Anthocyanin retention improvement of microencapsulated butterfly pea flower crude extract by using freeze drying and β-cyclodextrin

BY

Ms. Natnicha Veerathummanoon

ID. 5435109

A Special Project Submitted in Partial Fulfillment of the Requirement for the Degree of Bachelor of Science in Biotechnology

Assumption University

Academic Year 2015

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Natnicha Veerathummanoon

ABSTRACT

The study aimed to optimize the microencapsulation of butterfly pea extract by using freeze drying and β- cyclodextrin. The stability of the powder was also investigated. The butterfly pea flower was first dried at 45°C for 24 h. The dried petals were extracted by using distilled water and concentrated until reached the total soluble solid was 16 ± 0.5°Brix. The extract was then mixed with supporting materials using the ratio of the extract to supporting material as 1:1, 1:2, and 1:3. The supporting material used was the mixture of maltodextrin DE 20 and β-cyclodextrin (BCD), with the concentration of BCD as 25, 50, and 75%. Then the mixture was subjected to freeze drying under a temperature of -35°C for 24 hours. Finally, the encapsulated powder was kept in a covered airtight container to avoid the light. Chemical and physical properties of encapsulated powders, such as moisture content, total anthocyanin contents as well as microencapsulation yield (MY) and encapsulation efficiency (EE) were analyzed. The ratio of the extract to the supporting materials (75% maltodextrin and 25% β-cyclodextrin) as 1:1 and freeze drying method could retain the highest anthocyanin content as high as 88.4% with good color profile (L* = 38.62, a* = 2.67, and b* = - 21.07). An increasing of the supporting material proportion and β-cyclodextrin tended to cause the color fading and reduced the anthocyanin content in the powder. Moreover, the storage stability test was investigated at 35, 45, and 45°C and a_w of 0.53, 0.64, and 0.75) for 5 weeks. In each week, the quality of the encapsulated powder was evaluated. There are the effects of temperature and interaction of temperature and water activity on the first order kinetic reaction of anthocyanin. The most suitable condition of storage was 45°C with a_w of 0.53 and 0.64, providing the kinetic constant (k) of 0.1026 and 0.0881; and correlation coefficient (R^2) of 0.9806 and 0.9906 with $t_{half\text{-}life}$ of 6.76 and 7.93 weeks for a_w of 0.53 and 0.64, respectively.

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INTRODUCTION

The most striking feature of butterfly pea (*Cilteria ternatea*), a perennial climber found commonly in Southeast Asia, is its deep blue flower. For nature, the beautiful coloration acts as an attractant for plant physiology mechanism, but for us, it has held the potential to be used as a natural colorant applied in foods and cosmetics. Some studies incorporated the color extract into hard candy and sugar free ice cream and found the good possibility (Limsuwan *et al.*, 2014; Caroline and Padmavati, 2015), whereas in Thailand, the color of the flower is widely extracted and added into beverages and Thai desserts, such as Khanom-Chan, Chormuang, and Kanom Tuay.

The deep blue color of flower is associated with polyacylated anthocyanins pigment characterized as acelated delphinidin 3, 3'5'tri-glucosides. The properties of anthocyanins provide the solubility and beautiful hue; however, it also brings about the instability problem. The pigments are remarkably vulnerable and highly susceptible to degradation by various factors, including their chemical structure, pH, temperature, enzyme, light, oxygen, and other accompanying substances such as ascorbic acid, sugars, sulfur dioxide, and metallic ions (Welch et. al, 2008). When the structure is disrupted, it becomes colorless or brown colored compound. Consequently, this phenomenon restricts the utilization of the anthocyanins colorant.

Encapsulation is the promising technology to help protecting sensitive ingredient as anthocyanins from surrounding by embedding the molecule inside of the barrier material, and the component is released when they are desired. There are various matrixes that have been used to incorporate the active agents and cyclodextrin is one of them. β-cyclodextrin (β-CD) is one of a group of cyclic oligosaccharides consisting of six or more glucose units. This compound is able to complex with molecules via its unique structure. Many studies applied this compound in trapping the anthocyanin and found to ameliorate the stability. Tantituvanont *et al.* (2008) conducted the research on the effect of β-CD on thermal stability of the butterfly pea extract, which found to improve the samples in both solution and solid state. It also improved the retention of the chokeberry juice at pH 3.6 after 8 months (Howard *et al.*, 2013). Moreover, freeze drying is of interest for microencapsulation of butterfly pea flower crude extract due to low temperature used to avoid the degradation of anthocyanin by heat, leading to better anthocyanin retention. Therefore, this research was aimed to improve anthocyanin retention by using β-CD and

freeze drying for microencapsulation of butterfly pea flower crude extract. The stability of encapsulated powder was also investigated through 5 weeks storage.

OBJECTIVES

The objectives were as follows:

- To optimize the microencapsulation of the butterfly pea extract by using freeze drying and β- cyclodextrin
- To evaluate the stability of microencapsulated powder during 5 weeks

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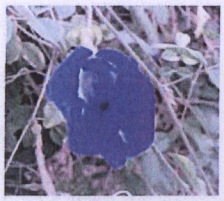
LITERATURE REVIEW

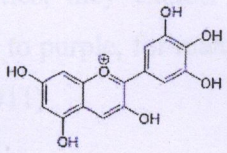
Butterfly pea flower

Botanically, butterfly pea (*Ciltoria ternatea L.*) is classified in *Fabaceae* family and *Pailionaceae* sub family. The plant is a perennial climber 90-162 cm tall with an erect habit. Its leaves are pinnate 5-9 leaflets whereas flowers are solitary and possess attractive deep blue color. Pods are flat, linear, and beaked, and contain 6-8 seeds, which turn to be black when mature. The plant is originated in Southeast Asia and widely distributed to other regions. Mainly *Ciltoria ternatea L.* is used as fodder, cover crop, and green manure as well as rotational grazing growing with tall grasses. It is also grown as an ornamental crop for garden lovers because of its beautiful coloration of flowers (Michael and Kalamani, 2003). Besides, the *Clitoria ternatea L.* is known as herbaceous plant as which every part of the plant can be utilized for medicinal purposes in a variety of aliment. These include constipation, indigestion, fever, arthritis, eye infection, headache, and antidote for snakebite poisoning (Alok *et al.*, 2015). It is believed to help enhancing memory and intelligence and provide good health effects such as antioxidative, antidiabetic, and hepatoprotective activities (Taranalli and Cheeramkuzhy, 2003; Rajamanickam *et al.*, 2015; Zingare *et. al*, 2013).

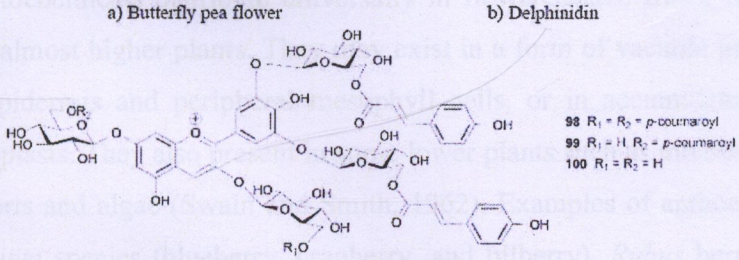
Among other parts of the butterfly pea, bluish colored flower (Figure 1a) is the most striking feature, which comes from an accumulation of the chemical compound called ternatin, polyacylated anthocyanins (Figure 1c). The flower stores six major anthocyanin ternatins (A1, A2, B1, B2, D1, and D2), characterized as malonylated delphinidin 3,3'5'-triglucosides with side chain at 3' and 5' with alternating D- glucose and ρ-coumaric acid unit (Terahara *et al.*, 1990; Francis, 1992). The Blue color results from three major constituents including delphinidin (Figure 1b), glycosylation, and acylation. Additionally, the existence of acyl moieties in the structure increases the stability of the color via intramolecular stacking (Tanaka and Brugliera, 2006).



a) Butterfly pea flower



b) Delphinidin



c) Ternatin, a group of (poly) acylated anthocyanin

Figure 1: Butterfly pea flower and its main chemical constituents

(Source: https://upload.wikimedia.org/wikipedia/commons/0/02/Delphinidin.png; http://pubs.rsc.org/services/images/RSCpubs.ePlatform.Service.FreeContent.ImageService.svc/ImageService/Articleimage/2009/NP/b800165k/b800165k-u17.gif)

Due to the soluble anthocyanin pigment and stabilization of acyl group, the color from the flowers can be extracted easily and is stable in weakly acidic or neutral solution. Some researchers conducted the study on incorporation of color extract into hard candy and sugar free ice cream and evaluated the quality (Limsuwan *et al.*, 2014; Caroline and Padmavati, 2015). They showed the good potential to be added into foods. The color extract is also applied in cosmetics and used as natural pH indicator for pharmaceutical field. In Thailand, the flower color is widely used throughout the countries. The color of butterfly pea is added to beverages and Thai desserts such as Khanom-Chan, Chor-muang, and Kanom Tuay.

Anthocyanin

- Background

Anthocyanins (ACNs) are the largest group of hydrosoluble pigments in nature, synthesized through a secondary metabolism of plant, phenyl propanoid pathway. They belong to the family of compounds known as flavonoids, which are sub-group of polyphenols. Their name is derived from two Greek words: *anthos* (flower) and *kyanose*

(dark blue), which refers to their coloring properties; they exhibit incredible colors, ranging from shade of red through crimson and blue to purple, for many plant parts, with odorless and nearly flavorless. (Raghvendra *et al.*, 2011)

These phytochemicals distribute universally in flower petals, fruits, leaves, and storage organs of almost higher plants. They may exist in a form of vacuole in outer cell layers, such as epidermis and peripheral mesophyll cells, or in accumulated vesicles called anthocyanoplasts. They also present in some lower plants such as mosses and ferns but not in liverworts and algae (Swain and Smith, 1962). Examples of anthocyanin-rich plants are *Vaccinium* species (blueberry, cranberry, and bilberry), *Rubus* berries (black raspberry, red raspberry, and blackberry), red cabbage, black rice and etc. (Raghvendra *et al.*, 2011). In most fruits and vegetables, generally, their concentration varies between 0.1 to 1% of the dry matter content (Swain and Smith, 1962). Pigment production is affected by two principal factors: one is the genetic variation of species to species; another is environmental factor including light, temperature, water, and growth medium. (Oancea and Oprean, 2011)

Besides, the ACNs of plants play a role in plant physiology. They serve as an attractant to persuade insects to pollinate flowers whereas for fruit, the colorful skin from pigments draws attention of animal, which eat the fruits and spread the seeds. They can also absorb blue-green and UV light, which protect the plants from cell damage due to photoinhibition or high light stress (Raghvendra, *et al.*, 2011).

- Structure

There are over 635 different anthocyanin structures that have been identified and isolated from plants; however, they all are chemically formed from same three components:

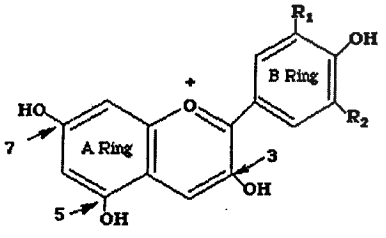
(1) Anthocyanidin or aglycone: this compound contributes to the unique characteristic of anthocyanins among other flavonoids. It is composed of C_6-C_3-C_6 skeleton as known as flavylium cation. There are 7 different binding positions for side groups, such as a hydrogen atom, a hydroxyl or a methoxyl-group. Naturally, there are twenty-two aglycones but only six are commonly distributed in plants and foods: cyanidin (Cy), delphinidin (Dp), peonidin

(Pn), petunidin (Pt), and malvidin (Mv). Table 1 shows the base structure and characteristics of six common anthocyanidins.

(2) Sugar moiety: aglycone is usually glycosylated with one or more sugar since it is more stable and frequently in the 3-OH, 5-OH, or 7-OH position. The natural sugar molecules in anthocyanins are glucose, rhamnose, xylose, galactose, arabinose, and fructose

(3) Organic acid: some aglycone is acylated with one or more molecules of acid or aliphatic acids through ester linkage with sugar. The common acids are coumaric, caffeine, ferulic, *p*-hydroxy benzoic, synaptic, acetic, succinic, and oxalic. Without the acylation, the compounds are called as non-acylated anthocyanins.

Table 1: General chemical structure of anthocyanidins (Delgado-Vargas and Paredes-Lopez, 2003; Jackman and Smith, 1992)

Basic Structure	Anthocyanidin	R_1	R_2	R_3	Color
	Pelargonidin (Pg)	-H	-OH	-H	Orange, salmon
	Cyanidin (Cy)	-OH	-OH	-H	Magenta and Crimson
	Delphinidin (Dp)	-OH	-OH	-OH	Purple, mauve, and blue
	Peonidin (Pn)	$-OCH_3$	-OH	-H	Magenta
	Petunidin (Pt)	-OH	-OH	$-OCH_3$	Purple
	Malvidin (Mv)	$-OCH_3$	-OH	$-OCH_3$	Purple

The diversity of anthocyanins is regarded to the component identity and its number and position on the basic anthocyanidin skeleton. These include the hydroxyl and methoxyl group, sugar moiety, and acylating agent. The increments in the number of hydroxyl groups intensify the bluish shade whereas methoxyl groups increase the redness.

Aside from constitutions, physiochemical factors, such as pH, temperature, type of solvent, and co-pigmentation, can affect to structural transformation (Mazza, 2007).

- Health benefits

Numerous studies indicate that the ACNs have the anti-oxidative effect and inflammatory properties and suggest the pigments could lower the risk of many chronic diseases, including cardiovascular disease, diabetes, arthritis, and cancer (Rechner and Kroner, 2005; Wang and Stoner, 2008), and improve the sight acuteness, enhancing overall vision and night vision (Lila, 2004). As a consequence, the pigments have a potential to be functional ingredient and therapeutic agents to supplement people (Shipp and Abdel-Aal, 2010).

Anthocyanin as a source of natural color additive

- Natural colorant

Chemically derived colorants had been preferred over time because they are ease of production, inexpensive, and well incorporated properties (Chaitanya Lakshmi, 2014). However, they were drawback due to safety concern. The criticism was started as long as the 1975s after Ben Feingold released his book *Why Your Child Is Hyperactive*, claiming that synthetic food additive can cause hyperactivity and learning disability in children. The information on health effects was spread widely, although there was no link found (Nachay, 2009). Afterwards, there are more studies shown the intake of synthetic colorant led to harm. These include cancer, DNA damage, reproductive damage, neuromuscular damage, and asthma, as well as learning and behavior problem. One study conducted with 803 students in public school in New York found that test scores increased dramatically resulting from inhibiting the use of artificial colors, flavors, and preservatives. In addition, synthetic colorants were also found to have synergistic effect when added with MSG and aspartame (Hersey and Heltzel, 2007). Consequently, it caught up the attention of government agencies to place restriction and consumer advocacy to call for bans. Many approved synthetic dyes are delisted and continued to reduce (Nachay, 2009). Consumers also significantly shift their demand to natural derived colorants, which show to benefit over synthetic color. In addition, products containing synthetic color have been banned to trade to Japan and all European countries (Chaitanya Lakshmi, 2014) whereas

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retailers in Europe and U.S. also encourage the consumption by having entire lines carry only products formulated without synthetic dyes (Nachay, 2009).

Natural colorants have a growing demand day by day in the international market with the 10% increase annually (EIBI, 2010). This is not only a result from the harmful health people perceive but also a result from the popular message "Natural is Better" (Rymbal *et al.*, 2011). Natural dyes are considered environmentally friendly, safe, less toxic, non-carcinogenic, and non-poisonous (Ramamoorthy, 2007). Some provide advantage on health as they carry potent antioxidant and improved visual acuity properties. Table 1 provides some examples of natural colorants from plants and animals. Nonetheless, the development of the natural colorants is still in progress in term of collection, extraction, and stability over certain factors including processing and storage (Rymbal *et al.*, 2011).

Table 2: Examples of naturally derived colorants (Chaitanya Lakshmi, 2014)

Natural colorant derived from plants		
Color	**Examples**	**Applications**
Red	Annatto	Cheese
	Paprika	Cosmetic and drug
	Beeta vulgaris (Betalains)	Yogurt and ice cream
	Grapes vitacea (Grape fruit)	Paint skin and feathers in historical time
	Alkanna tinctoria (Alkanna)	Cosmetics
Yellow-orange	*Curcuma longa* (Turmeric)	Dairy products, beverages, cereal, pickles, sausages, confectionaries, ice cream, bakery, and savory products
	Sanguinaria canadensis (Blood root)	-
	Tagetes erecta (Mexican Marigold)	Baked good, beverages, breakfast creals, chewing gum, dairy product analogs, egg products, fats and oils, sauces, infant and

		toddler foods
	Crocus sativus (Saffron)	Textile dye, Perfumery, and medicine
Brown	*Camellia thea* (Tea)	Cosmetics
Green	Plant, algae, and cyanobacteria	-
Blue-violet	*Indigofera tinctoria* (Indigotin)	Linen and hair dye
	Centaurea cyanus (Cornflower),	Sugar and confectionaries
	Sambucus nigra (Blueberry)	-
Natural colorant derived from animals		
Color	**Examples**	**Applications**
Red	*Dactylopius coccus* (Cochineal),	Food: Juice, ice cream, yogurt, and candy; Cosmetics: eye shadow and lipstick
	Monascus purpureus	Candy and red wine
Orange-red	*Sepia officinalis* (Female cuttlefish)	Spanish cuisine breaded and deep fried cuttlefish, black pasta, *Chocos com tinta,* and printing ink

- Anthocyanin derived colorant

Synthetic colorants have been major coloring additives in the market. In food industries, 90% dyes used are made chemically and added to variety of products, such as sausage, beverages, cereal, candy, snack foods, and pet food (CSPI, 2010). However, increased health awareness and controversy of synthetic dyes, together with their regulation restriction, boost up the finding of new alternatives and therefore anthocyanin based colorant are one of the ideal candidates to meet the demand.

Anthocyanins provide a wide broad range of red-purple-blue color and require small amount to portray the desired color in the system (Mateus and Freitas, 2009). They

are distributed in large number in fruits and vegetables. Colorant from grape extract as well as grape skin extract has been incorporated commercially in beverages, soft drinks, fruit yogurt, cheesecake, and confectionery products (Delgado-Vargas and Paredes-Lopez, 2003). The economic source is the grape pomace as a by-product from some industrial processes. This includes wine production that annually uses grapes around 60,000,000 metric tons. Other potential sources from fruit anthocyanins are elderberry, black currant, and cherry (Francis, 2002).

Radishes and red potatoes are examples of vegetable derived sources that provide natural red color similar to FD&C red No.4 (Allura red) (Giusti and Wrolstad, 2003). Their color has the tinctorial strength against pH due to high percentage of acylation in the structures, and because of this, it could be used in broader pH in foods (Stintzing FC, 2004). Additionally, FDA is proposing on banning the Allura red dyes so this could create higher chance of these color sources as substitutes in the near future (Melo *et al.*, 2009).

The application of anthocyanins as colorant is based on countries by countries. Many countries define the food products to be added and prohibit their use in milk and fruit juice. For instance, U.S. allows only the addition of coloring agents made from grape skin extract, grape color extract, fruit juice, and vegetable juice, which they are exempt from certification (Wrolstad, 2004). In the European union, they classify the anthocyanin-derived colorant as natural food additive with a code E163 whereas its permitted amount is 200 ppm (Melo *et al.*, 2009).

Stability of anthocyanin

The limitation of the application of anthocyanins as natural color additive is their relative low instability (Delgado-Vargas and Paredes-Lopez, 2003). The pigments are unstable and susceptible to degradation, resulting in changing in color. Consequently, in perspective of manufacture, it affects to production yield, quality, packaging, handling, and shelf life when added into the process as well as sold in a form of concentrate, powder, or extract. The destruction of anthocyanins are strongly influenced by its chemical structure, pH, temperature, enzyme, light, oxygen, and other accompanying substances such as ascorbic acid, sugars, sulfur dioxide, copigments, and metallic ions (Welch et. al, 2008).

- Effects of chemical structure

Anthocyanins can exhibit color because of its resonating structure but the resonance phenomena also bring about the intrinsic instability to the pigment (Delgado-Vargas, 2000). When it is disrupted by high pH medium or bleaching agent, as an example, the color is lost (Brouillard, 1982). Additionally, the number, position, and identity of the group attached to the structure have a substantially impact on the stability. Hydroxyl group is reactive and increased number of this group destabilizes the pigment. For methoxylation, it is capable of blocking the reactive hydroxyl group, which results in providing stability to the molecule, and this gives higher stability to peonidin, petunidin, and malvidin over pelargonidin, cyanidin, and delphinidin. Further, diglycosides are shown to be more stable than monoglucosides; however, the addition of sugar molecule makes the browning reaction favored. Acylation of sugar residue improves the stability of the anthocyanin by intramolecular interaction, as known as copigmentation. It showed to be more stable than nonacylated form (Delgado-Vargas, 2000).

- Effects of pH

Based on pH, structure of anthocyanins transforms to 4 different chemical forms: the blue quinoidol base (A), the red flavylium cation (AH+), the colorless carbonic base (B), and colorless chalcone (C), shown in Figure 2. In aqueous medium, at pH approximately 3 or lower, the anthocyanins exist in their most stable form, flavylium cation, with the appearance of orange-red to violet color. When the pH rises up to 4.5, the flavylium cation undergoes hydration reaction giving a colorless carbonic pseudo-base and due to its instability, it is converted to colorless chalcone. Once approached to pH 6, the color becomes purple whereas at pH 7, the proton transfer reaction occurs generating the quinonoidol base form, which has dull blue to green color. As pH is raised, anthocyanins turn from blue to green and then to yellow (Cavalcanti *et al.*, 2013; Kidmose *et al.*, 2002).

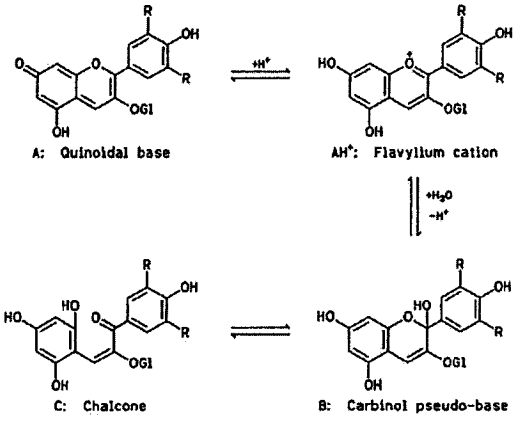

Figure 2: Structural transformation of anthocyanin toward pH change (Source: http://what-when-how.com/food-colors/natural-food-pigments-part-2/)

- Effects of temperature

Temperature is one of detrimental influence on the stability of anthocyanin pigment. The rise of temperature increases the rate of degradation. It has been reported that the increased temperature in arithmetic course can degrade anthocyanin exponentially by several studies (Markakis, 1982). The exact degradation mechanism is not fully studied. Markakis *et al.* (1957) proposed the opening of heterocyclic ring was the first degradation and latter pigment degraded to brown compounds. One suggested high temperature cleaves the glycosic bond followed by ring opening, forming chalcone, uncolored pigment (Adam and Ongley, 1973). Then the brown compounds formed (Markakis, 1982). Piffaut *et al.* (1994) proposed the hydrolysis of glycosidic bond forming the uncolored pigment and subsequently converted to other compounds such as aldyhyde compounds, benzoic acids and its derivatives. Besides, heating can also induce the oxidation reaction to occur (Cavalcanti *et al.*, 2013). According to Roobha *et al.* (2011), it was reported that anthocyanin pigments in *Musa acuminata* bract was stable at lower temperature and rapidly destructed at 40°C and higher. Increasing of temperature had a positive effect to the degradation rate of grape juice as well as the polymeric anthocyanin formation rate (Danisman *et al.*, 2015).

- Effects of enzymes

Three main anthocyanin-degrading enzymes are glycosidase, peroxidase, and phenolase. They exist originally in the plant, itself, or get contaminated from mold. The activity of glycosidase has a direct effect. It breaks the covalent bond between the sugar moiety and anthocyanidin, which in turn make the compound highly unstable (Melo *et al.*, 2009). Peroxidase and phenolase, with the presence of oxygen, catalyzes the oxidation of phenol compound into O-quinone, which reacts with anthocyanin forming brown condensation product (Markakis, 1982).

- Effects of moisture

Anthocyanin stability increases when water activity decreases. One reported that high water content increased the rate of degradation as well as accelerated the browning reaction in dehydrated strawberries (Aguldelo-Laverde *et al.*, 2013), whereas the research conducted by Obiegbuna *et al* (2014) showed that cocoyum flour's anthocyanin decreased with time and higher relative humidity.

- Other factors associated with anthocyanin stability

Light is the main key for biosynthesis of anthocyanins; however, after extraction, it tends to accelerate the degradation of the pigment. Anthocyanins in blackberry (*Rubus fruticosus*) was degraded around 2.5 times when irradiated with light 3968.30 lux of the extract in the absence of light, and increased intensity of light exposure also cause the gradual loss (Contreras-Lopez *et al.*, 2014).

Oxygen reacts to unsaturated bond of the anthocyanin structure, resulting in color loss, and the reaction is strengthened in the presence of ascorbic acid. Ascorbic acid, itself, may protect anthocyanin from polymerization due to O-quinone (Santos *et al.*, 2011). However, if it is oxidized, the hydrogen peroxide (H_2O_2) is produced. The H_2O_2 then open the pyrylium ring and produces colorless esters and coumarin derivatives (Jackman and Smith, 1992). From the research of De-Rosso and Mercadante (2007), the addition of ascorbic acid caused the fading color in a juice, which contained anthocyanin pigment.

The effect of sugar on anthocyanin stability depends on the anthocyanin structure, concentration, and type of sugar. At low concentration of sugar, water level is high and

able to react with flavylium cation, forming colorless carbonic base, whereas high level stabilize the anthocyanin due to low water activity (Jackman and Smith, 1992). In addition, if the sugar is degraded to furfural type compounds, furfural or hydroxylmethyfurfural, via maillard browning reaction, it can condense to anthocyanin and lead to formation of browned color or colorless compound (Lee and Schwartz, 2005).

Anthocyanin stability enhancement

Instability of anthocyanin drawbacks the practical use as natural derived colorant, but because of public's demand that is increasing growing up, many techniques and technologies have been applied in order to improve the tinctorial strength of the pigment. There are four basic principles involved: self-association, copigmentation, metal complexation, and encapsulation.

- Self-association

Self-association is a combination of two or more anthocyanins that form as a stacking like structure in vertical alignment. Figure 3 is an example of self-association of anthocyanins at C-4 position. The compounds are aggregated via the hydrophobic interaction, surrounded by the hydrophilic interaction of sugar moiety. Because of this interaction above, it prevents the molecules from hydration of anthocyanin that initiates the color loss (Hoshino, 1992). The mechanism was first described by Asen *et al.* (1972) that only increased 100 times of cyanidin 3,5 diglucosides concentration, from 10^{-4} to 10^{-2}, caused the increase in absorbance 300 folds. This did not rely on the Beer's Law and also proved the aggregation of molecules were occurred (Asen *et al.*, 1972). Accordingly, Timberlake (1980) reported the similar intensified color of tenfold increased concentration of malvidin 3-glucoside at pH 3.5, which associated with the complexation of anthocyanins. Concerning the stacking, it was initially assumed to be vertical or horizontal until early 1980's the result from circular dichoroism (CD) and H' NMR showed the stacking is vertical either a left or a right of the anthocyanin molecules (Matsumoto *et al.*, 1980). However, increased temperature can cause the dissociation of the pigments, leading to the loss in color quality, which found to occur in wine (Hoshino, 1992). The structural component in term of type and position also affects to the molecular stacking, which either can enhance or takeaway the association. Hydroxyl (-OH) and

methoxyl (-OCH_3) provide greater association of anthocyanins (Leydet *et al.*, 2012; Han and Xu, 2015). Hydroxylation at position C'3 position weakens the association whereas at the C'5 position helps the self-association (Han and Xu, 2015). The availability of information about the application of self-association in anthocyanins stability is low. One research confirmed that self-association affected to the hydration constant and color modification of 3-glucosides of malvidin, delphinidin, and peonidin, which are the wine grape anthocyanins (Gonzalez-Manzano *et al.*, 2008). It is also believed to partially impart in color of the wine during aging (Ribéreau-Gayon, 1982).

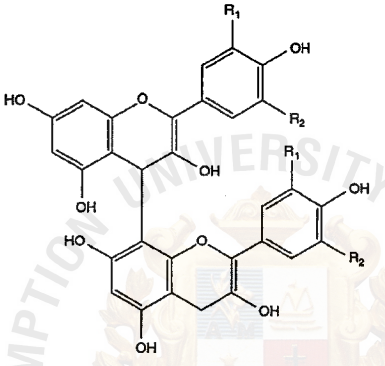

Figure 3: Self association of anthocyanin at C-4 position (Wallace and Giusti, 2009)

- Copigmentation

Copigmentation is a phenomenon involved with a complex formation between anthocyanin and other colorless organic molecules, as known as copigments, in solution. The mechanism results in improving the pigment stability and enhancing a greater color exhibition since it enables to prevent water attack on C2 and C4 and formation of uncolored chalcone (Brouillard, 1998; Asen *et al.*, 1972). When the copigmentation occurs, it can be observed through a bathochromic shift ($\Delta\lambda_{max}$), the shift of maximum absorption wavelength (λ_{max}) towards higher wavelength, which results from a hyperchromic effect, a pigment color changes from red to more blue color (Asen *et al.*, 1972). Therefore, naturally it occurs in reddish-blue flowers and fruits, which helps preventing their vegetal vacuole from hydration (Goto and Kondo, 1990; Brouillard, 1998).

Copigmentation can undergo through several interactions but their two main types are intramolecular and intermolecular complex formations. Intramolecular

copigmentation involves with the associations within the pigment, itself. It is the interaction of aromatic acyl group covalently bond to the sugar residue of anthocyanin and make it difficult for the hydration to occur to pyrilium ring of anthocyanin, which in turn, stabilize the pigment (Cavalcanti *et al.*, 2013). Examples of aromatic acyl group are ρ-coumaric, caffeic, ferulic, sinapic, gallic, or ρ-hydroxybenzoic acid (Giusti and Wrolstad, 2003). Moreover, due to the strength of the bond, this type of copigmentation is stronger and effective than intermolecular copigmentation and self-association (Brouillard, 1982).

Intermolecular complex formation is the association between anthocyanin and colorless compounds through hydrogen bond and hydrophobic interactions, as main driving forces. This results in overlapping arrangement of the two molecules, protecting the pigment against nucleophile agent attack (Rein, 2006). The colorless co-pigments could be flavonoids, and other polyphenols, alkaloids, polysaccharides, amino acid, and organic acids. Grape skin anthocyanins were stabilized with the addition of quercetagetin at different three pH values (Liu *et al.*, 2015).

However, there are also many factors influencing copigmentation: concentration of pigment, ratio of pigment to copigment, pH, extent of nonaqeous condition, and the anions in the solution (Boulton, 2001). The color enhancement is relatively fixed which requires specific condition.

- Metal complexation

Some metal ions with highly charge were found to form stable complex with anthocyanin, which called as metallocyanin. These include aluminum (Al), magnesium (Mg), Iron (Fe), Copper (Cu) and Tin (Sn) (Boulton, 2001). The complex formation also has a bluing effect similarly as self-association and copigmentation. Boulton (2001) reported the use of metal in blue juice and wine. However, it is believed that metal anthocyanin complex caused pinking in pears and peaches (Lee and Schwartz, 2005).

- Encapsulation

Since the anthocyanins are highly sensitive bioactive compounds, they comply with a primary purpose of encapsulation technology. It is an advance process to embed a susceptible agent within a barrier material to protect from any unwanted interaction. Not only the protection advantage but it also uses to mask unpleasant features, such as bitter

and astringent taste. The sensitive core, as an outcome, is in an adequate concentration with a uniform dispersion, and convenient for handling process. When utilized, the release rate is controlled over prolonged period, under a designated specific site (Desai and Park, 2005). The encapsulation has been applied to many vulnerable materials such as enzyme, probiotic microorganisms, flavoring compounds, ethylene gas, and natural pigment. The technology is rapidly expanding technology, highly specialized, with affordable costs (Jackson, 1991).

The selection of techniques and wall material composition are important in order to obtain maximum degree of protection and stability. Basically, they are chosen based on physiochemical properties of the core, the wall composition, core size, concentration, composition, required particle size release mechanism, cost, and final form of product (Dubey, 2009; Nedovic *et al.*, 2011; Murugesan and Orsat, 2012). There are numbers of techniques of encapsulation available, which usually based on drying (Nedovic *et al.*, 2011). They can be classified as: physical and chemical methods. The physical method includes spray drying, spray chilling, fluid bed coating, and pan coating, whereas chemical method includes simple/complex coacervation, solvent evaporation, interfacial polymerization, and matrix polymerization. Concerning protective wall, they might be used to entrap one or more core materials with single or multilayer with other type. Table 3 summarized microencapsulation materials and its classification based on origin and type.

Table 3: Classification of microencapsulation material based on origin and type (Wandrey *et al.*, 2010)

Origin	Carbohydrate polymer	Protein	Lipid
Plant	Starch and derivatives Cellulose and derivatives Plant exudates (Gum) Plant extracts Polysaccharide	Gluten (corn) Isolates (pea, soy)	Fatty acids/alcohols Glycerides Waxes Phospholipids

Marine	Carrageenan Alginate		
Microbial/animal	Xanthan Gellan Dextran Chitosan	Casein Whey proteins Gelatin	Fatty acids/alcohols Glycerides Waxes Phospholipids

Many researches employed the encapsulation technique to stabilize anthocyanin with the variation of methods and wall materials used. Table 4 summarized some studies of anthocyanin encapsulation.

Table 4: Examples of anthocyanin encapsulation in diverse methods and wall materials

Encapsulation method	Source of anthocyanin	Wall material	Observation	Reference
Spray drying	Black carrot	Maltodextrin (10DE, 20-23DE, and 28-31 DE)	- The inlet temperature affected the loss of anthocyanin in which the maltodextrin was found to be the great barrier protection. - Keeping the encapsulated powders at 4°C can prolong longer time than 25°C.	Ersus and Yurdagel, 2007
Spray drying	açai juice	Maltodextrin (10DE and 20DE), gum arabic, and tapioca starch	- High temperature and high water activity showed negatively affected to the storage time of the powder. - Anthocyanin retention was not highly influenced by the different matrix; however, maltodextrin showed the	Tonon *et al.*, 2009

			greater retention and good physical properties.	
Spray drying	Roselle	Maltodextrin, gum arabic, soluble starch, combination of maltodextrin and gum arabic	All encapsulating agents excluding soluble starch were able to increase the half-life of the roselle whereas the combination of maltodextrin and gum arabic improved the degradation rate the most at 60°C and 80°C.	Idham *et al.*, 2012
Spray drying	Black glutinous rice bran	Maltodextrin 10DE	- Increasing the inlet temperature of the spray drying condition provided good physical properties and encapsulation efficiency. However, it affected to total anthocyanin content and color quality	Kanha and Laokuldilok, 2014
Spray drying	Red cabbage	Gum arabic and Polydextrose	- The matrix protected the anthocyanin and antioxidant properties well from increasing the inlet temperature of spray dryer - The encapsulated powder is high solubility and relatively stable at $a_w < 0.2$	Anahi Bernstein and Zapata Noreña, 2015
Spray drying Freeze drying	Black glutinous rice bran	Black glutinous rice bran maltodexrin (10DE, 20DE, and 30DE), commercial	- Black glutinous rice maltodextrin DE20 was suggested to use with freeze drying and spray drying (inlet	Laokuldilok and Kanha, 2015

		maltodextrin 10DE	temperature 180°C). - It showed process yield than commercial maltodextrin.	
Gelation	Biberry	Whey protein	Whey protein was induced by acid and heat to trap the anthocyanins in which it was found that pH dependence interactions between whey proteins and BBE-constituents were detrimental affected. pH 1.5 was stronger stabilizing the pigment.	Betz and Kulozika, 2011
Freeze drying	Saffron petal	Gum arabic and maltodextrin (4-7DE & 16-20 DE)	- Capability in stabilizing of anthocyanin of different matrix used was not different and showed good protection after 10 weeks storage at 35°C. - The color retention efficiency can be ordered as AG > M20 > 7 identified by total color difference	Khazaei *et al.*, 2014

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Freeze drying and vacuum oven drying	Butterfly pea flower	Maltodextrin, combination of maltodextrin and arabic gum, and arabic gum	- It was found that vacuum oven drying was better than freeze drying due to lower moisture content and higher storage time - The combination of maltodextrin and arabic gum provided better protection than another two.	Hamzah *et al.*, 2013
Molecular inclusion	Roselle	β-CD	- Thermal degradation followed 1st order - The complexation with β-CD showed the decreased rate of degradation and results in doubled half time, and it showed the improvement in protection against oxidation.	Mourtzinos *et al.*, 2008

Encapsulation: Freeze drying technique

Freeze drying, also known as lyophilization or cryodesiccation, is one of the encapsulation techniques for drying heat labile substances, as anthocyanins, that are unstable in an aqueous solution (Poshadri and Kuna, 2010). It has been used to encapsulate water soluble essences, natural aromas, drugs as well as probiotics, enzymes and peptides. The following steps are the process, adapted from (Fang and Bhandari, 2012).

1) Dissolving: suspense sensitive core and carrier in the water.
2) Freezing: freeze the suspension at temperature that maximizes the crystallization of water and other solid compounds.

3) Primary drying (Main drying): dry at low pressure to transform ice to water, whereas the evaporated vapor is transferred to condensation system. This process is called as sublimation.
4) Secondary drying (Desorption drying): remove the additional bound unfrozen water from the solid after completion of ice sublimation.
5) Grinding (if necessary): break the brittle cakes into smaller pieces

Using freeze drying process, the selection of wall material and preparation of wall-core matrix solution should be well considered. They affect to the final characteristics, properties, storage condition, and shelf life. The process also applies a great deal of stresses onto, so the wall material should be able to provide the protection and stability for active agents. The common carriers used are proteins, maltodextrins, disachharides, gums, and etc..

Advantage: suitable for thermosensitive material encapsulation; controllable moisture content of final product; good retention of appealing color and texture; easy to rehydrate (Fang and Bhandari, 2012; Poshadri and Kuna, 2010).

Disadvantage: high energy consumption; high production cost; long processing time; open porous structure powder which might require vacuum packing or inert atmospheric condition (Fang and Bhandari, 2012; Zuidam and Shimoni, 2010; Nedovic *et al.*, 2011).

Maltodextrins

Maltrodextrins are the common protective wall applied in many encapsulation methods including spray drying, fluid bed spraying, fluid bed granular granulation, extrusion, etc. The compound is acid- or enzyme-catalyzed hydrosates with dextrose equivalent (DE) value less than 20 and molecular weight <4,000 g/mol. It is derived from partial starch hydrolysis of several starch sources, such as potato, corn, wheat, and tapioca. Properties of maltodextrin are that they are creamy white, low viscosity, high water solubility, tasteless, and form colourless solutions (Wandrey *et al.*, 2010; Ozkan and Bilek, 2014). The incorporation of maltodextrin can help protecting the ingredient from oxidation and heat, showed to be essential to preserve the integrity of anthocyanins (Shahidi and Han, 1993; Munin and Edwards-Levy, 2011). Some might also blend

maltodextrins with other ingredients including gum arabic and pectin (Khazaei *et al.*, 2014; Gurak *et al.*, 2013; Oidtmann *et al.*, 2013).

Beta-Cyclodextrin

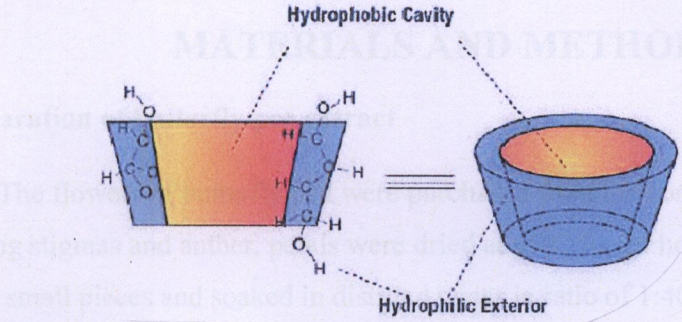
Cyclodextrins (CDs) are a family of macrocyclic polymers containing glucose molecules joined via glycosidic linkage. It is produced by the hydrolysis of amylose, amylopectin, and glycogen with the use of dilute acid or enzyme as a catalyst. After the degradation, the glucose units are cyclized, forming closed circular molecule, or known as truncated cone (Figure 4a). The interior of the cavity is hydrophobic due to the presence of CH_2 groups. The external region that is the cavity entrances contains numerous primary and secondary hydroxyl groups, contributing the solubility property. Of these, the structure can perform stable host-guest complexation in which the molecule is hold partly or entirely within the cavity by the hydrophobic-hydrophilic interaction and van deer waal forces. For the properties of CDs, they are crystalline, homogenous, and non-hygroscopic, readily dispersible, and resist shear forces (Wandrey *et al.*, 2010; Agrawal and Gupta, 2012). The encapsulation with this compound can stabilize and prevent the core against oxidation and evaporation (Zhou *et al.*, 2013).

There are three common forms of CDs (Figure 4b):

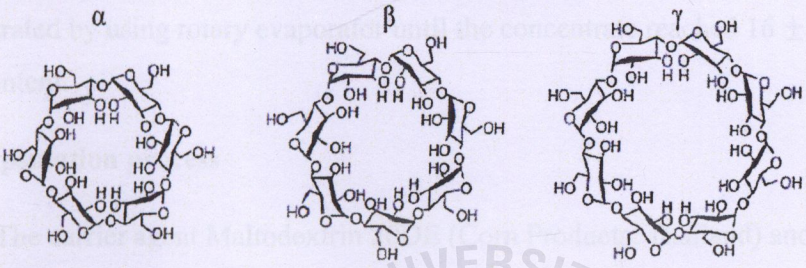
- α –CD: six sugar glucose residues, inner diameter 0.5 nm

- β-CD: seven sugar glucose residues, inner diameter 0.6 nm

- γ- CD: eight sugar glucose residues, inner diameter 0.8 nm



(a) Cyclodextrin - Hydrophilic external phase and Hydrophobic inner surface



(b) Chemcial structure of common forms (α, β and γ CD's)

Figure 4: Cyclodextrin and its three common forms (Agrawal and Gupta, 2012)

The complexation depends on CD dimension. Differences in internal diameter and its volume determine the capacity of the agent that can be incorporated. The greater size can hold bigger molecule. Based on these dimension, α –CD can hold only low molecular weight molecules or compounds with aliphatic side chains; β-CD binds with aromatic compounds and heterocycles; γ- CD has the largest space to trap bigger molecules such as macrocycles and steroids (Martin Del Valle, 2004). Moreover, molecular weight, size, and shape of agent, steric hindrance, chemical functionality, and polarity can influence the greater or lesser extent (Chranioti and Tzia, 2015).

β-CD is the most common CDs used in encapsulation and has been approved by USA, Japan, and Europe for the use as food additives (Magnusdottir and Loftsson, 2002). It has high availability and low price, and its cavity dimension is able to handle the core material (Cannava *et al.*, 2008). Many studies used β-CD to ameliorate the stability of the anthocyanins extract. Tantituvanont *et al.* (2008) conducted the research on the effect of β-CD on thermal stability of the butterfly pea extract, which found to improve the samples in both solution and solid state. It also improved the retention anthocyanin of the chokeberry juice at pH 3.6 after 8 months (Howard *et al.*, 2013).

MATERIALS AND METHODS

1. Preparation of butterfly pea extract

The flowers of butterfly pea were purchased from the commercial market. After removing stigmas and anther, petals were dried at 45°C for 24 hours. Dried petals were cut into small pieces and soaked in distilled water in ratio of 1:40 for 3 hours. The suspension was filtered through a filter paper (Whatman no.1), and the solution was concentrated by using rotary evaporator until the concentrate reached 16 ± 0.5% soluble solid content.

2. Encapsulation process

The carrier agent Maltodextrin 20DE (Corn Products, Thailand) and β-CD (Xi'an HongChang Phamaceuticals, China) were combined with the pigment extract as shown in Table 5 and stirred until all the materials were completely dissolved. Subsequently, the mixtures were homogenized at 600 rpm for 15 minutes. The resulting solutions were dried by using freeze dryer for 24 hours and then the extracts cakes were converted into powder.

Table 5: Formulation with different portion and composition of encapsulating agent

Treatment	Portion of core to wall material	Encapsulating agent	
		β-CD	MD
1	1:1	25	75
2		50	50
3		75	25
4	1:2	25	75
5		50	50
6		75	25
7	1:3	25	75
8		50	50
9		75	25

3. Stability evaluation of the encapsulated butterfly pea extract

Encapsulated powders were stored at 3 different temperatures (35°C, 45°C, and 55°C) and 3 different a_w (0.53, 0.64, and 0.75). The degradation was monitored for 5 weeks and chemical and physical analyses were conducted weekly. The half- life of the samples was determined as well.

4. Microencapsulation yield (ME)

The process yield is calculated using following formula:

$$\text{Yield (\%)} = \frac{\text{Weight of the encapsulated powder}}{\text{Total weight of all ingredients}} \text{ x } 100$$

5. Encapsulation efficiency (EE)

The microencapsulation efficiency is calculated using following formula:

$$\text{Encapsulation efficiency} = \frac{Total\ anthocyanin\ content\ in\ powder}{Total\ anthocyanin\ in\ extract} \text{x } 100$$

6. Chemical and physical analysis

6.1 Moisture content

Weighted samples (1.0 g) were oven dried at 105°C for 8 hours in pre-weighed moisture cans. The moisture cans were transferred to desiccators immediately, cooled and weighed. The moisture content was then calculated as follow:

$$\text{Moisture (\%)} = \frac{Loss\ in\ weight\ (g)}{Sample\ weight\ (g)}\ x\ 100$$

6.2 Total anthocyanin content

Total anthocyanin content (TAC) was evaluated using pH-differential method described by Lee *et al.* (2005). The test samples were diluted with potassium chloride (pH 1.0) and sodium acetate (pH 4.5). The absorbance was measured at 520 and 700 nm against the distilled water filled in a blank cell. The results were expressed as mg of

cyanidin-3-glucoside of 100 g of dry matter of sample, which calculated from following formula:

$$\text{Anthocyanin pigment} = \frac{A \, x \, MW \, x \, DF \, x \, 10^3}{\varepsilon \, x \, 1}$$

Where A = $(A520 - A720\ nm)_{pH1.0} - (A520 - A700\ nm)_{pH4.5}$; molecular weight (MW) = 440.2 g/mol; extinct coefficient (ε) = 26,900 L cm^{-1} mol^{-1}. Triplicate analysis was performed for each treatment.

6.3. Colorimetric analysis

The color of encapsulated powders were determined as L*, a*, and b* values using a HunterLab Miniscan EZ in triplicate. It was used to investigate all samples after freeze drying and during 5 week stability test. Moreover, in the stability test, total color differences (ΔE) was calculated by this following equation:

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2}$$

7. Statistical analysis

A randomized block design and 3x3 factorial designs with 2 replications were used. Statistical analysis of measured results was carried out by using ANOVA procedure, and significant differences were determined by using Duncan's multiple range test ($p \leq 0.05$) by IBM SPSS Statistics 20.0. Moreover, one sample t test was conducted to compare the difference between two samples.

RESULT AND DISCUSSION

1. Microencapsulation yield

The process yield (Table 1) was not affected by the difference concentrations of β-CD and its interaction with proportion of wall material. It was positive correlated with the proportion of core and wall materials. The highest yield (72.7%) was found at ratio 1:3 and 25% β-CD concentration and lowest yield (47.3%) was found at ratio 1:1 and 25% β-CD concentration. It might result from the body provided by the wall material. When the amount of the body increased, the yield of the powder obtained also increased. However, the lowest yield of the experiment was about 12% greater than the highest production yield (35.2%) of encapsulated butterfly pea using spray drying technique (Tantituvanont *et al.*, 2008). Freezing drying technique may prevent the loss that occurred in spray drying including the loss of fine particles as well as the residue left in the dryer or cyclone.

2. Encapsulation efficiency (%EE)

The encapsulation efficiency of all samples was in the range of 11.14 - 88.41%, in which both ratio of core to wall materials and β-CD concentration were significant affected ($p \leq 0.05$). From Table 6, it can be observed that the increase in wall material in the mixture tended to decrease %EE. The EE of 50% β-CD at 1:1 ratio was higher than that of 1:2 and 1:3 approximately 2 and 3 times, respectively. This might be the result from the lesser extract added and less distribution of the extract throughout the matrix.

Table 6: Some chemical and physical properties of encapsulated butterfly pea flower extract

Portion of core to wall material	Concentration of β-CD	Yield (%)	TAC (mg/100g of dry matter)	EE (%)	Moisture content (%)	L*	a*	b*
1:1	25%	$47.3 \pm 3.18^{c*}$	1126.0 ± 29.58^{a}	88.4 ± 2.32^{a}	7.4 ± 0.31^{bc}	38.62 ± 4.96^{a}	2.67 ± 0.10^{a}	-21.07 ± 0.79^{a}
	50%	51.2 ± 2.63^{c}	690.8 ± 5.63^{b}	54.2 ± 0.44^{b}	8.0 ± 0.18^{d}	44.71 ± 2.21^{b}	2.50 ± 0.10^{ab}	-20.13 ± 0.75^{ab}
	75%	48.9 ± 0.26^{c}	325.7 ± 3.81^{c}	25.6 ± 0.30^{c}	7.7 ± 0.13^{cd}	50.16 ± 2.46^{bc}	0.88 ± 0.10^{d}	-18.08 ± 0.45^{cd}
1:2	25%	63.7 ± 0.01^{b}	363.9 ± 13.56^{c}	28.6 ± 1.06^{c}	7.3 ± 0.25^{b}	50.43 ± 2.37^{bc}	2.21 ± 0.14^{b}	-19.07 ± 0.02^{bc}
	50%	63.5 ± 0.03^{b}	342.1 ± 13.84^{c}	26.9 ± 1.09^{c}	7.7 ± 0.09^{cd}	56.20 ± 2.60^{cd}	1.45 ± 0.40^{c}	-18.47 ± 0.31^{cd}
	75%	62.2 ± 1.54^{b}	227.1 ± 6.37^{de}	17.8 ± 0.55^{de}	7.3 ± 0.24^{b}	57.40 ± 3.10^{d}	0.33 ± 0.15^{e}	-17.62 ± 0.15^{cd}
1:3	25%	72.7 ± 2.67^{a}	267.0 ± 36.10^{d}	21.2 ± 2.83^{d}	6.7 ± 0.31^{a}	55.91 ± 0.03^{cd}	1.70 ± 0.04^{c}	-16.82 ± 0.04^{de}
	50%	72.0 ± 2.00^{a}	219.9 ± 8.42^{e}	17.3 ± 0.66^{e}	6.8 ± 0.09^{a}	58.26 ± 0.72^{d}	1.50 ± 0.01^{c}	-15.96 ± 0.01^{e}
	75%	69.0 ± 1.11^{a}	141.9 ± 18.37^{f}	11.1 ± 1.44^{f}	6.7 ± 0.32^{a}	58.36 ± 5.46^{d}	0.40 ± 0.12^{e}	-15.31 ± 0.12^{e}

*Means within a column with different letters are significantly different ($p \leq 0.05$).

Concerning the β-CD concentration, it also had negatively influenced on EE as well. For instance, regard to 1:1 ratio, when β-CD concentration increased from 25 to 50%, the anthocyanin content declined from 1126.0 to 690.8 mg/100 g of dry matter content, or approximately 34% EE; while decreasing from 50 to 25% of BCD concentration, % EE was reduced by 50% or approximately 369.35 mg/100 g of dry matter content. Of these, there might be some interaction between the anthocyanin and β-CD that led to the decline of % EE in the presence of β-CD. It has been found that β-CD was favored to interact with the colorless chalcone form, and it also catalyzed the structural transformation of callistephin (pelargonidin-3-O-glucoside) and chrysanthemin (cyanidin-3-O-glucoside), shifting the pigment hydration equilibrium toward the formation of more colorless anthocyanin. Higher concentration of β-CD induced greater effect (Dangles *et al.*, 1992; Fernandes *et al.*, 2013). Moreover, Frenandes *et al.* (2014) used ^{1}H NMR spectra to observe anthocyanin betacyclodextrin complex and described that there was a disruption of self-association of anthocyanin structure, which brought about the decrease in concentration.

The highest % EE among all 9 samples is the formula that has least matrix proportion and least β-CD in the mixture (ratio of the extract to the wall material containing 25% BCD concentration). It was shown that the same portion of core and wall material was sufficient to trap the anthocyanins of the butterfly extract and provided the highest EE of 88.41%. Besides, the effect of high matrix portion and high β-CD concentration made EE lessen to 11.14%.

3. Some chemical and physical of properties of encapsulated butterfly pea flower anthocyanin

3.1 Moisture content

The moisture content of the encapsulated powder is presented in Table 6. It varied from 6.72 - 8.0% which significantly influenced by the portion of core and matrix ($p \leq 0.05$), whereas there was no effect from the concentration of β-CD and its correlation with proportion of matrix ($p > 0.05$). The greater proportion of wall materials provided lower moisture content. Therefore, with the highest ratio of 1:3, it

gave the lowest moisture content of 6.72% for 25% and 50% β-CD and 6.78% for 75% β-CD. The moisture contents of encapsulated powder were not different to encapsulation of butterfly pea flower by spray drying method using HPMC/gelatin as wall materials and freeze drying method (Tantituvanont *et al.*, 2008); maltodextrin/maltodextrin and algenate/algenate (Hamzah *et al.*, 2013). Their moisture contents ranged from 5.5 - 8.3% and 6.5 – 7.2%, respectively. However, vacuum oven drying method provided lower moisture content (3.5 – 5.7%) (Hamzah *et al.*, 2013).

3.2 Color charactieristics

The color of encapsulated powder was measured by using HunterLab Model, which gives the result of three parameters including L* and two coordinates a* and b*. L* refers as the lightness that minimum value representing black is 0 and maximum value representing white is 100. Positive a* value indicates the direction of redness whereas negative a* value indicates greenness. For b*, yellowness is in the negative range and blueness is in the positive range. According to the result of all three parameters in Table 6, they were influenced by both wall material portion and β-CD quantity in the mixture ($p \leq 0.05$). The portion made the difference in L* providing 1:2 and 1:3 ratio lighter than 1:1, whereas the increased β-CD to 50 and 75% significantly yielded higher L* value. It was possible that the formula containing high quantity of wall material was lighter due to limited extract for trapping and well distribution. In addition, the interaction of anthocyanin β-CD complex did not only affect on the anthocyanin content trapped in the molecules but also the fading of the color. Lewis and Walker (1995) reported the decrease of anthocyanin color in both pH 2 and 4 in the presence of β-CD and the effect was increased with the increased concentration, whereas Dangles *et al.* (1992) reported the fading color of callistephin due to β-CD as the anti-copigment effect.

In accordance to the positive a* and negative b* shown in Table 6, all encapsulated powder samples exhibited the color ranging from purplish blue to dark slate blue. The variation occurred due to ratio of matrix and β-CD concentration, which was believed to cause by the same phenomena as L*. The high amount of

matrix had less extract to be trapped in the molecules, whereas the complexation with anthocyanin changed the blue color of butterfly pea to colorless.

From Figure 5, it can be seen that the color of butterfly pea samples was in corresponding to the result of L*, a*, and b*. The color faded down from dark blue to light slate blue when matrix portion increased and β-CD increased.

Based on all qualities, the formulation that had a ratio of core to wall material at 1:1 and 25% β-CD was the best condition. It encapsulated the highest anthocyanin content in the powder, with 88.4 % EE, and provided the dark slate blue with less color fading than other samples.

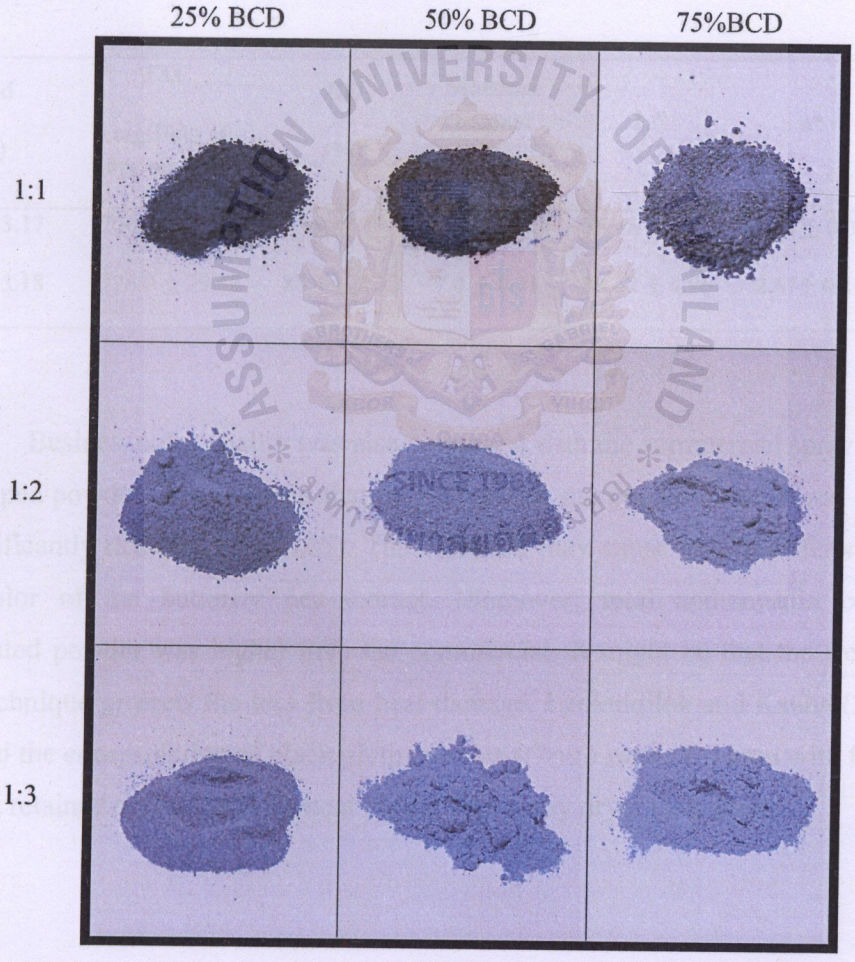


Figure 5: Encapsulated powders of butterfly pea flower extract using the mixture of maltodextrin and β-cyclodextrin and freeze drying method.

Comparing to control (0% β-CD and the ratio of core and matrix as 1:1), it was found that there were no significant differences on all attributes ($p > 0.05$), shown in Table 7. Although β-CD may cause the color diminishing effect or decreasing anthocyanin content, adding 25% β-CD does not remarkably affect the qualities of encapsulated powder. The complexation might also lead to the anthocyanin stabilization (Fernandes *et al.*, 2013).

Table 7: Some chemical and physical properties of encapsulated butterfly pea extract produced by using maltodextrin alone (control) and the mixture of maltodextrin and β-clyclodextrin

Attributes	Yield (%)	TAC (mg/100g of dry matter)	EE (%)	Moisture content (%)	L*	a*	b*
Control	46.8 ± 3.17	1206.4 ± 28.01	93.9 ± 2.20	7.5 ± 0.27	35.48 ± 2.97	3.10 ± 0.30	-21.28 ± 0.34
Sample	47.3 ± 3.18	1126.0 ± 29.58	88.4 ± 2.32	7.4 ± 0.31	38.62 ± 4.96	2.67± 0.10	-21.07 ± 0.79

Besides, color quality was also compared with the commercial spray dried butterfly pea powder. It was found that only a* value, the purple shade of red color, was significantly different ($p \geq 0.05$). The variation may cause by the difference in initial color of the butterfly pea extract. Moreover, total anthocyanin of the encapsulated powder was higher than the commercial. It might be that the freezing drying technique protects the loss from heat damage. Laokuldilok and Kanha (2015) also found the encapsulation of black glutinous rice (*Oryza sativa* L.) bran with freeze dryer was retained anthocyanin content higher than spray dryer technique.

Table 8: Color property of butterfly pea flower extract produced by using spray drying/maltodextrin (commercial) and freeze drying/maltodetrin-β-CD (encapsulated powder)

Attribute	TAC	L*	a*	b*
Commercial	945.7 ± 17.74	30.38 ± 0.21	4.88 ± 0.27	-18.11 ± 0.45
Sample	1126.0 ± 29.58	38.62 ± 4.96	2.67 ± 0.10*	-21.07 ± 0.79

* indicates means within a column are significantly different ($p \leq 0.05$).

4. Stability test

Stability of the microencapsulated butterfly pea extracts was evaluated during 5 weeks in three different temperatures (35, 45, and 55°C) and three different water activity levels (0.53, 0.64, and 0.75). In each week the samples were taken and analyzed. Table 9 summarized the results including half-life and all other parameters of the 5-week of the stability test.

For half-life, since many researches supported that the degradation of anthocyanin during the storage is the first order reaction (Cemeroglu *et al.*, 1994; Ma *et al.*, 2012; Moldovan *et al.*, 2012); therefore, the determination of half-life of encapsulated butterfly pea flower extract was based on the first order reaction. According to table 9, it was noticed that the encapsulated powder had half-life between 4.51-7.93 weeks, which was mainly influenced by temperature and its correlation with water activity ($p \leq 0.05$). For example, the half-life of encapsulated powder stored at 55°C/a_w 0.53 was less than that of at 35°C and 45°C about 2 weeks. Moreover, the interaction effect of water activity and temperature provided the longest half-life of temperature of 55°C at a_w 0.75 and decreasing a_w reduced the storage time. Jimenez *et al.* (2012) reported that the reduction in water activity from 0.99 to 0.34 at high temperature (100 - 140°C) increased the degradation rate of anthocyanin 4 folds. It resulted from polymerization reaction that favored to occur at low water activity. It is proposed that the same phenomenon might occur at 55°C when stored in a long time as well. Overall, the conditions that provide the highest half-life was at 35°C with a_w 0.53 and 0.75 and at 45°C with aw 0.53 and 0.64.

The changes in anthocyanin retention over 5 week stability test are shown in Figures 6 and 7. Figure 6 shows the effect of temperature on anthocyanin retention over time. It was found that temperature has a negative effect on anthocyanin, and higher heat intensity causes greater effect on its degradation. The influence of this factor has been found to cause the impact and it has been suggested to possibly cause by the destruction of glycosidic bond, which brings about the instability, or heat induced ring opening, converting the pigment to colorless form. Roobha *et al.* (2011) reported that anthocyanin pigments in *Musa acuminata* bract was stable at low temperature and rapidly destructed at 40°C and above, whereas the anthocyanin of grape juice degraded higher with the rise of temperature (Danisman *et al.*, 2015). In contrast, the water activity seemed not to have an influence on the retention as shown in Figure 7. Trends of three water activity levels were similar; however, the interaction of water activity and temperature was found. The anthocyanin content at 45°C and a_w 0.75 decreased more sharply than other two water activity levels. Therefore, it could be concluded that temperature affected on the retention of anthocyanin and increasing water activity up to 0.75 caused the greater destruction rate at 45°C. Moreover, Table 9 indicated that the highest anthocyanin retention conditions were 35°C with a_w 0.53 and 0.75; and at 45°C with a_w 0.53 and 0.64, which were similar to half-life results.

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Table 9: Degradation kinetic of anthocyanin degradation of encapsulated powder and color quality evaluation

Temperature (°C)	a_w	1st order reaction			L*	a*	b*	ΔE	% Moisture content
		$t_{1/2}$ (weeks)	Reaction constant (k)	Correlation coefficient (R^2)					
35	0.53	6.59^{abc*}	0.1052 ± 0.0002	0.9677	61.7 ± 0.53^{ab}	33.11 ± 1.20^{a}	2.13 ± 0.51^{a}	-12.89 ± 1.60^{bc}	12.8 ± 0.50^{c}
	0.64	5.32^{bcd}	0.1304 ± 0.0002	0.9557	52.3 ± 1.91^{bc}	36.67 ± 0.72^{ab}	1.60 ± 0.61^{d}	-11.99 ± 0.99^{d}	11.9 ± 0.28^{c}
	0.75	6.32^{abc}	0.1109 ± 0.0002	0.8672	55.6 ± 5.99^{abc}	37.94 ± 0.17^{bc}	1.63 ± 0.18^{cd}	-12.65 ± 0.28^{c}	11.9 ± 0.09^{c}
45	0.53	6.76^{ab}	0.1026 ± 0.0028	0.9548	60.8 ± 0.31^{ab}	38.12 ± 1.30^{bc}	1.86 ± 0.28^{bc}	-12.82 ± 0.55^{bc}	8.4 ± 0.30^{b}
	0.64	7.93^{a}	0.0881 ± 0.0110	0.9716	65.4 ± 2.88^{a}	39.35 ± 1.58^{bcd}	1.83 ± 0.05^{cd}	-13.33 ± 0.11^{ab}	8.0 ± 0.02^{ab}
	0.75	4.95^{cd}	0.1405 ± 0.0101	0.9519	50.9 ± 5.90^{bc}	36.67 ± 0.30^{ab}	2.08 ± 0.17^{ab}	-13.67 ± 0.50^{a}	8.5 ± 1.17^{b}
55	0.53	4.51^{d}	0.1537 ± 0.0005	0.9103	46.2 ± 2.26^{c}	40.71 ± 0.39^{cd}	0.01 ± 0.78^{e}	-0.70 ± 0.70^{g}	7.4 ± 0.68^{ab}
	0.64	4.85^{cd}	0.1525 ± 0.0539	0.9178	45.8 ± 11.39^{c}	43.01 ± 3.47^{d}	-0.02 ± 0.17^{e}	-3.85 ± 0.91^{e}	6.9 ± 0.40^{a}
	0.75	5.68^{bcd}	0.1220 ± 0.0046	0.9208	50.7 ± 0.45^{bc}	40.37 ± 0.30^{bcd}	0.11 ± 0.46^{e}	-1.41 ± 0.34^{f}	7.4 ± 0.07^{ab}

*Means within a column with different letters are significantly different ($p \leq 0.05$).

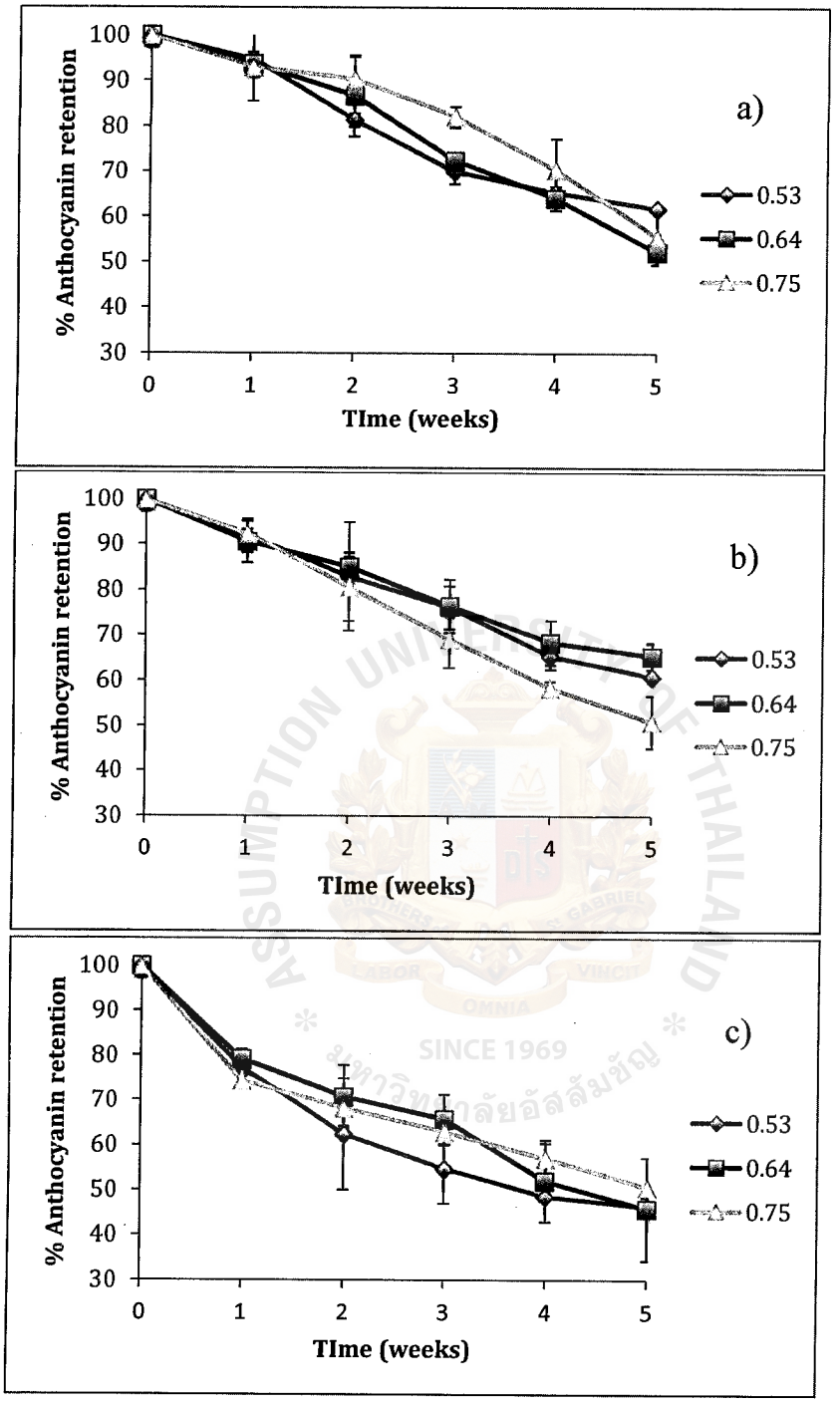

Figure 6: The effect of temperature on anthocyanin retention during 5 week stability test (a) 35°C, (b) 45°C, and (c) 55°C

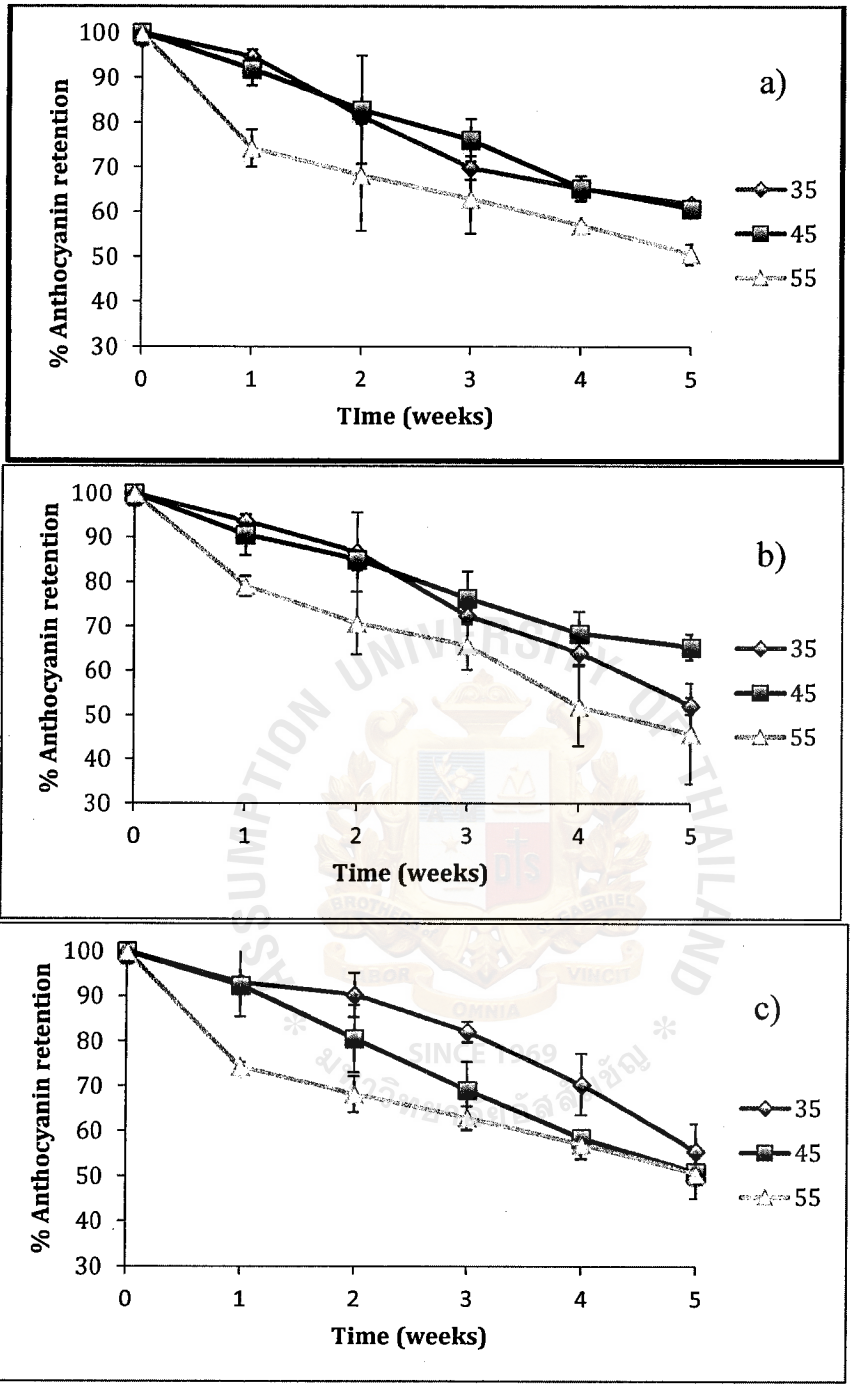

Figure 7: The effect of water activity on anthocyanin retention during 5 week stability test (a) 0.53, (b) 0.64, and (c) 0.75

The color quality was monitored by three parameters (L*, a*, and b*) whereby the average initial color attributes were L* = 38.14, a* = 2.43, and b* = -17.80. The impact of temperature on the lightness (L*) can be observed in Figure 8. It shows that each temperature has a marginal difference in trend of lightness change. At 35°C L* decreased until week 4 and rised up in week 5 whereas at 45°C the increasing of L* started at week 3. For 55°C, L* was slightly steady at the beginning except for a_w 0.53 that L* moderately declined. However, in the last three weeks, the lightness of all samples hiked up. Moreover, comparing the increasing and decreasing ranges of L*, it was found that lower temperature tended to give lower L* while higher temperature gave higher L*. In Figure 9, it can be observed that water activity did not cause the effect on the change of L* value but it had a particular interaction in reducing of L* at a_w 0.75/35°C (Figure 9c). Therefore, the temperature was positive correlated with the lightness, and some interaction with a_w might cause increasing and decreasing of L*. Accordingly, L* of the fifth week also elaborated the effect of the factors.

a* value as well as b* value was reduced during 5 weeks, which indicated the degradation of anthocyanin content and the loss of encapsulated powder's blue color. a* was influenced by temperature as seen in Figure 10. When the temperature increased, a* tended to reduce markedly. Especially at 55°C, a* declined dramatically within the first week and continue reducing steadily down to 0.01-0.02, and 0.11 at a_w 0.53, 0.64, and 0.75, respectively in the fifth week. For water activity, it negatively affected to a* only at 35°C whereas other temperatures caused no effect as shown in Figure 11. Regarding to b* value, in Figure 12, the high temperature (55°C) caused the reduction of b* value vastly, giving the lowest b* at the end whereas the decline of b* value in other two temperatures were slightly different from each other. On the other hand, the water activity caused the difference in change of b* over time at 55°C (Figure 13).

ΔE was calculated to identify the total color difference among all nine conditions. The data was reported in Table 9 indicating that the lowest change of color was found at 35°C/a_w 0.75 and 45°C at all a_w.

The final moisture contents of the encapsulated powders of all 9 conditions were reported in Table 9, which varied from 6.5–12.8%. There was the influence of temperature, which can be observed in Figure 14. At 35°C, the moisture content

sharply increased through 5 weeks at all water activity levels whereas at 45°C the moisture content increased until week 3 and then slightly decreased. For 55°C, the moisture content increased highly in the first week but afterwards it gradually decreased. The change in moisture content might be due to the porous structure of encapsulated powder created by freeze drying. The samples firstly absorbed moisture when firstly exposed to high humidity. Then if the temperature was low, the moisture would continually absorb, but if high, the encapsulated powder might be dried, leading to a reduction of moisture content. Moreover, water activity was found not to have the influence on moisture content as shown in Figure 15.

Based on all parameters, the temperature was negatively influenced on half-life, anthocyanin retention, and color quality of the encapsulated powder, whereas the water activity caused some effects corresponding to the temperature. At 35oC and 45°C with low water activity, the conditions provided high half-life and anthocyanin retention; however, at 45°C the color was less changed over time. It was proposed that there were two mechanisms involved during 5 week storage. Firstly, the polymerization occurred as the evidence of decreased L*, decreased b*, and increased a*. It might due to condensation of pigment with other phenolic compounds, the residual enzyme activity, and oxidation, which in turn changed the encapsulated powder's color. Anthocyanins in processed blueberries products and raspberry pulp were envisaged to polymerize during storage (Brownmiller *et al.*, 2008; Ochoa *et al.*, 1999). The second reaction might be the destruction of pigment due to heat, which converted anthocyanins to chalcone form. However, the compound generally would turn to be brown as claimed by (Markakis *et al.*, 1957; Markakis, 1982), but obviously in this case, because of the presence of β-CD, the degraded anthocyanin possibly complexed with the cyclic oligosaccharide compound, decreasing the lightness. Moreover, the lower temperature was favored polymerization and when the storage time increased, the heat was accumulated and caused the anthocyanin hydrolysis. Besides, at high temperature, the evolvement of chalcone might occur faster so that the increasing of lightness at 45°C underwent before those at 35°C. At 55°C the steady change of L* was observed although a* and b* decreased rapidly.

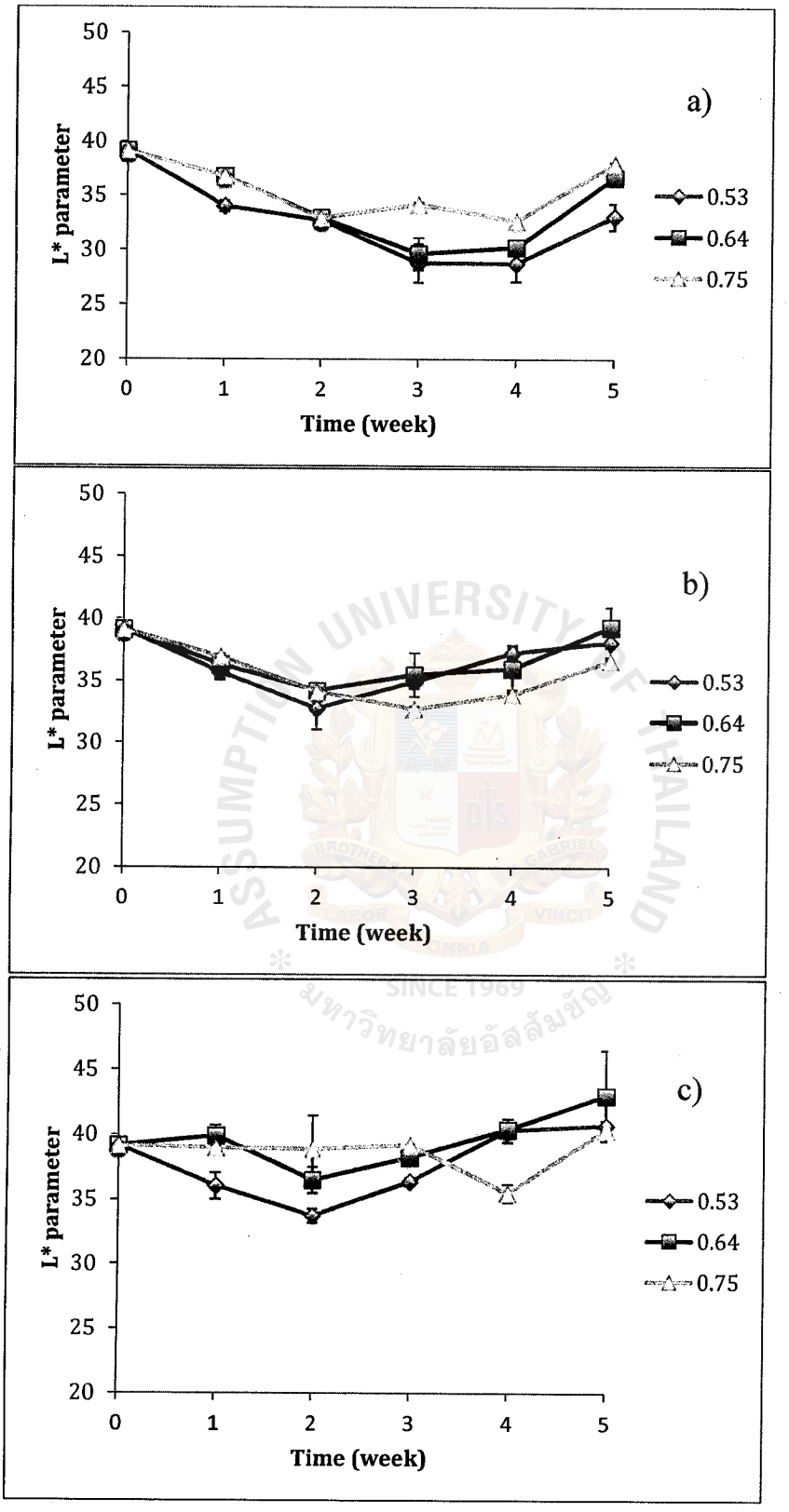

Figure 8: The effect of temperature on L* value (Lightness) during 5 week stability test (a) 35°C, (b) 45°C, and (c) 55°C

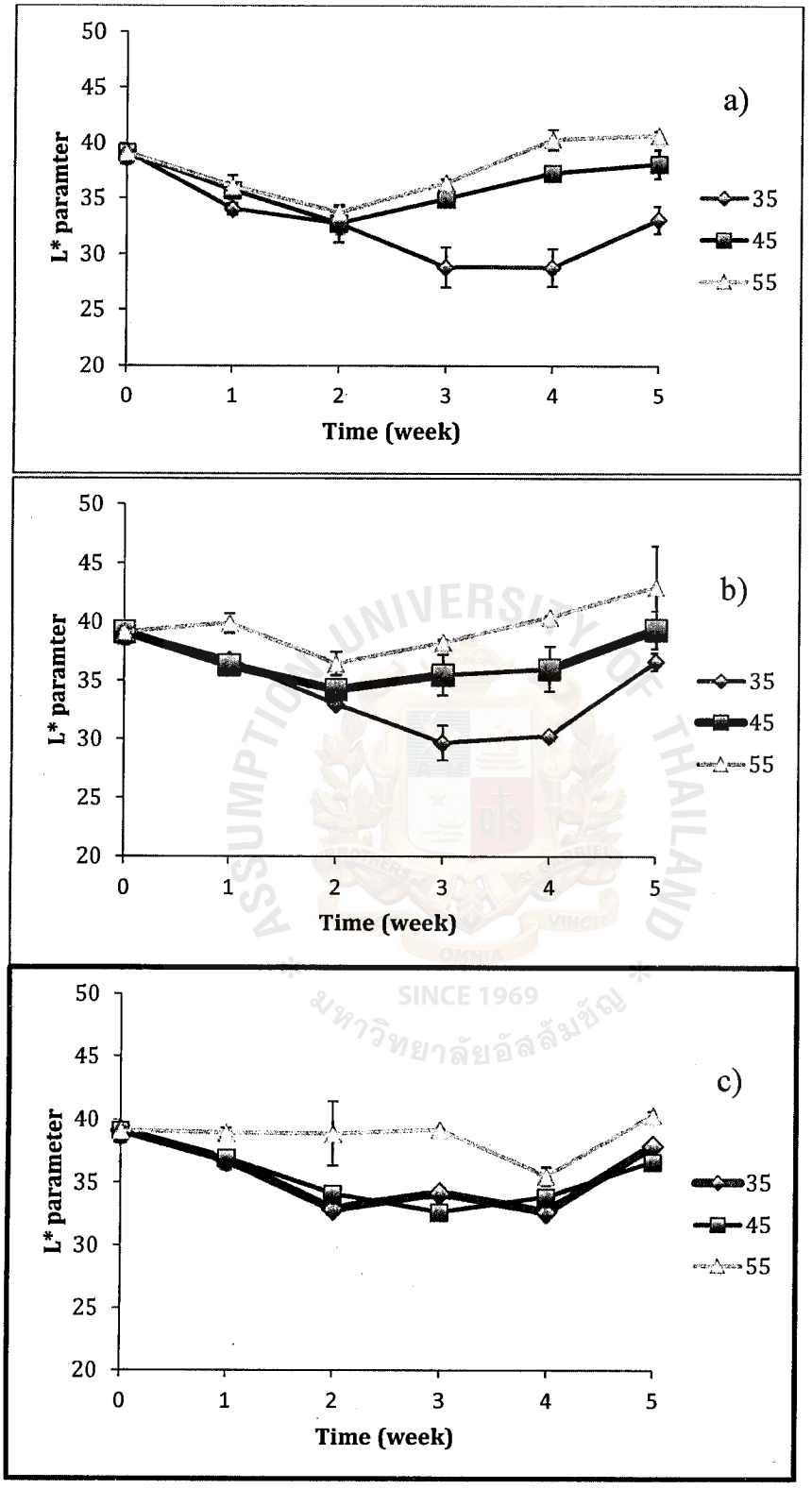

Figure 9: The effect of water activity on L* value (Lightness) during 5 week stability test (a) 0.53, (b) 0.64, and (c) 0.75

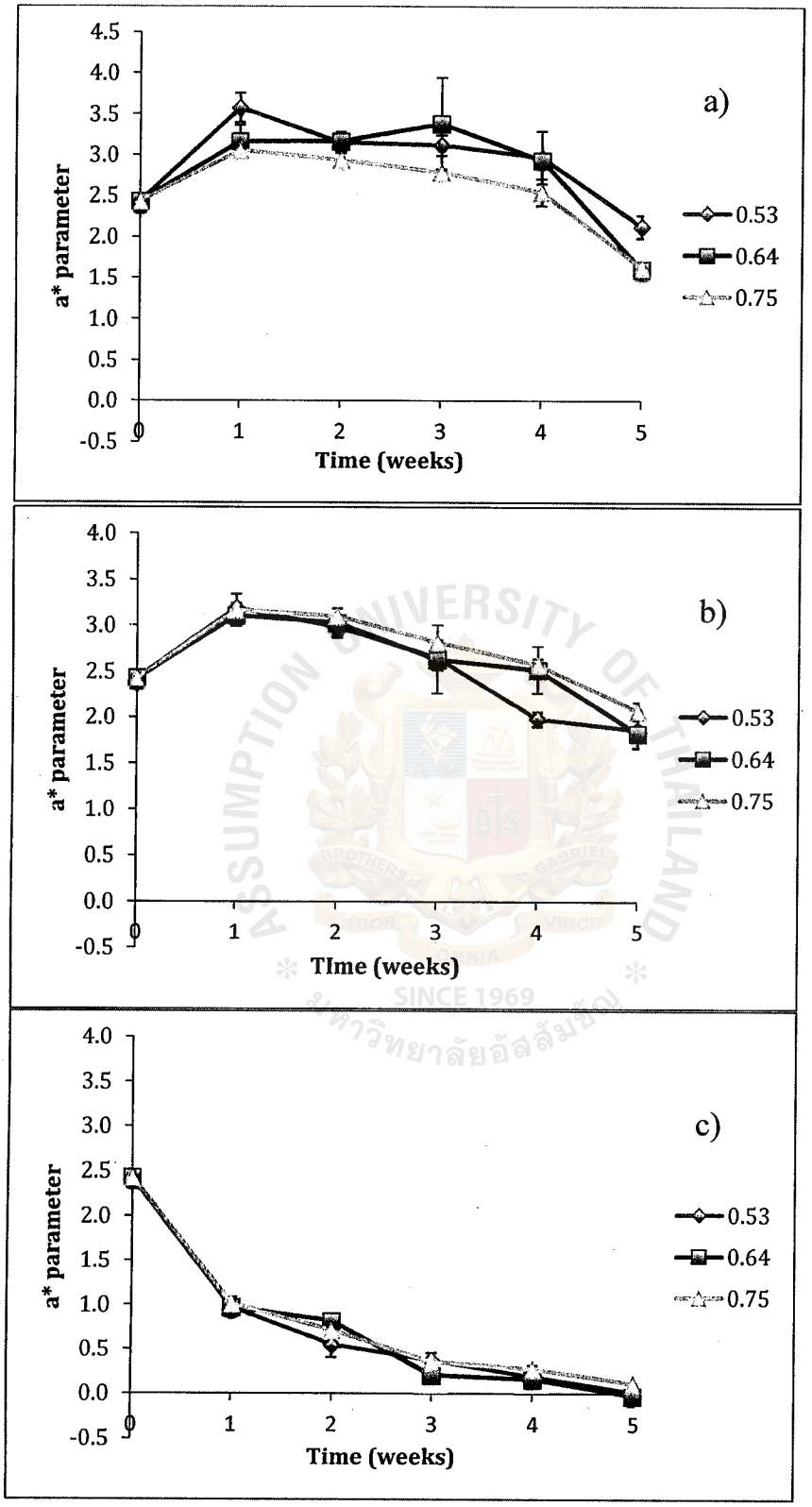

Figure 10: The effect of temperature on a* (redness) value during 5 week stability test (a) 35°C, (b) 45°C, and (c) 55°C

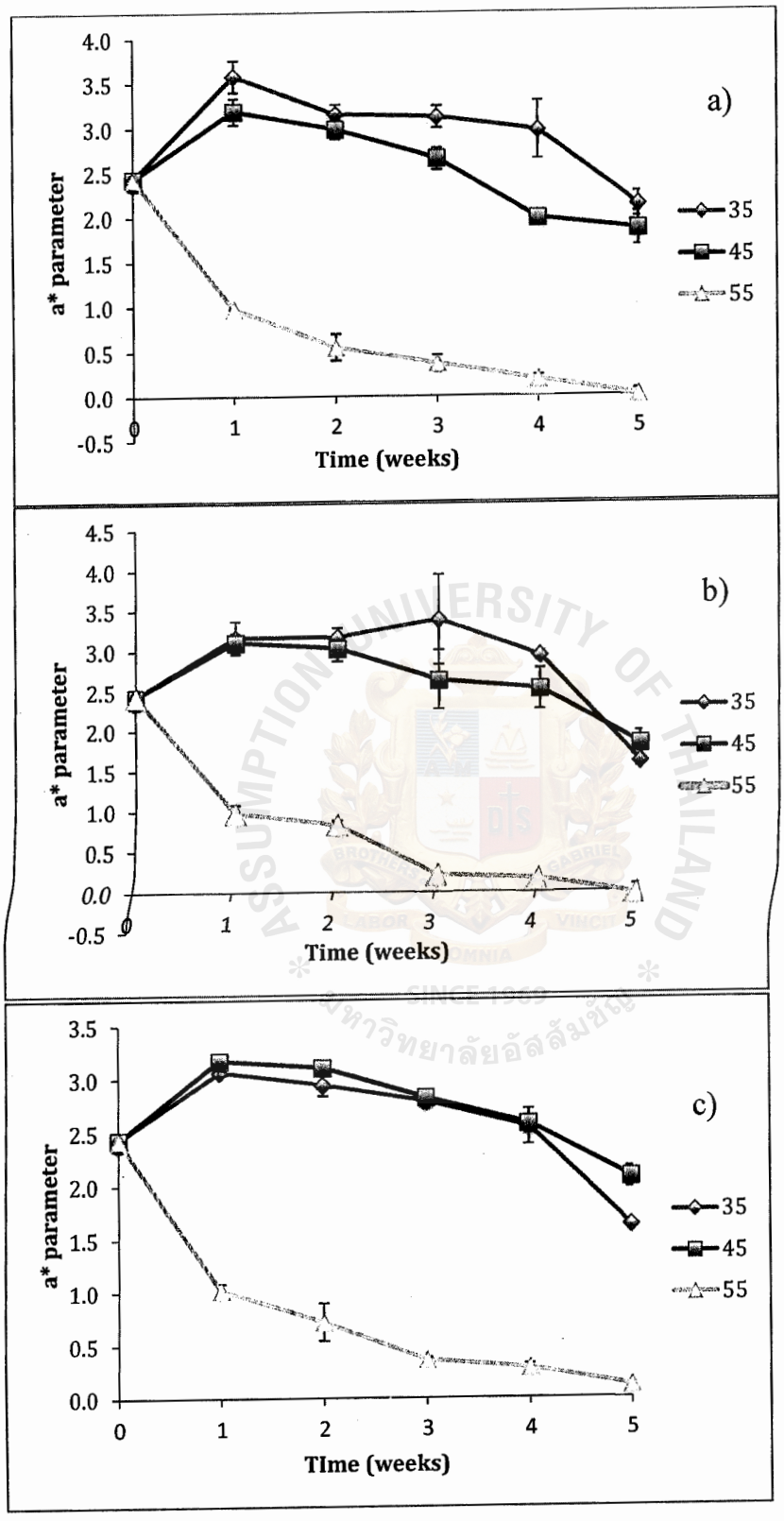

Figure 11: The effect of water activity on a* (redness) value during 5 week stability test (a) 0.53, (b) 0.64, and (c) 0.75

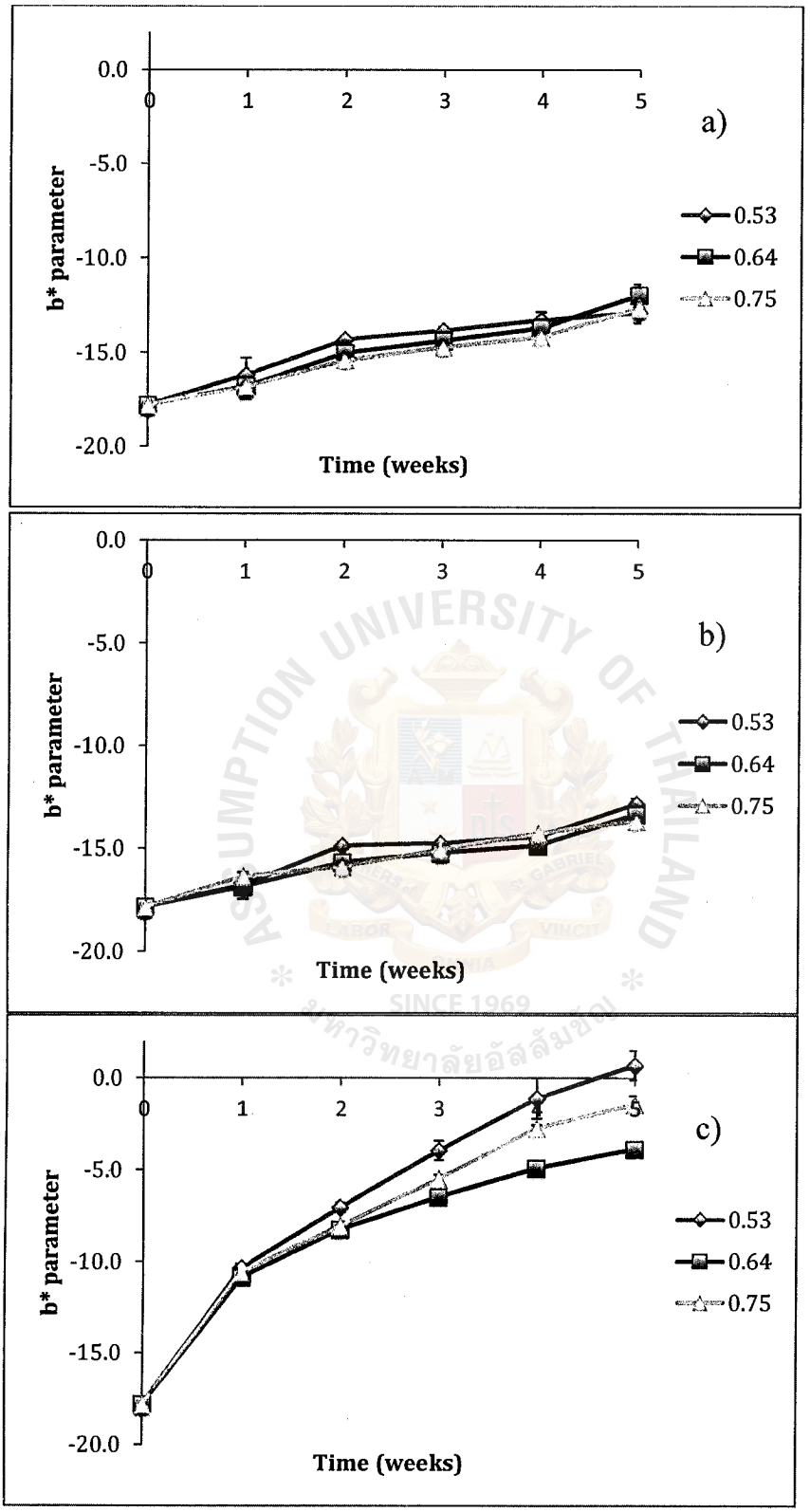

Figure 12: The effect of temperature on b* (blueness) value during 5 week stability test (a) 35°C, (b) 45°C, and (c) 55°C

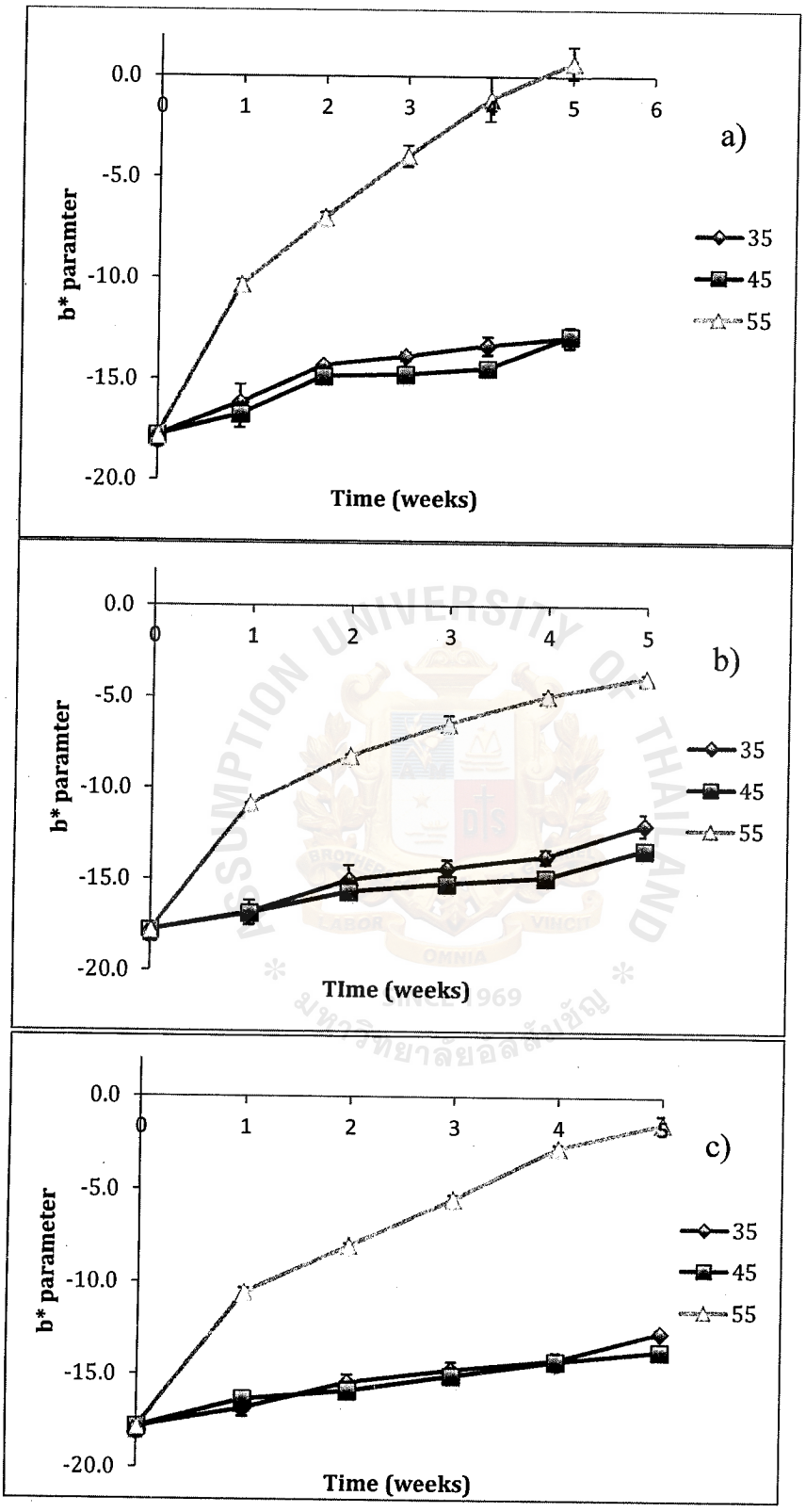

Figure 13: The effect of water activity on b* (blueness) during 5 week stability test (a) 0.53, (b) 0.64, and (c) 0.75

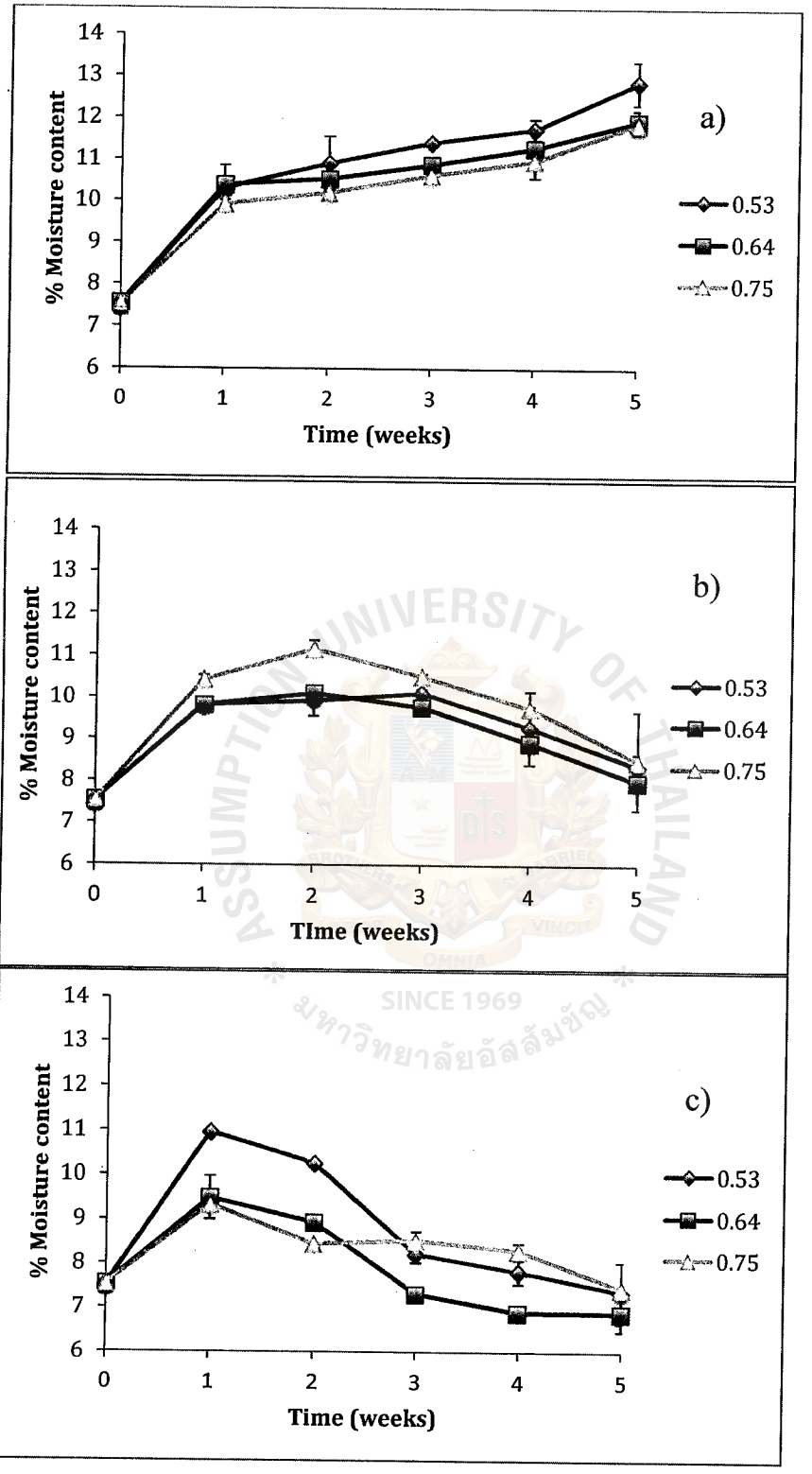

Figure 14: The effect of temperature on moisture content during 5 week stability test (a) 35°C, (b) 45°C, and (c) 55°C

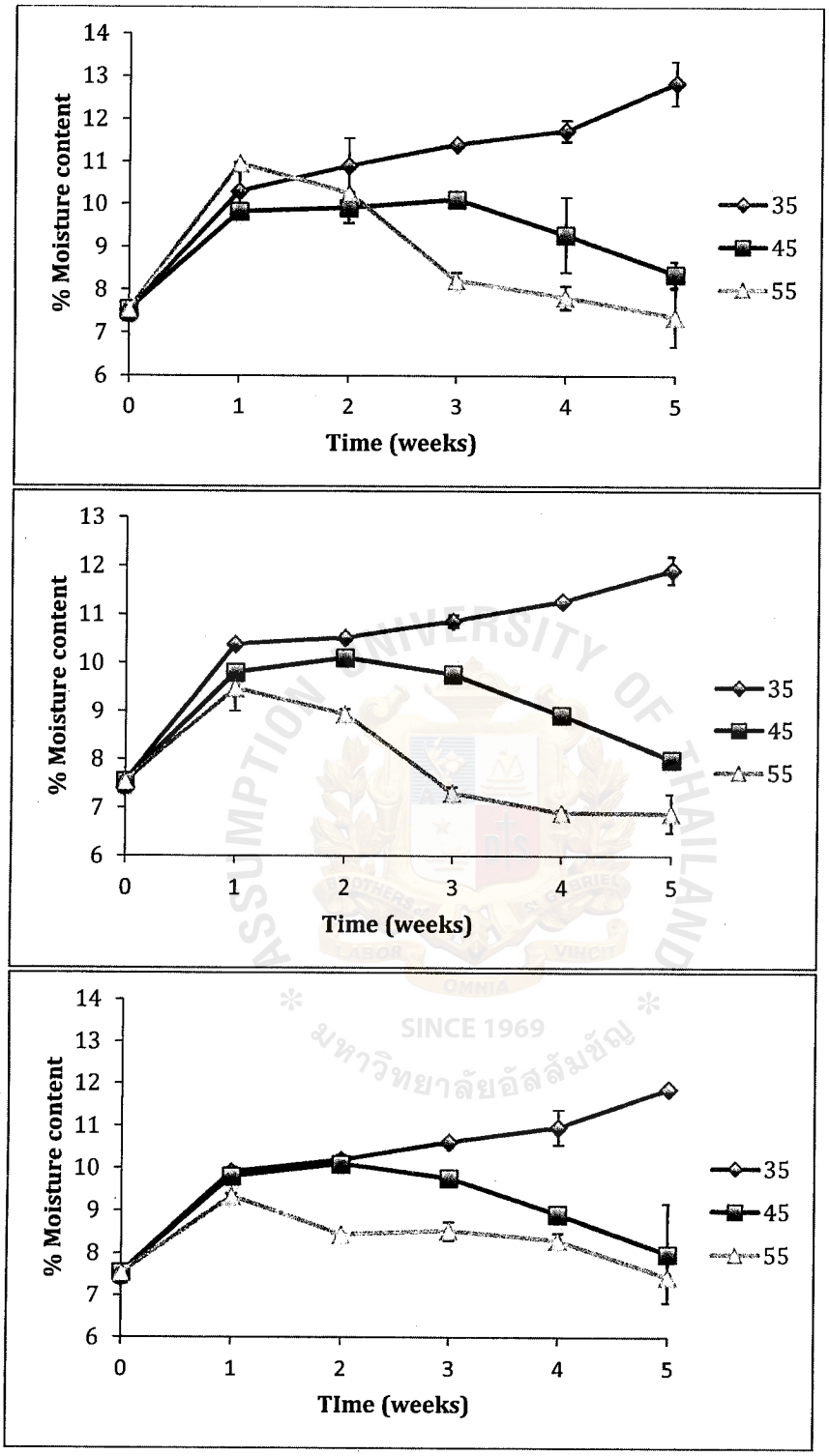

Figure 15: The effect of water activity on moisture content during 5 week stability test (a) 0.53, (b) 0.64, and (c) 0.75

CONCLUSION

Encapsulation of butterfly pea flower extract by using the ratio of the extract to the supporting materials (75% maltodextrin and 25% β-cyclodextrin) as 1:1 and freeze drying method could retain the highest anthocyanin content as high as 88.3% with good color profile (L* = 38.62, a* = 2.67, and b* = -21.07). An increasing of the supporting material proportion and β-cyclodextrin tended to cause the color fading and reduced the anthocyanin content in the powder. The temperature and its interaction with water activity gave significant effect on the first order kinetic reaction of anthocyanin during the storage. The most suitable condition of storage was 45°C with aw of 0.53 and 0.64, providing the kinetic constant (k) of 0.1026 and 0.0881; and correlation coefficient (R2) of 0.9806 and 0.9906 with half-life of 6.75 and 7.88 weeks for aw of 0.53 and 0.64, respectively.

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APPENDIX

SPSS analysis

Appendix Table 1: Moisture content of encapsulated powder

Dependent Variable: Moisture content

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.468[a]	8	.433	7.490	.003
Intercept	956.848	1	956.848	16532.703	.000
BCD	.375	2	.188	3.243	.087
Matrix	2.944	2	1.472	25.436	.000*
BCD * Matrix	.148	4	.037	.640	.647
Error	.521	9	.058		
Total	960.836	18			
Corrected Total	3.989	17			

a. R Squared = .869 (Adjusted R Squared = .753)

* The mean difference is significant difference at 95% confidential level

Appendix Table 2: Yield of encapsulated powder

Dependent Variable: %Yield

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1530.693[a]	8	191.337	53.952	.000
Intercept	67328.598	1	67328.598	18985.099	.000
BCD	14.257	2	7.128	2.010	.190
Matrix	1497.039	2	748.520	211.065	.000*

BCD * Matrix	19.397	4	4.849	1.367	.319
Error	31.918	9	3.546		
Total	68891.208	18			
Corrected Total	1562.610	17			

a. R Squared = .980 (Adjusted R Squared = .961)

* The mean difference is significant difference at 95% confidential level

Appendix Table 3: Total anthocyanin content of encapsulated powder

Dependent Variable: Total anthocyanin content

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1532781.211[a]	8	191597.651	564.192	.000
Intercept	3054236.696	1	3054236.696	8993.727	.000
BCD	378570.021	2	189285.010	557.382	.000*
Matrix	852347.640	2	426173.820	1254.942	.000*
BCD * Matrix	301863.550	4	75465.887	222.222	.000*
Error	3056.367	9	339.596		
Total	4590074.274	18			
Corrected Total	1535837.578	17			

a. R Squared = .998 (Adjusted R Squared = .996)

* The mean difference is significant difference at 95% confidential level

Appendix Table 4: Encapsulation efficiency of encapsulated powder

Dependent Variable: %Encapsulation efficiency

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	9448.969[a]	8	1181.121	564.187	.000
Intercept	18828.102	1	18828.102	8993.639	.000
BCD	2333.729	2	1166.864	557.377	.000*
Matrix	5254.372	2	2627.186	1254.931	.000*
BCD * Matrix	1860.868	4	465.217	222.221	.000*
Error	18.841	9	2.093		
Total	28295.913	18			
Corrected Total	9467.810	17			

a. R Squared = .998 (Adjusted R Squared = .996)

* The mean difference is significant difference at 95% confidential level

Appendix Table 5: Lightness (L*) of encapsulated powder

Dependent Variable: L*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	758.740[a]	8	94.842	9.709	.001
Intercept	49095.511	1	49095.511	5025.771	.000
BCD	152.643	2	76.322	7.813	.011*
Matrix	562.137	2	281.069	28.772	.000*
BCD * Matrix	43.959	4	10.990	1.125	.403
Error	87.919	9	9.769		
Total	49942.170	18			

Corrected Total	846.659	17			

a. R Squared = .896 (Adjusted R Squared = .804)

* The mean difference is significant difference at 95% confidential level

Appendix Table 6: a value (a*) of encapsulated powder

Dependent Variable: a*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	11.743[a]	8	1.468	52.681	.000
Intercept	41.365	1	41.365	1484.586	.000
BCD	9.049	2	4.524	162.379	.000*
Matrix	2.298	2	1.149	41.241	.000*
BCD * Matrix	.396	4	.099	3.552	.053
Error	.251	9	.028		
Total	53.358	18			
Corrected Total	11.994	17			

a. R Squared = .979 (Adjusted R Squared = .961)

* The mean difference is significant difference at 95% confidential level

Appendix Table 7: b value (b*) of encapsulated powder

Dependent Variable: b*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	56.447[a]	8	7.056	16.155	.000
Intercept	5870.815	1	5870.815	13441.941	.000
BCD	11.955	2	5.977	13.686	.002*

Matrix	42.656	2	21.328	48.833	.000*
BCD * Matrix	1.837	4	.459	1.051	.433
Error	3.931	9	.437		
Total	5931.193	18			
Corrected Total	60.378	17			

a. R Squared = .935 (Adjusted R Squared = .877)

* The mean difference is significant difference at 95% confidential level

Appendix Table 8: The one sample t test of moisture content compared with the control

One-Sample Test

	Test Value = 7.5011					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
% Moisture content	-.328	1	.798	-.0711000	-2.824535	2.682335

Appendix Table 9: The one sample t test of total anthocyanin content compared with the control

One-Sample Test

	Test Value = 1206.4466					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
TAC	-3.848	1	.162	-80.4806500	-346.258240	185.296940

Appendix Table 10: The one sample t test of % encapsulation efficiency compared with the control

One-Sample Test

	Test Value = 93.9306					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
% Encapsulation efficiency	-3.364	1	.184	-5.5255000	-26.392900	15.341900

Appendix Table 11: The one sample t test of yield compared with the control

One-Sample Test

	Test Value = 46.75					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Yield	.249	1	.845	.5597000	-28.036884	29.156284

Appendix Table 12: The one sample t test of L* (Lightness) compared with the control

One-Sample Test

	Test Value = 35.4767					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
L*value	.895	1	.535	3.1383000	-41.396948	47.673548

Appendix Table 13: The one sample t test of a* (redness) compared with the control

One-Sample Test

	Test Value = 3.1033					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
a*value	-6.190	1	.102	-.4333000	-1.322734	.456134

Appendix Table 14: The one sample t test of b* (blueness) compared with the control

One-Sample Test

	Test Value = -21.2833					
	t	df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
b*value	.375	1	.772	.2100000	-6.905475	7.325475

Appendix Table 15: The one sample t test of total anthocyanin content compared with the commercial powder

One-Sample Test

	Test Value = 945.6597					
	t	df	Sig. (2-tailed)	Mean Difference	99% Confidence Interval of the Difference	
					Lower	Upper
TAC	8.620	1	.074	180.3062500	-1151.211353	1511.823853

Appendix Table 16: The one sample t test of L* (Lightness) compared with the commercial powder

One-Sample Test

	Test Value = 30.38					
	t	df	Sig. (2-tailed)	Mean Difference	99% Confidence Interval of the Difference	
					Lower	Upper
L	2.350	1	.256	8.2350000	-214.881878	231.351878

Appendix Table 17: The one sample t test of a* (redness) compared with the commercial powder

One-Sample Test

	Test Value = 4.88					
	t	df	Sig. (2-tailed)	Mean Difference	99% Confidence Interval of the Difference	
					Lower	Upper
a	-31.571	1	.020	-2.2100000	-6.665972	2.245972

Appendix Table 18: The one sample t test of b* (blueness) compared with the commercial powder

One-Sample Test

	Test Value = -18.11					
	t	df	Sig. (2-tailed)	Mean Difference	99% Confidence Interval of the Difference	
					Lower	Upper

b	-5.292	1	.119	-2.9633000	-38.611075	32.684475

Appendix Table 19: half life of encapsulated powder

Dependent Variable: Half life of First order

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	19.684[a]	8	2.460	4.376	.020
Intercept	621.901	1	621.901	1106.081	.000
aw	.494	2	.247	.439	.658
Temp	7.387	2	3.693	6.569	.017*
aw * Temp	11.804	4	2.951	5.248	.018*
Error	5.060	9	.562		
Total	646.645	18			
Corrected Total	24.744	17			

a. R Squared = .795 (Adjusted R Squared = .614)

* The mean difference is significant difference at 95% confidential level

Appendix Table 20: % Anthocyanin retention of fifth week

Dependent Variable: Total anthocyanin content of fifth week

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	776.040[a]	8	97.005	4.004	.027
Intercept	53226.605	1	53226.605	2197.158	.000
aw	44.888	2	22.444	.926	.431
Temp	436.262	2	218.131	9.004	.007*

aw * Temp	294.891	4	73.723	3.043	.076
Error	218.027	9	24.225		
Total	54220.672	18			
Corrected Total	994.067	17			

a. R Squared = .781 (Adjusted R Squared = .586)

* The mean difference is significant difference at 95% confidential level

Appendix Table 21: L* value of fifth week

Dependent Variable: L* value of fifth week

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	131.127[a]	8	16.391	7.951	.003
Intercept	26594.337	1	26594.337	12900.941	.000
aw	16.827	2	8.413	4.081	.055
Temp	90.630	2	45.315	21.982	.000*
aw * Temp	23.670	4	5.918	2.871	.087
Error	18.553	9	2.061		
Total	26744.017	18			
Corrected Total	149.680	17			

a. R Squared = .876 (Adjusted R Squared = .766)

* The mean difference is significant difference at 95% confidential level

Appendix Table 22: a* value of fifth week

Dependent Variable: a* value of fifth week

Source	Type III Sum of Squares	df	Mean Square	F	Sig.

Corrected Model	13.751[a]	8	1.719	143.896	.000
Intercept	28.033	1	28.033	2346.732	.000
aw	.123	2	.062	5.161	.032*
Temp	13.306	2	6.653	556.931	.000*
aw * Temp	.322	4	.081	6.746	.009*
Error	.108	9	.012		
Total	41.892	18			
Corrected Total	13.859	17			

a. R Squared = .992 (Adjusted R Squared = .985)

* The mean difference is significant difference at 95% confidential level

Appendix Table 23: b* value of fifth week

Dependent Variable: b* value of fifth week

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	541.111[a]	8	67.639	374.319	.000
Intercept	1491.246	1	1491.246	8252.688	.000
aw	5.925	2	2.963	16.396	.001*
Temp	518.813	2	259.406	1435.578	.000*
aw * Temp	16.372	4	4.093	22.651	.000*
Error	1.626	9	.181		
Total	2033.983	18			
Corrected Total	542.737	17			

a. R Squared = .997 (Adjusted R Squared = .994)

* The mean difference is significant difference at 95% confidential level

Appendix Table 23: Total color difference of fifth week

Dependent Variable: Delta E

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	510.973[a]	8	63.872	102.518	.000
Intercept	1588.154	1	1588.154	2549.079	.000
aw	13.123	2	6.562	10.532	.004*
Temp	489.455	2	244.727	392.801	.000*
aw * Temp	8.395	4	2.099	3.369	.060
Error	5.607	9	.623		
Total	2104.734	18			
Corrected Total	516.580	17			

a. R Squared = .989 (Adjusted R Squared = .979)

* The mean difference is significant difference at 95% confidential level

Appendix Table 24: % Moisture content of fifth week

Dependent Variable: Moisture content of fifth week

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	84.522[a]	8	10.565	39.089	.000
Intercept	1538.323	1	1538.323	5691.431	.000
aw	1.046	2	.523	1.936	.200
Temp	82.690	2	41.345	152.967	.000*
aw * Temp	.785	4	.196	.726	.596
Error	2.433	9	.270		
Total	1625.277	18			
Corrected Total	86.954	17			

a. R Squared = .978 (Adjusted R Squared = .958)

* The mean difference is significant difference at 95% confidential level

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