can be seen in Figure 5. Treatments with different a_w gave almost the same rate of moisture increasing. Moreover, different a_w gave no significant different in final moisture content ($p>0.05$) (Table 10).

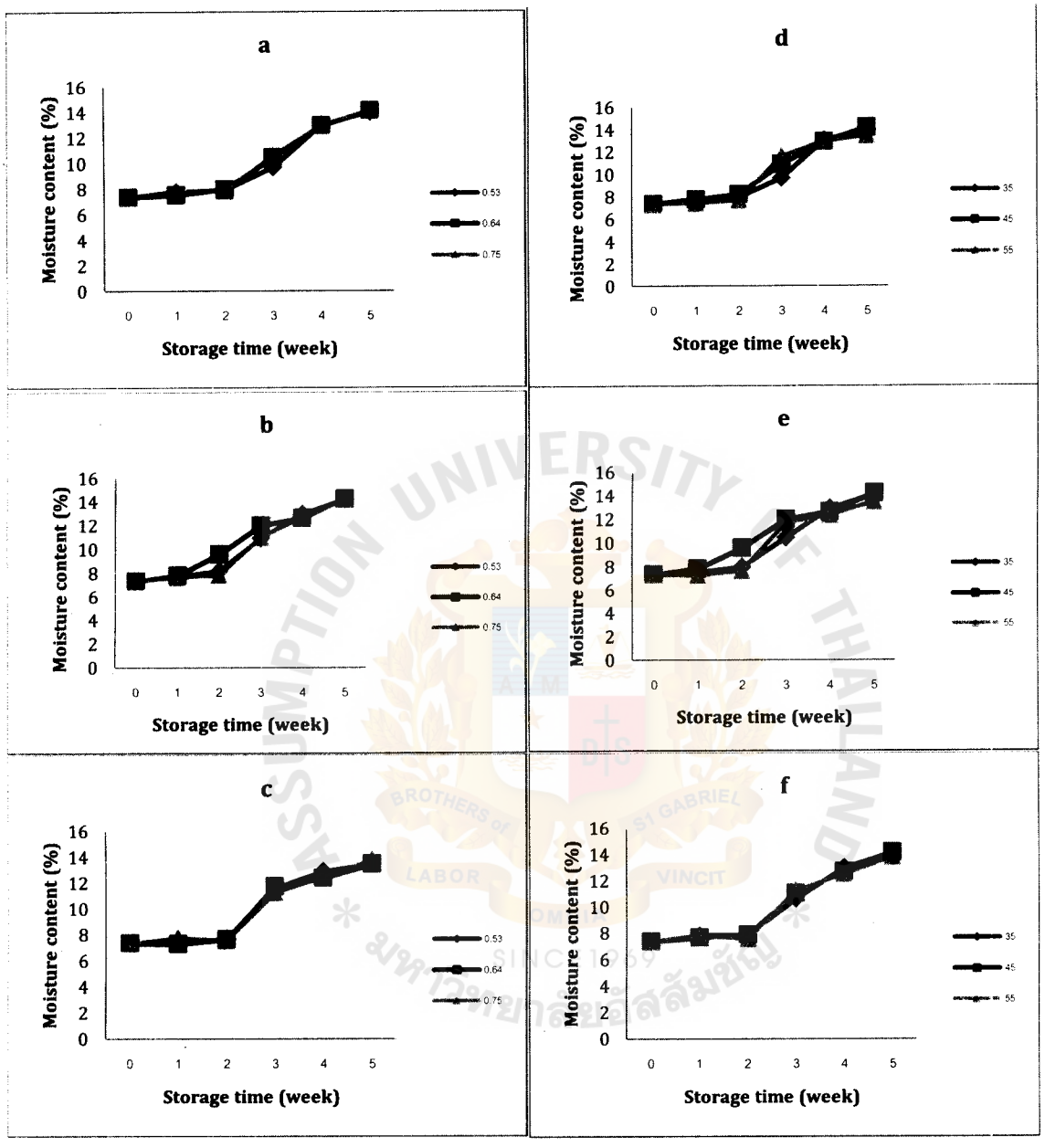

Figure 5: Moisture content of encapsulated natural vanilla extract during the storage of 5 weeks at different temperatures and a_w (a=35°C, b=45°C, c=55°C, d=0.53, e=0.64, and f=0.75)

For vanillin retention, at varied temperatures for all a_w (Figure 6), the same trend was observed. Vanillin retentions were gradually decreased during the first week. Except for at 55°C, for all a_w, that vanillin retention was sharply reduced in the first week. Then in the second week, vanillin retentions were dropped dramatically for all temperatures. After that the rates of reduction were reduced to almost constant. The final vanillin contents for every temperature seemed to be almost the same except for 55°C/a_w 0.75 that seemed to be lower than the others. At varied a_w, every a_w exhibited the same trend as at 35°C and 45°C, vanillin retention sharply reduced in the second week and then the rates were slowed down. While at 55°C for in every a_w the rate of reduction seemed to be faster from the first week. These results indicated that vanillin degradation occurred mostly in the second week of storage at all conditions, except at 55°C that the rate seemed to be faster at the first week. These might occur from the fact that vanillin was normally degraded to vanillic acid by thermal oxidation at high temperature (Mourtzinos *et al.*, 2009). So lower temperatures (35°C and 45°C) required more time to decompose vanillin. While higher temperature as 55°C, the decomposition occurred faster. However, the decomposition rates were slowed down in the third week to the fifth week because vanillin concentration at those weeks was already low made the reaction occur harder.

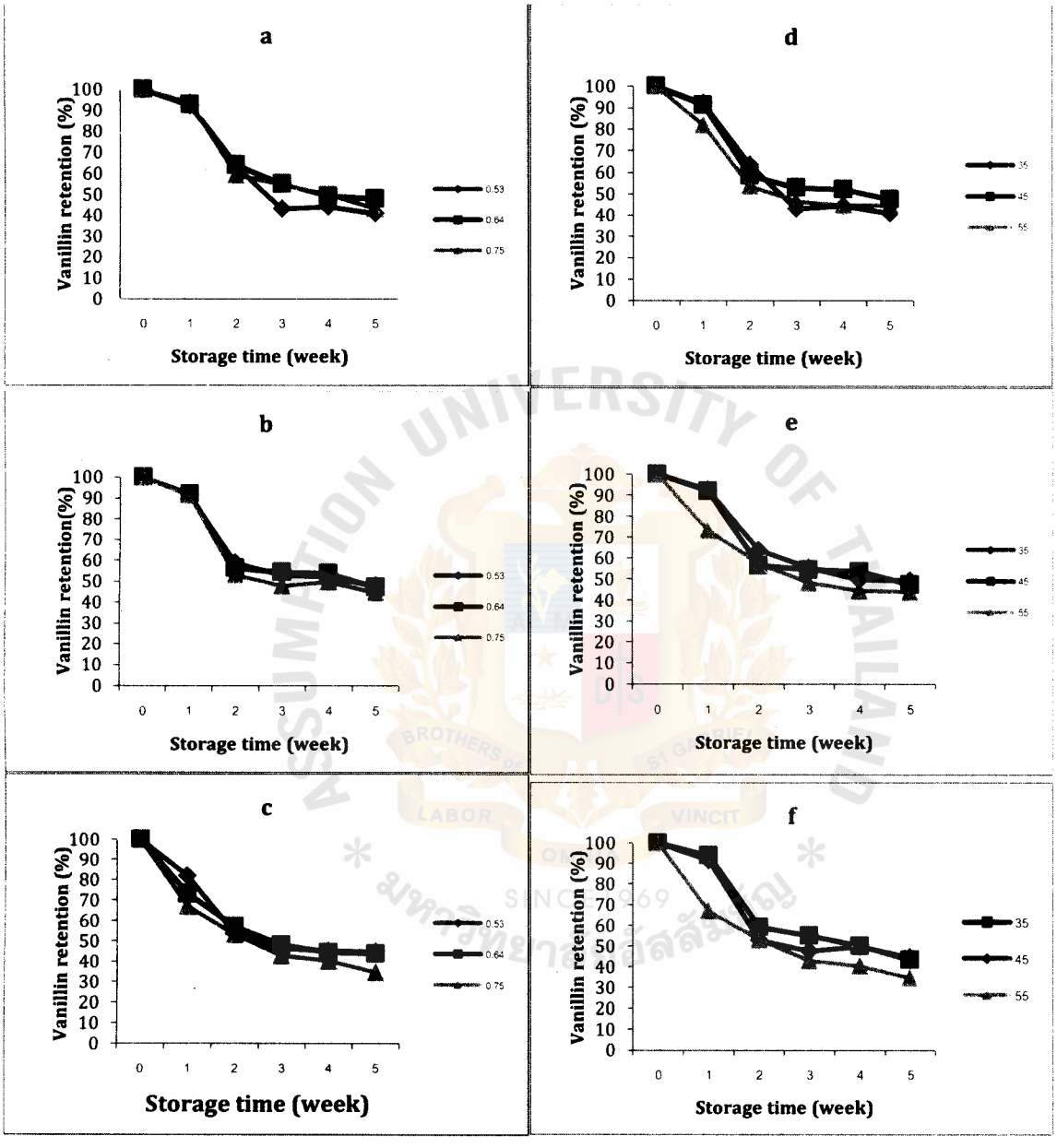

Figure 6: Vanillin content of encapsulated natural vanilla extract during the storage of 5 weeks at different temperatures and a_w (a=35°C, b=45°C, c=55°C, d=0.53, e=0.64, and f=0.75)

Table 10: Final vanillin retention and moisture content of the encapsulate vanilla powder stored at different temperatures and a_w

Temperature (°C)	a_w	Vanillin retention (%)	Moisture content (%)
35	0.53	40.80±5.90 [ab*]	13.96±0.05 [a]
	0.64	48.10±5.50 [a]	14.07±0.05 [a]
	0.75	43.34±1.49 [ab]	14.11±0.04 [a]
45	0.53	47.63±4.82 [a]	14.14±0.00 [a]
	0.64	47.32±6.04 [a]	14.19±0.15 [a]
	0.75	44.45±2.38 [a]	14.14±0.04 [a]
55	0.53	44.62±0.14 [a]	13.45±0.00 [b]
	0.64	43.91±0.89 [ab]	13.45±0.44 [b]
	0.75	34.45±4.00 [b]	13.79±0.32 [ab]

* The same letter indicates no significant difference at 95% confidential level.

To determine the stability of encapsulated vanilla powder, half-life of the powder was determined. The half-life of the powder related to vanillin content was calculated based on second order of reaction as Fargues *et al.* (1996). They had reported that the rate of oxidation reaction of vanillin at pH<12 was second order reaction. The half-life of encapsulated vanilla powder was varied according to different temperatures and a_w. From statistical analysis, both temperature and a_w alone had no significant effect on the half-life of the powder ($p \geq 0.05$), however, the interaction between temperature and a_w had significant effect ($p<0.05$). The reason that the half-life of the powder depended on the interaction of the two factors might be that normally vanillin was decomposed by thermal oxidation at temperature above 85°C (Mourtzunos *et al.*, 2009). However, with the effect of high a_w, the degradation temperature may be reduced and vanillin was degraded at much lower temperature. From the result in Table 11, the highest half-life (4.54 weeks) was found at temperature of 35°C and a_w 0.64; while the lowest half-life (2.71 weeks) was found at temperature of 55°C and a_w 0.75. The conditions of 35°C and a_w 0.64 gave the highest half-life as the effect of low temperature, so thermal oxidation occurred lower. Moreover, from a study of Beristian *et al.* (2002) who found that the encapsulated

orange peel oil powder stored at a_w 0.628 showed the greatest stability against oxidation after 30 days of storage. They concluded that at this a_w the system was under rubbery stage making the oxidation reaction occur harder. So, with the encapsulated vanilla powder, the same phenomenal might be occurred at a_w 0.64. For the conditions of 55°C with a_w 0.75 which gave the lowest half-life, might be the result of high temperature that caused more thermal oxidation and high water activity, leading to formation of paste-like mass that destroyed the microcapsules.

Table 11: Half-life of encapsulated vanilla powder stored at varied temperatures and a_w

Temperature (°C)	a_w	2nd order reaction		
		R^2	K	Half life
35	0.53	0.96±0.02	0.0035±0.0001	2.85±0.15^{c*}
	0.64	0.92±0.01	0.0024±0.0006	4.54±0.65^{a}
	0.75	0.93±0.03	0.0028±0.0001	3.68±0.11^{abc}
45	0.53	0.89±0.01	0.0024±0.0002	4.30±0.49^{abc}
	0.64	0.92±0.00	0.0027±0.0001	3.13±0.06^{c}
	0.75	0.87±0.01	0.0027±0.0002	3.73±0.25^{abc}
55	0.53	0.87±0.04	0.0027±0.0001	3.69±0.14^{abc}
	0.64	0.93±0.04	0.0027±0.0001	3.78±0.20^{bc}
	0.75	0.98±0.01	0.0038±0.0005	2.71±0.36^{c}

* The same letter indicates no significant difference at 95% confidential level.

Conclusion and Recommendation

Ethanol concentration and amount of vanilla pods had positive influence on the amount of vanillin in vanilla extract. The highest vanillin was 341.23 mg/100 mL of vanilla extract when the extraction was performed by using 55% ethanol and the ratio of vanilla to ethanol as 1:4. .

The encapsulation of vanilla extract in β-cyclodextrin was done by using kneading method. The amount of vanilla extract and kneading time had significant effect on the encapsulation efficiency. The optimized condition was 9% of vanilla extract and 10 minutes kneading time, resulting in 94.50% encapsulation efficiency.

The stability of encapsulated vanilla powder was determined under accelerated conditions. Moisture absorption of the powder was affected by only temperature. The lowest moisture content after 5 weeks of storage was found at 55°C for both a_w 0.53 and 0.64 which was 13.45%. However, there was no significant effect from both temperature and a_w alone on half-life of the powder. While, there was interaction effect between temperature and a_w, giving significant influence on the half-life of the powder. The highest half-life of the powder was about 1 month under the condition of 35°C and a_w of 0.64, with 40.10% of vanillin retained in the powder after 5 weeks of storage.

Further research on the microencapsulation of natural vanilla extract was recommended to investigate the control release rate of the encapsulated vanilla powder. Also this encapsulated vanilla powder was promising on their use in food and cosmetic products which could be a goal of further study.

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Appendix

Table 12: Analysis of variance (ANOVA) for vanillin content in natural vanilla extract

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Ethanol	2	0.86123648	0.43061824	22.85	<.0001*
Ratio	2	4.33299893	2.16649947	114.98	<.0001*
Ethanol*Ratio	4	0.11476520	0.02869130	1.52	0.2377

R square = 0.94 (* indicates statistically significant at 95% confident level)

Table 13: Analysis of variance (ANOVA) for encapsulation efficiency of natural vanilla extract encapsulation

Source	DF	Anova SS	Mean Square	F Value	Pr > F
%vanilla	2	24256.34023	12128.17011	128.16	<.0001*
Kneading time	2	2182.41055	1091.20527	11.53	0.0006*
%vanilla*kneading time	4	112.66488	28.16622	0.30	0.8757

R square = 0.94 (* indicates statistically significant at 95% confident level)

Table 14: Analysis of variance (ANOVA) for moisture content of encapsulated vanilla powder

Source	DF	Anova SS	Mean Square	F Value	Pr > F
%vanilla	2	0.64021599	0.32010799	0.24	0.7890
Kneading time	2	1.18271427	0.59135714	0.44	0.6486
%vanilla*kneading time	4	1.96539769	0.49134942	0.37	0.8279

R square = 0.13 (* indicates statistically significant at 95% confident level)

Table 15: Analysis of variance (ANOVA) for production yield of encapsulate vanilla powder

Source	DF	Anova SS	Mean Square	F Value	Pr > F
%vanilla	2	389.0753578	194.5376789	5.23	0.0162*
Kneading time	2	250.5017355	125.2508677	3.37	0.0573
%vanilla*kneading time	4	178.4693098	44.6173274	1.20	0.3454

R square = 0.55 (* indicates statistically significant at 95% confident level)

Table 16: Analysis of variance (ANOVA) for final moisture content of encapsulated vanilla powder after five weeks of storage

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Temperature	2	1.19351541	0.59675770	16.56	0.0010*
Aw	2	0.08630473	0.04315236	1.20	0.3459
Temperature*Aw	4	0.09768715	0.02442179	0.68	0.6244

R square = 0.81 (* indicates statistically significant at 95% confident level)

Table 17: Analysis of variance (ANOVA) for final vanilla retention of encapsulated vanilla powder after five weeks of storage

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Temperature	2	2.61825757	1.30912878	11.47	0.0034*
Aw	2	0.34153817	0.17076909	1.50	0.2749
Temperature*Aw	4	0.99695286	0.24923821	2.18	0.1521

R square = 0.79 (* indicates statistically significant at 95% confident level)

Table 18: Analysis of variance (ANOVA) for half life of encapsulated vanilla powder

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Temperature	2	0.39745323	0.19872662	1.88	0.2072
Aw	2	0.58081052	0.29040526	2.75	0.1167
Temperature*Aw	4	5.04418463	1.26104616	11.96	0.0012*

R square = 0.86 (* indicates statistically significant at 95% confident level)

Figure 7: Natural vanilla extract/β-cyclodextrin powder prepared by kneading method (treatment 1-9)