# Microencapsulation of curcumin (*Curcuma longa*) extract by using hydroxylpropyl-$\beta$-cyclodextrin and its application in the pickled green mustard

By

Ms. Anantanuch Intajak

ID. 6119703

Thesis

A master's thesis submitted to the faculty of Biotechnology, Assumption University in partial satisfaction of the requirement for the degree of Master of Science in Biotechnology

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| By: | Ms. Anantanuch Intajak |
| Advisor: | Asst. Prof. Dr. Wunwisa Krasaekoopt |
| Level of Study: | Master of Science |
| Department: | Food Biotechnology |
| Faculty: | Biotechnology |
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(Asst. Prof. Dr. Wunwisa Krasaekoopt)
Advisor, Faculty of Biotechnology

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# Abstract

Curcumin is herb that used as natural colorant in pickled green mustard to improve the product's color. Unfortunately, the yellow color of curcumin is not sTable in the product due to its photodegradation. Therefore, this research was aimed to study the encapsulation of curcumin crude extract by using hydroxypropyl-β-cyclodextrin and kneading method to improve its color stability as well as its application in pickle green mustard as the natural colorant. Curcumin podwer was extracted by using the mixture of ethanol and water with the ratio of ethanol to water as 100:0, 75:25, and 50:50 for 1.5 h. The ratio of ethanol to water as 75:25 provided the highest curcumin content as 62.98%. Then the curcumin crude extract was encapsulated by using HP-β-CD and kneading method with the molecular ratio of curcumin crude extract to HP-β-CD as 1:1, 1:1.5, and 1:2 with the kneading time of 5, 10, 15, and 20 min. The mixture was then dried at 40°C for overnight and then ground into powder. Some properties of encapsulated curcumin powder, such as color release and powder stability, were investigated as well as encapsulation efficiency (EE) and encapsulation yield (EY). The molecular ratio of 1:2 gave the highest EE and EY as 99.26 and 41.08%, respectively. Moreover, the curcumin was retained on the encapsulated powder as 54% under shaking condition for 20 min. Thermal degradation of encapsulated power was found to be zero-order with $R^2$ of 0.901, while photodegradation was found to be the first-order with $R^2$ of 0.964. The color of encapsulated curcumin powder was pH dependence. In addition, the encapsulated curcumin powder was applied as colorant in pickled green mustard and then kept at 55°C for 30 days as accelerated storage condition. Although, there was no significant difference ($p>0.05$) in color due to lower dosage used for microencapsulated powder, pickled green mustard containing encapsulated curcumin powder had better score than control in flavor, texture, and overall liking throughout the storage with product acceptance as 68.89% at the end of accelerated storage test, which was equal to 6 months.

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# Chapter 1

# Introduction

Curcumin (Cur) or Turmeric (*Curcuma longa*) is a major phytopolyphenolic compound derived from turmeric root which is the rhizomes of *Curcuma longa*, a plant in the ginger family (Siri et al., 2014). Curcumin (Cur), a common oriental spice and medicinal herb that has been used in India for thousands of years which gives curry powder its bright yellow color. It is also frequently used in Asian cooking, especially Indian, Pakistani, and Thai (Prasad et al., 2014). Since ancient time, curcumin has been applied in many fields for several proposes because curcumin has a wide range of biological and pharmacological effects, including antioxidant, antitumor, anti-inflammatory, and anticancer properties to use for medicinal purposes. Moreover, curcumin has been used for external body as a treatment of skin diseases, wound healing, and cosmetics to alter skin pigmentation (Suwannateep et al., 2012).

Curcumin also has been commonly applied as a coloring agent in several food industries because of its powerful properties. It has strong in yellow color and unique flavor as a functional food, which is nontoxic to body and legally in Thailand. Moreover, the curcumin is popularly used in many fermented vegetable products as a coloring agent in Thailand, which is following Thai regulation. The pickled green mustard is the product that use curcumin as yellow colorant. However, there is a major problem of this product during long storage, which is fading of yellow color to brown color, contributing to low bioavailability of curcumin. Nevertheless, the main reasons attributing to the low bioavailability are poor solubility and/or dispersibility of curcumin in aqueous phase at acidic pH as well as rapid hydrolytic degradation caused by light, which is called photodegradation. There are various factors that cause the degradation of curcumin including UV light/light, thermal and pH value. To restrain curcumin degradation and loss during processing and storage, thus, microencapsulation technique is implied.

In recent years, several methods have been proposed to enhance stability and bioavailability of curcumin, among them microencapsulation seems to be preferred. Encapsulation is defined as a technology of packaging solids, liquids, or gaseous materials (core material) in miniature, sealed capsules (coating material) that can release their contents under specific conditions (Fang and Bhandari, 2010). In general, it is the inclusion of one thing within another thing which allows them to pass through biological barriers. These are made from a wide array of biocompatible materials that can be used in food and pharmaceutical industries. Therefore, encapsulation is a method that can enhance bioavailability of curcumin and prevent their degradation during processing and storage.

Cyclodextrin molecules are cyclic oligosaccharides having six, seven or eight glucose units linked by α-1,4-glucosidic bonds, called, respectively, α-, β- and γ-Cyclodextrins. The special characteristic of cyclodextrins is the ability to form an inclusion complex, increasing the water solubility of sparingly soluble compounds and can also enhance the stability of flavors to oxygen, heat, or light (Sambasevam et al., 2013). Moreover, there are some alternative to α-, β- and γ-cyclodextrins such as hydroxypropyl-β-cyclodextrin (HP-β-CD) which can extend the physicochemical properties such as improve solubility and increase capacity of inclusion complexation (Challa et al., 2005). So, inclusion complexation using hydroxypropyl-β-cyclodextrin (HP-β-CD) is readily available and widely used for encapsulation due to its simplicity, low cost and nontoxic for oral use. There are many methods to form the inclusion complexation (Gould and Scott, 2005). In this study, kneading or paste method was selected in which core material is mixed with hydroxypropyl-β-cyclodextrin (HP-β-CD) by using shear force.

Thus, the aim of this percent study was to solve the color problem of curcumin in pickled green mustard products by using encapsulation technique with hydroxypropyl-β-cyclodextrin (HP-β-CD) via kneading method. The solubility, stability, the encapsulation yield (EY) as well as encapsulation efficiency (EE) values were also investigated.

# Objectives

1. To study the extraction condition of crude curcumin extract.
2. To study the microencapsulation of crude curcumin extract by using hydroxypropyl-β-cyclodextrin (HP-β-CD) and kneading method.
3. To study the application of microencapsulated curcumin in pickled green mustard as a food colorant.

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# Chapter 2

# Literature review

## 2.1. Curcumin

### 2.1.1. Plant information of curcumin

Turmeric, scientific name of turmeric is *Curcuma longa* (1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione as IUPAC Name), Cur, (Figure. 1), is a member of family Zingiberaceae as a ginger. It is a traditional Indian spice and has been used in traditional medicines for many conditions including skin diseases, wounds, intestinal worms, diarrhea inflammation, breathing problems, rheumatism, serious pain, and fatigue. It is also used as food preservative in India and ancient Egypt since last 6000 years (Tripathi et al, 2010). Turmeric is a plant distributed throughout tropical and subtropical regions of the world, especially in India and Asia countries. It is a native to tropical plant and needs temperatures between 20°C and 30°C, and a considerable amount of annual rainfall to thrive. Characteristic of *C. longa* plant is identifiable by both its tuberous root and the leaves that extend upward from erect, thick, and short stems arising from the root. The plant's leaves are divided, lance-shaped and narrower at each end. The flowers arise from those leaves, and are a pale-yellow color, growing in groupings of three to five. Turmeric root is actually fleshy oblong tuber 2–3 inches (5–10 cm) inches length, and close to 1 inches (2.54 cm) wide. Turmeric root contains a bitter volatile oil, brown coloring matter, gum, starch, calcium chloride, woody fiber and a yellowish coloring material that is known as curcumin as an active compound (Figure 2.1) (Annonymous, 2016). Curcumin is a bioactive polyphenol compound derived from turmeric root, the rhizomes of *C. longa*, which the chemical formula defined $C_{21}H_{20}O_6$. Curcumin comprises curcumin I (curcumin-C, 94%), curcumin II (demethoxycurcumin - DMC, 6%) and curcumin III (bisdemethoxycurcumin - BDMC, 0.3%). Its pKa value is 8.54. It has a melting point at 176–177°C. Rhizomes of *C. longa* or turmeric contain carbohydrates (60–70%), protein (6–8%), essential oil (3–7%), fixed oils

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(5-10%), fiber (2-7%), minerals (3-7%), moisture (13.1%), and pigments known as curcuminoids (2-6%) (Siri et al., 2014).

**Figure 2.1: Turmaric Plant (Anonymous, 2016)**

### 2.1.2. Active compounds in curcumin

Curcuminoids are natural polyphenol compounds derived from turmeric, its primary active ingredients with yellow color that used as food coloring agent, cosmetics colorant and traditional medicine. The curcuminiod are mainly composed of curcumin (C), demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) (Tripathi et al, 2010). Demethoxy and bisdemethoxy derivatives of curcumin have also been isolated from turmeric.

2.1.2.1. Curcumin (C)

Curcumin (Cur) contains two methoxy groups at its ortho position (Figure 2.2). The Molecular weight of curcumin is 368.38 g/mol. Molecular formula is $C_{21}H_{20}O_6$. Curcumin is the major curcuminoid phenolic compound which practically insoluble in water at acidic and natural pH. It is also soluble in alkaline conditions and in oil. Curcumin has high decomposition rate in alkaline conditions and decomposes under exposure to light or occur photodegradation in organic solvent condition. The use of curcumin is limited because of these properties. According to the research, the curcumin could have improved solubility and stability in complex formations, which is one technique in encapsulation methods by using cyclodextrin as a wall material

(Surojanametakul, et al., 2010). Moreover, curcumin provide antioxidant properties and it prevents lipid peroxidation. The antioxidant properties are the result of the double carboxyl groups along with hydroxyl groups (Jovičić et al., 2017).

2.1.2.2. Demethoxycurcumin (DMC)

Demethoxycurcumin (DMC), an analogue of CUR, is one of such compounds. In comparison with CUR, the structure of demethoxycurcumin (DMC) contains only one methoxy group directly linking to the benzene ring (Figure 2.2). Molecular formula of DMC is $C_{20}H_{18}O_5$ and molecular weight is 338.3539 g/mol. The chemical characteristics of DMC is more sTable than CUR. As a research, the studies have reported that DMC has several biological activities including anti-inflammation and anti-cancer activities. DMC could inhibit renal, breast cancer cell growth and prostate cancer. Also its increase stability and better aqueous solubility are found at physiological pH (Ni, et al., 2012). In addition, DMC showed higher potency in suppressing inflammation in lipopolysaccharide (LPS)-induced nitric oxide synthase (iNOS)/nitric oxide (NO), cycloxygenase-2 (COX-2) and nuclear factor-kB than that of bisdemethoxycurcumin (BDMC). Moreover, DMC exhibited the strongest inhibitory activity on NO/nitric oxide and TNF-α production in LPS-activated microglia compared to curcumin (CUR) and BDMC, an important factor for colon cancer development (Somchit et al., 2014).

2.1.2.3. Bisdemethoxycurcumin (BDMC)

Bisdemethoxycurcumin (BDMC) is a derivative and represents one of the major active components of curcuminoid products isolated from *Curcumae sp.* Bisdemethoxycurcumin (BDMC) has similar anti-inflammatory properties with demethoxycurcumin. It can inhibit LPS-induced nitric oxide (NO) production and expression of iNOS and COX2 in RAW264.7 cells by blocking NF-kB activation as like as DMC. Bisdemethoxycurcumin also displays unique properties in that it enhances Abeta clearance by upregulating expression MGAT3 and TLR genes. BDMC is chemically more sTable than curcumin and DMC in physiological media, (Subramanian, et al., 2017). From Figure 2.2, bisdemethoxycurcumin (BDMC) contains none of methoxy groups in their structure. Empirical formula is $C_{19}H_{16}O_4$ and

Molecular weight of BDMC is 308.33 g/mol. BDMC suppresses proliferation in cancer cells which BDMC could inhibit the proliferation and survival of several types of tumor cells including breast cancer cells, colon cancer cells and leukemia cells. Moreover, BDMC is better increasing stability and improved nuclear cellular uptake compared to curcumin (Ying-Bo et al., 2013).

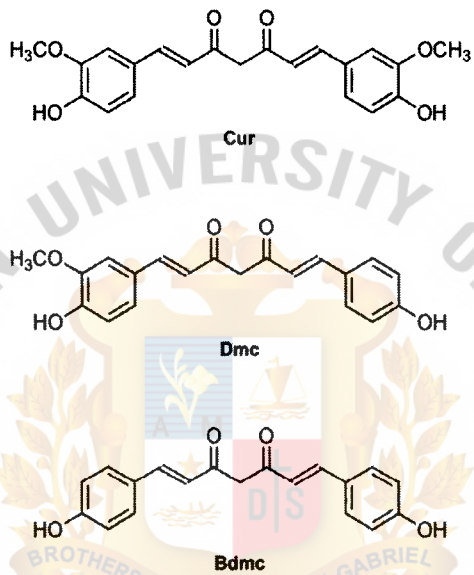


**Figure 2.2: Structures of curcumin (Cur), demethoxycurcumin (Dmc), and bisdemethoxycurcumin (Bdmc) (Sato, 2014)**

## 2.2. Benefits of curcumin

### 2.2.1. Medicinal plant

Although, curcumin is used as food additive (E100), but also used in traditional medicine to treat disorders such as anorexia, biliary complaints, cough, hepatic diseases and sinusitis (Hewlings and Kalman, 2017). There has been enormous interest in curcumin because of its potent antioxidant, anti-inflammatory, anti-proliferative, anti-bacterial, anti-carcinogenic anti-mutagenic, anticoagulant, anti-infective, and anti-angiogenic activities. Curcumin and turmeric products have been characterized as safe by the Food and Drug Administration (FDA) in USA. The average intake of curcumin is approximately 2-2.5 g for 60 kg/weight, which corresponds to a daily intake of approximately 60-100 mg of curcumin. Curcumin can also be consumed in high dose

(Nguyen et al., 2017). It is a food additive that can prevent cancer in animal tumor models. As a research, curcumin can reduce the proliferation rate of cell lines, causing a 96% decrease by 48 h that can show the ability of ant-cancer (Jovičić, et al., 2017).

2.2.1.1. Anti-inflammatory activity

Tumor necrosis factor α (TNF-α) is a major mediator of inflammation in most diseases. This effect is regulated by the activation of a transcription factor and nuclear factor (NF)-κB. Whereas TNF-α is the most potent NF-κB activator, and the expression of TNF-α is also regulated by NF-κB. Tumor necrosis factor α is also activated by most inflammatory cytokines including gram-negative bacteria, various disease-causing viruses, environmental pollutants, chemical, physical, mechanical, and psychological stress, high glucose, fatty acids, cigarette smoke and other disease-causing factors that are causing the several diseases to human body. Therefore, curcumin can block the NF-κB activation by reducing the response of specific proteins - cytokines that occur in the processes of inflammation, such as TNF-α (Hewlings and Kalman, 2017).

2.2.1.2. Antioxidant properties

Curcumin shows antioxidant properties as a food additive in many food categories as dietary supplement and drug. Curcumin binds free radicals and it also donates a hydrogen atom that responsible for its antioxidant properties. Curcumin has a property of donating electrons to neutralize free radicals by creating sTable products (Hewlings and Kalman, 2017). Thus, breaking a chain reaction of creating free radicals in a living organism. The ability of capturing hydrogen peroxide of curcumin is better than the commercial antioxidants such as BHA, BHT and vitamin E at the same concentration. Also, curcumin reduces the loss of these vitamins C and E during sleep time. Curcumin prevents oxidation and lipid modification of low density lipoproteins which contributes to the formation of thrombosis and arteriosclerosis (Jovičić et al., 2017).

2.2.1.3. Anticancer activity

Cancer is uncontrolled cell growth that give a terrible disease to human. From the studies, it shows that it can contribute to the death of cancerous cells, reduce angiogenesis and inhibit the growth of tumors in test animals. As above in anti-inflammatory activity, the NF-κB has the main role in the creation of tumors. Thus, curcumin inhibits the factors that causing cancer. For example, curcumin analog BAT3 selectively inhibits NF-κB dependent gene expression by binding to chromatin DNA (Jovičić et al., 2017). Curcumin also inhibits the growth of androgen-independent prostate cancer cells. Ability of curcumin can inhibit carcinogenesis at three stages: angiogenesis, tumor promotion, and tumor growth (Nasri et al., 2014).

2.2.1.4. Antimicrobial Activity

Curcumin can inhibit the growth of a variety of bacteria, pathogenic fungi, and parasites. For example, curcumin is highly effective in suppressing *Helicobacter pylori* which is a bacterial damage cell wall in the stomach (Nasri et al, 2014). Curcumin shows significant antimicrobial properties in many studies, it can prevent on gram positive bacterial such as *Bacillus cereus* and *Staphylococcus aureus* as well as gram negative as *Escherichia coli* and *Yersinia enterocolitica* (Jovičić, et al., 2017).

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**2.2.2. Natural colorant**

Curcumin is used as coloring agent for food and cosmetic products because of curcuminiod, which is an active compound responsible for yellow pigment in curcumin. It is a natural safety colorant for human that promotes health and well-being by preventing or even healing diseases. Unlike synthetic dyes such as tartrazine and carmoisine that may impair liver function and cause oxidative stress (Martins R.M., et al, 2013). The main purpose of curcumin is coloring products in the range of yellow to red, depending on the pH of the product. At pH 2.5 to 7.0, it has an intense yellow color, and above pH 7.0 it is red. As an additive, curcumin is sTable during thermal treatment and in dry food. In food industry, the amount of curcumin used is 5-500 mg/kg, depending on the food category. Curcumin provide antioxidant property that can

prevent rancidity of foods and provide foodstuffs containing less oxidized fat or free radicals. The powerful of curcumin has an important role in storage period for a long time without it turning rancid (Stankovic, 2004).

### 2.2.3. Food ingredient

Curcumin is used as a spice in curries for South Asian and Middle Eastern cuisine. It has been also used in India for thousands of years as a spice. It is commonly known as curry powder that give color and relatively mild flavor into foods. There are nutrient-rich ingredients to use in kitchen for all. Its yellowness of curcumin powder makes it a great addition to foods as well as to pickles, soups and some sauces. Also it contains many health advantages as a supplement. Curry powder is necessary in Indian curry dishes and Asian countries (Basnet and Skalko-Basnet, 2011).

**Figure 2.3: Curcumin powder (Anonymous,2016)**

## 2.3. Curcumin as natural colorant in food

Curcumin is a natural yellow pigment compound primarily used in food and chemical industries as coloring, flavoring and preservative. The yellow color of curcumin is mainly due to the presence of polyphenolic compound as curcuminoids. Curcuminoid known as diferuloylmethane is major constituent of curcumin that present in turmeric and responsible for yellow color. Also, the other two curcuminoids are demethoxycurcumin (DMC) and bis-demethoxycurcumin (BDMC), which are the

derivatives of curcumin isolated from turmeric. As a food additive, its E number is E100. It also can be in form of solid and liquid. The shade of color depends on the pH range; at pH 2.5- 7.0 it is yellow color and at above pH 7.0 it is red color which is widely used for many product categories (Stankovic, 2004). Curcuminoid exhibit strong absorption between 420 and 430 nm in an organic solvent because of widely electronic delocalization inside the molecules (Amalraj, et al., 2017).

According to their color, curcumin is used in many products to give the yellow color and help to improve the color of commercial product such as pickled green mustard, as a famous product for Asian countries. In Thailand, the industries use yellow color of curcumin to give the golden yellow to pickled product, especially in pickled green mustard for consumer acceptance. Examples of the product that use curcumin as a coloring agent:

2.3.1. Pickle

Fermentation of vegeTables or pickles is one of the traditional methods for food preservation. There are various vegeTables used to make pickle such as cabbage, lettuce, yellow tea melon, cucumber, carrots, and green mustard. In Thailand, pickle is also called "pak dong", which is including with several vegeTables. Green mustard (pak gahd kiew in Thai or gai choy in Chinese) is available at most Asian markets and farmer markets. Normally, Thai industrials use curcumin as a coloring agent in pickled green mustard, following Thai regulation. Curcumin powder gives the golden yellow in pickled green mustard that make the accepted color for consumer (Figure 2.4) (Breidt and Pérez-Díaz1, 2013).

**Figure 2.4: pickled mustard green (Elaine, 2014)**

2.3.2. Beverages

Many beverage products contain curcumin as a food additive to give yellow color and stability to the product. According to research, curcumin was used with whey-based beverage which can provide yellow color, antioxidant activity and increase shelf life of the product without addition of preservatives (Ankitha, et al., 2018). Thus, curcumin is popular to use in beverage as commercial products that provide many advantages to the products and consumer.

2.3.3. Dairy product

Curcumin also can be added to dairy products such as milk, cheese, and ice cream. From research, the curcumin powder could be added for butterscotch flavor at the maximum of 0.5% in the preparation of natural color ice cream without much effects on product acceptability (Manoharan, et al., 2012). Moreover, combination of curcumin with annatto has been used to color cheeses, dry mixes, salad dressings, winter butter, and margarine. Curcumin also plays an important role as food preservative in cheeses by preventing bacterial growth and prolong shelf life of the product (Hassan and Algarni, 2016).

2.3.4. Confectionery

Generally, curcumin is accepTable in all flour and sugar confectionery. Flour confectionery is traditional to use yellow color from curcumin in cake and biscuit. In sugar confectionery industries, curcumin is used about 20 ppm to prepare as stock solution to mix with sugar mass. Confectionery product should be protected from prolonged exposure to light by suiTable packaging (Houghton and Hendry, 1996).

## 2.4. Curcumin Extraction

According to chemical structure, curcumin has less water solubility at acidic and neutral pH, but it can be soluble in organic solvent such as ethanol, methanol, acetone, alkali, ketone, dimethyl sulfoxide, and chloroform. The extraction process for curcumin from turmeric is solvent method, which is the simplest and cheapest technique. There are several methods and ratios to extract curcumin as shown in Table 2.1.

**Table 2.1: Method/parameters, solvent ratio and curcumin content of turmeric extraction.**

| Method/Parameter | Solvent ratio | Curcumin content (%) | Reference |
|---|---|---|---|
| Ultrasound generation probe | 7:3(Ethanol: water) ; 3 grams cucumin powder | 3.08 | Martins et al., 2013 |
| Solvent extraction | 1:7.5(Cur: Solvent) ; Hexane (B.P=69°C), Chloroform (BP = 61°C), Ethyl acetate (B.P=77°C), Methanol (B.P=65°C), and Acetone (B.P=56.53°C). | Hexane 6.5 Chloroform 19.7 Ethylacetate 18.76 Methanol 15.68 and Acetone 22.8 | Revathy et al., 2011 |
| NA | Ethanol and water (1:1) ;Cur: Sovent ratio 1:30, 1:40 and1:50 extraction temperature 27°C, 50°C and 70° C extraction time 2 hr. | 14.79 | Surojanametakul et al., 2010 |
| Soxhlet apparatus | 1 : 42 (cur: solent) ; Solvent (Chloroform, Ethylacetate, Methanol and Acetone) | Chloroform 4.3 Ethylacetate 4.5 Methanol 5.6 Acetone 4.6 | Kulkarn et al., 2017 |
| Borosilicate vessel and a glycerin bath | 1:6 (Cur: Ethanol) ; extraction time 24 h, agitation speed 70 rpm, extraction temperature 80 °C and ethanolic strength of 96% | 62.6 | Paulucci etal., 2012 |
| Double-jacketed flasks | NA | 4.49-12.89 | Sogi etal., 2010 |
| Pressurized hot water extraction (PHWE) and Soxhlet extraction | 1:9 (Cur: water) ; extraction temperature 150 °C | 17.0 | Euterpio et al., 2011 |
| Soxhlet apparatus | 1:10 (Cur: Solvent) Solvent; ethanol, methanol, acetone and ethylacetate | 4.2 | Patil et al., 2018 |

From Table 2.1, there are many methods and parameters which several solvents used for curcumin extraction. Solvent used mostly are ethanol and acetone, these solvents give high amount of curcumin content.

## 2.5. Curcumin degradation

There also has some limitation of curcumin due to curcumin is a hydrophobic polyphenol which is extremely low in water solubility, low stability, poor absorption, and rapid metabolism that severely reduce bioavailability (Sarkar, et al., 2016).

There are many factors that cause the degradation of curcumin by following:

2.5.1. UV light / Light

Photo degradation normally occurs in curcumin pigment. As a research, the rate of photo degradation in the first-order kinetics showed that curcumin pigment has the highest rate compared to demethoxycurcumin (DMC) and bis-demethoxycurcumin (BDMC). Curcumin is also more stable in organic solvent than acid brine solution (Price and Buescher et al., 1996). The degradation of curcumin is an autoxidation process, free radical-driven incorporation of $O_2$. Bicyclopentadione is a major product of the autoxidation of curcumin which come form oxygenation and double cyclization of the heptadienedione chain connecting the two methoxyphenol rings. Vanillin, vanilic acid and ferulic acid are minor products. Autoxidation is introduced by $O_2$ serving as the initial electron acceptor, for example; a reaction of curcumin with molecular oxygen: for every molecule of curcumin converted, one molecule of oxygen is consumed (Schneider et al., 2015).

2.5.2. Thermal

Changes in temperature can affect to stability of curcumin. From the research, curcumin was heated at 180 °C up to 70 minutes and was analyzed by using HPLC-PDA. The result of heated curcumin showed the degradation products affecting to reduce bioactivity of the curcumin (Esatbeyoglu. et al., 2015).

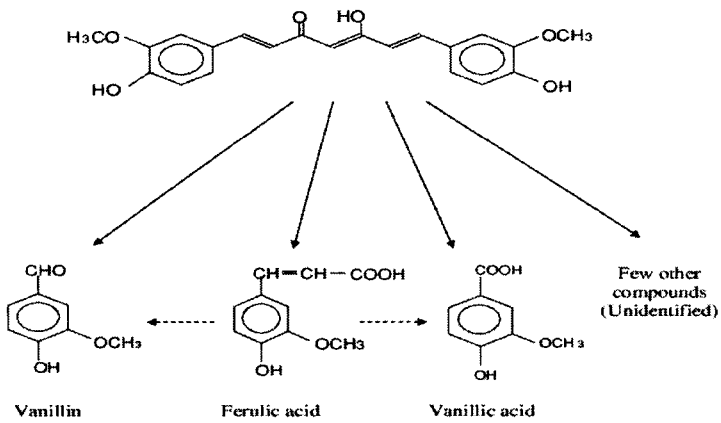


**Figure 2.5: Thermal degradatin of curcumin (Suresh and Srinivasan, 2009)**

2.5.3. pH

Curcumin is sensitive to higher pH conditions. Curcumin is not sTable at neutral (pH 7) and alkaline (pH 7-14) for longer period. It is easily degraded into compounds like vanillin and ferulic acid. The initial degradation products are formed after 5 minutes. As a research, curcumin is representative for alkaline degradation when increasing pH to 8.5 after 28 hours. Under acidic condition, curcumin is more sTable and slower in degradation. The kinetics of hydrolytic degradative reactions of compound over the pH range 1- 11 was studied. The result showed that water solubility is very low in pH 1-7 range and solutions are yellow color. At pH more than 7.5, the color changes to red (Kumavat et al, 2013).

Thus, low bioavailability of curcumin is important to concern because it affects to product stability, for example pickled green mustard. Poor solubility and dispersibility of curcumin in aqueous phase are occurred at acidic and neutral pH with rapid hydrolytic degradation. There are various factors that cause the degradation of curcumin, including UV light, light, thermal and pH value due to production process, storage period, packaging material. These factors have influence on the product quality, for example, the pickled green mustard color is faded and turned to brown color in long term storage (3-6 months) as an undesirable product. To restrain curcumin degradation and loss during processing and storage period, thus, microencapsulation technique is implied.

## 2.6. Encapsulation of curcumin

Microencapsulation is a process by which very tiny droplets or small particles of liquid , solid material or gas are packed or coated with a continuous film of polymeric material (secondary material) to form microcapsule. Normally, the packaged components (small particle inside) are called as the core, active component or fill, and the secondary material which encapsulates the core has been called as encapsulant, membrane, wall, external phase, matrix or shell. Microencapsulation provides several benefits and many functions such as providing environmental protection, controlling the release, structurating and functionalizing by entrapment of an active compound inside. The size of microcapsule is about 1 - 1000 $\mu m$ in diameter. Thus, microencapsulation technology is widely accepted in food industries and pharmaceutical and other fields (Bansode et al., 2010).

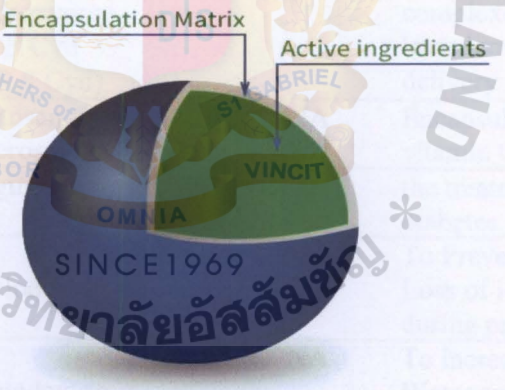


**Figure 2.6: Microencapsulation structure (Anonymous, 2006)**

As a research, the summarized Table 2.2 demonstrates several methods, core material, wall material, EE, EY and application of encapsulation. Encapsulation has been used in many proposes to enhance the property of active compounds, it can improve bioavailability as solubility and stability. Encapsulation are mostly used in pharmaceutical filed for drug delivery into body such as encapsulation of vitamin C which use chitosan as a coating material to improve activity of vitamin C. Moreover, EE or encapsulation efficiency is defined by the concentration of the incorporated

material which gives an idea about the percentage of drug or percentage of active compound that is successfully entrapped/adsorbed into nanoparticles. Thus, EE is necessary for encapsulation to demonstrate effectiveness of the encapsulation process.

**Table 2.2: Methods, core material, wall material, encapsulation efficiency, encapsulation yield and their application**

| Method | Core material | Wall Material | EE (%) | EY (%) | Application | Reference |
|---|---|---|---|---|---|---|
| NA | Curcumin | Sophorolipid micelles | 82 | 14 | NA | Peng et al., 2018 |
| Centrifugation | Curcumin | Exosomes | NA | NA | Drug Delivery System: The Anti-inflammatory Activity | Sun et al., 2010 |
| Evaporation method | cucurbitacin I | PLGA | 48.79 | NA | Increase activity | Alshamsan, 2014 |
| Co-evaporation and sealed-heating methods | liposomes | $\beta$-cyclodextrin ($\beta$ Cyd) and hydroxypropyl-$\beta$ Cyd (HP $\beta$ Cyd) | 26.8 and 33.8 | NA | liposomes encapsulating cyclodextrin complexes for transdermal drug delivery | Maestrelli et al.,2005 |
| Spray drying | vitamin C | chitosan microspheres | 58.30 | NA | Encapsulation of vitamin C | Desai et al., 2005 |
| NA | Lipoinsulin | Alginate | 21.5 | NA | the treatment of diabetes | Ramadas et al.,2000 |
| Spray drying | Menthol | $\beta$-cyclodextrin | 20-30 | NA | To Prevent the Loss of l-Menthol during process | LIU et al., 2014 |
| Spray drying and freeze-drying | Nutraceutical Monoterpenes | $\beta$-cyclodextrin and Modified starch | 60-80 | NA | To increase Water solubility of the active compounds. | Mourtzinos et al., 2008 |
| Electrospinning | vanillin | Cyclodextrin | 40 | NA | Prolonged shelf-life and high temperature stability of vanillin | Kayaci and Uya,2012 |
| Kneading method | Meloxicam | α-cyclodextrin and β-cyclodextrin | NA | 85.5 for α-cyclodextrin, 81.4 for β-cyclodextrin | To increase its solubility in water | Miclea et al., 2010 |

## 2.7. Inclusion complexation encapsulation

### 2.7.1. Definition

The combination of the cyclodextrin and active compound to be complexed which formally was called as inclusion complexation technique, it is in term of chemical process used for encapsulation (called as emulsion formation in physical term) (Madene et al., 2006). Inclusion complexation is the only method of encapsulation that takes place on a molecular level. It is accomplished using cyclodextrins, typically $\beta$-cyclodextrin which consists of 7 glucose units linked by 1, 4 glucosidic bonds. The inclusion complexes contain a guest molecule is held within the cavity of the cyclodextrin host molecule (host-guest complexes) (Figure 2.7). Basically, cyclodextrin has a hollow, hydrophobic center with a hydrophilic outer surface. In aqueous solution, molecules that are less polar will replace the water molecule that is held in the center of the cyclodextrin. The CDs can form inclusion complex with hydrophobic active compound by entrapping the entire molecules or nonpolar part of molecules inside the lipophilic cavity. This complex becomes less soluble and will precipitate out of solution. According to molecular structure and cone shape of cyclodextrin, their posscess have unique ability to act as molecular containers by entrapping guest molecules (core or active compound) in their internal cavity. It is also not covalent bonds forming or broken during CDs complex formation. In aqueous solution, the complexes readily dissociate and free molecules inside remain in equilibrium with the molecules bound within the CD cavity (Tirucherai and Mitra, 2003).

### 2.7.2. Advantages of cyclodextrin inclusion complexation

- Enhancement of solubility: CDs increase the aqueous solubility of many poorly soluble component by forming inclusion complexes with their apolar (non-polor) molecules or functional groups.
- Improvement of stability: CD complexation improved the chemical, physical and thermal stability. For an active molecule to degrade upon exposure to

oxygen, water, radiation or heat, chemical reactions must take place. When a molecule is entrapped within the CD cavity, it is difficult for the reactants to diffuse into the cavity and react with the protected guest.

- Reduction of irritation: As the complex gradually dissociates and the free active compound is released, it gets absorbed into the body and its local free concentration always remains below the levels that might be irritating to the mucosa.
- Odor and taste masking: Unpleasant odor and bitter taste of components or drugs can be masked by complexation with CDs. Molecules or functional groups that cause unpleasant tastes or odors can be hidden from the sensory receptors by encapsulating them within the CD cavity.
- Material handling benefits: Substances that are oils/liquids at room temperature can be difficult to handle into sTable solid dosage forms. Complexation with CDs may convert such substances into amorphous powders which can be conveniently formulated into solid dosage forms by conventional production processes and equipment (Martin Del Valle, 2003).


**Figure 2.7: Cyclodextrin Inclusion Complexation (Ayala-zavala J.F., et al, 2008)**

2.7.3. Cyclodextrin as wall material

Coating materials (wall materials) are protein, carbohydrates, lipid, gum, cellulose and modified starch used for encapsulating the active compound, are showed in Table 3. The coating materials are the external layer of microcapsule which can called in many names such as encapsulant, membrane, carrier, wall, external phase, matrix or shell. It should be capable of forming a continuous film that is connected to the core or

active material. The coating should be nonreactive with active compound inside and it should provide the desired coating properties including strength, flexibility, impermeability, optical properties, and stability (Poncelet, 2005).

**Table 2.3: Food-grade materials used for encapsulation of food, (Nedovic, 2011)**

| Material class | Type of materials |
|---|---|
| Proteins | Milk proteins - caseins and whey proteins<br>Soya proteins<br>Wheat proteins<br>Egg proteins<br>Zein<br>Hydrolysed protein |
| Polysaccharides | Starch and starch products - maltodextrins, dextrins, starches,<br>resistant starch, modified starches<br>Gums - agar, alginates, carrageenan, gum acacia, gum arabic, pectin,<br>carboxymethyl cellulose<br>Chitosan |
| Lipids | Natural fats and oils<br>Fractionated fats<br>Mono- and di-glycerides<br>Phospholipids<br>Glycolipids<br>Waxes - beeswax, carnauba wax |

Each class of materials (lipids, biopolymers - polysaccharides and proteins) have their own unique characteristics that influence the processes used and the applications for which they are used. Lipids are water insoluble and are good barriers to water and normally used to coat solid cores. For biopolymers (polysaccharides and proteins), they are water soluble materials which commonly used to produce microcapsules loaded with fats, oil-soluble vitamins, and solvent-miscible flavors.

Cyclodextrin (CD) is one type of carbohydrate or polysaccharides that used to produce microcapsules. The characteristics of this type of materials are gelling, emulsion stabilization, film forming, and ability to form glassy solids on dehydration.

Cyclodextrins (CDs) are a group of structurally related natural products formed which usually produced from enzymatically hydrolyzed starch. Cyclodextrins (CDs) are cyclic oligosaccharides containing glucopyranose units joined by $\alpha$ (1, 4) glucosidic linkage and consisting of 6, 7 and 8 glucose units are called $\alpha$ -, $\beta$ - and $\gamma$ - cyclodextrin, respectively. They also contain a somewhat hydrophobic/lipophilic central cavity and a hydrophilic outer surface. They are non-toxic compounds that have many useful properties and widely used in foods, cosmetics, pharmaceuticals and agrochemicals (Gould and Scott, 2005). Cyclodextrins (CDs) have mainly been used as complexing agents or encapsulating agent to increase aqueous solubility of active compound and to increase their bioavailability, stabilization of guests or core against the degradative effects of environment such as oxidation, light and heat, control of volatility and sublimation, physical isolation, taste modification by masking off flavors, odor elimination and controlling of drug and flavor release for food industrial products. Therefore, cyclodextrins can be applied to several fields as cosmetics, food products, agricultural chemicals products and pharmaceuticals which are an environment safety and contain huge of advantages to use it.

Cyclodextrins can be classified into three types: $\alpha$ -cyclodextrin, $\beta$ -cyclodextrin, $\gamma$ - cyclodextrin and alternative (HP- $\beta$ -CD), referred to as the first generation and differentiate by number of glucose units. The main properties of three main different cyclodextrins are given in Table 2.4 (Martin Del Valle, 2003).

**Table 2.4: The properties of three main type of cyclodextrins. (Martin Del Valle, 2003)**

| Property | $\alpha$ - cyclodextrin | $\beta$ - cyclodextrin | $\gamma$ - cyclodextrin |
|---|---|---|---|
| Number of glucopyranose units | 6 | 7 | 8 |
| Molecular weight (g/mol) | 972 | 1135 | 1297 |
| Solubility in water at 25 °C (%, w/v) | 14.5 | 1.85 | 23.2 |
| Outer diameter (Å) | 14.6 | 15.4 | 17.5 |
| Cavity diameter (Å) | 4.7-5.3 | 6.0-6.5 | 7.5-8.3 |
| Height of torus (Å) | 7.9 | 7.9 | 7.9 |
| Cavity volume (Å$^3$) | 174 | 262 | 427 |

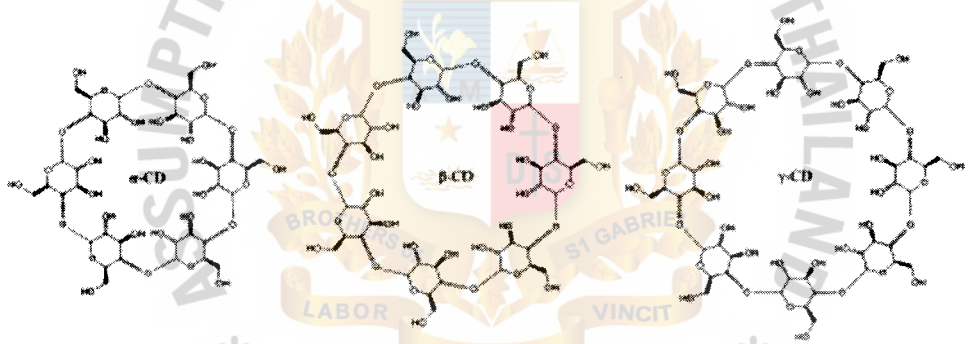


**Figure 2.8: Structure of: α - cyclodextrin, β - cyclodextrin and γ – cyclodextrin (Skowron, 2006)**

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2.7.3.1. α -cyclodextrin

Cyclodextrins are classified according to the numbers of glucopyranose units they have in their structure. Alpha cyclodextrin is hexasaccharide derived from glucose. It is the smallest of cyclodextrin (Harata, 1977).

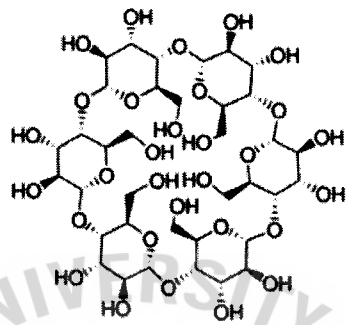

**Figure 2.9: α –cyclodextrin structure (Caligur, 2008)**

2.7.3.2. γ – cyclodextrin

Gamma-cyclodextrin is the largest of cyclodextrin family which consisting of 8 glucose units compared with α- and β-cyclodextrins. γ-cyclodextrin also has a larger internal cavity, higher water solubility, and more bioavailability. From research, more economic production processes for γ-cyclodextrin have been developed using improved γCGTases as a unique enzyme capable of converting starch and appropriate complexing agents (Li., 2007).

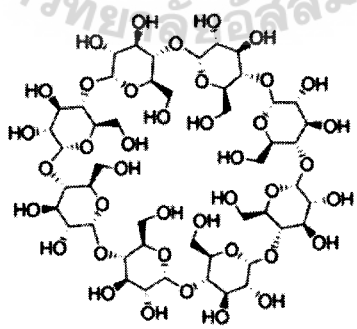

**Figure 2.10: γ-cyclodextrin structure (Caligur, 2008)**

2.7.3.3. β-Cyclodextrin

β -Cyclodextrin (BCD) are the most commonly used compounds in pharmaceutical preparations and food industries. β -Cyclodextrin (BCD) is widely used because its cavity size fit to common guests with molecular weights between 200 and 800 g/mol. They are also available and reasonable price to use (Haiyee et al., 2009).

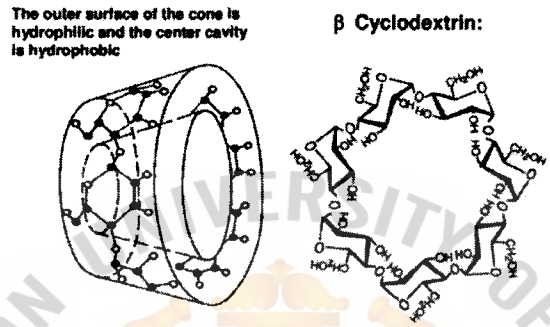


**Figure 2.11: β –Cyclodextrin (BCD) structure (Haynes, 2009)**

2.7.3.4. Hydroxypropyl- β -cyclodextrin (HP- β -CD)

Hydroxypropyl- β -cyclodextrin (HP- β -CD) (Figure 2.12 ) is an alternative to parent cyclodextrin ( $\alpha$ -cyclodextrin, $\beta$ -cyclodextrin and γ - cyclodextrin) as a hydroxyalkyl derivative with improve solubility in aqueous phase (above 60 g in 100 ml water at 25°C more than BCDs), low toxicity, increase stability and extend to inclusion capacity (Rakmai et al., 2018). Hydroxypropyl- β -cyclodextrin is a chemically modified β -CD that have been known for 20 years ago. Hydroxypropyl- β -cyclodextrin (HP- β -CD) enhances the solubility and dissolution and permeability of drugs which is popular to use in pharmaceutical products with inclusion complexation method. Moreover, the stability of CDs depends on the nature and effect of the included functional group of active compounds. The alternative CD or hydroxypropyl- β -cyclodextrin (HP- β -CD) can reduced photodegradation of complexation that means the inclusion complexation of HP- β -CD can protect the active compound from the light and also increase antibacterial activity of some active compounds (Challa et al., 2005). The main advantage of hydroxypropyl- β -cyclodextrin (HP- β -CD) complexation is the enhanced aqueous phase soluble of guest, which are also depends on the pH: at pH 2 the

molar ratio of host:guest is dominantly 2:1 mole/mole, while at pH 7 the malar ratio is 3:1 mole/mole complexes (Peeters et al. 2002). Therefore, the lower order of complexation observed at lower pH was related to substructure protonation, which reduced HP- β -CD interaction. When hydroxypropyl- β -cyclodextrin (HP- β -CD) encapsulate in the core material, the particle size is smaller than free HP- β -CD, also forming clusters in solution by hydrogen bond (Rakmai et al., 2018).


**Figure 2.12: Hydroxypropyl- β -cyclodextrin (HP- β -CD) structure (Celebioglu and Uyar, 2011)**

## 2.8. Inclusion Complexation Techniques

The encapsulation technique of choice depends on the type and physical properties of the core (active compounds) and shell material in each study. Thus, the selected encapsulation technique should give a high encapsulation efficiency and loading capacity of actives. Therefore, there are several techniques that can be applied to produce microcapsules, including the solution method, the co-precipitation method, neutralization method, spray dry method, kneading method, evaporation method and grinding method.

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2.8.1. Co-precipitation method

Co-precipitation technique is suiTable for non-water-soluble substances. This method provided very poor yield because of the competitive inhibition from organic solvents used as the precipitant (depend on solvent used). The guest is dissolved in organic solvents such as chloroform, benzene and diethyl ether. The wall material dissolved in water is also added with agitation. The solution is cooled and complex crystals occur. The crystals are washed with organic solvent and then dried. This technique is normally applied for drug or pharmaceutical products (Madene et al., 2006).

2.8.2. Evaporation method

In this method, the lipid micropaticles which contains active compound entrapped with a high melting point lipid, such as fatty alcohols, fatty acids, fatty acid esters, waxes, etc. So, a high melting point lipid is dissolved in an organic solvent, then the mixture is emulsified with an aqueous phase at ambient temperature. The solid particles are then formed by evaporation of the organic solvent. By the way, size of solid lipid particles is smaller than the initial oil droplets, thus temperature controlled emulsification is needed, as a solid lipid microparticles are of the same size as the initial oil droplets. As a research, delivery of cyclosporine A (CyA) by using a co-solvent evaporation method showed addition of concentrated polymeric solutions in organic phase to aqueous phase in and provided the optimum condition in terms of carrier diameter and CyA loading in MePEO-b-PCL micelles (Aliabadi et al., 2007).

2.8.3. Kneading method or paste method

The kneading method is the simplest technique because the liquid or dissolved solid guest is added into moistened wall material and continuously mixed and kneaded by kneader or mixer (in mortar) as a paste or dough mixture and then the paste is dried. The obtained solid is washed with a small amount of solvent to remove the free particles and then dried in drier. Kneading technique is suiTable for poorly water-soluble guests. According to research, the kneading method is also suiTable for inclusion complex formation of cyclodextrins because it affords a very good yield of inclusion formation, but it is unsuiTable for large scale preparation (Cheirsilp and Rakmai, 2016).

2.8.4. Spray dry method

For this method, a wall material and guest molecule are dissolved in deionized water and then the core/wall material mixture is fed into a spray dryer where it is atomized through a nozzle or spinning wheel. The dried particles or microcapsules fall to the bottom of the spray dryer and are collected (Risch, 2018). Moreover, the spray-dryer is operated under the most appropriate conditions such as inlet temperature and sample feeding speed, for example; as temperatures of 50 - 70°C are used, this technique is only used for thermosTable molecules. Spray drying is still the most economical and widely used method of encapsulation. Equipment is readily available and production costs are lower in large scale production (Madene et al., 2006).

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# Chapter 3

# Materials and Methods

## 3.1. To study the suitable condition of curcumin crude extraction

Curcumin powder (2 g) was mixed with 30 mL of the mixture of ethanol and water with the ratio of ethanol to water as 100:0, 75:25, and 50:50 for 1.5 h. The crude extract was filtered. The curcumin content was measured by using spectrophotometer at 425 nm. and calculated as the following equation.

Curcumin content (g/100 g) = (0.0025 x Absorbance at 425 nm x volume made up x Dilution factor) / (0.42 x weight of sample x 1000) x 100

Since 0.42 absorbance at 425 nm = 0.0025 g of curcumin (Bagchi, 2012).

The extraction condition that provided the highest curcumin extract was used to produce concentrated curcumin crude extract by using rotary evaporation method (VV22000) and the curcumin crude extract was kept at -80°C for further experiment.

## 3.2. Curcumin encapsulation using inclusion complexation technique

Curcumin-HPBCD complex was produced by using kneading method (Sapkal et al., 2007). Curcumin crude extract was added into previously moistened HPBCD in the molar ratios of 1:1, 1:1.5, and 1:2. The mixtures ratios were mixed by kneader with the kneading time of 5, 10, 15, and 20 min. The mixture was then dried at 40°C for overnight. Then the dried mixture was crushed, sieved (mesh 100) and stored at temperature of -80°C.

### 3.2.1. Determination of encapsulation yield (EY)

The dried powder was weighed and the respective yields were calculated by using the following equation:

$$\%EY = \frac{\text{Amount of encapsulated curcumin obtained}}{\text{Total Amount of curcumin and polymer}} \times 100$$

3.2.2. Determination of encapsulation efficiency (EE)

The encapsulation efficiency of the powder was determined by analyzing the supernatant of the sample that the powder was removed from it by centrifugation at 3,000×g for 15 min. For the estimation of curcumin present in the supernatant, the absorbance was measured spectrophotometric at 425 nm (Mukerjee and Vishwanatha, 2009). The amount of the curcumin encapsulated and the % encapsulation in the powder was given by

$$\%EE = \frac{\text{Curcumin encapsulated}}{\text{Total amount of curcumin}} \times 100$$

*Curcumin encapsulated = Curcumin total – Curcumin filtrate (by using spectrophotometer to determine curcumin content)

*Curcumin encapsulated should be washed the surface by water before determination

**3.3. To study some properties of encapsulated curcumin powder**

3.3.1. Color release determination

The color release was performed by preparing 2 g encapsulated curcumin extract/solution. Solutions were centrifuged at 3000 rpm at 0, 5, 10, and 20 mins. The supernatant of encapsulated curcumin extract concentration was determined by using UV spectrophotometry at 427 nm (Arango-Ruiz et al, 2018). The curcumin retention was calculated as the following equation:

$$\text{Curcumin retention (\%)} = \frac{Curcumin_{initial} - Curcumin_{supernatant}}{Curcumin_{initial}} \times 100$$

3.3.2. Stability determinations

3.3.2.1. Light stability

To evaluate the effect of carriers on the stability of Cur in the nanoparticles against UV photolysis, stability of free Cur and the encapsulated Cur in HPBCD particles were tested following the method reported by Xiao et al. (2015). Briefly, 5 mL of freshly prepared Cur-HPBCD were placed into transparent glass vials. Then samples were put in a controlled light for 4 days. The sampling was carried out at designed time intervals of 0, 1, 2, 3, and 4 days. The quantity of Cur remained was determined through spectrophotometry analysis. The retention % of Cur was reported. Light stability test was performed tripplicate.

Condition:

Light source = Panasonic LED lamp Noon daylight 50/60Hz 6000K – 6500K

Light intensity = 7W 100 – 240V 56mA 540lm0

Temperature = 28-30°C

3.3.2.2. pH Stability

The pH is measured by using pH meter (Kamdee , *et al.,* 2014) Approximately 10 ml solutions, stored at room temperature were tested at pH 1 to 14 to investigate their pH stability. The different pH can be obtained with 1 mol/l sodium hydroxide solution or HCl added in the solutions.

3.3.2.3. Thermal stability

Briefly, 5 g of freshly prepared encapsulated curcumin were placed into transparent glass vials and incubated in water bath at different temperatures (25 (RT), 50, 60, 70, 80, 90 and 100°C) for 30 min and cooled down to room temperature (25°C). The quantity of curcumin/HP-β-CD complex remained in samples was then determined by using spectrophotometer.

### 3.4. Food application in pickled mustard green by using encapsulated curcumin as natural colorant.

The encapsulated curcumin and curcumin powder (control) were applied in the production of pickled green mustard as food colorant. The powder was added in the flavored solution before packing. Five packs of pickle green mustard of encapsulated powder and curcumin powder were kept at -18°C and used as the control for comparing with the sampling one.

The samples were kept at 55°C for 1 month for accerelated process (ASL = 6 mouths). The samples were collected weekly to determine the changes of some properties of the product as following:

#### 3.4.1. Color

The color of pickled green mustard was measured by using Hunter lab color scale. L*a*b* color measuring system (HunterLab Miniscan EZ) (León and Mery, 2006) and expressed as. L*, a* and b* values The Total color differences (**ΔE**) was calculated by using the following equation:

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

Remarks: ΔL* (L* sample minus L* standard) = difference in lightness and darkness

(+ = lighter, - = darker)

Δa* (a* sample minus a* standard) = difference in red and green

(+ = redder, - = greener)

Δb* (b* sample minus b* standard) = difference in yellow and blue

(+ = yellower, - = bluer)

ΔE* = total color difference

The color differences (ΔL*, Δa*, Δb*) were obtained from the first day of storage (Day 0) subtracted by the last day of storage period (Day 30).

3.4.2. Total Acidity

The acidity content was evaluated by using titration method. The sample was titrated with 0.1 N NaOH to an end point using phenolphthalen as an indicator. Then percentage acidity was calculated by using the following formula:

$$\%\text{acid}=\frac{[\text{mls NaOH used}] \times [0.1\text{ N NaOH}] \times [\text{milliequivalent factor}] \times [100]}{\text{grams of sample}}$$

Where: milliequivalent factor of critic acid = 0.064

3.4.3. Salt

Salt content of the product was measured by using salometer. This method determines the salt content of a substance based on its refractive index. Refractive index was determined by passing a light through a prism into a sample and measuring how the light bends and establishing the critical angle.

3.4.4. Sensory evaluation

The samples were sensory evaluated every week for 1 months. Four sensory attributes were evaluated as flavor, texture, color and overall palatability by using a 9-point hedonic scale where 9 excellent and1extremely poor and 30 panelists.

3.4.5. Curcumin content

Curcumin content was determined by using spectrophotometer at 425 nm and calculated using the following equation:

Curcumin content (g/100 g) = (0.0025 x Absorbance at 425 nm x volume made up x Dilution factor) / (0.42 x weight of sample x 1000) x 100

Since 0.42 absorbance at 425 nm =0.0025 g of curcumin (Bagchi, 2012).

### 3.5. Statistical Analysis

A randomize complete block design and and a 3x4 factorial design were used in this experiment. Data were evaluated using analysis of variance ANOVA and means were compared by using Least significant (LS) test and Turkey ($p < 0.05$) using the software SAS 9.4.

# Chapter 4

# Results and Discussions

## 4.1. To study the suitable condition of curcumin crude extraction

For this study, the method for extracting curcumin from turmeric powder was the use of ethyl alcohol as an organic solvent, which was the simplest way to extract active (crude) compounds as curcuminoids. Moreover, ethanol (EtOH) or absolute alcohol was used as a solvent in this experiment. EtOH is a chemical compound, natural's colorless alcohol produced by fermentation processes from yeast, so ethanol is an edible compound or a food-grade that can use with food products.

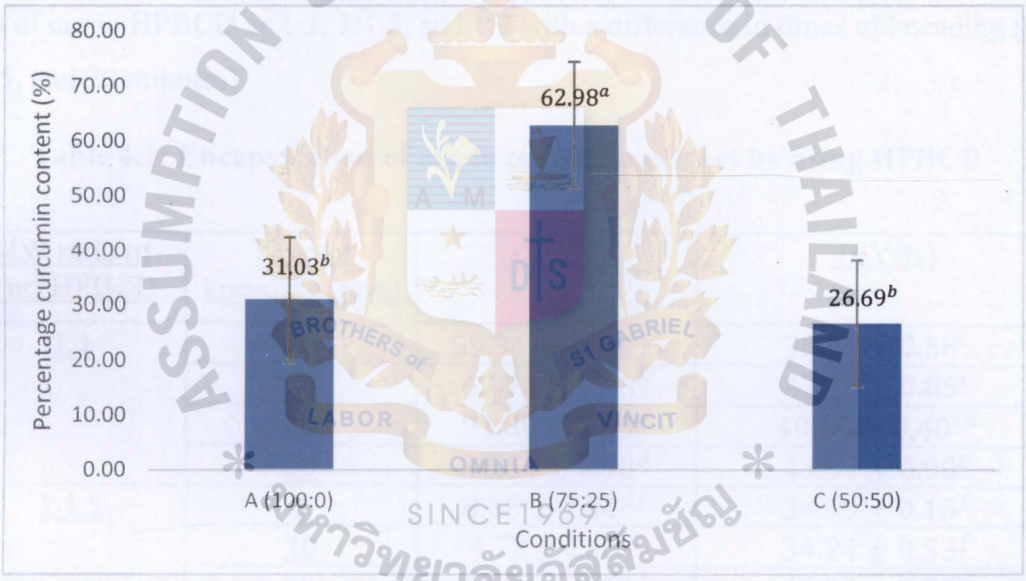


***Figure 4.1: Percentage curcumin content of three conditions (A(100:0) , B (75:25), C(50:50) ; EtOH: water).***

For this experiment, three ratio of ethanol to water were used as 100:0, 75:25, and 50:50. According to result from Figure 4.1, it was noticed that the percentage curcumin content of three treatments were 31.03 %, 62.98 % and 26.69% as the following condition A, B, and C, respectively. Based on the curcumin content of crude extract, the ratio of ethanol to water as 75:25 provided the highest curcumin content which was significantly higher than the other ($p \leq 0.05$). It was also because of the chemical structure of curcumin, as a liposoluble compound that likely dissolves in an organic solvent but also some parts of curcumin structure practically dissolves in water

for a little amount too When using a mixture of ethanol and water as a solvent, it provided higher percentage of curcumin content than using ethanol alone. Thus, crude extract from 75:25 (EtOH: water) condition was selected to use as crude curcumin extraction for further study.

## 4.2. Curcumin encapsulation by using inclusion complexation technique

Curcumin crude extract was encapsulated by using the kneading method or paste method as an inclusion complexation technique and HPBCD as a wall material. Kneading method is suitable for poorly water-soluble active compounds and it affords very great yields (Cheirsilp and Rakmai, 2016). In this section, the experimental design used was a 3x4 factorial design. Encapsulated curcumin was produced by using 3 MW ratios of cur to HPBCD as 1:1, 1:1.5, and 1:2 with a difference in times of kneading (5, 10, 15, and 20 minutes).

**Table 4.1: Encapsulation of crude curcumin extract by using HPBCD**

| MW ratio of Cur: HPBCD | Time of kneading (min) | (EE %) | (EY %) |
|---|---|---|---|
| 1:1 | 5 | $59.34 \pm 2.29^{bc*}$ | $38.37 \pm 0.58^{e}$ |
| | 10 | $62.70 \pm 6.73^{b}$ | $42.18 \pm 0.85^{c}$ |
| | 15 | $91.80 \pm 7.83^{a}$ | $40.60 \pm 0.40^{cd}$ |
| | 20 | $34.64 \pm 0.90^{d}$ | $43.89 \pm 0.90^{b}$ |
| 1:1.5 | 5 | $8.68 \pm 2.84^{gf}$ | $34.99 \pm 0.18^{f}$ |
| | 10 | $4.72 \pm 2.61^{g}$ | $34.24 \pm 0.53^{f}$ |
| | 15 | $16.84 \pm 0.51^{f}$ | $40.35 \pm 0.35^{d}$ |
| | 20 | $19.90 \pm 2.52^{ef}$ | $38.21 \pm 0.75^{e}$ |
| 1:2 | 5 | $49.43 \pm 0.64^{c}$ | $40.06 \pm 0.20^{d}$ |
| | 10 | $65.03 \pm 2.60^{b}$ | $40.17 \pm 0.34^{d}$ |
| | 15 | $99.26 \pm 6.23^{a}$ | $41.08 \pm 0.64^{cd}$ |
| | 20 | $29.35 \pm 1.24^{ed}$ | $45.81 \pm 0.24^{a}$ |

** The different letters indicate significantly different at 95% confidential level.*

As a result (Table 4.1), encapsulation efficiency (EE %) was used as the major criterion for encapsulation, which was determined by using curcumin content. From the statistical analysis, it was recognized that both ratio and kneading time had significant ($p>0.05$) effects on %EE. It was recognized that as the keading time increased from 5 to 10 min, and it was further increased to reach the peak at 15 min of kneading. After

that, %EE declined, although longer time of kneading was used, which appeared at the ratio 1:1 and 1:2. It might be caused by that the longer keading time used, the higher of broken particles occurred due to the mechanical force applied for encapsulation. On the other hand, it was remarkedly noticed that at the ratio 1:1.5, %EE was dramatrically dropped in all kneading times, which its mechanism is still unclear for this phenomenon. Also, encapsulation yield (EY %) was 41.08 % that can be calculated by the amount of encapsulated curcumin obtained divided by total amount curcumin and nanoparticle weight, which is the point to the loading capacity of encapsulation (Mukerjee and Vishwanatha, 2009). In addition, it appeared that 15 minutes of kneading process was a perfect time for rearrangement of the structure of the hydrophobic active compound by entrapping the nonpolar part of molecules inside the lipophilic cavity (Tirucherai and Mitra, 2003).

Moreover, it was observed that %EE at the ratio 1:1 and 1:2 was not significantly different in all kneading times. Due to the high cost of curcumin crude extract, the ratio 1:2 of curcumin crude extract to HPBCD was chosen for the further study because of higher amount of encapsulated powder obtained.

## 4.3. To study some physical properties of encapsulated curcumin/ HP-β-CD complex

In this study, some physical properties of encapsulated powder were investigated. There was color release, thermal stability, pH stability, and light stability.

### 4.3.1. Color release

Color release is a property that indicates the ability of encapsulated powder to release pigment into the solvent. The encapsulated curcumin was dissolved in distilled water for 0, 5, 10, and 20 minutes and then centrifuged. The supernatant was taken to determine the curcumin retention in the encapsulated powder (Table 4.2). It was noticed that when the mechanical force was applied as shaking condition, the curcumin remained in the encapsulated powder approximately in the range of 51.4-54.0%. The longer time of shaking had no effect on the releasing of curcumin. The loss of curcumin might be caused by the surface curcumin on the nanoparticle. This was also implied that the curcumin was well protected within the structure of HPBCD and had slow releasing mechanism. This property had ensured that when the encpausulated powder was applied in food, the curcumin should be release slowly and maintain yellow color

of the product during storage. Moreover, it was recognized that encapsulated curcumin/ HP-β-CD was well disperse in water. The HP-β-CD enhanced curcumin ability to soluble in water by increasing the aqueous solubility of many poorly soluble component by forming inclusion complexes with their non-polar molecules or functional groups (Martin Del Valle, 2003). Application of curcumin powder in clear solution of pickle green mustard creates turbidity of the solution due to low water solubility, leading to the sedimentation of curcumin powder in the product. Therefore, the encapsulated powder of curcumin extract can solve this problem. In addition, it was noticed that the color release reached the maximum level when the shaking time was 10 mins. And reached constant after that.

**Table 4.2: Curcumin retention of encapsulated powder in distilled water at 3,000 rpm.**

| Times (min.) | Curcumin retention (%) |
|---|---|
| 0 | 51.4[c] |
| 5 | 53.1[b] |
| 10 | 53.7[a] |
| 20 | 54.0[a] |

** The different letters indicate significantly different at 95% confidential level.*

4.3.2. Thermal stability

Thermal change is one of the factors affecting on curcumin stability. Thus, the degradation of encapsulated curcumin/ HP-β-CD by heat was investigated at different temperatures as 25, 50, 60, 70, 80, 90, and 100°C. As shown in Table 4.3. It was observed that as the temperature increased, the curcumin retention sifnificantly ($p>0.05$) reduced. From the kinetic study of thermal degradation of curcumin, $R^2$, the linear regression coefficient of zero order, first order, second order, and third order was compared. It was recognized that $R^2$ of zero order was 0.925, which was high and close to 1.0, indicating that thermal degradation of encapsulated powder was zero order. Thermal degradation of curcumin is caused by the vulnerability of Diketone bridge in curcumin molecules, which is sensitive to heat. This degraded compound was

characterized as vanillin, vanillic acid, and ferulic acid as a product (Suresh and Srinivasan,2009).

**Table 4.3: Thermal stability of encapsulated curcumin differences temperatures**

| Temperature (°C) | Percentage of curcumin content (%) | Zero order C | First-order Ln C | Second -order 1/C | Third-order $1/C^2$ |
|---|---|---|---|---|---|
| 25 (RT) | 86.00[a] | 86.000 | 4.454 | 0.012 | 0.000 |
| 50 | 85.80[a] | 85.800 | 4.452 | 0.012 | 0.000 |
| 60 | 80.05[b] | 80.050 | 4.383 | 0.012 | 0.000 |
| 70 | 78.82[bc] | 78.820 | 4.367 | 0.013 | 0.000 |
| 80 | 77.33[bc] | 77.330 | 4.348 | 0.013 | 0.000 |
| 90 | 74.57[d] | 74.570 | 4.312 | 0.013 | 0.000 |
| 100 | 69.85[e] | 69.850 | 4.246 | 0.014 | 0.000 |
| $R^2$ | | 0.901 | 0.892 | 0.881 | 0.868 |
| k | | -0.216 | -0.002 | 0.000 | 0.000 |

** The different letters indicate significantly different at 95% confidential level.*

4.3.3. pH stability

Normally curcumin is not stable at neutral (pH 7) and alkaline (pH 7-14) but it is stable at very low pH (Kumavat et al., 2013) which means the stability was more in acidic pH and low as the pH increases. Hence, the appearance of the curcumin/ HP-β-CD complex in homogeneous solution was studied as a consisting of pH at 2.1, 3, 3.7, 4.7, 6.2, 7, 7.7, 9.8, 11.2 and 14. The results indicated that the color of the sample solution changed as the pH changed at pH 2.1, 3, and 3.7, the color changed to light yellow, which was stable color required in this study. On the other hand, the color of the solution at pH 4.7- 7.7 gave orange-red color and at pH 9.8 above had a dark red color as shown in Figure 4.2. This was implied that when the encapsulated curcumin powder was applied in the pickle green mustard that has low pH of not greater than 4.6, the color of encapsulated powder would remain the same as yellow color for the acceptant appearance from the consumer.

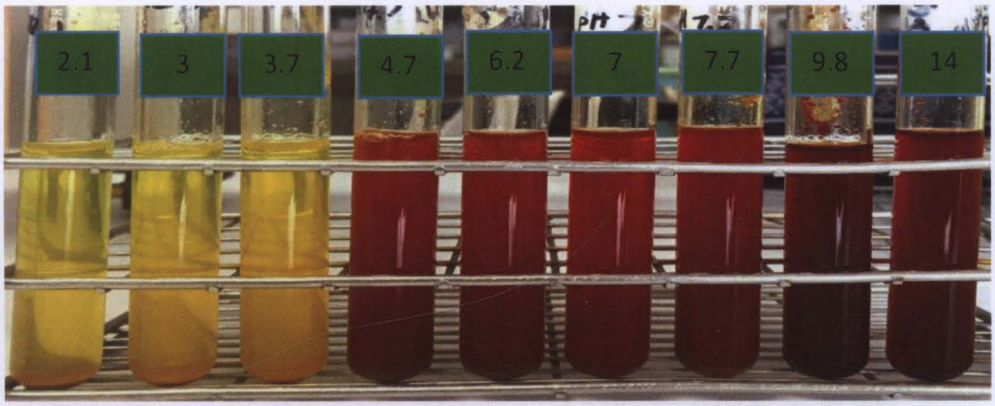


***Figure 4.2: Effect of pH on the stability of yellow color of encapsulated curcumin powder***

4.3.4. Light stability

It is generally known that curcumin is unable where it is exposured to sunlight. According to the fact that curcumin absorbs strongly in the visible wavelength range, which making it pre-activated to degradation (Jankun. Et al., 2016). In this experiment, the curcumin/HP-β-CD complex was tested into the light chamber that mimics daylight at room temperature within 4 days and the color change was obseaved by using a spectrophotometer at 425 nm. As a result of Table 4.4, it was noticed that curcumin content declined during 4 day - light exposure by 8.89 %. The result was parallel to the study of Mangolim. et al. (2014) who reported that the pure curcumin decreased by 18% after 30-day light exposure.

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**Table 4.4: Light stability: Percentage of curcumin content in encapsulated curcumin/HP-$\beta$-CD within 4 days.**

| Day | Curcumin content (%) | Zero order C | First-order Ln C | Second-order 1/C | Third-order $1/C^2$ |
|---|---|---|---|---|---|
| Day 0 | $86.00^a$ | 86.000 | 4.454 | 0.012 | 0.000 |
| Day 1 | $81.38^b$ | 81.380 | 4.399 | 0.012 | 0.000 |
| Day 2 | $79.85^c$ | 79.850 | 4.380 | 0.013 | 0.000 |
| Day 3 | $77.36^d$ | 77.360 | 4.348 | 0.013 | 0.000 |
| Day 4 | $77.11^d$ | 77.110 | 4.345 | 0.013 | 0.000 |
| $R^2$ | | 0.903 | 0.964 | 0.927 | 0.872 |
| k | | -2.180 | -0.462 | 0.123 | 0.079 |

* *The different letters indicate significantly different at 95% confidential level.*

When the kinetic was considered, it was noticed that photodegradation of curcumin was the first order reaction as $R^2$ was 0.964, which was close to 1.00. The result was in parallel with the study of Castillo et al., (2015) which pure curcumin was degrades 50% after 6 days it can demonstrates that encapsulated powder can protect the degradation of curcumin content inside cavity. In addition, the first order reaction in this study was also parallel to Tønnesen et al, (2002) who reported that the destabilization of curcumin had ocurred because several factors such as light, pH, temperature and also concentration of medium, i.e. the increasing concentration of cyclodextrin affected to destabilize of curcumin over the time passed because by intermolecular hydrogen bond formation that was under further investigation.

## 4.4. Food applications in pickled mustard green by using encapsulated curcumin as natural colorant.

In general, pickled green mustard in Thailand uses curcumin powder as a natural colorant to provide the golden yellow color of the products. Unfortunately, the golden yellow of the product changes to darker color due to curcumin photodegradation during storage, leading to product rejection Thus, in this experiment, the curcumin/HP-$\beta$-CD complex was used in this product to replace the pure curcumin at the same curcumin

concentration but the dosage of encapsulated was added to product was only 0.076% while the pure curcumin used was 0.2% in product. The pickled green mustard with curcumin/HP-$\beta$-CD complex was kept for 30 days as accelerated storage at 55°C. The sample was collected every 5 days. The quality of the product was evaluated as color, acidity, and salt, curcumin content and sensory.

### 4.4.1. Color

According to the pickled green mustard products, the color of this product is important in this study. So, the color in pickled green mustard was determined by using Hunter Lab CIE L*a*b* system and the result data was obtained as Lightness (L*), a* and b*. This theory assumes that the receptors in the human eye perceive color. The results were shown as following (Hunter, 1948).

Lightness or L* values

The lightness values obtained from HunterLab CIE L*a*b* system has a scale from 0 to 100 points. The zero (0) point indicates the darkness of color in the sample and the hundred (100) point indicates the brightest white color in the sample.

In this study, the lightness of pickled green mustard changed during long term storage because of the occurring of browning reaction during the storage period. As a result from Table 4.5, the lightness value of pickled green mustard slightly decreased during storage even though the encapsulated curcumin powder was added as a coloring agent. On the other hand, the control (curcumin powder) was moderately decreased and slightly fluctuated more than the curcumin/HP-$\beta$-CD. The pickled green mustard with curcumin/HP-$\beta$-CD complex was more constant and had stable lightness values which found at day 15 to day 30, the lightness value was higher than pickle with pure curcumin (control). Although, L* of control and sample were significantly different ($p \leq 0.05$) at the beginning of the storage, but at the end of storage there were not significantly different ($p \geq 0.05$). Conversely both were declined throughout the storage period, it was propably due to browning reaction or photodegradation still occurred in pickled green mustard during long storage. However, the lightness value of sample was 44.85 at day 30 in pickled green mustard with curcumin/HP-$\beta$-CD complex, indicating the a bit bright color of the product over time.

a* values

In general, a* or a scale is redness and greenness scale where a positive number indicates red (+) and a negative color indicates green (-). From Table 4.5, there are all negative results for a* value which means that the pickled mustard green product is all green color scale. It was noticed that a* of control and sample were not significantly different throughout the storage ($p \geq 0.05$), but at the last day of storage there were significantly different ($p \leq 0.05$). It might becaused by browning reaction occurred during storage. On the other hand, the sample at the last day was -4.25 which had more green scale than control (-2.98), it was implied that the encapsulated powder can protect the discoloration of green mustard. However, they had changed a little bit of scale, which was parallel to the color of the product.

b* values

The color channels b* or b scale represent true neutral gray values at b* = 0 and the yellow/blue opponent colors are represented along the b* axis, with blue at negative (-) b* values and yellow at positive (+) b* values (Hunter, 1948). As a result from Table 4.5, All of products have yellow color because of the pure curcumin (control) and curcumin/HP-$\beta$-CD complex (sample) used as a coloring agent and the b* values indicate in positive. Conversely, long-term storage had effect on b* value too. From Table 4.10, b* values were rapidly decreased and continue slightly decreased until at day 15 and it was st able at day 25 to day 30. Therefore, the products had a yellow color as the eyes perceive. On the other hand, there was a decrease in positive b* value during long term storage after the darkness had appeared. Although, b* of control and sample seemed to be not significant different ($p \geq 0.05$) throughout the storage especially at day 0 and day 30, b* values of both were declined during storage as same as L* and a* which might be caused by browning reaction and photo-degradation.

ΔE values

The total color differences (ΔE) is ability of perception, which is normally range from 0 to 100 (Brainard, 2003). For the study, the total color differences indicates how a difference of the product throughout storage period. ΔE values of both control and sample increased until mid of storage period and then dropped after Day 20. At day 30, it was noticed that control (cur) had Δ E as 20.18, and sample (cur/ HP-$\beta$-CD) had Δ E

as 21.44. In this case, ΔE of sample cur/ HP-β-CD had more color (L*, a* and b*) change than control (Table 4.5). ΔE of both control and sample were significantly different ($p \leq 0.05$) during the storage, it was propably due to the color changes and color stability of product during storage.

In addition, it could not to be clearly seen the difference of the color in product bwtween control and sample (Figure 4.3) because the dosage of powder used was different due to the intention to present the same concentration in both of control and sample. On the other hand, the benefit for using lower dose than normal is lower cost and increase efficiency of production. For recommendation, the using the encapsulated powder for 0.2%, it shall show more yellow color in the product that would be interesting for futher study.

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**Table 4.5: Color changes of treatments of pickled green mustard during accerelated storage at 55°C for 30 days.**

| Color factor | Treatment | Storage (days) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 15 | 20 | 25 | 30 |
| L* | Control | 51.0± 1.44$^{a}$ | 45.18±0.41$^{b}$ | 45.42±5.68$^{a}$ | 38.89±6.83$^{a}$ | 38.41±5.13$^{b}$ | 41.73±2.46$^{a}$ | 42.34±2.78$^{a}$ |
| | Encap | 48.82±2.45$^{b}$ | 46.91±0.43$^{a}$ | 44.58±3.45$^{a}$ | 43.24±3.14$^{a}$ | 45.45±3.71$^{a}$ | 43.16±4.79$^{a}$ | 44.85±2.29$^{a}$ |
| a* | Control | -6.49± 0.44$^{a}$ | -3.7±0.4$^{a}$ | -3.03±0.19$^{a}$ | -3.75±0.36$^{a}$ | -4.34±0.39$^{b}$ | -3.17±0.35$^{a}$ | -2.98±0.42$^{a}$ |
| | Encap | -6.59± 0.33$^{a}$ | -4.10±0.4$^{a}$ | -3.80±0.54$^{b}$ | -3.64±0.36$^{a}$ | -3.83±0.37$^{a}$ | -3.34±0.84$^{a}$ | -4.25±0.35$^{b}$ |
| b* | Control | 31.64±2.70$^{a}$ | 13.0±1.50$^{b}$ | 9.47±1.50$^{b}$ | 9.13±4.18$^{a}$ | 12.26±4.25$^{a}$ | 9.40±1.60$^{b}$ | 13.74±2.67$^{a}$ |
| | Encap | 34.74±2.25$^{a}$ | 15.90±2.5$^{a}$ | 11.77±2.31$^{a}$ | 10.43±1.86$^{a}$ | 13.89±2.62$^{a}$ | 13.65±3.35$^{a}$ | 13.80±1.85$^{a}$ |
| ΔE | Control | 0.00 | 19.67$^{a}$ | 23.12$^{b}$ | 25.70$^{a}$ | 23.20$^{a}$ | 24.32$^{a}$ | 20.18$^{b}$ |
| | Encap | 0.00 | 19.10$^{b}$ | 23.52$^{a}$ | 25.12$^{b}$ | 21.30$^{b}$ | 21.10$^{b}$ | 21.44$^{a}$ |

Remark * the different letters mean significant different at 95% confidential level and the analysis was done in each day.

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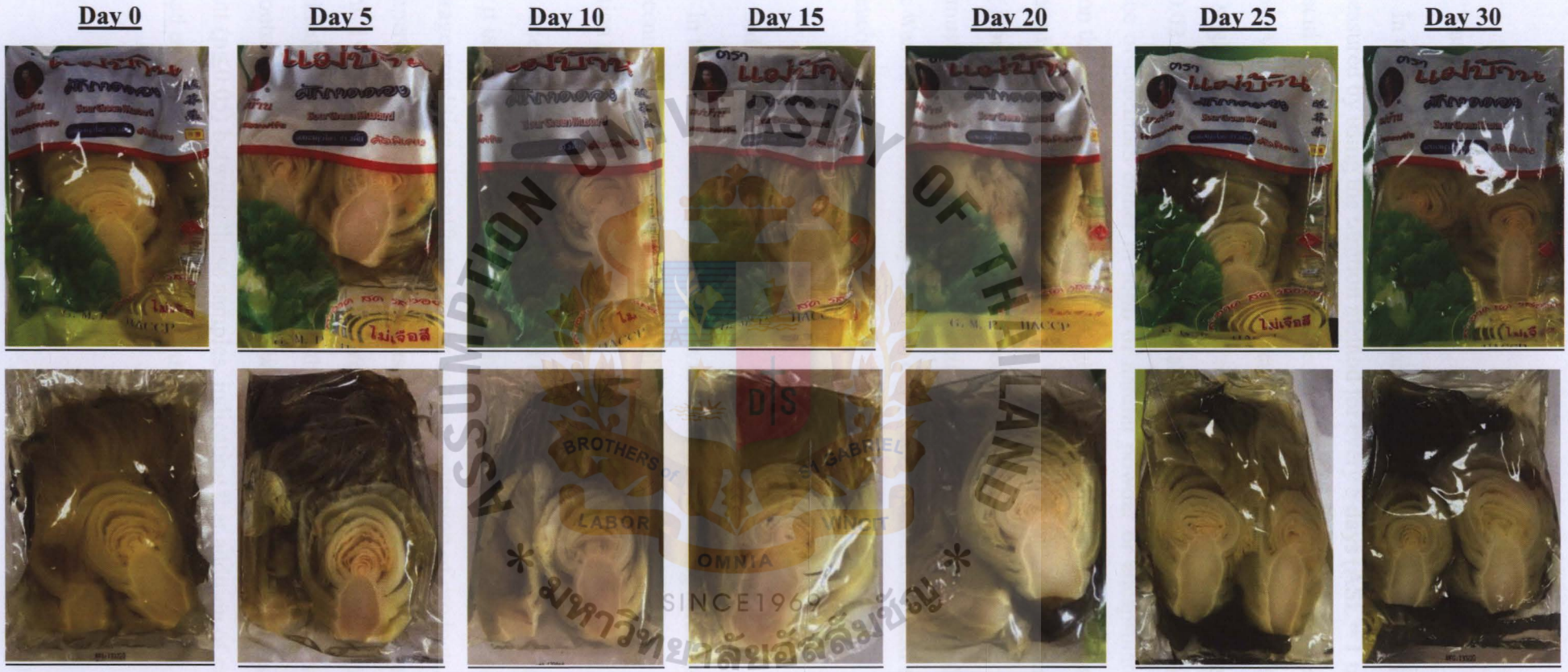


*Figure 4.3: Color changes of pickled green mustard during accelerated storage at 55℃ for 30 days, *A = Control (Curcumin powder), *B = Sample (Encapsulated powder)*

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### 4.4.2. Total Acidity

In this experiment, the total titratable acidity of pickled mustard green products was measured by using the titration method for every 5 days (ASL = 1 month) by using lactic acid (LA) as a predominant acid because the product is the fermented product. It was observed that acidity of control and sample were significant different ($p \leq 0.05$) and it was slightly declined from 1.49±0.06 to 1.18±0.01 % for control and 1.77±0.01 to 1.29±0.02 % for sample, respectively (Table 4.6). The result was also implied that the presence of curcumin either in form of natural powder or encapsulated powder had effect on the acidity of the product. It might be due to the error of processing when acid was added into both control and sample. On the other hand, the decreasing of acidity during storage might be cause by acid equilibrium. Due to the large size of pickled green mustard, the acid equilibrium occurred long time to reach. It also noticed that sample was no change in acidity between day 25 and 30 due to the acid equilibrium had been reached.

### 4.4.3. Salt

In this study, salt concentration was measured by using salometer and reported as percentage. The concentration of salt has met the specification of factory and regulation (3%-12%) (FAO, 1997).

The range of salt concentration of both treatments was in the range of 4.3 to 5.9 % that it is not much different in each other (Table 4.6). Moreover, the temperature of the storage condition also affected to the salt concentration of products, the increasing the temperature increased both the salt passage and the permeate flow rate (Bastaki and Qahtani, 1994). Day 0 (the first day before storage) had a lower percentage of salt than the pickled product after 1 month. After that the level of salt was relatively constant in both control and sample.In addition, it was recognized that there was no significant different ($p \leq 0.05$) between these samples, indicating that addition of curcumin in any form had no influence on salt content of the product.

### 4.4.4. Curcumin content

In this experiment, Table 4.6 indicated the curcumin content in the solution of the product over time passed. There was not significant different ($p \geq 0.05$) of curcumin content throughout the storage due to the added concentration of curcumin in control and sample product was in parallel. Curcumin content also declined after day 10 until the end of storage which means long-term storage affect to curcumin concentration of the product that can cause by temperature, lightness and solubility of curcumin as low in bioavailability.

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**Table 4.6: Some chemical properties of pickled green mustard colored with encapsulated curcumin and natural curcumin powder during the accelerated storage at 55°C for 30 days.**

| Treatments | | day0 | day5 | day10 | day15 | day20 | day25 | day30 |
|---|---|---|---|---|---|---|---|---|
| Acidity (%) | Control | 1.49±0.06[b] | 1.45±0.07[b] | 1.36±0.01[b] | 1.68±0.01[a] | 1.73±0.01[a] | 1.17±0.03[a] | 1.18±0.01[b] |
| | Sample | 1.77±0.01[a] | 1.78±0.03[a] | 1.47±0.04[a] | 1.52±0.01[b] | 1.43±0.15[a] | 1.27±0.03[a] | 1.29±0.02[a] |
| Salt (%) | Control | 4.3±0.00[a] | 4.9±0.00[a] | 4.7±0.00[a] | 4.9±0.00[a] | 5.9±0.00[a] | 4.60±0.00[a] | 4.9±0.00[a] |
| | Sample | 4.87±0.29[a] | 4.7±0.2[a] | 5.37±0.40[a] | 4.43±0.15[b] | 5.00±0.10[b] | 5.07±0.31[a] | 4.3±0.50[a] |
| Cur Content (%) | Control | 0.02±0.002[a] | 0.02±0.004[a] | 0.02±0.002[a] | 0.01±0.00[a] | 0.00±0.001[a] | 0.00±0.001[a] | 0.01±0.007[a] |
| | Sample | 0.02±0.004[a] | 0.02±0.005[a] | 0.02±0.007[a] | 0.01±0.009[a] | 0.01±0.008[a] | 0.01±0.011[a] | 0.01±0.001[a] |

Remarks: *the different letters mean significant different at 95% confidential level and the analysis was done in each day.

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### 4.4.5. Sensory evaluation

Sensory evaluation of pickle green mustard was evaluated by using preference test 9-point hedonic scale and 30 panelists. Four attributes were evaluated as color, flavor, texture, and overall liking. Sensory evaluation is a necessary part of both product development and quality control. There were two products (control and sample) which test every 5 days (ASL = 1 month). As a result of Table 4.7, there were no significant difference ($p \leq 0.05$) between sample and control in all attributes between days 0 to day 15. It was interested that there were significant different ($p \geq 0.05$) at day 20 to the end of storage. For flavor and overall liking attributes of sample, there were higher score than control at day 20 to day 30, which indicated that the cosumer satisfied the encapsulated powder in the product. For the color attributes, it seem like no difference between control and sample.

On ther other hand, the liking score for four attributes conforms to the same direction that declined every week when the time passed due to the low quality of the product over time.

In addition (Table 4.8), product acceptance was investigated weekly. It was observed that the sample had higher product acceptance than that of control through the storage test at accerelated 55°C for 30 days. There were significant different ($p \leq 0.05$) of acceptance throughout the storage. At the end of the test, the control was rejected (35.56%), while the sample was accepted as 68.89%. In consumers' opinion, the control had a more sour taste with very soft texture, dark in color, and bad smell than the sample (cur/HP-β-CD complex). This was implied that the addition of encapsulated curcumin powder as coloring agent had improved product acceptance significantly, which would be the positive benefits to the producer.

**Table 4.7: Liking score of control product and sample (using encapsulated curcumin/HP-β-CD as colorant)**

| Attributes | Day | Day 0 | Day 5 | Day 10 | Day 15 | Day 20 | Day 25 | Day 30 |
|---|---|---|---|---|---|---|---|---|
| **Color** | Control | 7.02±1.14[a] | 7.20±1.95[a] | 7.04±1.31[a] | 7.11±1.28[a] | 6.67±1.26[a] | 6.73±1.70[a] | 6.00±2.28[a] |
| | Sample | 7.00±1.43[a] | 6.90±1.52[a] | 6.89±1.53[a] | 6.58±1.53[b] | 6.91±1.20[a] | 6.29±2.06[b] | 5.84±2.22[a] |
| **Flavor** | Control | 6.71±1.27[a] | 6.90±1.29[a] | 6.60±1.05[a] | 6.02±1.82[a] | 5.58±1.53[b] | 5.73±2.04[b] | 4.78±2.09[b] |
| | Sample | 6.58±1.47[a] | 6.60±1.60[a] | 6.38±1.35[a] | 6.27±1.60[a] | 6.27±1.57[a] | 6.31±2.13[a] | 5.80±1.90[a] |
| **Texture** | Control | 7.07±1.21[a] | 7.20±1.39[a] | 6.53±1.56[a] | 6.51±1.66[a] | 6.47±1.69[b] | 6.73±2.08[a] | 5.00±2.13[b] |
| | Sample | 7.31±1.29[a] | 6.90±1.55[a] | 6.53±1.39[a] | 6.29±1.89[a] | 6.91±1.47[a] | 6.29±2.08[b] | 5.40±1.98[a] |
| **Overall liking** | Control | 6.80±1.20[a] | 7.10±1.03[a] | 6.53±1.32[a] | 6.56±1.55[a] | 5.91±1.53[b] | 6.09±2.38[a] | 4.89±2.10[b] |
| | Sample | 7.02±1.31[a] | 6.70±1.41[a] | 6.69±1.24[a] | 6.38±1.51[a] | 6.56±1.18[a] | 6.36±1.81[a] | 5.67±1.57[a] |

Remarks: *the different letters mean significant different at 95% confidential level and the analysis was done in each day.

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**Table 4.8: Percentage consumer acceptance of control and sample (using encapsulated curcumin/HP-$\beta$-CD) in every 5 days for 30 days (ASL=6months)**

| Day | Control acceptability (%) | Sample acceptability (%) |
|---|---|---|
| 0 | $86.67^{a}$ | $86.67^{a}$ |
| 5 | $93.33^{a}$ | $91.11^{b}$ |
| 10 | $86.67^{b}$ | $91.11^{a}$ |
| 15 | $73.33^{b}$ | $82.22^{a}$ |
| 20 | $53.33^{b}$ | $91.11^{a}$ |
| 25 | $55.56^{b}$ | $71.11^{a}$ |
| 30 | $35.56^{b}$ | $68.89^{a}$ |

Remarks: *the different letters mean significant different at 95% confidential level and the analysis was done in each day.

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# Chapter 5

# Conclusion

The extraction process of curcumin with ethanol: water mixture (75:25) was the best condition in the study. The crude curcumin (active compound) was encapsulated in HP-$\beta$-CD polymer (as called as an inclusion complexation technique) by kneading method. The proper ratio for kneading method is 1:2 (cur: HP-$\beta$-CD) with 15 minutes which gave the highest in Encapsulation efficiency (EE %) as 99.26 %.

The addition encapsulated curcumin/HP-$\beta$-CD complex instead of pure curcumin powder as a natural colorant in the pickled green mustard increased consumer acceptance during long term storage. However, the color of the product were no significant between control and sample ($p \geq 0.05$).

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# Chapter 6

# Recommendation

For recommendation, the amount of encapsulated curcumin/HP-$\beta$-CD complex used in the product should add more to observe the differences of color apperance from control. Moreover, the texture analysis should be measure due to the result from sensory evaluation and liking score were significant different between control and sample which are a recommended for futher study.

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# Appendix

**Appendix Table 1: ANOVA of extraction of curcumin powder in different ratio.**

ANOVA

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| **Between Groups** | 0.181067 | 2 | 0.090533 | 1.335519 | 0.331315 | 5.143253 |
| **Within Groups** | 0.406733 | 6 | 0.067789 | | | |
| **Total** | 0.5878 | 8 | | | | |

**Appendix Table 2: ANOVA of Encaupsulation efficiency of encapsulated Curcumin/HP-$\beta$-CD complex**

| Source | DF | Sum of Squares | Mean Square | Error DF | F Value | Pr > F |
|---|---|---|---|---|---|---|
| **Times** | 3 | 8239.286689 | 2746.428896 | 24 | 181.44 | <.0001 |
| **Treatment** | 2 | 19146 | 9573.228233 | 24 | 632.46 | <.0001 |
| **Times*Treatment** | 6 | 4985.366311 | 830.894385 | 24 | 54.89 | <.0001 |
| **Residual** | 24 | 363.277533 | 15.136564 | . | . | . |

**Appendix Table 3: ANOVA of Encaupsulation Yield of encapsulated Curcumin/HP-$\beta$-CD complex**

| Source | DF | Sum of Squares | Mean Square | Error DF | F Value |
|---|---|---|---|---|---|
| **Times** | 3 | 121.676475 | 40.558825 | 24 | 133.31 |
| **Treatment** | 2 | 168.894867 | 84.447433 | 24 | 277.56 |
| **Times*Treatment** | 6 | 68.020867 | 11.336811 | 24 | 37.26 |
| **Residual** | 24 | 7.302067 | 0.304253 | . | . |

**Appendix Table 4: ANOVA of Solubility of encapsulated Curcumin/HP-$\beta$-CD complex**

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.00156867 | 0.00078433 | 4.81 | 0.0568 |
| **Treatment** | 3 | 0.04041092 | 0.01347031 | 82.53 | <.0001 |

**Appendix Table 5: ANOVA of Thermal stability of encapsulated Curcumin/HP-$\beta$-CD complex**

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.00012838 | 0.00006419 | 0.03 | 0.9726 |
| **Treatment** | 6 | 2.07583057 | 0.34597176 | 150.20 | <.0001 |

**Appendix Table 6: ANOVA of Light stability of encapsulated Curcumin/HP-$\beta$-CD complex**

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.00161830 | 0.00080915 | 1.18 | 0.3551 |
| **Treatment** | 4 | 0.50462840 | 0.12615710 | 184.23 | <.0001 |

**Appendix Table 7: ANOVA of Lightness (L*) of pickled mustard green product during storage time**

Day 0

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 31.59266667 | 6.31853333 | 3.64 | 0.0914 |
| **Treatment** | 1 | 14.12670000 | 14.12670000 | 8.13 | 0.0358 |

Day 5

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 15.97246667 | 3.19449333 | 2.43 | 0.1762 |
| **Treatment** | 1 | 8.94413333 | 8.94413333 | 6.80 | 0.0478 |

Day 10

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 114.1407667 | 22.8281533 | 1.07 | 0.4704 |
| **Treatment** | 1 | 28.3976333 | 28.3976333 | 1.33 | 0.3003 |

Day 15

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 201.8299750 | 40.3659950 | 2.50 | 0.1691 |
| **Treatment** | 1 | 56.8980750 | 56.8980750 | 3.52 | 0.1196 |

Day 20

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 167.2957417 | 33.4591483 | 5.09 | 0.0493 |
| **Treatment** | 1 | 148.8960750 | 148.8960750 | 22.65 | 0.0051 |

Day 25

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 110.5335417 | 22.1067083 | 3.20 | 0.1138 |
| **Treatment** | 1 | 6.1776750 | 6.1776750 | 0.89 | 0.3878 |

Day30

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 19.07850000 | 3.81570000 | 0.42 | 0.8210 |
| **Treatment** | 1 | 18.80003333 | 18.80003333 | 2.05 | 0.2116 |

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**Appendix Table 8: ANOVA of a* value of pickled mustard green product during storage time**

Day 0

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 0.99560000 | 0.19912000 | 1.90 | 0.2485 |
| **Treatment** | 1 | 0.03413333 | 0.03413333 | 0.33 | 0.5926 |

Day 5

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 1.26674167 | 0.25334833 | 2.48 | 0.1712 |
| **Treatment** | 1 | 0.37807500 | 0.37807500 | 3.69 | 0.1126 |

Day 10

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 0.72174167 | 0.14434833 | 0.77 | 0.6116 |
| **Treatment** | 1 | 1.80187500 | 1.80187500 | 9.56 | 0.0271 |

Day 15

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 0.91446667 | 0.18289333 | 2.33 | 0.1877 |
| **Treatment** | 1 | 0.03853333 | 0.03853333 | 0.49 | 0.5150 |

Day 20

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 1.23266667 | 0.24653333 | 5.30 | 0.0455 |
| **Treatment** | 1 | 0.78030000 | 0.78030000 | 16.79 | 0.0094 |

Day 25

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 1.84286667 | 0.36857333 | 0.80 | 0.5947 |
| **Treatment** | 1 | 0.08670000 | 0.08670000 | 0.19 | 0.6829 |

Day 30

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 0.45326667 | 0.09065333 | 0.44 | 0.8046 |
| **Treatment** | 1 | 4.88963333 | 4.88963333 | 23.82 | 0.0045 |

**Appendix Table 9: ANOVA of b* value of pickled mustard green product during storage time**

Day 0

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 16.99504167 | 3.39900833 | 0.38 | 0.8440 |
| **Treatment** | 1 | 28.79900833 | 28.79900833 | 3.22 | 0.1327 |

Day 5

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 27.14864167 | 5.42972833 | 1.76 | 0.2756 |
| **Treatment** | 1 | 24.56740833 | 24.56740833 | 7.95 | 0.0371 |

Day 10

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 29.71116667 | 5.94223333 | 3.64 | 0.0913 |
| **Treatment** | 1 | 15.91603333 | 15.91603333 | 9.74 | 0.0262 |

Day 15

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 55.00237500 | 11.00047500 | 1.11 | 0.4556 |
| **Treatment** | 1 | 5.10907500 | 5.10907500 | 0.52 | 0.5048 |

Day 20

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 93.24410000 | 18.64882000 | 2.99 | 0.1273 |
| **Treatment** | 1 | 7.93813333 | 7.93813333 | 1.27 | 0.3105 |

Day 25

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 42.45766667 | 8.49153333 | 1.60 | 0.3094 |
| **Treatment** | 1 | 54.01763333 | 54.01763333 | 10.17 | 0.0243 |

Day 30

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 18.03197500 | 3.60639500 | 0.52 | 0.7557 |
| **Treatment** | 1 | 0.01020833 | 0.01020833 | 0.00 | 0.9709 |

**Appendix Table 10: ANOVA of $\Delta E$ of pickled mustard green product during storage time**

Day 0

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 0 | 0 | . | . |
| **Treatment** | 1 | 0 | 0 | . | . |

Day 5

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 0.00000000 | 0.00000000 | 0.00 | 1.0000 |
| **Treatment** | 1 | 0.97470000 | 0.97470000 | 6.1E14 | <.0001 |

Day 10

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 0.00000000 | 0.00000000 | Infty | <.0001 |
| **Treatment** | 1 | 0.48000000 | 0.48000000 | Infty | <.0001 |

Day 15

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 0.00000000 | 0.00000000 | 0.00 | 1.0000 |
| **Treatment** | 1 | 1.00920000 | 1.00920000 | 3.07E14 | <.0001 |

Day 20

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 0.00000000 | 0.00000000 | Infty | <.0001 |
| **Treatment** | 1 | 10.83000000 | 10.83000000 | Infty | <.0001 |

Day 25

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 0.00000000 | 0.00000000 | . | . |
| **Treatment** | 1 | 14.78520000 | 14.78520000 | Infty | <.0001 |

Day 30

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 5 | 0.00000000 | 0.00000000 | Infty | <.0001 |
| **Treatment** | 1 | 4.76280000 | 4.76280000 | Infty | <.0001 |

**Appendix Table 11: ANOVA of Total acidity of pickled mustard green product during storage time**

Day 0

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.00423333 | 0.00211667 | 0.98 | 0.5039 |
| **Treatment** | 1 | 0.12615000 | 0.12615000 | 58.67 | 0.0166 |

Day 5

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.00130000 | 0.00065000 | 0.15 | 0.8691 |
| **Treatment** | 1 | 0.15681667 | 0.15681667 | 36.33 | 0.0264 |

Day 10

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.00253333 | 0.00126667 | 1.46 | 0.4063 |
| **Treatment** | 1 | 0.01706667 | 0.01706667 | 19.69 | 0.0472 |

Day 15

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.00013333 | 0.00006667 | 0.33 | 0.7500 |
| **Treatment** | 1 | 0.03840000 | 0.03840000 | 192.00 | 0.0052 |

Day 20

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.02123333 | 0.01061667 | 0.88 | 0.5330 |
| **Treatment** | 1 | 0.14106667 | 0.14106667 | 11.64 | 0.0762 |

Day 25

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.00143333 | 0.00071667 | 0.70 | 0.5865 |
| **Treatment** | 1 | 0.01601667 | 0.01601667 | 15.75 | 0.0580 |

Day 30

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.00103333 | 0.00051667 | 10.33 | 0.0882 |
| **Treatment** | 1 | 0.01815000 | 0.01815000 | 363.00 | 0.0027 |

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**Appendix Table 12: ANOVA of Salt of pickled mustard green product during storage time**

Day 0

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.08333333 | 0.04166667 | 1.00 | 0.5000 |
| **Treatment** | 1 | 0.48166667 | 0.48166667 | 11.56 | 0.0767 |

Day 5

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.04000000 | 0.02000000 | 1.00 | 0.5000 |
| **Treatment** | 1 | 0.06000000 | 0.06000000 | 3.00 | 0.2254 |

Day 10

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.16333333 | 0.08166667 | 1.00 | 0.5000 |
| **Treatment** | 1 | 0.66666667 | 0.66666667 | 8.16 | 0.1038 |

Day 15

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.02333333 | 0.01166667 | 1.00 | 0.5000 |
| **Treatment** | 1 | 0.32666667 | 0.32666667 | 28.00 | 0.0339 |

Day 20

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.01000000 | 0.00500000 | 1.00 | 0.5000 |
| **Treatment** | 1 | 1.21500000 | 1.21500000 | 243.00 | 0.0041 |

Day 25

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.09333333 | 0.04666667 | 1.00 | 0.5000 |
| **Treatment** | 1 | 0.32666667 | 0.32666667 | 7.00 | 0.1181 |

Day 30

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.27000000 | 0.13500000 | 1.00 | 0.5000 |
| **Treatment** | 1 | 0.54000000 | 0.54000000 | 4.00 | 0.1835 |

**Appendix Table 13: ANOVA of Curcumin content of pickled mustard green product during storage time**

Day 0

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.00000448 | 0.00000224 | 0.13 | 0.8831 |
| **Treatment** | 1 | 0.00001204 | 0.00001204 | 0.71 | 0.4878 |

Day 5

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.00005169 | 0.00002585 | 2.10 | 0.3221 |
| **Treatment** | 1 | 0.00007073 | 0.00007073 | 5.76 | 0.1385 |

Day 10

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.00003200 | 0.00001600 | 0.46 | 0.6855 |
| **Treatment** | 1 | 0.00000561 | 0.00000561 | 0.16 | 0.7272 |

Day 15

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.00007500 | 0.00003750 | 1.05 | 0.4874 |
| **Treatment** | 1 | 0.00000683 | 0.00000683 | 0.19 | 0.7044 |

Day 20

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.00004854 | 0.00002427 | 0.71 | 0.5850 |
| **Treatment** | 1 | 0.00010251 | 0.00010251 | 3.00 | 0.2256 |

Day 25

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.00009161 | 0.00004581 | 0.65 | 0.6062 |
| **Treatment** | 1 | 0.00004320 | 0.00004320 | 0.61 | 0.5157 |

Day 30

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.00004996 | 0.00002498 | 1.34 | 0.4269 |
| **Treatment** | 1 | 0.00000241 | 0.00000241 | 0.13 | 0.7536 |

**Appendix Table 14: ANOVA of liking score of Color attribute of pickled mustard green product during storage time**

Day 0

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 116.4888889 | 2.6474747 | 3.82 | <.0001 |
| **Treatment** | 1 | 0.0111111 | 0.0111111 | 0.02 | 0.8998 |

Day 5

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 208.2222222 | 4.7323232 | 3.43 | <.0001 |
| **Treatment** | 1 | 1.8777778 | 1.8777778 | 1.36 | 0.2493 |

Day 10

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 147.4000000 | 3.3500000 | 4.76 | <.0001 |
| **Treatment** | 1 | 0.5444444 | 0.5444444 | 0.77 | 0.3838 |

Day 15

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 131.8222222 | 2.9959596 | 3.02 | 0.0002 |
| **Treatment** | 1 | 6.4000000 | 6.4000000 | 6.46 | 0.0146 |

Day 20

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 110.4888889 | 2.5111111 | 4.77 | <.0001 |
| **Treatment** | 1 | 1.3444444 | 1.3444444 | 2.55 | 0.1171 |

Day 25

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 285.4888889 | 6.4883838 | 10.00 | <.0001 |
| **Treatment** | 1 | 4.4444444 | 4.4444444 | 6.85 | 0.0121 |

Day 30

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 341.9555556 | 7.7717172 | 3.35 | <.0001 |
| **Treatment** | 1 | 0.5444444 | 0.5444444 | 0.23 | 0.6303 |

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**Appendix Table 15: ANOVA of liking score of Flavor of pickled mustard green product during storage time**

Day 0

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 90.62222222 | 2.05959596 | 1.20 | 0.2752 |
| **Treatment** | 1 | 0.40000000 | 0.40000000 | 0.23 | 0.6318 |

Day 5

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 119.4888889 | 2.7156566 | 1.82 | 0.0249 |
| **Treatment** | 1 | 1.8777778 | 1.8777778 | 1.26 | 0.2679 |

Day 10

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 67.48888889 | 1.53383838 | 1.09 | 0.3876 |
| **Treatment** | 1 | 1.11111111 | 1.11111111 | 0.79 | 0.3789 |

Day 15

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 191.6222222 | 4.3550505 | 2.90 | 0.0003 |
| **Treatment** | 1 | 1.3444444 | 1.3444444 | 0.89 | 0.3495 |

Day 20

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 151.9555556 | 3.4535354 | 2.54 | 0.0013 |
| **Treatment** | 1 | 10.6777778 | 10.6777778 | 7.85 | 0.0075 |

Day 25

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 347.9555556 | 7.9080808 | 10.09 | <.0001 |
| **Treatment** | 1 | 7.5111111 | 7.5111111 | 9.58 | 0.0034 |

Day 30

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 301.4888889 | 6.8520202 | 6.09 | <.0001 |
| **Treatment** | 1 | 23.5111111 | 23.5111111 | 20.90 | <.0001 |

**Appendix Table 16: ANOVA of liking score of Texture of pickled mustard green product during storage time**

Day 0

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 108.2888889 | 2.4611111 | 3.59 | <.0001 |
| **Treatment** | 1 | 1.3444444 | 1.3444444 | 1.96 | 0.1683 |

Day 5

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 146.4000000 | 3.3272727 | 3.33 | <.0001 |
| **Treatment** | 1 | 2.5000000 | 2.5000000 | 2.50 | 0.1210 |

Day 10

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 147.4000000 | 3.3500000 | 3.28 | <.0001 |
| **Treatment** | 1 | 0.0000000 | 0.0000000 | 0.00 | 1.0000 |

Day 15

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 234.6000000 | 5.3318182 | 5.35 | <.0001 |
| **Treatment** | 1 | 1.1111111 | 1.1111111 | 1.11 | 0.2970 |

Day 20

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 174.2888889 | 3.9611111 | 3.74 | <.0001 |
| **Treatment** | 1 | 4.4444444 | 4.4444444 | 4.20 | 0.0464 |

Day 25

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 350.4888889 | 7.9656566 | 11.11 | <.0001 |
| **Treatment** | 1 | 4.4444444 | 4.4444444 | 6.20 | 0.0166 |

Day 30

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 335.4000000 | 7.6227273 | 8.97 | <.0001 |
| **Treatment** | 1 | 3.6000000 | 3.6000000 | 4.24 | 0.0455 |

**Appendix Table 17: ANOVA of liking score of overall liking of pickled mustard green product during storage time**

Day 0

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 89.28888889 | 2.02929293 | 1.83 | 0.0243 |
| **Treatment** | 1 | 1.11111111 | 1.11111111 | 1.00 | 0.3228 |

Day 5

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 92.88888889 | 2.11111111 | 2.99E16 | <.0001 |
| **Treatment** | 1 | 0.00000000 | 0.00000000 | 0.00 | 1.0000 |

Day 10

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 89.88888889 | 2.04292929 | 1.64 | 0.0532 |
| **Treatment** | 1 | 0.54444444 | 0.54444444 | 0.44 | 0.5125 |

Day 15

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 145.4000000 | 3.3045455 | 2.41 | 0.0021 |
| **Treatment** | 1 | 0.7111111 | 0.7111111 | 0.52 | 0.4751 |

Day 20

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 115.6000000 | 2.6272727 | 2.35 | 0.0027 |
| **Treatment** | 1 | 9.3444444 | 9.3444444 | 8.36 | 0.0059 |

Day 25

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 333.5555556 | 7.5808081 | 5.52 | <.0001 |
| **Treatment** | 1 | 1.6000000 | 1.6000000 | 1.17 | 0.2862 |

Day 30

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 44 | 272.5555556 | 6.1944444 | 9.12 | <.0001 |
| **Treatment** | 1 | 13.6111111 | 13.6111111 | 20.04 | <.0001 |

**Appendix Table 17: ANOVA of % Acceptance of pickled mustard green product during storage time**

Day 0

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0 | 0 | . | . |
| **Treatment** | 1 | 0 | 0 | . | . |

Day 5

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.00000000 | 0.00000000 | Infty | <.0001 |
| **Treatment** | 1 | 7.39260000 | 7.39260000 | Infty | <.0001 |

Day 10

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.00000000 | 0.00000000 | Infty | <.0001 |
| **Treatment** | 1 | 29.57040000 | 29.57040000 | Infty | <.0001 |

Day 15

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.0000000 | 0.0000000 | 0.00 | 1.0000 |
| **Treatment** | 1 | 118.5481500 | 118.5481500 | 2.09E15 | <.0001 |

Day 20

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.000000 | 0.000000 | 0.00 | 1.0000 |
| **Treatment** | 1 | 2140.992600 | 2140.992600 | 4.71E15 | <.0001 |

Day 25

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.0000000 | 0.0000000 | Infty | <.0001 |
| **Treatment** | 1 | 362.7037500 | 362.7037500 | Infty | <.0001 |

Day 30

| Source | DF | Anova SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Block** | 2 | 0.000000 | 0.000000 | 0.00 | 1.0000 |
| **Treatment** | 1 | 1666.333350 | 1666.333350 | 7.33E15 | <.0001 |



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