Production of Coconut Juice Powder

By

Miss Preeyaporn Dummuak
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A SPECIAL PROJECT SUBMITTED TO THE SCHOOL OF BIOTECHNOLOGY, ASSUMPTION UNIVERSITY IN PART OF FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF BACHELOR OF SCIENCE IN BIOTECHNOLOGY
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Faculty	:	Biotechnology
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Advisor

(Dr. –Ing. Tatsawan Tipvarakarnkoon)

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Abstract

The aim of this study was to formulate and process development for the production of coconut juice powder using freeze dry method. Due to the chemical composition of coconut juice there was a possibility to obtain coconut juice powders by freeze drying at -30°C for 48 hours when 5%, 10% and 20% of maltodextrin DE10-12 were added. Quality parameters of fresh coconut water was determined to study, consist of weight, pH, Soluble Solid, Turbidity, color and Total Acidity. Weight, pH and Soluble solid value of fresh coconut water within 402.59 g, 4.98 pH and 6.50°Brix respectively, which is the range 7-9 month of coconut age. Analysis color of fresh coconut water related to turbidity of it, L* value of fresh coconut water quite low mean low turbidity. Physiochemical (moisture content and color L*a*b*) and powder properties (hygroscopicity, Bulk, Tapped, Porosity, and Carr's index (%)) of coconut juice powder were determine to study the effect of maltodextrin concentration. Color properties (L* a* b*) of the coconut juice powder was significantly affected by the difference concentration of maltodextrin. Analysis color of coconut juice powder shows, 15% maltodextrin give highest L* (mean most bright) but lowe in b* (mean more yellow). Bulk density and Tapped density of the powders decreased with increase in concentration of the maltodextrin. Carr's index (%) related to flowability of powder product, from the result coconut juice powder have high percentage of Carr's index which is very poor in flowability. The study effect of Total soluble solid on sweetness of coconut juice powder show that 66.67% of panel prefer 10°Brix of rehydrated coconut juice. Difference concentration of maltodextrin have effect on preference score of consumers, 83% of panel prefer 5% maltodextrin added. Moreover, sensorial properties of rehydrated coconut juice powder were study by use sorting analysis. The result shows, rehydrated coconut juice powder like commercial brand "Cocomax", which similar in 100% coconut aroma, clear color, young and high coconut flavor, and quite low body.

Keywords: Fresh coconut water, Freeze drying, Maltodextrin, powder properties.

Objectives

1. To study physiochemical properties of fresh coconut water.
2. To study effect of total soluble solid on sweetness of coconut juice powder.
3. To study effect of maltodextrin concentrations on properties of coconut juice powder.
4. To investigate sensorial properties of rehydrated coconut juice powder using flash profiling rapid method.

Acknowledgements

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Miss Preeyaporn Dummuak

May, 2018

1.Introduction

Economic value of coconut water.

From U.S. Plant-based Waters Market Size, By Product, 2013 – 2024 (Fig.1), coconut water is one of the biggest market in terms of plant-based water when compare with another plant-based waters. From the statistictical data, in 2016 the coconut water was around 80% of 0.7 USD Billion when compare with other plant-based waters. Moreover, the percentages of coconut water double dramatically increase in 2024 when compare with the percentages of coconut water in 2016. (www.gminsights.com, 2017)

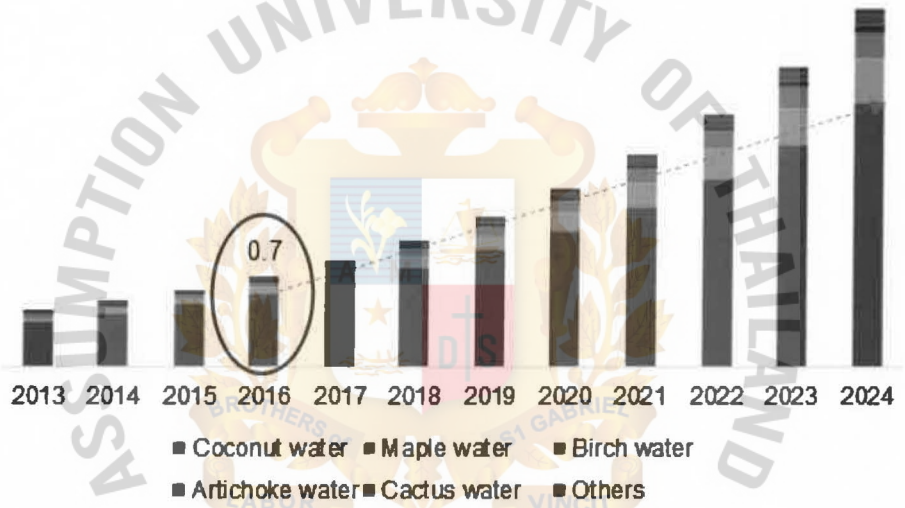


Figure 1 U.S. Plant-based Waters Market Size, By Product, 2013 - 2024 (USD Billion)

From United association of Philippines, show that in Philippines coconut-water were exports more than 66.3 million litters on 2015 which increase around 105% from previous year. (https://www.wsj.com, Lucy Craymer Updated April 7, 2016)

Thailand was agricultural country which has main products is a coconut. Thailand's coconut is very famous in the world. The big markets are Hong Kong, Malaysia, Singapore and Europe.The market still needs a lot of coconut. Currently, domestic consumption 50% export 50%, but the market price is only 12-15 baht per one of coconut, the price should be higher. But the middleman is buying too much. The sale price of the airport is 80-90 baht per one coconut, in some store it around 40 baht per one of coconut, so the price is too different. (www.prachachat.net, 2017).

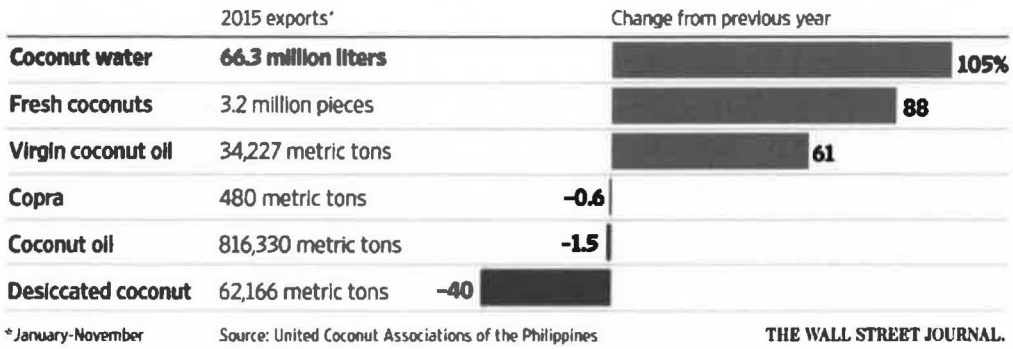


Figure 2 Export of coconut water on Philippines

Benefit of coconut water for Athlete.

Coconut water provide a higher concentration of electrolytes which help to maintain proper hydration status and nerve, heart and muscle function (http://www.stack.com/a/benefits-of-coconut-water, October 08, 2012). Also, less than one percent of natural sugar which important as carbohydrates for athletic endeavours without overdoing. It's low in calories, and it might help prevent you from feeling too full. Most important, coconut water deriver vitamins which can aid in recovery, including vitamin C to boosts immune system, folate (B9) helps body create new cells, and vitamin B6 helps build muscle by producing amino acids. However, every people can drink coconut water include vegan and gluten-free.

Shelf life of fresh coconut water

Fresh coconut water is quick to begin decomposing once the coconut is cut, so with little ability to preserve it, the water was often discarded as an unmarketable by-product. Highest shelf life of unopened to opened coconut water around 1-3 weeks in refrigerator. In some case of coconut water process will include heat in their process, it effect directly to loss of nutrient in the coconut juice also effect to coconut juice flavor. (www.theguardian.com, 2017)

Weight of coconut per one coconut are too heavy that have effect to transportation, so it difficult to export in another country include own country. Moreover, there are many limitation of the coconut juice product in Thailand. Thus, I will solve this problem by use Freeze dry. Freeze dry will help to reduce the problem of nutrition loss, keep taste, aroma, and flavor of coconut water similar to original, and keep coconut juice in longer time in room temperature and solve transportation problem because it have light weight. Therefore, I consider doing the coconut juice powder.

The aim of this study was to formulate and process development for the production of coconut juice powder using freeze dry method. To determine the effect of different amounts of maltodextrin concentration on the physical and powder properties of coconut juice powder. Moreover, to determine the quality of fresh coconut water which have age around 7-9 months. This would develop the Coconut juice powder from freeze dry process to the market which also prolong shelf life of coconut water, easy to transport and convenient to consume. However, it can be the ingredient of sport drink for athletes.

2. Literature review

Freeze-Drying of Aqueous Solutions

Freeze-drying is a two-step dehydration process, first is frozen step and second is water removed by sublimation under vacuum. Freeze dry are involve the water or other solvent removal from a frozen product by using sublimation process. Sublimation occurs when a frozen liquid goes directly to the gaseous state without passing through the liquid phase. Freeze dry is the food preservation method, which is very popular in now. The advantages of freeze drying are make food are easy to transport at temperatures around 25-30°C, help to keep food for a long period of time, help to reduce the preparing method of food for consumer. Moreover, the characteristic and taste of freeze-dried foods are similar to the original, and make those product more natural. Drying above collapse temperature gives a distorted (collapsed) product with poor solubility, low drying rates, uneven drying, and loss of texture and volatile substances. Collapse Temperature is the temperature, which the material are softens to the point of not being able to support its own structure. The increasing solute molecular weight gives higher collapse temperatures, and predicts observed collapse temperatures quantitatively. Freezing causes a micro separation of an aqueous solution into a two-phase mixture of ice and a solute-rich phase. Some solutes crystallize readily, and for such solutions a eutectic mixture of ice and crystallized solute forms. The solutions of eutectic-forming solutes collapse at their eutectic temperature during sublimation. The Eutectic Temperature is the temperature at which the solutes material melts, preventing any structure from

forming after the solvent has been removed. Commonly, many organic solutes and especially mixed solutes such as occur in natural substances are kinetically inhibited from exhibiting eutectic behavior. Freezing these solutions gives a micro scale mixture of ice needles and concentrated amorphous solution (CaS). The CaS remains an amorphous aqueous solution or a glass even below -78C. Glass Transition is the temperature at which the glass first exhibits a changes in viscosity from a brittle solid into a soft solid. The solute concentration beyond which ice will not nucleate represents an upper limit the concentration of solutions to be freeze-dried.[1]

Bulking Agents

Several auxiliary or bulking agents have been utilized during spray and/or freeze-drying to prevent nano-crystal aggregation (Van Eerdenbrugh et al., 2008b,c). Typical matrix formers or bulking agents utilized in spray and/or freeze-drying of nano-crystalline suspensions are as follows.

- Low molecular weight sugars (such as, sucrose and trehalose)
- Sugar alcohol (such as, mannitol)
- High molecular weight sugar polysaccharides (such as, maltodextrins).[2]

The addition of additives such as maltodextrin is necessary to apply this technique to juices, adding amounts not exceeding the operational limits of the equipment (it could increase the viscosity and negatively affect performance) or altering the flavor. Currently, maltodextrin is the most widely used additive to obtain fruit juice powders, since it satisfies the demands and is reasonably cheap (Bhandari, Datta, & Howes, 1997; Bhandari et al., 1993; DibTaxi, Santos, Menezes, & Grosso, 2000; Figueiredo, 1998; Ribeiro, 1999). Production of Maltodextrins may be manufactured either by acid or by acid–enzyme processes. It made from corn, rice, potato starch or wheat.

- Acid process produced by acid conversion of starch from dent corn contain a high percentage of linear fragments, which may slowly re-associate into insoluble

compounds causing haze in certain applications. Haze formation, which results from retrogradation, can be overcome by use of alpha-amylases.

- Enzymatic process using alpha-amylases Alpha-amylases preferentially cleave the alpha-l,4-D-glucosidic bonds of amylose and amylopectin, leaving a higher proportion of branched fragments, decreasing the ability of the fragments to reassociate. Maltodextrins made from waxy corn starch also have a lower tendency to haze, because such starch is composed almost entirely of the highly branched molecule, amylopectin.Thus, I prefer to use maltodextrin as a bulking agent for naturalness of juice.

Maltodextrin has 4 calories per gram which is same amount of calories as sucrose, or table sugar. Like sugar, your body can digest maltodextrin quickly, so it's useful for who need a quick boost of calories and energy. However, maltodextrin's GI is higher than table sugar, ranging from 106 to 136. This means that it can raise your blood sugar level very quickly.[4]

Coconut

There are only two distinct varieties of coconut, the tall and the dwarf. The tall cultivars that are extensively grown are the West Coast Tall and East Coast Tall. The dwarf variety is shorter in stature and its life span is short as compared to the tall.

Tall Varieties Characteristic has 15m to 18m of terr. The nut is medium to big in size varying in shape from spheroid to linear-oblong and with colors varying from green, yellow and orange to shades of brown. About 6,000 nuts yield a ton of copra. Popular to use in coconut milk or oil processing because of thicker meat than dwarf varieties characteristic.

Dwarf Varieties Characteristics has small in stature (5-7 m).Short variety which producing green, orange and yellow nuts. Nuts are small in size and ovoid or round in shape. Nut weighs about 3 oz (85 gm) with 65 per cent oil content. Popular to consume fresh coconut water (Aromatic coconut in Thailand)[5]

3. Material and methods

3.1 Raw materials

Fresh coconut water (7-9 month or two layer's coconut meat) obtained from coconut farm in Amphawa, Samut Sonhkhram, Thailand. Maltodextrin (MD) with 10-12 DE value was use as bulking agent which

3.2 Preparation of fresh coconut water.

Collect coconut water after break coconut's shell. Pour coconut water into sieve to prevent contamination of physical hazard. Fresh coconut water have to weight before mix with MD every time to determine concentration of MD would use, which divided in to 3 conc. 5%, 10%, and 15% of MD. Mix MD with fresh coconut water by overhead stirrer at 400 rpm for 15 min. Coconut water solution was dried by freeze dry machine.

3.3 Freeze drying

Freeze drying were performed at Durian freezedry co.,Ltd. for 48 hours. Fresh coconut water was mix with maltodextrin 5%, 10%, and 15% to around 1lites of solution. The freeze dry was operated under vacuum at -30°C condenser temperature. One lite of solution were pour into each freeze dried tray. Powder was stored in air tight package in cold storage before physiochemical analysis and sensor test.

3.4 Physiochemical analysis of fresh coconut water

Quality parameters of fresh coconut water was determined to study are as follows.The weight of fresh coconut water was determined with 2-digit balance. The pH was determined by Hanna pH 211 Microprocessor pH Meter at 26-27 °C. Soluble Solid of fresh coconut water measured with refractometer. The color (L*, a*, b*values) measured with Color spectrophotometer (Miniscan EZ-4500L spectrophotometer, Hunter Lab co.,Ltd, USA) at D65/10standard light source (outdoor daylight) and 45°/0° angle of illumination/observe. The L* values is measure of lightness which ranges between 0 to 100. The a* value represent green to red color from negative to positive values

respectively. Moreover, b* value represent negative value in yellow color and positive value in blue color.

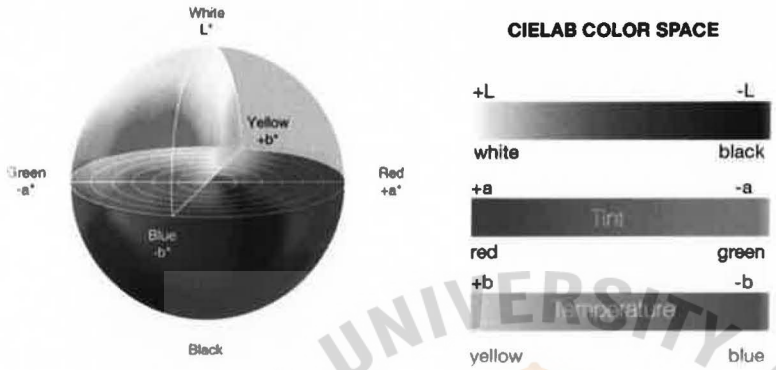


Figure 3 CIE LAB color space (http://obsessive-coffee-disorder.com/tag/cielab/., Nov 16, 2014)

The percentage of titratable acidity was determined by use titration technique according to the (AOAC 2000).

Calculate from $\%TA = \frac{NxMxV_1x100}{V_2x\ 1000}$

N = normality of NaOH used
M = Malic acid factor (67.05)
V_1= Tritated volume
V_2 = Volume of coconut water added

3.5 Analysis of the powder properties.

The different concentration of maltodextrin of coconut juice powder were analyzed for the different physical properties such as moisture content, bulk density, trapped density, porosity, %Carr's compressibility, color, hygroscopicity, and degree of caking.

3.5.1 Moisture content

Dried empty can for 2-3 hours. An accurately weighed 5g of coconut juice powder was taken in empty can. The can along with sample was placed in the oven, maintained at 105±1°C until a constant.

Moisture content (%) = [(W2 – W) / (W1 – W)] ×100

Where;
W = Weight of empty Petri dish
W1 = Weight of Petri dish with sample before drying;
W2 = Weight of Petri dish with sample after drying to constant weight.

3.5.2 Bulk density, Trapped density, porosity, and %Carr's compressibility

Weight 5 g of coconut juice powder and pour into 25ml cylinder and record the bulk volume to calculate bulk density. Tap the powder about 100 times and record the tapped volume to calculate tapped density. Then, calculate for % carr's compression. Further, calculate porosity

Bulk density = Weigh/ Bulk volume

Tapped density = Weigh/ True volume

Carr's index (%) = (Poured or bulk density/ Tapped density) × 100

Porosity = (1- Bulk density/True density)

Flow description	%Compressibility
Excellent Flow	5-15
Good	16-18
Fair	19-21
Poor	22-35
Very poor	36-40
Extremely poor	>40

Figure 4 Relationship between powder flowability and % compressibility.

3.5.3 Hygroscopicity

Hygroscopicity of powder samples was determined according to the method given by Caparinoet al.(O.A. Caparino, J. Tang, C.I. Nindo, S.S. Sablani, J.R. Powers, J.K. Fellman, Effect of drying methods on the physical properties and microstructures of mango powder, J. Food Engg. 111 (2012) 135–148.)with slight modifications. Approximately One gram of powder sample was placed in a can, with three replicate samples for each concentration of maltodextrin coconut juice powder, put separately in desiccators containing saturated NaCl solution (75.5% humidity) and stored at 25°C for 7 days. Hygroscopicity expressed as grams of adsorbed moisture per 100 g dry solids (g/100 g) was calculated, using the following equation:

Hygroscopicity = [Δm / (M+Mi) / (1+ Δm/M)]

Where;
Δm (g) is the increase in weight of powder after equilibrium
M is the initial mass of powder.
Mi (%, wb) is the free water contents of the powder before exposing to the humid air environment

3.5.4 Color Measurement

Color measurement of the powder samples was carried out using a Hunter Lab Color spectrophotometer (Miniscan EZ-4500L spectrophotometer, Hunter Lab co.,Ltd, USA) at D65/10standard light source (outdoor daylight) and 45°/0° angle of illumination/observe. The results were expressed in terms of L*, a* and b* value, where 'L' was represent lightness, 'a' to redness and greenness and 'b' to yellowness and blueness.

3.6 Sensory Analysis

3.6.1 The study effect of different Total soluble solid (°Brix) on sweetness of rehydrated coconut water and effect of maltodextrin concentration on rehydrated coconut water.

The sensory analysis was done by 30 untrained panelists for both experiments. Panels were asked to rate the liking score by using the 9-point hedonic scale and just about right scale to measure the appropriateness of the level of an attribute. There are 6 attribute that use in both experiments follow figure.

No. .....

Rehydrated Coconut Juice

Date: .....

Instruction: Please taste the sample; remember to rinse your plate with water or saltine cracker before tasting the other. Rate the sample based on your preference using a 9-point hedonic scale. Please rate the level of a sensory attribute of the sample by using √ symbol.

1 = Dislike extremely 2 = Dislike very much 3 = Dislike moderately
4 = Dislike slightly 5 = Neither like nor dislike 6 = Like slightly
7 = Like moderately 8 = Like very much 9 = Like extremely

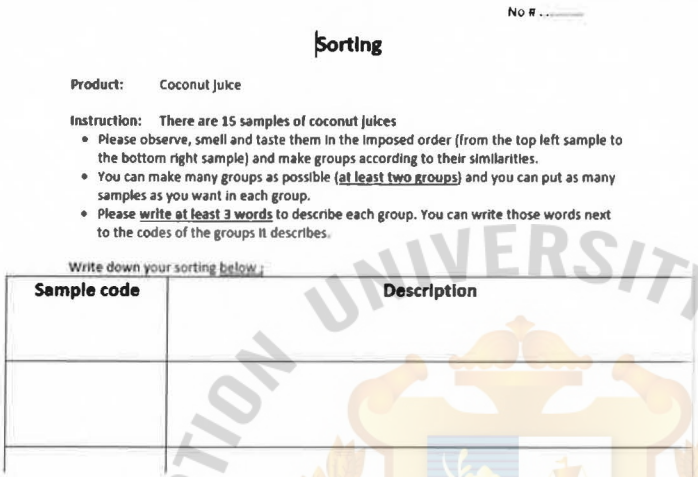
Attribute	Liking Score Sample 415	Just About Right Test				
		Much too weak	Somewhat too weak	Just about right	Somewhat too strong	Much too strong
Turbidity (ความขุ่น)		O	O	O	O	O
Aroma (กลิ่น)		O	O	O	O	O
Flavor (รสชาติ)		O	O	O	O	O
Sweetness (ความหวาน)		O	O	O	O	O
Body		O	O	O	O	O
Overall liking (ความชอบโดยรวม)						

Figure 5 a 9-point hedonic scale and JAR scale

The sensory analysis was carried out in individual booths, under white light and the temperature from 23 to 25°C. Each rehydrated coconut juice has temperature below 10°C and served with water for cleaning the palate between assessments was provided.

3.6.2 The investigate sensorial properties of rehydrated coconut juice powder using sorting method

The sensory was done by 50 untrained panelists. Panels were asked to group 14 commercial brand with rehydrated coconut juice by sorting form by follow figure.



No # .....

Sorting

Product: Coconut Juice

Instruction: There are 15 samples of coconut juices

- Please observe, smell and taste them in the imposed order (from the top left sample to the bottom right sample) and make groups according to their similarities.
- You can make many groups as possible (at least two groups) and you can put as many samples as you want in each group.
- Please write at least 3 words to describe each group. You can write those words next to the codes of the groups it describes.

Write down your sorting below :

Sample code	Description

Figure 6 Sorting form of coconut water

The sensory analysis was carried out in individual booths, under white light and the temperature from 23 to 25°C. Each rehydrated coconut juice has temperature below 10°C and served with water for cleaning the palate between assessments was provided.

Table 1 Description of 14 commercial coconut water brand.

Categories	**Product Brand Names**	**Product Description**	**Picture**
Fresh coconut water	Cocomax	100% coconut water -No Preservative - Overall calories 80kcal/350ml.	

	Maprao	100% Organic coconut water -No Preservative - Overall calories 63kcal/250ml.	
	Icoco	100% coconut water -No Preservative	
	Tipco	100% coconut water - Overall calories 225kcal/1L.	
	Chaokho	100% coconut water -No Preservative - Overall calories 180kcal/1L.	
	Chabaa	100% pure coconut water - Overall calories 175kcal/1L.	

	Malee coco	100% coconut water -No Preservative - Overall calories 225kcal/1L.	
	UFC refresh	100% coconut water - Overall calories 225kcal/1L.	
	Coc'eau	100% coconut water - Overall calories 80kcal/310ml.	
	If	100% coconut water	
	All coco	100% coconut water	

Rosted coconut water	If	99.9% rosted coconut water	
	Magic farm	80% rosted coconut water 5% sugar	
	Foco life plus	98% coconut water 2% sugar	

3.7 Experimental design and statistical analysis

The study physiochemical properties of fresh coconut water effect of Total soluble solid on sweetness (5 to 10°Brix), effect of maltodextrin concentrations (5% to 15%) on properties of coconut juice powder, investigate sensorial properties of rehydrated coconut juice powder using sorting method was evaluated by analysis of variance (ANOVA). Duncan's multiple range test at a 95% confidence level was use to determine the differences between means. All data were analyzed using ANOVA in R program version 2.15.3.

4.Results and discussion

4.1 The study of physiochemical properties of fresh coconut water

This physicochemical properties of fresh cut coconut water was investigated to evaluate average age of coconut in each lot before using coconut water in freeze dry process and also compared some attribute with rehydrated coconut juice to use in sensory test to know acceptance of consumer. However, age of coconut can estimate from their physical by observe from color of outer layer, fibrous husk or coconut meat (endosperm).

From the Table 2 shows, all attribute of coconut water in this lot were not significant different ($p>0.05$), which ranged from xxx to xxx.

Weight, pH value, and °Brix can be used to indicate the age of fresh coconut water. The result in table 2, range of weight, pH, and °Brix indicated that age of coconut water should be around 7 to 9 months. Weight of coconut water in 7 to 9 months must around 250 to 550 gram (*Jose´ C Jackson,1 * Andre´ Gordon, 2004*). pH value and °Brix of coconut water in 7 to 10 month must be around 4.5 to 5.3 and 5.5 to 8.0 respectively (*Coconut Hand book, ©Tetra Pak 2018*).

Percentage of titratable acidity refers to the total concentrations of free protons and undissociated acids in a solution that can react with a strong base and be neutralized. In 7 to 9 month of coconut water, %TA should be around 0.07 to 0.09 but from the experiment %TA around 0.15, which can summarize that in difference area, geographic, quality of soil or environment factor can effect to different of free protons and undissociated acids in coconut water.

Color measurement of this experiment related to turbidity of fresh coconut water. From my assumption, L* value represent the brightness of objective, from the result L*value around 3.29±2.85 means fresh coconut water quite turbid because it can absorb most of black color from black cover.

Table 2 Physiochemical properties of fresh coconut water.

	Attributes	Mean	Min	Max
	Weight (g)	402.594±32.64^{ns}	357.50	446.33
	pH	4.98±0.12 ^{ns}	4.80	5.11
	Brix	6.50±0.50 ^{ns}	6.00	7.00
	%TA	0.15±0.01 ^{ns}	0.14	0.17
Color	L*	3.29±2.85 ^{ns}	1.58	8.26
	a*	-0.25±0.05 ^{ns}	-0.29	-0.17
	b*	-0.82±0.24 ^{ns}	-1.12	-0.50

4.2 The study affect of Total soluble solid on sweetness of coconut juice powder.
Result of sensory analysis

Rehydrated coconut juice samples were tested using a 9-point hedonic scale preference test and just about right analysis by 30 panelists on six attributes – Turbidity, Aroma, Flavor, Sweetness, Body, Overall liking. Coconut juice powder were dissolved by cold water to required total soluble solid contents (5,7, and 10°Brix). From pie chart showed the preference score of rehydrated coconut juice with different °Brix , 67% of 30 panelists prefer 10°Brix. At 10°Brix showed higher liking score at all attributes than 5°Brix and 7°Brix except turbidity. The scores of 10°Brix of rehydrated coconut juice for aroma, sweetness, body, and overall liking were 6.5 ± 1.11, 7.07 ± 1.39, 7.27 ± 1.55, 6.73 ± 1.70, 7.23 ± 1.19, respectively. From penalty analysis Fig.9 showed that there are two attributes that effect to 10°Brix rehydrated coconut juice, one attribute is much too strong body which gave effect mean drop around 1.4 and 0.3 % respondent. xxx give this attribute effect to the sample, and much too strong flavor effect mean drop around -0.6 and 0.4 % respondent give this attribute effect to the sample which also effect liking score of rehydrated coconut juice.

Table 3 Mean scores for sensory attribute of rehydrated coconut juice by 30 panelists.

	Turbidity^{ns}	Aroma	Flavor	Sweetness	Body	Overall liking
5°Brix	6.7 ± 1.7	5.9 ± 1.1^{b}	5.6 ± 1.5^{b}	5.7 ± 1.8^{b}	5.8 ± 1.8^{b}	6 ± 1.4^{b}
7°Brix	7 ± 1.4	6.47±1^{a}	6.4 ± 1.1^{a}	6.3 ± 1.5^{b}	6.4± 1.4^{a}	6.6 ± 0.9^{a}
10°Brix	7.1 ± 1.6	6.5 ± 1.1^{a}	7.1± 1.4^{a}	7.3 ± 1.6^{a}	6.7± 1.7^{a}	7.2 ± 1.12^{a}

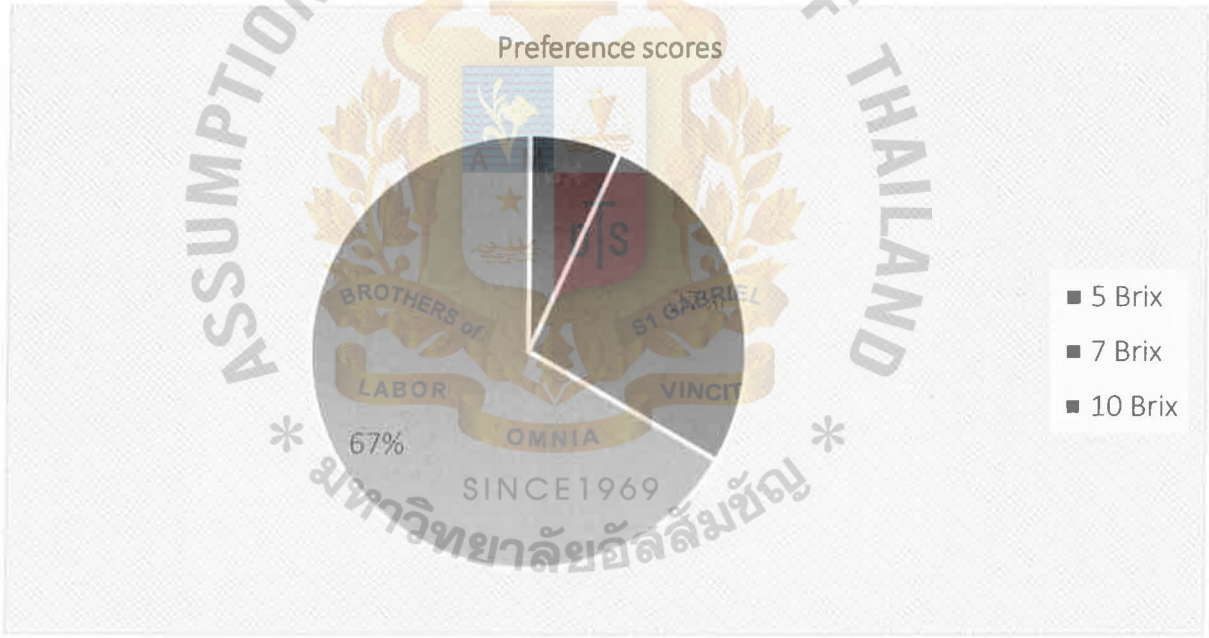


Figure 7 Pie chart of preference score of rehydrated coconut juice by 30 panelists

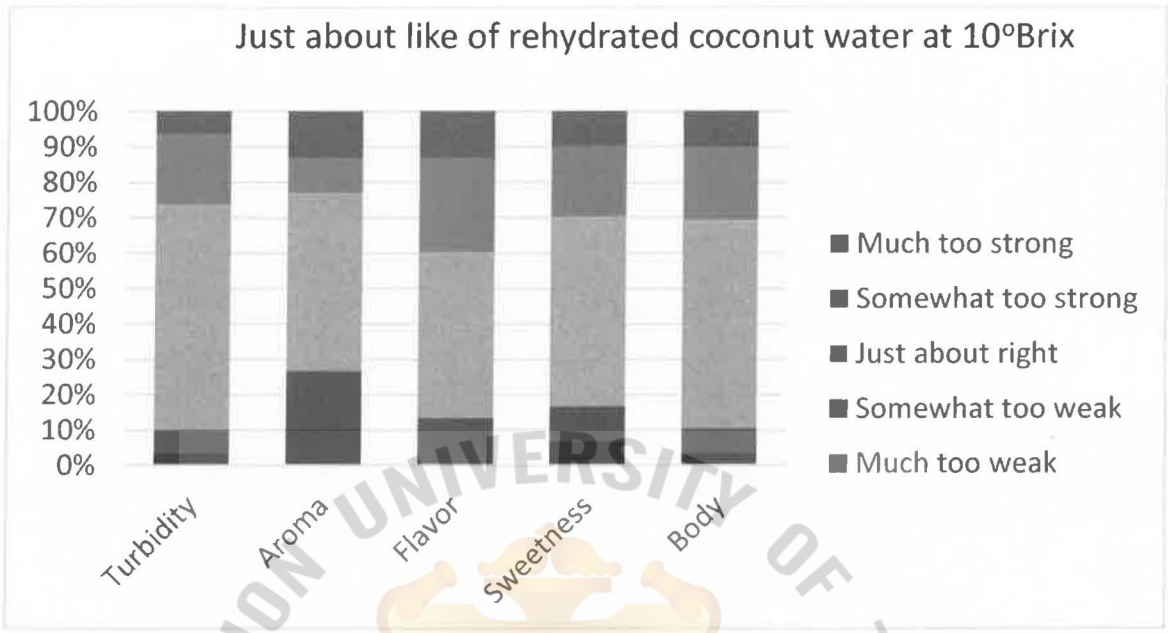


Figure 8 Just about like of rehydrated coconut water at 10°Brix

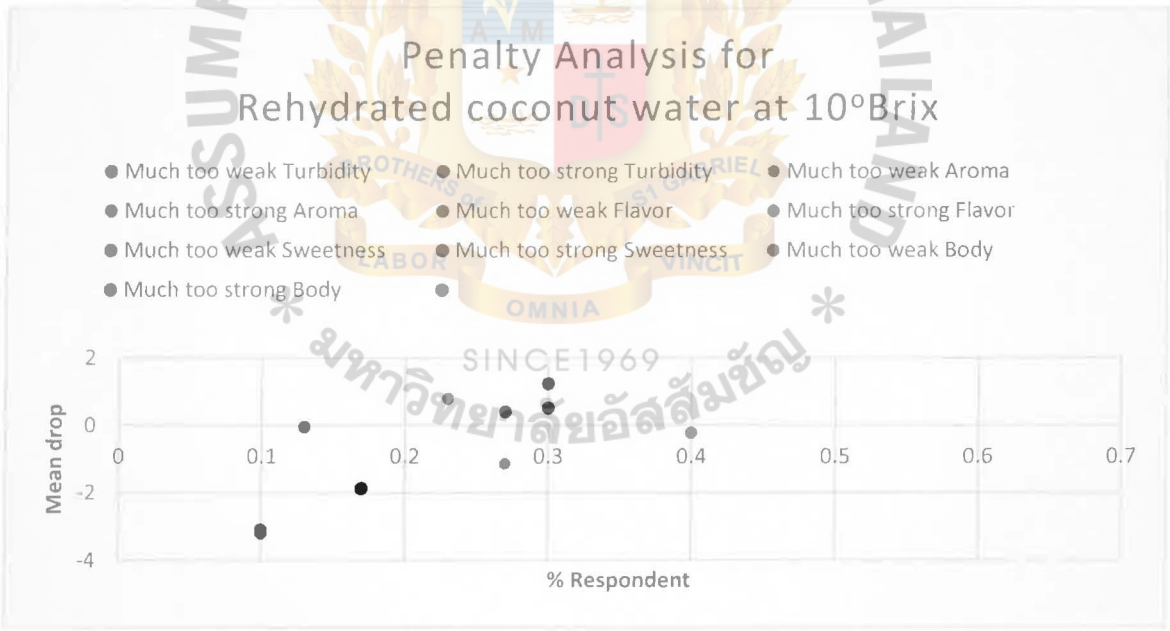


Figure 9 Penalty Analysis for rehydrated coconut water at 10° Brix

4.3 The study effect of maltodextrin concentrations on properties of coconut juice powder.

Result of sensory analysis

Rehydrated coconut juice samples were tested using a 9-point hedonic scale preference test and just about right analysis by 30 panelists on six attributes – Turbidity, Aroma, Flavor, Sweetness, Body, Overall liking. Different concentration of maltodextrin (5%, 10%, and 15%) in coconut juice powder were dissolve by cold water to required total soluble solid contents at 10°Brix. From pie chart show the preference score of rehydrated coconut juice with different concentration of maltodextrin at 10°Brix, 83% of 30 panelists prefer 5% maltodextrin. At 5% maltodextrin showed higher liking score at all attributes than 10% maltodextrin and 15% maltodextrin except turbidity. The scores of 5% maltodextrin of rehydrated coconut juice for Aroma, Sweetness, Body, and Overall liking were 6.9 ± 1.16, 6.97 ± 1.38, 7.07 ± 1.41, 7 ± 1.08, 7.37 ± 1.3, respectively. From penalty analysis figure show, most of attribute nearly 0 mean drop and % respondent quite low around 0.1% respondent.

Table 4 Mean scores for sensory attribute of rehydrated coconut juice with different concentration of maltodextrin by 30 panelists.

	Turbidity^{ns}	Aroma	Flavor	Sweetness	Body	Overall liking
5%MTD	6.9 ± 1.3	6.9 ± 1.2^{a}	7 ± 1.34^{a}	7.0 ± 1.4^{a}	7 ± 1.1^{a}	7.4 ± 1.3^{a}
10%MTD	6.9 ± 1.1	5.7 ± 1.3^{b}	6 ± 1.3^{b}	6 ± 1.4^{b}	5.8 ± 1.4^{b}	6.0 ± 1.3^{b}
15%MTD	6.7 ± 1.3	5.5 ± 1.3^{b}	5.5 ± 1.5^{b}	5.5 ± 1.67^{b}	5.4 ± 1.6^{b}	5.4 ± 1.6^{b}

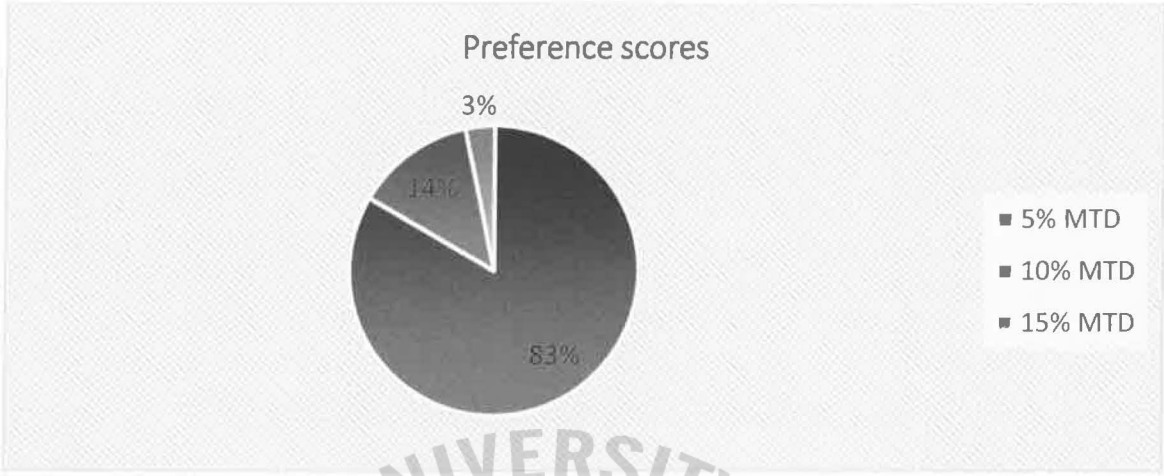


Figure 10 Pie chart of preference score of rehydrated coconut juice by 30 panelists.

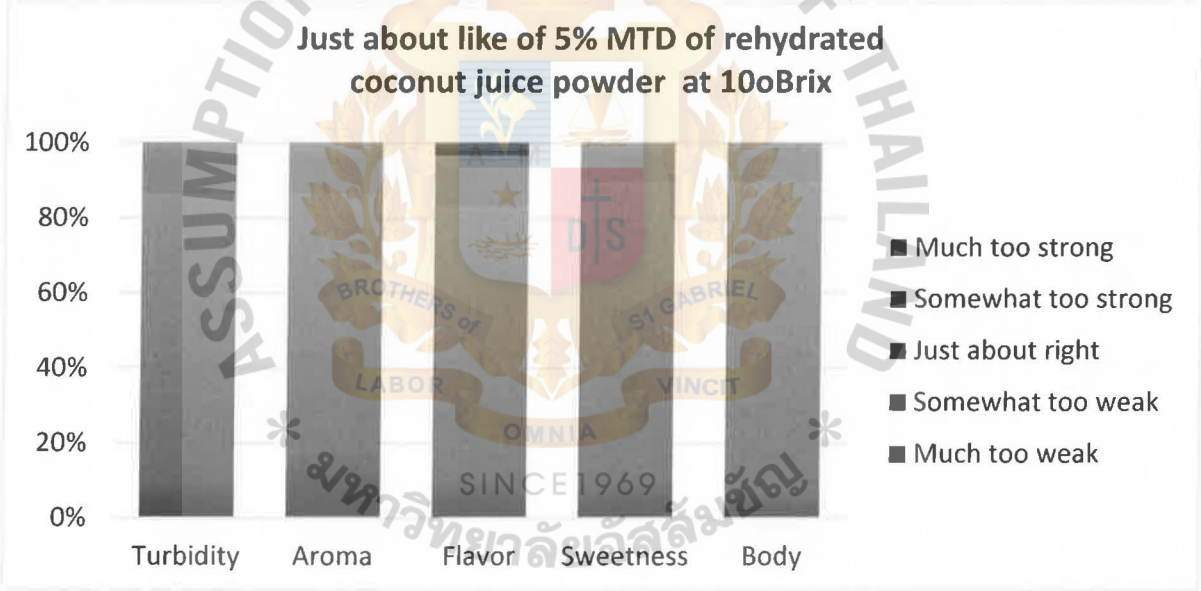


Figure 11 Just about like of 5% MTD of rehydrated coconut juice powder at 10o Brix

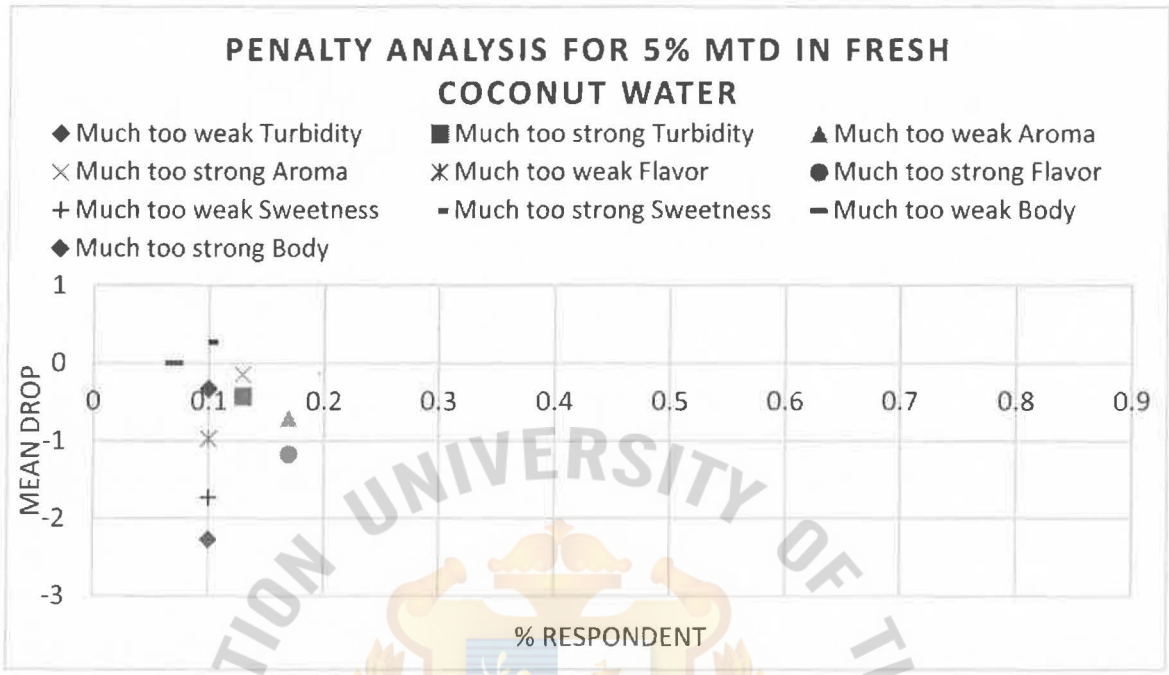


Figure 12 Penalty Analysis for 5% MTD in fresh coconut water.

Physical properties of coconut juice powder

Hygroscopicity is one of the measurements which indicated the shelf life of product by ability of the food powder to absorb environmental moisture also show effect of maltodextrin on absorption of moisture from environment. Result shown in table 5, the hygroscopic of coconut juice powder ranged between 0.04 to 0.05 g/100g, which means maltodextrin DE 10-12 have effect to low hygroscopicity of coconut juice powder that can help product have longer shelf life. Additionally, while the less hygroscopic product was obtained by increasing amount of maltodextrin at lower drying temperature, the more hygroscopic product obtained at a higher temperature (de Souza, Thomazini, de Carvalho Balieiro, & Favaro-Trindade, 2015)

Moisture content activity of coconut juice powder ranged between 4.37% to 4.68%. From table 5 shown, with high concentration of maltodextrin provide low % moisture but it is not significant different when compare with 5% and 10% maltodextrin

Bulk density, Tapped density of the powders decreased with ranged between 0.4 to 0.64, 0.53 to 0.77, respectively, which related to increasing in concentration of the maltodextrin.

Carr's index (%) related to flowability of powder product, from the result coconut juice powder have high percentage of Carr's index which is very poor in flowability the ranged around 76% to 84.21%.

Color is an important parameter which indicates the quality of powder. Color of the coconut juice powder was significantly affected ($P > 0.05$) by the difference concentration of maltodextrin. Analysis color of coconut juice powder shows, 15% maltodextrin give highest L* (mean most bright) but lowest in b* (mean more yellow)

Table 5 Physical properties of coconut juice powder with different concentration of maltodextrin.

		5%	10%	15%
		Mean	Mean	Mean
Hygroscopicity (g/100g)		0.04^{ns}	0.03^{ns}	0.05 ± 0.04^{ns}
Moisture content %	5 hours	4.68 ± 0.34^{ns}	4.49 ± 0.13^{ns}	4.37 ± 0.37^{ns}
Bulk density (g/ml)		0.64^{ns}	0.53^{ns}	0.40^{ns}
Tapped density		0.77^{ns}	0.63^{ns}	0.53^{ns}
Carr's index (%)		83.33^{ns}	84.21^{ns}	76.00^{ns}
Porosity		0.47^{ns}	0.75^{ns}	1.14^{ns}
Color	L*	87.70 ± 4.07^{b}	87.63 ± 0.73^{b}	92.48 ± 0.16^{a}
	a*	-0.78 ± 0.07^{b}	-0.64 ± 0.06^{a}	-0.65 ± 0.07^{a}
	b*	5.62 ± 0.33^{a}	3.16 ± 0.24^{b}	3.28 ± 0.07^{b}

4.4 The investigate sensorial properties of rehydrated coconut juice powder using sorting analysis.

From compromise and confidence interval shown, 7 groups of coconut water are as follow

1. Sample and Cocomax which have similar in 100% coconut aroma, clear color, young and high coconut flavor, and quite low body attribute.
2. Maprao and All coco which have similar in 100% coconut aroma, clear color, young and high coconut flavor, Natural taste, and quite low body attribute.
3. Coc'eau, Icoco, If aromatic coconut water, Focolife+ which have similar in Mild coconut aroma, good aroma, good taste, sweet aroma, fruity aroma, floral aroma, diluted coconut juice, cler in color, high sweet, fresh coconut flavour, Natural, and Low body attribute.
4. If roasted coconut water and magic farm which have similar in Fermented aroma, vanilla aroma, roasted coconut water, strong and stranger flavour, clear yellow color, vanilla and plastic flavour, high body, and strong after taste attribute.
5. Chaba and Chaokoh which have similar in Roasted coconut aroma, strong, strang and vanilla aroma, opuqe, turbid, and high body
6. Tipco which have Like coconut aroma, turbid, coconut flavour, sweet and sour taste, wood flavour, and sweet after taste attribute.
7. Refresh and Malee coco which have similar in coconut shell flavour, and sweet and sour taste.

The result shows, rehydrated coconut juice powder was the same group with commercial brand "Cocomax", which similar by follow attribute 100% coconut aroma, clear color, young and high coconut flavor, and quite low body.

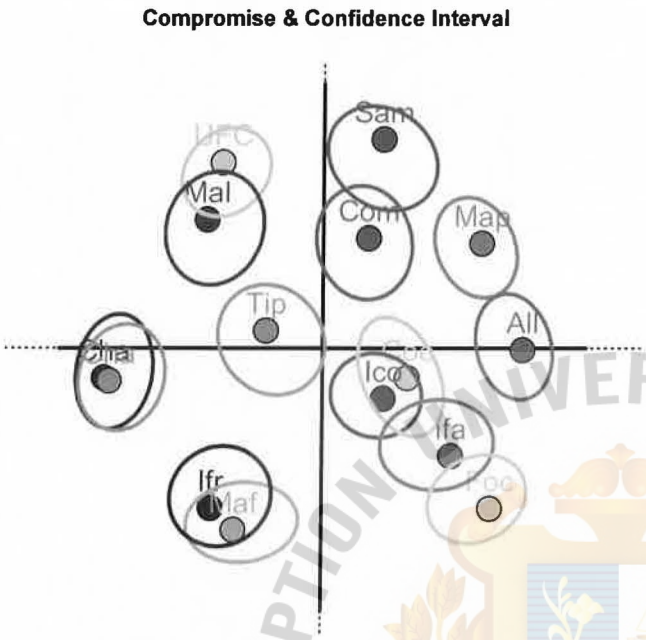


Table 6 Name and symbol of commercial coconut water

Brand Name	Symbol
Chaba	Cha
Tipco	Tip
UFC Refresh	UFC
Chaokoh	Cha
Malee coco	Mal
Cocomax	Com
Maprao	Map
Icoco	Ico
Coc'eau	Coc
If	Ifa
Allcoco	All
Foco life+	Foc
Magic farm	Maf
If rosted	Ifr
Sample	Sam

5. Conclusion

The study physiochemical properties of fresh coconut water show that weight, pH and Soluble solid value of fresh coconut water within 402.59 g, 4.98 pH and 6.50°Brix respectively, which is the range 7-9 month of coconut age.

The study the effect of TSS (Total soluble solid) on sweetness of coconut juice powder show that 66.67% or 20 penal prefer 10oBrix of rehydrated coconut juice.

The study effect of maltodextrin on properties of coconut juice powder show that color properties (L* a* b*) of the coconut juice powder was significantly affected by the difference concentration of maltodextrin. Bulk density and Tapped density of the powders decreased with increase in concentration of the maltodextrin.

The investigate sensorial properties of rehydrated coconut juice powder using flash profiling rapid method show that rehydrated coconut juice similar to commercial

bran " Cocomax" which have 100% coconut aroma, clear color, young and high coconut flavor, and no-low body.

6. Reference

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Appendix

Appendix A : Statistical analysis and sensory result of coconut juice powder

1.The study of physiochemical properties of fresh coconut water.

1.1 Weight

```
> summary(RCBD)
            Df Sum Sq Mean Sq F value Pr(>F)
trt          1    907   906.7   0.811  0.434
Residuals    3   3356  1118.6
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

1.1.2 pH

```
> summary(RCBD)
            Df  Sum Sq  Mean Sq F value Pr(>F)
trt          1 0.00312 0.003121   0.161  0.715
Residuals    3 0.05827 0.019423
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

1.3 °Brix

```
> summary(RCBD)
            Df Sum Sq Mean Sq F value Pr(>F)
trt          1     0  0.0000       0      1
Residuals    3     1  0.3333
---
```

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

1.4 %TA

> summary(RCBD)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
trt	1	9.79e-05	0.0000979	0.295	0.625
Residuals	3	9.97e-04	0.0003323		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

1.5 Color (L* value)

> summary(RCBD)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
trt	1	14.17	14.17	2.334	0.224
Residuals	3	18.21	6.07		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

1.6 Color (a* vale)

> summary(RCBD)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
trt	1	0.000014	0.0000144	0.005	0.95
Residuals	3	0.009413	0.0031376		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

1.7 Color (b* value)

```
> summary(RCBD)
            Df Sum Sq Mean Sq F value Pr(>F)
trt          1 0.1153 0.11535    2.83  0.191
Residuals    3 0.1223 0.04075
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2. The study of affect of Total soluble solid on sweetness of coconut juice powder.

Sensory test (30 panelists)

2.1Turbidity

```
> summary(RCBD)
            Df Sum Sq Mean Sq F value Pr(>F)
trt          1   2.02   2.017   0.836 0.3631
rep          1   8.79   8.794   3.645 0.0595 .
Residuals   87 209.91   2.413
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

2.2 Aroma

```
> summary(RCBD)
            Df Sum Sq Mean Sq F value  Pr(>F)
trt          1   5.40   5.400   4.973 0.02832 *
rep          1   8.61   8.614   7.933 0.00601 **
Residuals   87  94.47   1.086
```

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Means with the same letter are not significantly different.

Groups, Treatments and means

a 3 6.5

a 2 6.467

b 1 5.9

1.2.3 Flavor

> summary(RCBD)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
trt	1	33.75	33.75	18.490	4.45e-05 ***
rep	1	0.07	0.07	0.039	0.843
Residuals	87	158.80	1.83		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Means with the same letter are not significantly different.

Groups, Treatments and means

a 3 7.067

a 2 6.433

b 1 5.567

1.2.4 Sweetness

```
> summary(RCBD)
            Df Sum Sq Mean Sq F value   Pr(>F)
trt          1  36.82   36.82  14.718 0.000236 ***
rep          1   7.34    7.34   2.935 0.090229 .
Residuals   87 217.63    2.50
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Means with the same letter are not significantly different.


Groups, Treatments and means
a      3      7.267
b      2      6.267
b      1      5.7
```

1.2.5 Body

```
> summary(RCBD)
            Df Sum Sq Mean Sq F value Pr(>F)
trt          1  11.27  11.267   4.261  0.042 *
rep          1   0.69   0.686   0.259  0.612
Residuals   87 230.05   2.644
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Means with the same letter are not significantly different.

Groups, Treatments and means

a 3 6.733

a 2 6.4

a 1 5.867

1.2.6 Overall liking

```
> summary(RCBD)
            Df Sum Sq Mean Sq F value   Pr(>F)
trt          1  24.07  24.067  16.905 8.88e-05 ***
rep          1   1.47   1.468   1.031    0.313
Residuals   87 123.85   1.424
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Means with the same letter are not significantly different.

Groups, Treatments and means

a 3 7.233

a 2 6.633

b 1 5.967

3.The study effect of maltodextrin concentrations on properties of coconut juice powder.

3.1 Physical properties of coconut juice powder

3.1.1 Hygroscopicity

> summary(RCBD)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
trt	1	0.000067	6.67e-05	0.105	0.757
rep	1	0.000600	6.00e-04	0.942	0.369
Residuals	6	0.003822	6.37e-04		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

3.1.2 Moisture content

> summary(RCBD)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
trt	1	4.518	4.518	7.003	0.0141 *
rep	1	0.038	0.038	0.058	0.8115
Residuals	24	15.484	0.645		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Means with the same letter are not significantly different.

Groups, Treatments and means

a	3	4.496
ab	2	3.803
b	1	3.494

3.1.2.1 Moisture 5 hours

> summary(RCBD)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
rep	1	0.0000	0.0000	0	0.998
Residuals	7	0.8918	0.1274		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

3.1.3 Color (L* value)

> summary(RCBD)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
trt	1	57.17	57.17	8.457	0.0131 *
rep	1	7.27	7.27	1.076	0.3201
Residuals	12	81.12	6.76		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Means with the same letter are not significantly different.

Groups, Treatments and means

a	3	92.48
b	1	87.7
b	2	87.63

3.1.4 Color (a* value)

> summary(RCBD)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
trt	1	0.04489	0.04489	7.905	0.0157	*
rep	1	0.00016	0.00016	0.029	0.8682	
Residuals	12	0.06815	0.00568			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Means with the same letter are not significantly different.

Groups, Treatments and means

a	2	-0.644
a	3	-0.646
b	1	-0.78

3.1.5 Color (b* value)

> summary(RCBD)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
trt	1	13.736	13.736	26.510	0.000241	***
rep	1	0.038	0.038	0.074	0.790701	
Residuals	12	6.218	0.518			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Means with the same letter are not significantly different.

Groups, Treatments and means

a 1 5.62

b 3 3.276

b 2 3.156

3.2 Effect of maltodextrin on consumer acceptance of coconut juice powder. Sensory test (30 panelists)

3.2.1 Turbidity

> summary(RCBD)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
trt	1	0.60	0.600	0.393	0.532
rep	1	1.02	1.022	0.669	0.415
Residuals	87	132.78	1.526		

3.2.2 Aroma

> summary(RCBD)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
trt	1	28.02	28.017	18.001	5.49e-05 ***
rep	1	8.40	8.401	5.398	0.0225 *
Residuals	87	135.40	1.556		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Means with the same letter are not significantly different.

Groups, Treatments and means

a 1 6.9

b 2 5.7

b 3 5.533

3.2.3 Flavor

> summary(RCBD)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
trt	1	33.75	33.75	18.950	3.64e-05	***
rep	1	13.71	13.71	7.696	0.00677	**
Residuals	87	154.94	1.78			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Means with the same letter are not significantly different.

Groups, Treatments and means

a 1 6.967

b 2 5.967

b 3 5.467

3.2.4 Sweetness

> summary(RCBD)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
trt	1	35.27	35.27	16.125	0.000125	***
rep	1	6.86	6.86	3.135	0.080148	.

Residuals 87 190.28 2.19

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Means with the same letter are not significantly different.

Groups, Treatments and means

a 1 7.067

b 2 6

b 3 5.533

3.2.5 Body

> summary(RCBD)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
trt	1	38.40	38.40	20.142	2.19e-05	***
rep	1	1.34	1.34	0.702	0.404	
Residuals	87	165.86	1.91			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Means with the same letter are not significantly different.

Groups, Treatments and means

a 1 7

b 2 5.8

b 3 5.4

3.2.6 Overall liking

> summary(RCBD)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
trt	1	58.02	58.02	33.354	1.17e-07	***
rep	1	14.25	14.25	8.194	0.00526	**
Residuals	87	151.33	1.74			

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Means with the same letter are not significantly different.

Groups, Treatments and means

a 1 7.367

b 2 6.033

b 3 5.4

Appendix B : Just about Right analysis and Penalty analysis result of rehydrated coconut juice powder.

1. The study effect of total soluble solid on sweetness of coconut juice powder.

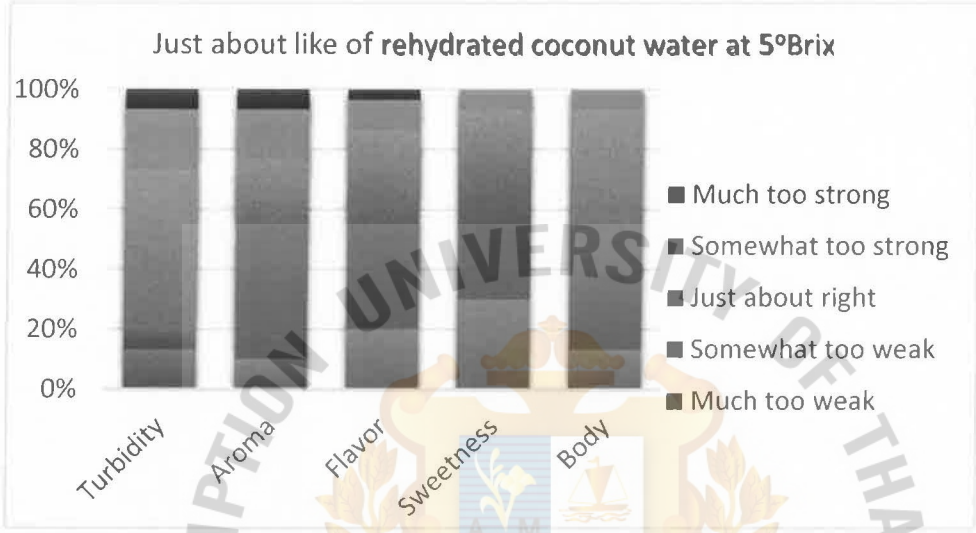


Figure 1 Just about right 5% MTD of rehydrated coconut juice powder at 5°Brix

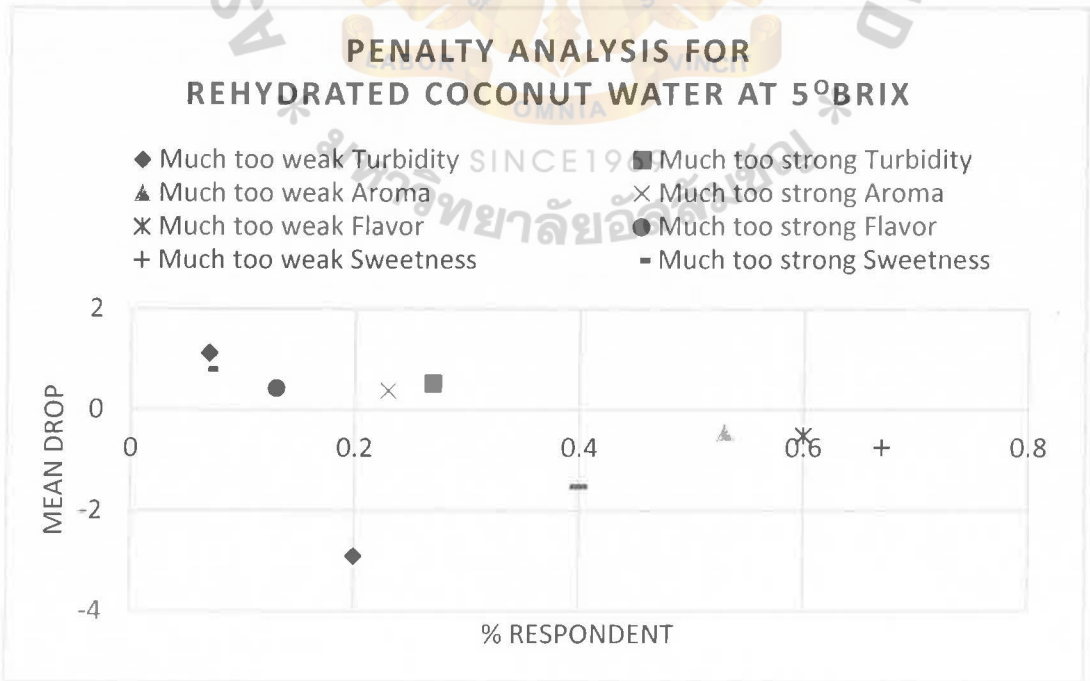


Figure 2 Penalty Analysis for 5% MTD of rehydrated coconut juice powder at 5°Brix

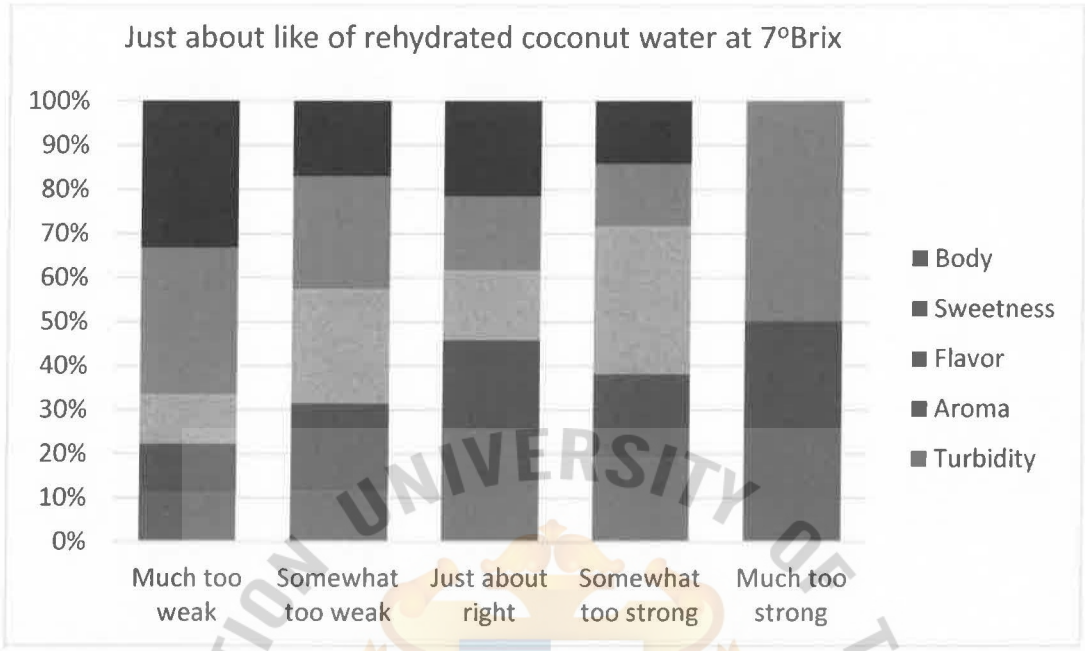


Figure 3 Just about right 10% MTD of rehydrated coconut juice powder at 7°Brix

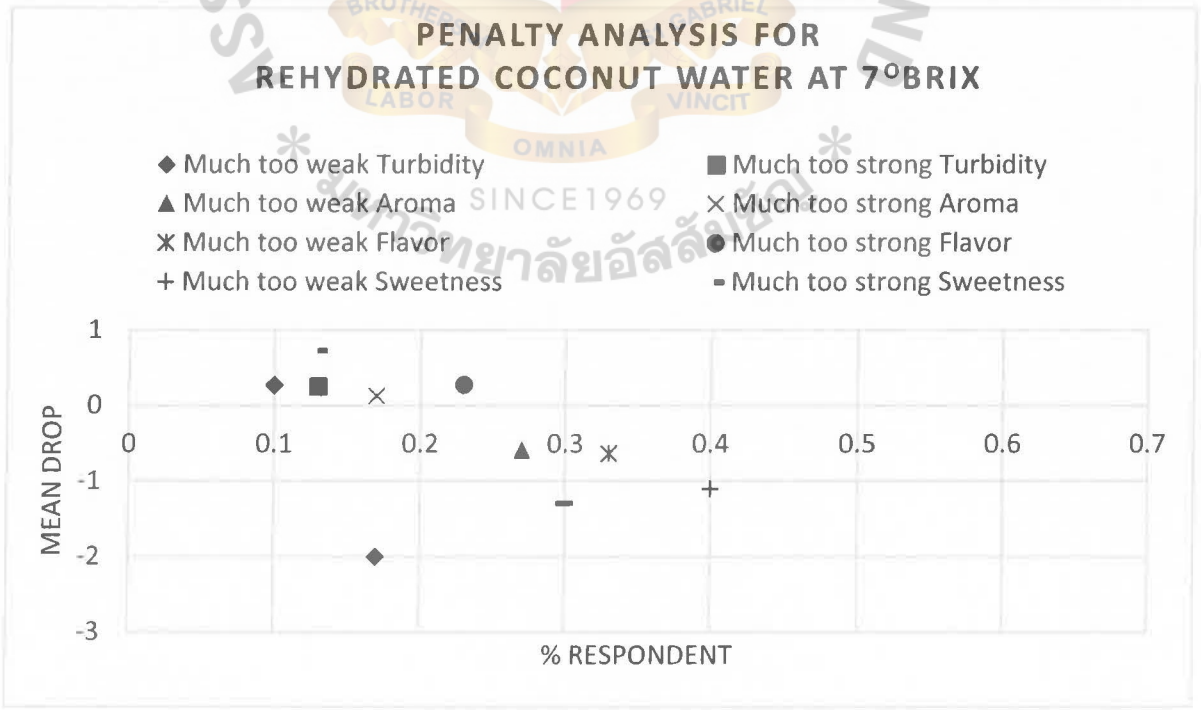


Figure 4 Penalty Analysis for 5% MTD of rehydrated coconut juice powder at 7°Brix

2. Effect of maltodextrin on consumer acceptance of coconut juice powder.

Sensory test (30 panelists)

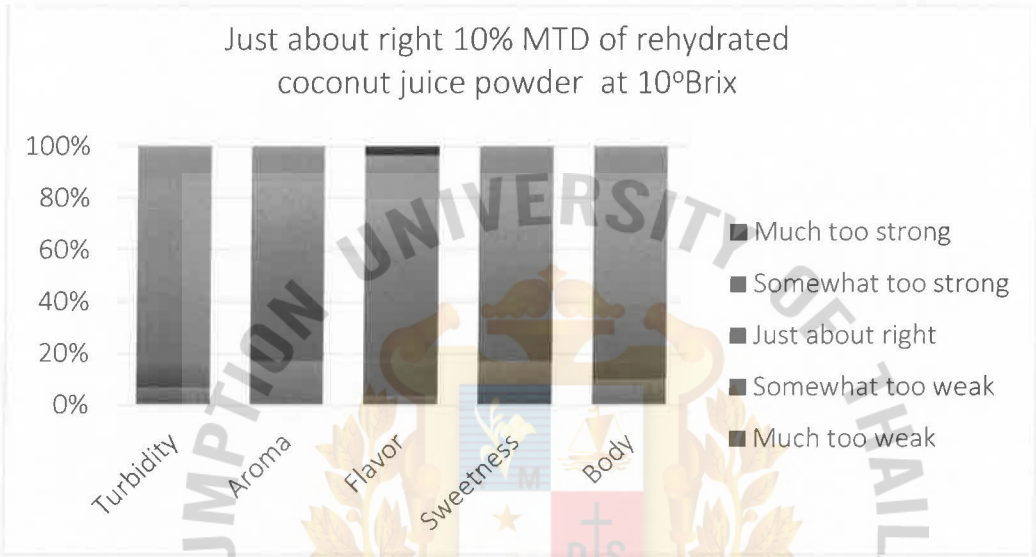


Figure 5 Just about right 10% MTD of rehydrated coconut juice powder at 10°Brix

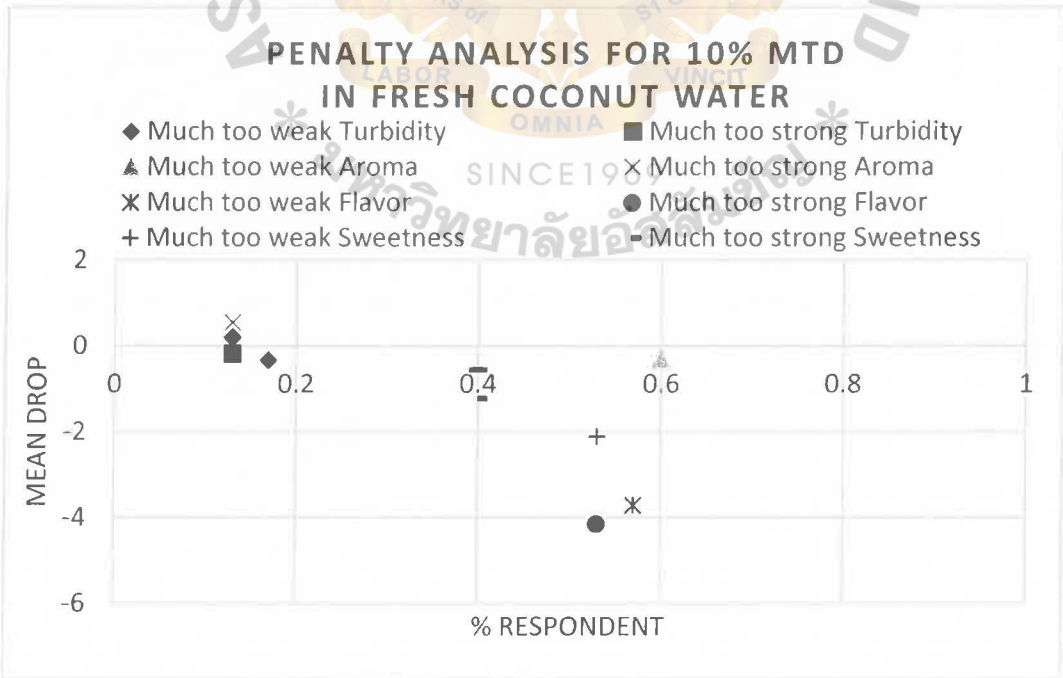


Figure 6 Penalty Analysis for 10% MTD of rehydrated coconut juice powder at 10°Brix

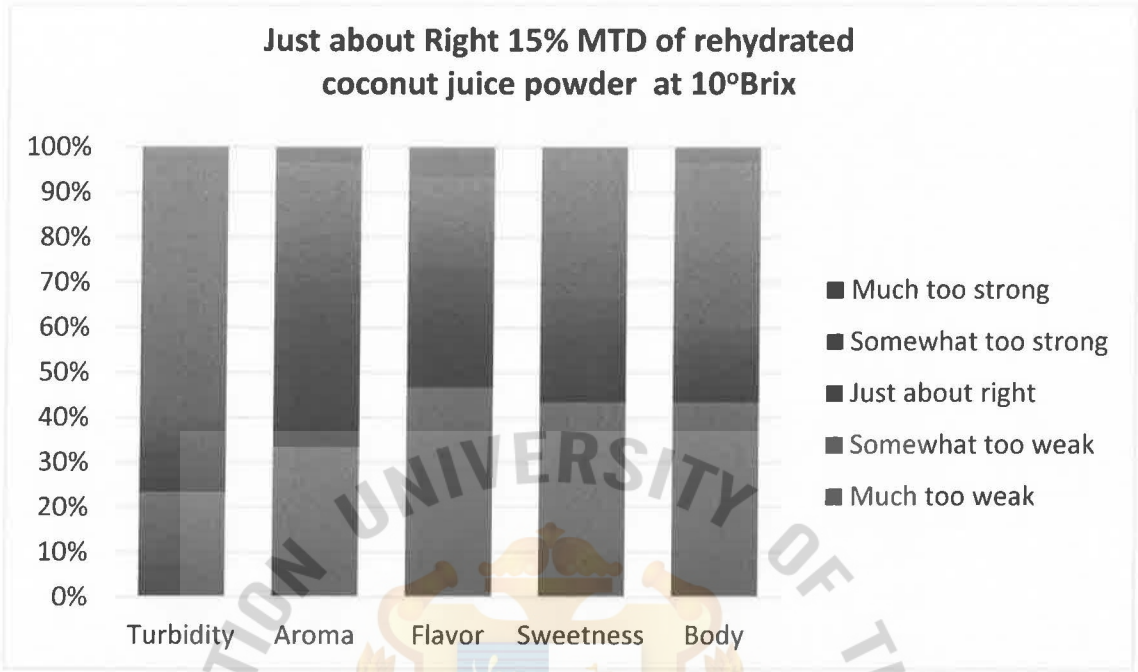


*Figure 7 Just about Right 15% MTD of rehydrated **coconut juice powder at 10°Brix***

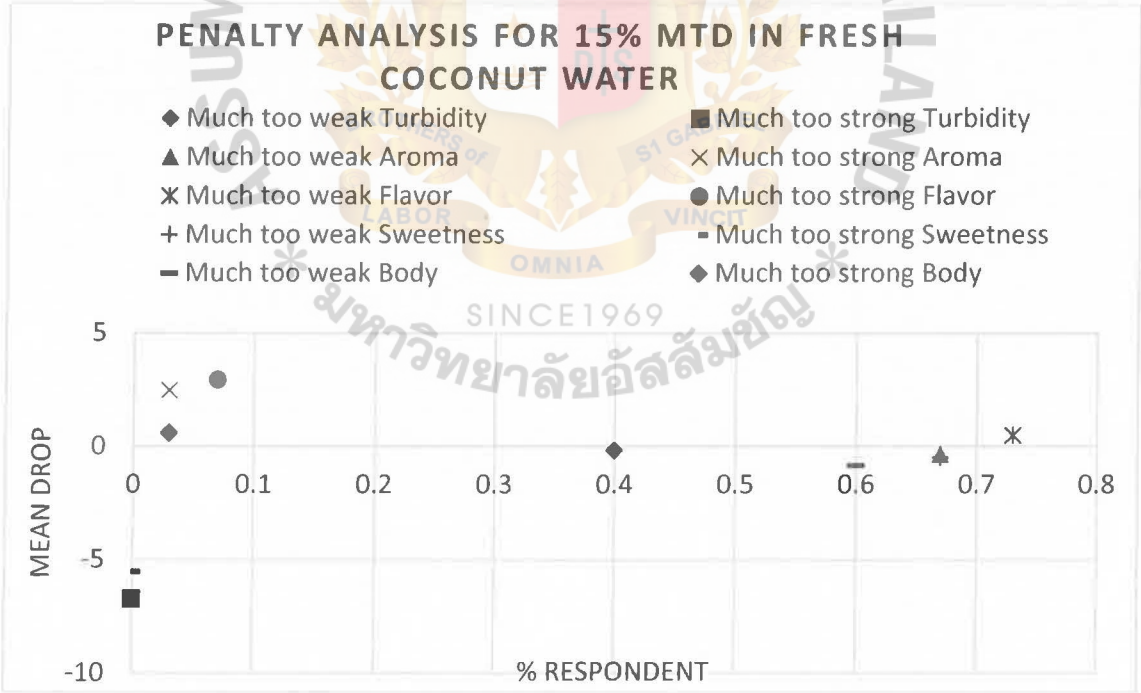
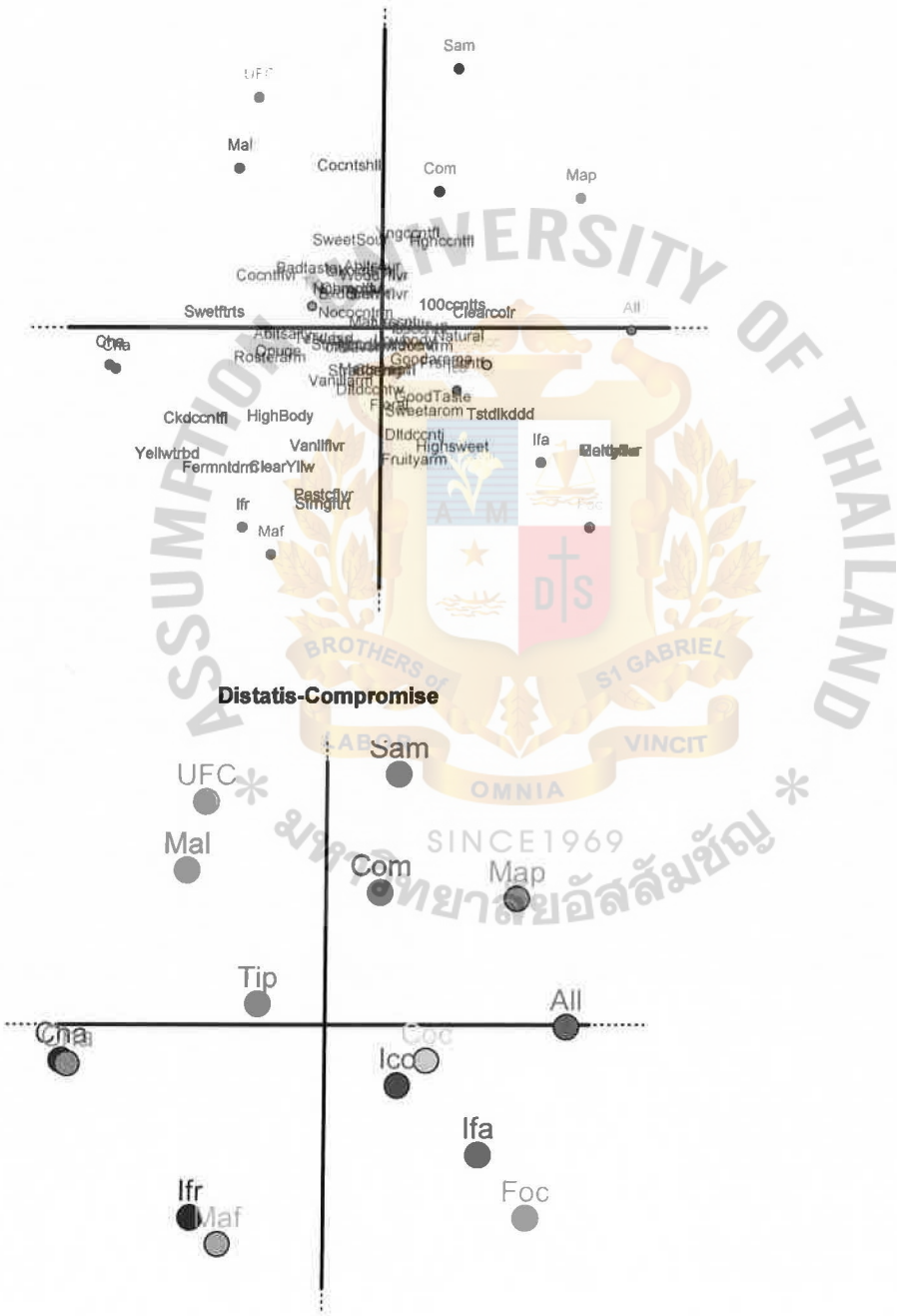


Figure 8 Penalty Analysis for 15% MTD of rehydrated coconut juice powder at 10°Brix

Appendix C : Sorting analysis result from comparison of 15 samples (14 commercial coconut water brand and 1 sample)



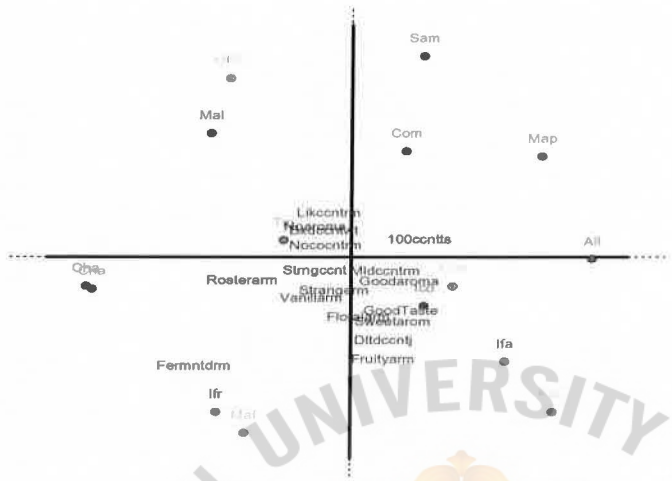


Figure 9 Sorting graph for aroma attribute.

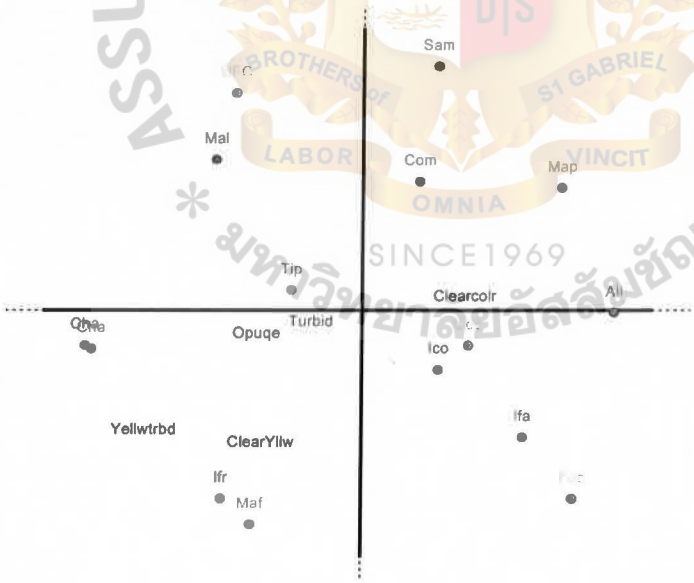


Figure 10 Sorting graph for color attribute.

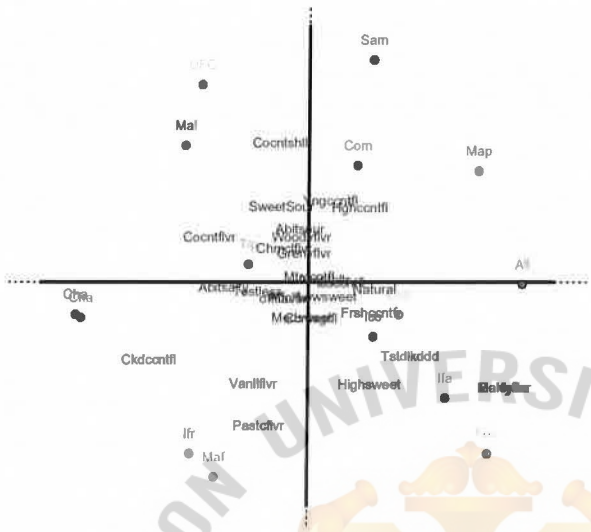


Figure 11 Sorting graph for flavor and taste attribute.

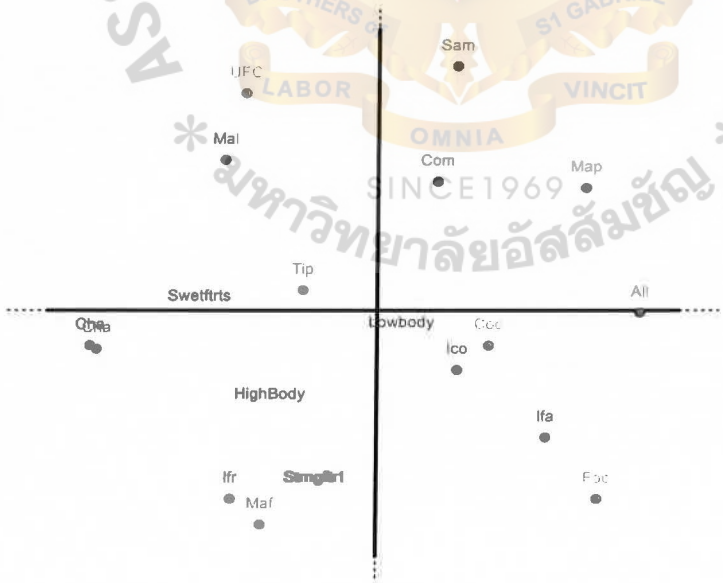
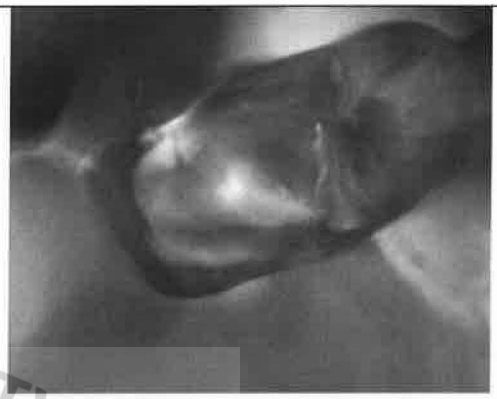


Figure 12 Sorting graph for mouth feel attribute.

Appendix D : Micrographs of coconut juice powder with difference concentration of maltodextrin (5%, 10%, and 15%)

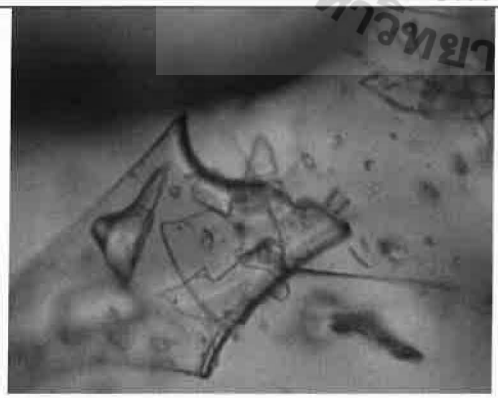
Micrographs of coconut juice powder with 5 % maltodextrin (magnifications of 10x)



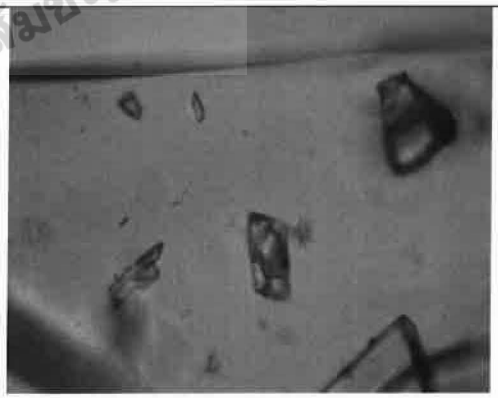
Micrographs of coconut juice powder with 5 % maltodextrin (magnifications of 40x)

Micrographs of coconut juice powder with 10 % maltodextrin (magnifications of 10x)

Micrographs of coconut juice powder with 10 % maltodextrin (magnifications of 40x)



Micrographs of coconut juice powder with 15 % maltodextrin (magnifications of 10x)



Micrographs of coconut juice powder with 15 % maltodextrin (magnifications of 40x)

Appendix E : Standard of coconut water

1. Standard of coconut water from FAO/WHO Food standard programme21st session

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

21st SESSION

OF

CODEX COMMITTEE ON PROCESSED FRUITS AND VEGETABLES

AT

TEXAS, U.S.A.

FROM

23-27 SEPTEMBER 2002

Government of India position

ON

Agenda no. 1, 3(a) to 3(e) , 4(a), 4(b)

AGENDA ITEM NO. 3(e): Draft Codex Standards for Aqueous Coconut Products

(CL 2002/19-PFV)

Government of India is of the view that one more category relating to tender coconut water may be added under Para-2 concerning description.

Para 2.1.7 - Tender coconut water

The UN Food & Agriculture Organisation (FAO) has applied for a patent on a new technology that would allow manufacturers to bottle coconut water i.e., biologically pure, tasty and full of salts, sugars and vitamins. The United Kingdom has granted a patent to FAO on the new technology. The new technology holds tremendous promise for tropical countries. Countries that process or export coconuts and small farmers who grow them will be the main beneficiaries of the newly patented technology. The companies in the beverage industry have already shown interest.

Today, most coconut water is still consumed fresh in tropical coastal areas. Once exposed to air, the liquid rapidly loses most of its nutritional characteristics and begins to ferment. But, the production of coconut beverages such as tender coconut water will benefit the small farmers as well as countries that are exporting coconut products. A document showing the benefits and nutritional composition of coconut water is enclosed.

TENDER COCONUT WATER

The water of tender coconut, technically the liquid endosperm, is the most nutritious wholesome beverage that the nature has provided for the people of the tropics to fight the sultry heat. It has caloric value of 17.4 per 100gm.

"It is unctuous, sweet, increasing semen, promoting digestion and clearing the urinary path," says Ayurveda on tender coconut water (TWC).

Numerous medicinal properties of tender coconut water reported are:-

- Good for feeding infants suffering from intestinal disturbances.
- Oral rehydration medium
- Contains organic compounds possessing growth promoting properties
- Keeps the body cool
- Application on the body prevents prickly heat and summer boils and subsides the rashes caused by small pox, chicken pox, measles, etc.
- Kills intestinal worms
- Presence of saline and albumen makes it a good drink in cholera cases
- Checks urinary infections.
- Excellent tonic for the old and sick
- Cures malnourishment.
- Diuretic
- Effective in the treatment of kidney and urethral stones
- Can be injected intravenously in emergency case.
- Found as blood plasma substitute because it is sterile, does not produce heat, does not destroy red blood cells and is readily accepted by the body.
- Aids the quick absorption of the drugs and makes their peak concentration in the blood easier by its electrolytic effect.
- Urinary antiseptic and eliminates poisons in case of mineral poisoning.

"It's a natural isotonic beverage with the same level of electrolytic balance as we have in our blood. It's the fluid of life, so to speak," says Mr.Morton Satin, Chief of FAO's Agricultural Industries and Post Harvest Management Service.

The major chemical constituents of coconut water are sugars and minerals and minor ones are fat and nitrogenous substances.

Analysis of Mature and Tender Coconut Water

	Mature Coconut Water	Tender Coconut Water
Total solids%	5.4	6.5
Reducing sugars %	0.2	4.4
Minerals %	0.5	0.6
Protein %	0.1	0.01
Fat %	0.1	0.01
Acidity mg %	60.0	120.0
pH	5.2	4.5
Potassium mg%	247	290
Sodium mg%	48	42
Calcium mg%	40	44
Magnesium mg %	15	10
Phosphorous mg%	6.3	9.2
Iron µ g%	79	106
Copper µ g%	26	26

Source: Satyavati Krishnankutty (1987)

Sugars

Sugars in the forms of glucose and fructose form an important constituent of the tender nut water. The concentration of sugars in the nut water steadily increases from about 1.5 per cent to about 5 - 5.5 per cent in the early months of maturation and then slowly falls reaching about 2 per cent at the stage of the full maturity of the nut. In the early stages of maturity sugars are in the form of glucose and fructose (reducing sugars) and sucrose (non-reducing sugar) appears only in later stages which increases with the maturity while the reducing sugars fall. In the fully mature nut approximately 90 per cent of the total sugars is sucrose.

Minerals

Tender coconut water contains most of the minerals such as potassium, sodium, calcium, phosphorous, iron, copper, sulphur and chlorides. Among the minerals more than half is potassium the concentration of which is markedly influenced by potash manuring. Tender coconut water being rich in potassium and other minerals plays a major role to increase the urinary output.

Protein

Coconut water contains small amounts of protein. The percentage of arginine, alanine, cystine and serene in the protein of tender coconut water are higher than those in cow's milk. Since it does not contain any complex protein the danger of producing shock to the patients is minimised.

Amino Acid Composition of Coconut Water
(% of total protein)

Alanine	2.41
Arginine	10.75
Aspartic acid	3.60
Cystine	0.97 - 1.17
Glutamic acid	9.76 - 14.5
Histidine	1.95 - 2.05
Leucine	1.95 - 4.18
Lysine	1.95 - 4.57
Proline	1.21 - 4.12
Phenylalanine	1.23
Serine	0.59 - 0.91
Tyrosine	2.83 - 3.00

Vitamins

Tender coconut water contains both ascorbic acid and vitamins of B group. The concentration of ascorbic acid ranges from 2.2 to 3.7mg per ml, which gradually diminishes as the kernel surrounding the water begins to harden.

Vitamins of B Group in Coconut Water

Nicotinic acid	0.64 microgram / ml
Pantothenic acid	0.52 ,,
Biotin	0.02 ,,
Riboflavin	< 0.01 ,,

Folic acid	0.003 ,,
Thiamine	Trace ,,
Pyridoxine	Trace ,,

2. Standard of Coconut Water from Thai Community Product Standard

มาตรฐานผลิตภัณฑ์ชุมชน

น้ำมะพร้าว

๑. ขอบข่าย

๑.๑ มาตรฐานผลิตภัณฑ์ชุมชนนี้ครอบคลุมเฉพาะน้ำมะพร้าวพร้อมดื่มที่ทำจากน้ำมะพร้าวเป็นส่วนประกอบหลักบรรจุในภาชนะบรรจุ ผ่านการฆ่าเชื้อโดยวิธีพาสเจอร์ไรซ์ เก็บรักษา ขนส่ง และวางจำหน่ายโดยการแช่เย็น
เพื่อรักษาคุณภาพของผลิตภัณฑ์

๒. บทนิยาม

ความหมายของคำที่ใช้ในมาตรฐานผลิตภัณฑ์ชุมชนนี้ มีดังต่อไปนี้

๒.๑ น้ำมะพร้าวแท้ หมายถึง เครื่องดื่มที่ได้จากการนำน้ำมะพร้าว ซึ่งอาจเติมเนื้อมะพร้าว ไปฆ่าเชื้อโดยวิธี
พาสเจอร์ไรซ์ก่อนหรือหลังบรรจุ และต้องเก็บรักษาโดยการแช่เย็น

๒.๒ น้ำมะพร้าวปรุง หมายถึง เครื่องดื่มที่มีน้ำมะพร้าวแท้ไม่น้อยกว่าร้อยละ ๒๐ โดยน้ำหนัก ผสมกับน้ำเชื่อม
อาจเติมเนื้อมะพร้าว นำไปฆ่าเชื้อโดยวิธีพาสเจอร์ไรซ์ก่อนหรือหลังบรรจุ และต้องเก็บรักษาโดยการแช่เย็น

๒.๓ วิธีพาสเจอร์ไรซ์ หมายถึง กรรมวิธีการฆ่าเชื้อด้วยความร้อนเพื่อลดปริมาณจุลินทรีย์ที่ก่อให้เกิดโรคให้อยู่

ในระดับที่ปลอดภัยต่อผู้บริโภค โดยทั่วไปใช้อุณหภูมิต่ำกว่า ๑๐๐ องศาเซลเซียส และใช้ระยะเวลาที่เหมาะสมแล้วทำให้เย็นลงทันที

๓. ชนิด

๓.๑ น้ำมะพร้าว แบ่งออกเป็น ๒ ชนิด คือ

๓.๑.๑ ชนิดน้ำมะพร้าวแท้

๓.๑.๒ ชนิดน้ำมะพร้าวปรุง

๔. คุณลักษณะที่ต้องการ

๔.๑ ลักษณะทั่วไป ต้องเป็นของเหลวขุ่นตามธรรมชาติของน้ำมะพร้าว กรณีมีเนื้อมะพร้าวต้องไม่เปื่อยยุ่ย หรือเละการทดสอบให้ทำโดยการตรวจพินิจ

๔.๒ สี ต้องมีสีที่ดีตามธรรมชาติของน้ำมะพร้าวและส่วนประกอบที่ใช้

๔.๓ กลิ่นรส ต้องมีกลิ่นรสที่ดีตามธรรมชาติของน้ำมะพร้าวและส่วนประกอบที่ใช้ ไม่มีกลิ่นรสอื่นที่ไม่พึงประสงค์ เช่น กลิ่นรสเปรี้ยวบูด เมื่อตรวจสอบโดยวิธีให้คะแนนตามข้อ ๙.๑ แล้ว ต้องไม่มีลักษณะใดได้ ๑ คะแนน จากผู้ตรวจสอบคนใดคนหนึ่ง

๔.๔ สิ่งแปลกปลอม ต้องไม่พบสิ่งแปลกปลอมที่ไม่ใช่ส่วนประกอบที่ใช้ เช่น เส้นผม ดิน ทราย กรวด ชิ้นส่วนหรือสิ่งปฏิกูลจากสัตว์การทดสอบให้ทำโดยการตรวจพินิจ

๔.๕ วัตถุเจือปนอาหาร ห้ามใช้สีสังเคราะห์และวัตถุกันเสียทุกชนิดการทดสอบให้ปฏิบัติตาม AOAC หรือวิธีทดสอบอื่นที่เทียบเท่า

๔.๖ จุลินทรีย์

๔.๖.๑ จุลินทรีย์ทั้งหมด ต้องไม่เกิน ๑ × ๑๐๔ โคโลนีต่อตัวอย่าง ๑ มิลลิลิตร

๔.๖.๒ ซาลโมเนลลา ต้องไม่พบในตัวอย่าง ๒๕ มิลลิลิตร

๔.๖.๓ สตาฟิโลค็อกคัส ออเรียส ต้องน้อยกว่า ๑๐ โคโลนีต่อตัวอย่าง ๑ มิลลิลิตร

๔.๖.๔ บาซิลลัส ซีเรียส ต้องไม่เกิน ๑๐๐ โคโลนีต่อตัวอย่าง ๑ มิลลิลิตร

๔.๖.๕ คลอสตริเดียม เพอร์ฟริงเจนส์ ต้องไม่เกิน ๑๐๐ โคโลนีต่อตัวอย่าง ๑ มิลลิลิตร

๔.๖.๖ ลิสเทอเรีย มอนอไซโทจีเนส ต้องไม่พบในตัวอย่าง ๒๕ มิลลิลิตร

๔.๖.๗ โคลิฟอร์ม โดยวิธีเอ็มพีเอ็น ต้องน้อยกว่า ๒.๒ ต่อตัวอย่าง ๑๐๐ มิลลิลิตร

๔.๖.๘ เอสเชอริเชีย โคไล ต้องไม่พบในตัวอย่าง ๑๐๐ มิลลิลิตร

๔.๖.๕ ยีสต์และรา ต้องไม่เกิน ๑๐๐ โคโลนีต่อตัวอย่าง ๑ มิลลิลิตร
การทดสอบให้ปฏิบัติตาม AOAC หรือ BAM (U.S.FDA) หรือวิธีทดสอบอื่นที่เทียบเท่า

๕. สุขลักษณะ

๕.๑ สุขลักษณะในการทำน้ำมะพร้าว สถานประกอบการต้องได้รับอนุญาตจากกระทรวงสาธารณสุข และให้
เป็นไปตามภาคผนวก ก.

๖. การบรรจุ

๖.๑ ให้บรรจุน้ำมะพร้าวในภาชนะบรรจุที่สะอาด ปิดได้สนิท และสามารถป้องกันสิ่งปนเปื้อนจากภายนอกได้
การทดสอบให้ทำโดยการตรวจพินิจ
๖.๒ ปริมาตรสุทธิของน้ำมะพร้าวในแต่ละภาชนะบรรจุ ต้องไม่น้อยกว่าที่ระบุไว้ที่ฉลาก
การทดสอบให้ใช้เครื่องวัดปริมาตรที่เหมาะสม

๗. เครื่องหมายและฉลาก

๗.๑ ที่ภาชนะบรรจุน้ำมะพร้าวทุกหน่วย อย่างน้อยต้องมีเลข อักษร หรือเครื่องหมายแจ้งรายละเอียดต่อไปนี้
ให้เห็นได้ง่าย ชัดเจน
(๑) ชื่อผลิตภัณฑ์ (ตาม มผช.) อาจตามด้วยชื่อเรียกผลิตภัณฑ์ เช่น น้ำมะพร้าวอ่อน
(๒) ส่วนประกอบที่สำคัญ เป็นร้อยละของน้ำหนักโดยประมาณและเรียงจากมากไปน้อย
(๓) ปริมาตรสุทธิ เป็นมิลลิลิตรหรือลิตร
(๔) วัน เดือน ปีที่ทำ และวัน เดือน ปีที่หมดอายุ หรือข้อความว่า "ควรบริโภคก่อน (วัน เดือน ปี)"
(๕) ข้อแนะนำในการเก็บรักษา เช่น ต้องเก็บไว้ในตู้เย็น
(๖) เลขสารบบอาหาร
(๗) ชื่อผู้ทำหรือสถานที่ทำ พร้อมสถานที่ตั้ง หรือเครื่องหมายการค้าที่จดทะเบียน
ในกรณีที่ใช้ภาษาต่างประเทศ ต้องมีความหมายตรงกับภาษาไทยที่กำหนดไว้ข้างต้น

๘. การชักตัวอย่างและเกณฑ์ตัดสิน

๘.๑ รุ่น ในที่นี้ หมายถึง น้ำมะพร้าวชนิดเดียวกัน ที่มีส่วนประกอบเดียวกัน ทำโดยกรรมวิธีเดียวกัน ในระยะเวลา
เดียวกัน

๘.๒ การชักตัวอย่างและการยอมรับ ให้เป็นไปตามแผนการชักตัวอย่างที่กำหนดต่อไปนี้

๘.๒.๑ การชักตัวอย่างและการยอมรับ สำหรับการทดสอบลักษณะทั่วไป สี กลิ่นรส สิ่งแปลกปลอม การบรรจุ และเครื่องหมายและฉลาก ให้ชักตัวอย่างโดยวิธีสุ่มจากรุ่นเดียวกัน จำนวน ๓ หน่วยภาชนะบรรจุ เมื่อตรวจสอบแล้วทุกตัวอย่างต้องเป็นไปตามข้อ ๔.๑ ถึงข้อ ๔.๔ ข้อ ๖. และข้อ ๗. ทุกรายการ จึงจะถือว่าน้ำมะพร้าวรุ่นนั้นเป็นไปตามเกณฑ์ที่กำหนด

๘.๒.๒ การชักตัวอย่างและการยอมรับ สำหรับการทดสอบวัตถุเจือปนอาหาร ให้ชักตัวอย่างโดยวิธีสุ่มจากรุ่น
เดียวกัน จำนวน ๓ หน่วยภาชนะบรรจุ เพื่อทำเป็นตัวอย่างรวม โดยมีปริมาตรรวมไม่น้อยกว่า ๓๐๐ มิลลิลิตร กรณีตัวอย่างไม่พอให้ชักตัวอย่างเพิ่มโดยวิธีสุ่มจากรุ่นเดียวกันให้ได้ตัวอย่างที่มีปริมาตรรวมตามที่กำหนด เมื่อตรวจสอบแล้วตัวอย่างต้องเป็นไปตามข้อ ๔.๕ จึงจะถือว่าน้ำมะพร้าวรุ่นนั้นเป็นไปตามเกณฑ์ที่กำหนด

๘.๒.๓ การชักตัวอย่างและการยอมรับ สำหรับการทดสอบจุลินทรีย์ ให้ชักตัวอย่างโดยวิธีสุ่มจากรุ่นเดียวกัน
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๘.๓ เกณฑ์ตัดสิน
ตัวอย่างน้ำมะพร้าวต้องเป็นไปตามข้อ ๘.๒.๑ ข้อ ๘.๒.๒ และข้อ ๘.๒.๓ ทุกข้อ จึงจะถือว่าน้ำมะพร้าวรุ่นนั้นเป็นไปตามมาตรฐานผลิตภัณฑ์ชุมชนนี้

๙. การทดสอบ

๙.๑ การทดสอบสีและกลิ่นรส

๙.๑.๑ ให้แต่งตั้งคณะผู้ตรวจสอบ ประกอบด้วยผู้ที่มีความชำนาญในการตรวจสอบน้ำมะพร้าว ๕ คน แต่ละ

คนจะแยกกันตรวจและให้คะแนนโดยอิสระ

๙.๑.๒ เทตัวอย่างน้ำมะพร้าวลงในแก้วใสโดยมีกระดาษสีขาวเป็นฉากหลัง ตรวจสอบโดยการตรวจพินิจและชิม

๙.๑.๓ หลักเกณฑ์การให้คะแนน ให้เป็นไปตามตารางที่ ๑

ตารางที่ ๑ หลักเกณฑ์การให้คะแนนในการทดสอบสีและกลิ่นรส

(ข้อ ๙.๑.๓)

ลักษณะที่ตรวจสอบ	ระดับการตัดสิน	คะแนนที่ได้รับ
สี	สีดีตามธรรมชาติของน้ำมะพร้าวและส่วนประกอบที่ใช้	๓
	สีพอใช้ใกล้เคียงกับสีตามธรรมชาติของน้ำมะพร้าวและส่วนประกอบที่ใช้	๒
	สีผิดปกติหรือมีการเปลี่ยนสี	๑
กลิ่นรส	กลิ่นรสดีตามธรรมชาติของน้ำมะพร้าวและส่วนประกอบที่ใช้	๓
	กลิ่นรสพอใช้ใกล้เคียงกับกลิ่นรสตามธรรมชาติของน้ำมะพร้าวและส่วนประกอบที่ใช้	๒
	กลิ่นรสผิดปกติหรือมีกลิ่นรสอื่นที่ไม่พึงประสงค์ เช่น กลิ่นรสเปรี้ยวบูด	๑

ภาคผนวก ก.

สุขลักษณะ

(ข้อ ๕.๑)

ก.๑ สถานที่ตั้งและอาคารที่ทำ

ก.๑.๑ สถานที่ตั้งตัวอาคารและที่ใกล้เคียง อยู่ในที่ที่จะไม่ทำให้ผลิตภัณฑ์ที่ทำเกิดการปนเปื้อนได้ง่าย โดย

ก.๑.๑.๑ สถานที่ตั้งตัวอาคารและบริเวณโดยรอบ สะอาด ไม่มีน้ำขัง แฉะ และสกปรก

ก.๑.๑.๒ อยู่ห่างจากบริเวณหรือสถานที่ที่มีฝุ่น เขม่า ควัน

ก.๑.๑.๓ ไม่อยู่ใกล้เคียงกับสถานที่น่ารังเกียจ เช่น บริเวณเพาะเลี้ยงสัตว์ แหล่งเก็บหรือกำจัดขยะ

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ความสะอาด และสะดวกในการปฏิบัติงาน โดย
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หรือไม่เกี่ยวข้องกับการทำอยู่ในบริเวณที่ทำ
ก.๑.๒.๓ พื้นที่ปฏิบัติงานไม่แออัด มีแสงสว่างเพียงพอ และมีการระบายอากาศที่เหมาะสม
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ก.๒ เครื่องมือ เครื่องจักร และอุปกรณ์ในการทำ
ก.๒.๑ ภาชนะหรืออุปกรณ์ในการทำที่สัมผัสกับผลิตภัณฑ์ ทำจากวัสดุมีผิวเรียบ ไม่เป็นสนิม ล้างทำความ
สะอาดได้ง่าย
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ผลิตภัณฑ์
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ก.๔ การสุขาภิบาล การบำรุงรักษา และการทำความสะอาด

H376

Title:	Bovine serum albumin nanoparticles for enhancing bioavailability of curcumin
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Department:	Agro - industry
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Abstract

Curcumin is considered to be a powerful bioactive agent, which is extensively known for its medicinal use. Despite its being excellent therapeutic agent and natural antioxidant, curcumin shows poor solubility and low bioavailability. Therefore, the development of formulation of BSA-curcumin nanoparticles (BCN) to improve its aqueous-phase solubility and low absorption is beneficial. In this study, BSA nanoparticles were prepared by desolvation method and the effects of BSA on enhancing bioavailability of curcumin were investigated. The characteristic properties of the prepared nanoparticles were observed by various techniques. The %encapsulation efficiency of curcumin in BCN was 84.3 ± 2.1 measured by UV-Vis spectrophotometer. Additionally, particle content was determined by dry weight method. The particle size and morphology were studied by Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The particle size of BCN measured by SEM and TEM were 212.70 ± 0.04 nm and 77.20 ± 0.02 nm, respectively. Moreover, functional groups of BCN were identified by Fourier Transform Infrared Spectroscopy (FTIR). An antioxidant activity (AA) was carried out using a 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. At the curcumin concentration of 10 mg/ml, %AA of BCN was higher than that of crude curcumin extract 10% approximately. However, there was no significant difference between BCN and crude curcumin ($p>0.05$). The results indicated BSA-curcumin nanoparticles could play more important role in biomedical fields in the future.

Keywords: BSA-curcumin nanoparticles, curcumin, antioxidant activity

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Abbreviation Full Nomenclature

BCN	BSA-curcumin nanoparticles
BSA	bovine serum albumin
°C	degree Celsius
DNA	deoxyribonucleic acid
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FT-IR	Fourier transform infrared Radiation
GTA	Glutaraldehyde
h	hour
mg	milligram
min	minute
mL	millimeter
mM	millimolar
MRI	Magnetic Resonance Imaging
NaCl	Sodium chloride
nm	nanometer
NaOH	sodium hydroxide
RPM	Revolutions per minute
SEM	scanning electron microscopy
TEM	transmission electron microscopy
UV-VIS	Ultraviolet–visible spectroscopy

Chapter 1

Introduction

1.1 Background

Nanoscience and nanotechnology are the study and application of extremely small things at nanoscale, which is about 1 to 100 nanometers. Moreover, it can be used across all the other science fields, such as chemistry, biology, physics, materials science, and engineering [1].

Nanoscience and nanotechnology have potentially provided new or alternative solutions in the development of functional foods, in particular the integration of bioactive compounds with no effect to the sensory perception of the consumer and improving the uptake of certain compounds [2]. Nanoscience has also been used in the medical field for example delivery of drugs to target tissues and to increase stability against degradation by enzymes. One of the major problems associated with drugs is poor solubility drugs and very low bioavailability. Therefore, nanosparticles are an interesting solution. Nanoparticles are ultra-small objects with dimensions measured in nanometers (nm). Nanoparticles typically have at least one dimension less than 100 nm in size [3].

Recently, there are many researches about development of high-performance delivery carriers for the encapsulation and protection of biologically active substances of food origin, using nanotechnology approaches. Many kinds of nutrients, bioactive ingredients and phytochemicals can be loaded into biocompatible and biodegradable nanoparticles, which will improve their aqueous solubility, stability, bioavailability, and circulation time in the body. Compared with micrometer-sized systems produced by traditional microencapsulation techniques, nanometer sized delivery systems provide more surface area and have the potential to improve solubility, enhance bioavailability, improve controlled release, and enable greater precision targeting of entrapped compounds. By carefully choosing the molecular components, it seems to be possible to design nanoparticles with different surface properties and to deliver active compounds directly to appropriate sites [2].

Curcumin is the active ingredient of turmeric, and is also found in limited amounts in ginger. It is an anti-inflammatory molecule with anti-cancer properties, and similar to fish oil, it seems to be an effective metabolic syndrome band-aid [4]. However, a poor solubility of curcumin in water at physiological pH and low bioavailability limited the absorption of curcumin after administration [5]. To overcome the problems of poor solubility and low bioavailability, recently several nanotechnology approaches have been reported for the encapsulation of curcumin into nanoparticles such as liposomes, polymeric nanoparticles,

micelles, nanogels, cyclodextrins, dendrimers, silvers and solid lipids as one of the useful alternatives [6]. Although these biocompatible nanocarriers hold a promise to enhance the bioavailability and deliver therapeutic concentrations of curcumin the safety and toxicity issues cannot be ignored. Therefore, this study is aimed to study the effects of BSA on enhancing bioavailability of curcumin. The objectives of this study are as follow;

1.2 Aim of study

This research was undertaken with the following specific objectives:

1. To synthesize BSA-curcumin nanoparticles (BCN).
2. To determine the percentage of curcumin's encapsulation efficiency in BSA nanoparticles and investigate their characteristic properties.
3. To study %Antioxidant activity of BCN.

Chapter 2

Literature Review

2.1 Nanoparticles

Nanoparticles are sub nanosized colloidal structures composed of synthetic or semisy nthetic polymers. Nanospheres are solid core spherical nanoparticulates containing substance embeded within the matrix or adsorbed onto the surface.

Nanoparticles are ultra-small objects with dimensions measured in nanometers (nm). Nanoparticles typically have at least one dimension less than 100nm in size [3]. Fine particles cover a range between 100 and 2500 nanometers, while ultrafine particles are sized between 1 and 100 nanometers. Nanoparticles may or may not exhibit size-related properties that are seen in fine particles. Despite being the size of the ultrafine particles individual molecules are usually not referred to as nanoparticles [7].

2.1.1 Nanoparticle research and uses

Nanoparticle research is currently the most studied branch of science with the number of uses of nanoparticles in various fields. The particles have wide variety of potential applications in biomedical, optical and electronic fields [7].

Nanoparticles have a very large surface area compared with their volume. So they are often able to react very quickly. This makes them useful as catalysts to speed up reactions. For example, they can be used in self-cleaning ovens and windows. Furthermore, they can be medium for drug delivery to target organs in medical field or improving solubility of certain substances [8].

2.1.2 Characteristics of nanoparticles

- Self-reassembling capability
- Pole free structure
- High conduction
- Utilization diversity in all the work areas
- Reliability and sustainability
- 100 nm size or more dimensions.
- More surface area as compared to other particles.

2.1.3 Applications of nanoparticles

A list of some of the applications of nanomaterials to biology or medicine is given below:

- Fluorescent biological labels [9-11]
- Drug and gene delivery [12,13]
- Detection of pathogens [14]
- Detection of proteins [15]
- Probing of DNA structure [16]
- Tissue engineering [17,18]
- Tumor destruction via heating (hyperthermia)[19]
- Separation and purification of biological molecules and cells [20]
- MRI contrast enhancement [21]
- Pharmacokinetic studies [22]

2.1.4 Advantages of nanoparticles in medicine

- The size and surface characteristics of nanoparticles can be easily manipulated. This could be used for both passive and active drug targeting
- Nanoparticles can be made to control and sustain release of the drug during the transportation as well as the location of the release. Since distribution and subsequent clearance of the drug from the body can be altered, an increase in drug therapeutic efficacy and reduction in side effects can be achieved.
- Choosing an appropriate matrix also helps in increasing the efficacy and reducing side effects
- Targeted drugs may be developed
- Various routes of administration including oral, nasal, injection, intra-ocular (within the eyes) etc. can be used. [7]

2.1.5 Historical background

Scientist always wanted to fine out the extra ordinary abilities of elements and natural materials so that better structures could be developed. In the early 1980s the concept of nanotechnology rose up with the nanoparticles. It was seen for the first time that by just reassembling the particles at nanoscales new excellent components can be formed. Nanoparticles have major contribution in finding out brilliant resource elements form natural materials such as carbon, which was the first natural material which was treated at nanoscales to find out new components and carbon nanotubes are ultimately the type of advanced nanoparticles [23].

2.2 Preparation of Nanoparticles

There are two basic methods for preparation of nanoparticles:

Emulsification method: Initially, its method was set forth by Scheffel and his coworkers (1972) in order to prepare albumin sphere to a high volume of pre-heated oil (over 120°C) drop by drop. This process will result a rapid evaporation of existed water and albumin irreversible destruction. This process will also cause formation of nanoparticles. The resulted suspension was put into cold- ice bath [24].

This method is based on the partial miscibility of an organic solvent with water. An o/w emulsion is obtained upon stabilizing agent (i.e. poloxamer) under mechanical stirring, followed by high pressure homogenization. The emulsion is then diluted with a large amount of water to overcome organic solvent miscibility in water. Polymer precipitation occurs as a result of the diffusion of organic solvent into water, leading to the formation of nanoparticles. This method is suitable for hydrophobic drug and showed high percentage of drug entrapment. The major drawbacks of this method include harsh processing conditions (e.g., the use of organic solvents) and the high shear forces used during nanoparticle preparation [25].

Desolvation method: The disadvantage of the emulsion methods for particle preparation is the need for applying organic solvents, for the removal both of the oily residues of the preparation process and of surfactants required for emulsion stabilization. Therefore, as an alternative method for the preparation of protein nanoparticles a desolvation process derived from the coacervation method of microencapsulation was developed. In this method, particles in aqueous will formed by coacervation process and later on will be stabilized by cross linking agent such as glutaraldehyde [24].

Desolvation technique is mainly used for the preparation of nanoparticles for proteins and polysaccharides [26]. Desolvation is a thermodynamically driven self-assembly process for polymeric materials to prepare nanoparticles [27]. The polymeric molecules form particles of different sizes depending on the preparation conditions such as protein content, pH, ionic strength, concentration of cross-linking agent, agitation speed, amount of desolvating agent etc [28].

This method was offered by Marty and his coworkers (1978) the foundation of this method was using a desolvation factor such as natural salts or alcohol which should be added to protein solution slowly. By adding this factor, protein third structure will changed. When we have reached to a certain level of a desolvation, protein clump will be formed. In the next stage, nanoparticles will result by this polymerization clump crosslinkage with a chemical factor that is glutaraldehyde [28]. In order to obtain dispersed nanoparticles not in a mass form, we must stop the system before particles start to accumulate. System turbidity will be increased owing to this desolvation factor. Particles accumulation will form alone

with increasing system's turbidity. In order to stop such kind of accumulation and creating ideal nanodispersion, we must use a resolvating agent.

2.3 BSA

Bovine serum albumin (also known as BSA or "Fraction V") is a serum albumin protein isolated from cows. Albumin is synthesized by the liver using dietary protein, the highest concentration protein in plasma. Albumin performs many functions such as maintaining the osmotic pressure that causes fluid to remain within the blood stream instead of leaking out into the tissues. Albumin also transports many small molecules in the blood, including fatty acids, bilirubin, calcium, progesterone, and many drugs. Liver disease, kidney disease, and malnutrition are the major cause of low albumin. A diseased liver produces insufficient albumin. Diseased kidneys sometimes lose large amounts of albumin into the urine faster than the liver can produce it (this is termed nephrotic syndrome) [29].

BSA has been used in many fields such as immunology, biochemistry and biotechnology. BSA immunoassay applications include ELISA (Enzyme-linked Immunosorbent Assay), immunoblots (western blot and dot blot), and immunocytochemistry (immunohistochemistry and immunofluorescence microscopy). BSA is also used as a nutrient in cell and microbial culture. In molecular biology BSA is used to stabilize some restriction enzymes during digestion of DNA and to prevent adhesion of the enzyme to reaction tubes, pipet tips, and other vessels. BSA is considered to be a universal blocking reagent in many applications. This is because BSA does not affect the functions of other proteins (enzymes) that do not need it for stabilization. BSA is also commonly used to determine the quantity of other proteins, by comparing an unknown quantity of protein to known amounts of BSA in, for instance, the Bradford Protein Assay. BSA is used because of its stability to increase signal in assays, its lack of effect in many biochemical reactions, and its low cost, since large quantities of it can be readily purified from bovine blood, a byproduct of the cattle industry [30].

2.3.1 Structure of BSA

The substantial information on serum albumin has led to some contradictory results and discussions. Based largely on hydrodynamic experiments (Hughes, 1954; Squire et al., 1968; Wright and Thompson, 1975) and low-angle X-ray scattering (Bloomfield, 1966), serum albumin was postulated to be an oblate ellipsoid with dimensions of 140 × 40Å. Experiments have continued to support these dimensions (Bendedouch and Chen, 1983; Feng et al., 1988) compiled a diverse variety of data and constructed a model of albumin as having the shape of a cigar.

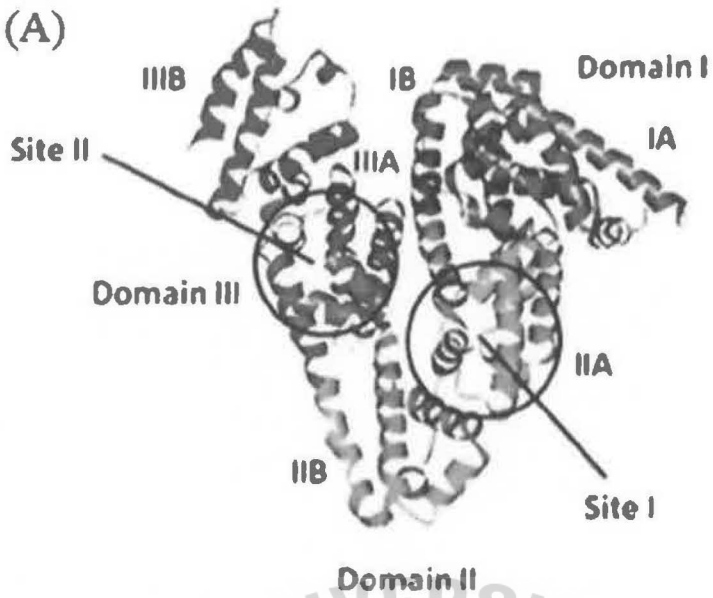

Figure 1 : Crystal structure of BSA [31]

2.3.2 Physical properties

The physical properties of BSA [32]

pH of 1% solution:	5.2 – 7
Stokes Radius:	3.48 nm
Sedimentation constant	4.5 monomer, 6.7 dimer
Diffusion constant, $D_{20,w}$ x10^7	5.9
Partial specific volume, V_{20}	0.733
Intrinsic viscosity, h	0.0413
Frictional ratio, f/f0	1.30
Overall dimensions	Å 40 x 140
Optical absorbance, A279nm 1 gm/L	0.667
Estimated a-helix, %	54
Estimated b-form, %	18

2.3.3 Examples of BSA application

Albumin from bovine serum was used for:

- Blocking of nonspecific immunoglobulin during immunohistochemical reactions.
- Coating microplates for blocking non-specific binding during I-ELISA (Indirect Enzyme-linked Immunosorbent Assay)
- As molecular mass standard in analytical gel filtration chromatography for Superdex® 75 10/300 GL gel filtration column.
- Measuring fluorescence for PR1 (phytofluor red 1) by using Fluorescence correlation spectroscopy (FCS)
- As a reference mixture along with horse Mb in Size Exclusion Chromatography. It can be used as a gel filtration molecular weight marker. In medical studies it can be used to quantify binding capacity and adsorption rate of protein enrichment and separation medium critical in proteomic research.

2.4 Encapsulation

Encapsulation may be defined as a process to entrap one substance (active agent) within another substance (wall material). The encapsulated substance, except active agent, can be called the core, fill, active, internal or payload phase. The substance that is encapsulating is often called the coating, membrane, shell, capsule, carrier material, external phase, or matrix [33, 34].

In the food industry, encapsulation process can be applied for a variety of reasons. Encapsulation is a useful tool to improve delivery of bioactive molecules (e.g. antioxidants, minerals, vitamins, phytosterols, lutein, fatty acids, lycopene) and living cells (e.g. probiotics) into foods [33,35]. In most cases, encapsulation refers to a technology in which the bioactive components are completely enveloped, covered and protected by a physical barrier, without any protrusion of the bioactive components [35]. Also, encapsulation has been defined as a technology of packaging solids, liquids, or gaseous materials in small capsules that release their contents at controlled rates over prolonged periods and under specific conditions [36]. Produced particles usually have diameters of a few nm to a few mm [33].

Encapsulation was originally introduced in the area of biotechnology to make production-processes more efficient as the matrix around the cells allows for rapid and efficient separation of the producer cells and the metabolites. Such technologies developed

approximately 60 years ago, are of significant interest to the pharmaceutical sector (especially for drug and vaccine delivery), but also have relevance for the food industry. In recent years, the food industry requires the addition of functional compounds in products. These compounds are usually highly susceptible to environmental, processing and/or gastrointestinal conditions and therefore, encapsulation has imposed an approach for effective protection of those. Functional compounds are used to control flavor, color, texture or preservation properties. Bioactive compounds with various potential health benefits are included, too. There is a multitude of possible benefits of encapsulated ingredients in the food industry. Encapsulation aims to preserve stability of the bioactive compounds during processing and storage and to prevent undesirable interactions with food matrix. Mainly, bioactive food compounds are characterised by rapid inactivation. These compounds would profit from an encapsulation procedure, since it slows down the degradation processes (e.g., oxidation or hydrolysis) or prevents degradation until the product is delivered at the desired sites [37]. Thus, the bioactive component would be kept as fully functional. Also, this technology may provide barriers between sensitive bioactive materials and the environment, and thus, to allow taste and aroma differentiation, mask bad tasting or smelling, stabilize food ingredients or increase their bioavailability.

In addition to the above, encapsulation can be applied for modification of physical characteristics of the original material in order to (a) allow easier handling, (b) help separate the components of the mixture that would otherwise react with one another, (c) provide an adequate concentration and uniform dispersion of an active agent [36].

2.4.1 Encapsulation techniques

There are number of techniques available for encapsulation of food compounds. Since encapsulating compounds are very often in a liquid form, many technologies are based on drying. Different techniques like spray drying, spray-bed-drying, fluid-bed coating, spray-chilling, spray-cooling or melt injection are available to encapsulate active agents [38,39].

Spray drying is one of the oldest and the most widely used encapsulation technique in the food industrial sector. It is a flexible, continuous, but more important an economical operation. It produces particles of good quality, which size is less than 40 μm [39]. Although spray-dryers are widespread in the food industry, there are several disadvantages of this technique such complexity of the equipment, non-uniform conditions in the drying chamber and it is not always easy to control particle size. About 80–90% of encapsulates are spray-dried ones, rest of them are mostly prepared by spray-chilling, freeze-drying, melt extrusion and melt injection [40,41].

Extrusion methods consists of dropping droplets of an aqueous solution of polymer (most often this is 0.6-3 wt% sodium alginate) and active into a gelling bath (in case of

alginate, gelling bath is 0.05-1.5 M calcium-chloride solution). The dripping tool can be simply a pipette, a syringe, a vibrating nozzle, a spraying nozzle, jet cutter or atomizing disk [33]. In comparison to other extrusion techniques, JetCutter was found to be the best technology for large-scale/industrial applications [42]. Electrostatic extrusion is especially effective for production of very small particles, down to 50 µm. An alternative extrusion technology is co-extrusion. It might be utilized to prepare spherical microbeads with a hydrophobic core and a hydrophilic or hydrophobic shell [43].

Another frequently used technique is emulsification. It is utilised in case of water soluble food active agents and there are two combinations of emulsions: water/oil emulsions or oil/water emulsions and water/oil/water double emulsions. An oil-in-water emulsion can be dried by different drying methods (Viktor Nedovic et al. / Procedia Food Science 1 (2011) 1806 – 1815 1809) such as spray- or freeze-drying, and thus to produce a powder. Such dried emulsions might be encapsulates or an instant formulation for numerous food products [43].

2.5 Freeze drying

Freeze drying is the removal of ice or other frozen solvents from a material through the process of sublimation and the removal of bound water molecules through the process of desorption. Lyophilization and freeze drying are terms that are used interchangeably depending on the industry and location where the drying is taking place. Controlled freeze drying keeps the product temperature low enough during the process to avoid changes in the dried product appearance and characteristics. It is an excellent method for preserving a wide variety of heat-sensitive materials such as proteins, microbes, pharmaceuticals, tissues & plasma [44].

Thoroughly understanding the concept of sublimation is a key building block to gaining knowledge of freeze drying. Sublimation is when a solid (ice) changes directly to a vapor without first going through a liquid (water) phase. As shown below on the phase diagram for water, low pressures are required for sublimation to take place. Sublimation is a phase change and heat energy must be added to the frozen product. Sublimation in the freeze drying process can be described simply as:

a. FREEZE - The product is completely frozen, usually in a vial, flask or tray.
b. VACUUM - The product is then placed under a deep vacuum, well below the triple point of water.
c. DRY – Heat energy is then added to the product causing the ice to sublime. [44]

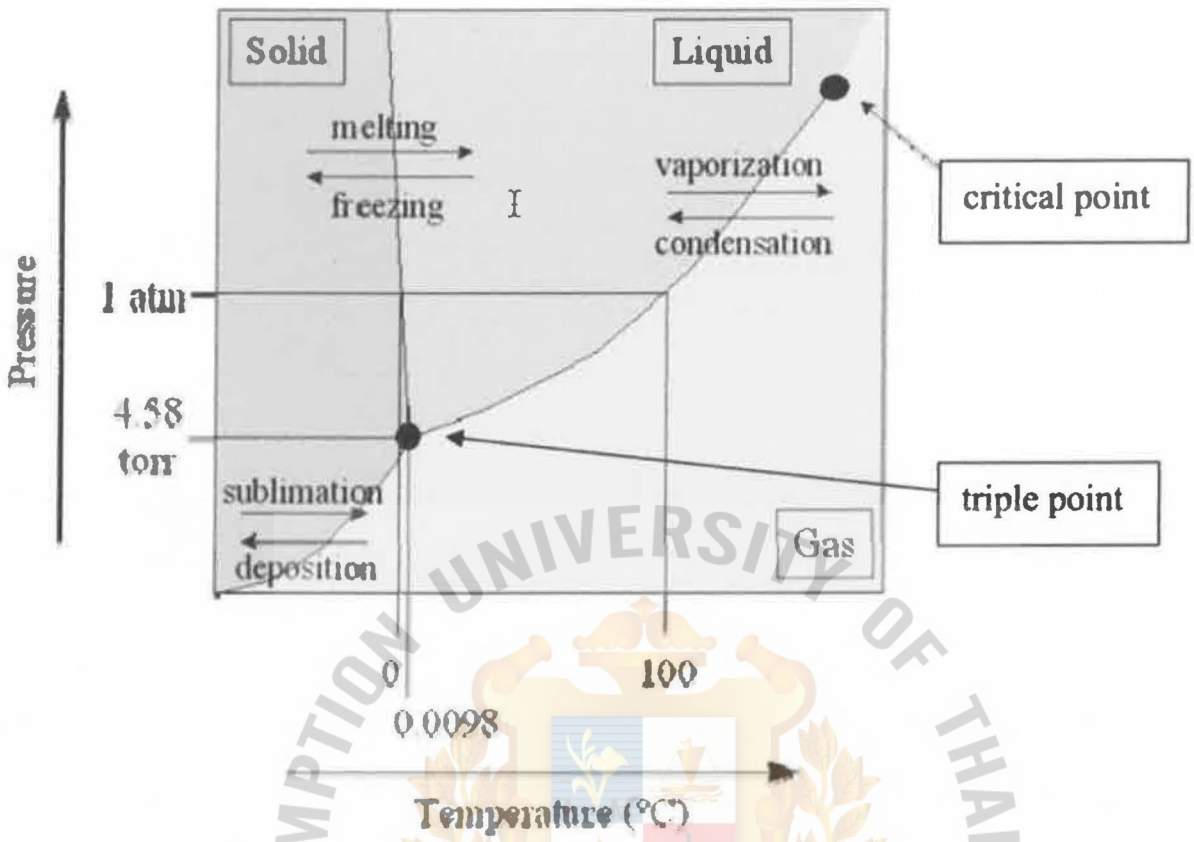

Figure 2 : Phase diagram for water [44]

The steps required to lyophilize a product in a batch process can be summarized as follows [44]:

- Pretreatment / Formulation
- Loading / Container (Bulk, Flask, Vials)
- Freezing (Thermal Treatment) at atmospheric pressure
- Primary Drying (Sublimation) under vacuum
- Secondary Drying (Desorption) under vacuum
- Backfill & Stoppering (for product in vials) under partial vacuum
- Removal of Dried Product from Freeze Dryer

2.6 Curcumin

Curcuma longa L. is a perennial herb, which belongs to family Zingiberaceae, and commonly known as turmeric. It occurs in tropical and sub-tropical regions throughout the world. It is commonly cultivated in Asian countries, mostly in India and China and is extensively used in ayurveda, unani, and siddha systems of medicine as one of the household therapies to alleviate different diseases [44-47]. Curcumin suppresses the activity of many bacteria such as Staphylococcus aureus, Salmonella paratyphi [48] and Bacillus subtilis, B. macerans, B. licheniformis, and Azotobacter [49]. Curcumin is also found to be effective against 20 types of Candida species [50]. It has been observed by trials on human and mouse that oral consumption of curcumin shows less bioavailability and it undergoes intestinal metabolism [51,52]. These obstacles of curcumin can be eliminated by synthesis of curcumin nanoparticles (Nano curcu), liposomes, micelles, and phospholipid complexes which can be used for the purpose of longer circulation, permeability and increased resistance to metabolic processes [47,53,54].

The use of curcumin-loaded nanoparticles for medical applications are currently under investigation by several experts. Curcumin loaded in poly (lactic-coglycolic acid) (PLGA) nanospheres was synthesized by using solid/oil/water emulsion solvent evaporation technique [55]. PLGA-loaded nanocurcumin are one of the efficient tools which can be used in the cancer therapy [55]. Curcumin-loaded hydrogel nanoparticles can act as an adjuvant in malarial treatment which reduces the use of antimalarial drugs [56].

Polymeric synthesis of curcumin-loaded nanoparticles was found effective against malignant brain tumors by inhibiting the growth of brain tumor cells [57]. In vitro study of synthesized nanoparticles by fatty acid coacervation technique revealed that they are effective in the treatment of cancer [58]. Also, curcumin-loaded PLGA nanoparticles were found to be novel candidates in the treatment of ovarian cancer [59]. It was demonstrated from the study that Nano curcu can stop the primary stage of metastasis, and therefore, it can be used as one of the novel treatments in cancer [60]. It was reported that the solid lipid Nano curcu was useful to enhance the oral bioavailability of curcumin [61].

Antibacterial activity of Nano curcu was tested against different types of Gram-positive and Gram-negative bacteria [62]. Silver nanocomposite film of curcumin was very effective material for antibacterial applications [63]. The researchers have also prepared sodium carboxylmethyl cellulose (SCMC) silver nanocomposite films [63]. Curcumin chitosan-poly (vinyl alcohol)-silver nanocomposite film was prepared by Vimla et al. (2011) in order to increase applications as antibacterial packaging, wound dressing, and antibacterial materials. Different nanoparticles like curcumin, silver and chitosan nanoparticles were synthesized and it was found that Nano curcu along with silver and chitosan nanoparticles showed anti-parasitic activity against *Giardia lamblia* [64].

Chapter 3

Research methodology

3.1 Materials and Equipment

3.1.1 Chemicals

All the materials used in this study were analytical grade with no further purification.

- 8% aqueous glutaraldehyde solution
- BSA
- *Curcumin from turmeric extract*
- 0.1 mM DPPH solution in methanol
- Ethanol
- Ethylene glycol
- Mannitol
- 10 mM NaCl solution
- 0.1 n NaOH
- Sucrose

3.1.2 Glassware and Equipment

- Beaker
- Erlenmeyer flask
- Test tubes and plastic tubes
- Magnetic bars
- Centrifuge (PLC-012, Harmonic Series, Gemmy Industrial Corporation, Taiwan)
- Freeze drying machine
- Heating and Drying Oven (Memmert, Metrology Technology Co., Ltd.)
- Hot place stirrer (HTS-1003, Harmany, Laboratory & Medical Supplies)
- Magnetic Stirrer (MS400, Bante Intrument)
- SEM (S-4300, Hitachi)
- Spectrophotometer (T80 UV/VIS Spectrometer, PG instruments Ltd.) and UNICO spectrophotometer Model : 1200
- FTIR spectrophotometer (Perkin Elmer)
- TEM (200CX, JEOL)
- Vortex mixer (wise Mix® VM – 10 wised laboratory instruments)
- Weighing balance (Ohaus, Jebsen & Jessen (Thailand) Ltd. And Ohaus, Ohaus Corporation, USA

3.2.2 Encapsulation efficiency

The curcumin loaded BSA nanoparticles were separated from the reaction medium by centrifugation and the UV absorbance of free curcumin was measured at a wavelength of 422 nm. The concentration of curcumin was calculated with reference to a regression equation (linear plot with slope 0.0308) obtained from constructed calibration curve of curcumin in absolute ethanol solution. The relative encapsulation efficiency was calculated based on the following equation

$$\%EE = (Wt/Wi) \times 100$$

Where

Wt is the total amount of curcumin in NP

Wi is the total quantity of curcumin added initially during preparation

3.2.3 Particle content identification

The particle yield in the suspension was determined by microgravimetry. An aliquot (1 ml) of the nanoparticle sample was put in an aluminum pan and dried for 2 h at 80oC. After drying the pan was cooled off in desiccators for 30 min and subsequently weighted with an analytical balance. The particle content (mg/ml) was calculated from the difference of the empty and filled sample. (Weight before dying – Weight after dying)

3.2.4 Fourier transform infrared spectroscopy (FT-IR)

BSA nanoparticles and BSA-curcumin nanosuspensions were characterized by Nexus-870 FT-IR spectrometer (Thermo Nicolet Corporation) equipped with a deuterated triglycine sulphate (DTGS) detector, a KBr beam splitter, zinc selenide (ZnSe) attenuated total reflectance (ATR) accessory at room temperature. The resolution was 4 cm^{-1} and the scanning range 4000-400 cm^{-1}

3.2.5 Antioxidant activity

1 ml of 0.1 mM DPPH solution was added into methanol with 1 ml of sample extract solution. Subsequently, experimental control was prepared by mixing 1 ml methanol and 1 ml DPPH solution. Furthermore, all samples was mixed using vortex and incubated for 30

minutes in the dark then measured their absorbance at 517 nm using UV-Vis spectrophotometer. Methanol was used as blank to measure the zero baseline of analyte level. Finally, the radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation

$$\% \text{ inhibition of DPPH activity} = [(AC - AA)/AC] \times 100$$

Where AA is the absorbance values of the test

AC is a control sample

3.2.6 Freeze-drying process

BSA-curcumin nanosuspension samples were separated into 2 groups (with and without sucrose) then 3% sucrose solution was added into a first section (10:1). Then all samples were put into a freeze-drying machine. The shelf temperature was reduced from 5 to -20 °C at a rate of 1 ° C/min. The pressure was 60 mT (=0.08 mbar). These parameters were held for 5 h. Furthermore, temperature was increased from – 20 to 5 ° C at 0.5 ° C/min the primary drying was achieved while the pressure remained unchanged. At the end of the primary drying heat ramp, a pressure rise test (PRT) was performed. Moreover, the secondary drying followed by increasing the temperature at a rate of 0.2 ° C/min to 15 ° C. This temperature was held for 2 h at a pressure of 60 mT (=0.08 mbar).

3.2.7 Transmission electron microscopy (TEM)

The size and shape of the nanoparticles were monitored by transmission electron microscopy (TEM). The stock solution of the nanoparticles was diluted 10 fold. A drop of sample was placed on a copper TEM grid that was then air dried and scanned in TEM operating at an accelerating volt-age of 80 kV.

3.2.8 Scanning electron microscopy (SEM)

Particle size and morphology of the nanoparticles were measured by scanning electronic microscopy (SEM). The freeze dried samples were placed on sample holder and coated with gold. The sample was observed under SEM at the electron acceleration of 60 kV.

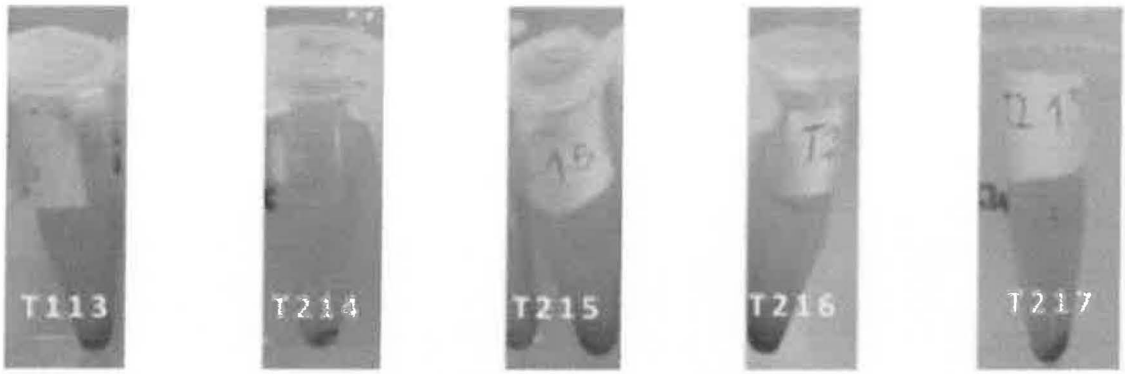

Figure 3 : Appearances of BSA nanoparticles (T111-T217) at different purification time

The result has shown that with the increasing of purification time, BSA solution was clearer and more precipitation occurred. The summary of BSA nanoparticle appearances can be observed in Table 2.

Table 2 : Appearances description for each treatment

Appearances of BSA nanoparticles after purification			
Sample code	Centrifugation speed (RPM)	Centrifugation time (min)	Result
T113	12000	8	Moderately precipitated
T214	12000	10	Precipitated more than the previous one
T215	12000	12	Moderately precipitated
T216	12000	15	Mostly precipitated
T217	12000	18	Mostly precipitated

According to Table 2, the most effective treatments were T216 and T217 due to the clearance of solution and amount of precipitants. However, the results of these two treatments were not clearly different or in other word their differences were pretty slightly.

Thus, because of its time efficiency treatment T216 was chosen as the optimal condition for purification of BSA nanoparticles. It can be concluded that the optimal condition for the purification process was centrifugation speed at 12000 RPM and 15 minutes for centrifugation time.

Since the optimal condition was acquired, the experimental samples were prepared into 3 different formulations which is shown in Table 3

Table 3 : Formulations for sample preparation

Sample preparation								
Sample code	BSA (mg)	NaCl (ml)	pH adjusted	BSA solution (ml)	EtOH (ml)	Curcumin in EtOH	8% GTA (µl)	Incubation time (hrs.)
T1	400	8	8.41	2.5	8	-	235	24
T1C	400	8	8.42	2.5	8	10 mg/ml	235	24
TEC	800	8	8.35	2.5	8	10 mg/ml	470	24

- T1 : BSA nanoparticles
- T1C, TEC : BSA-curcumin nanoparticles (BCN)

There were two main types of sample: BSA nanoparticles (T1) and BSA-curcumin nanoparticles (T1C and TEC). Their characteristics were shown in Figure 4;

BSA nanoparticles | BCN after centrifugation | BCN after purification

Figure 4 : Characteristics of T1 and T1C

Table 4 : pH of nanosuspension

Sample	pH
T1	7.57
T1C	7.49
TEC	7.77

Table 4 shows the pH values of each nanosuspension treatment after purification and it indicated that pH of all samples were neutral although the increasing amount of BSA and glutaraldehyde might increase the overall pH of TEC nanosuspension. The pH of blood is usually slightly basic with a value of pH 7.36 [68]. Therefore, the BSA-curcumin nanoparticles (T1C) could retain stably at physiological pH in blood circulation system and normal tissues.

4.2 UV-Vis spectrum

In this step, UV-Vis spectrophotometry was used to determine the λ_{max} of each sample and stock curcumin solution. The result of measurements was illustrated in Figure 5;

Figure 5 : UV-Vis spectrum of T1, T1C, TEC, stock curcumin solution

From the result of these measurements, it was clear that there were some interferences those interfered the λ max measurement of UV-Vis spectrum. Theoretically, λ max of BSA itself is 220 nm [69] and λ max of curcumin is 424 nm [70]. However, the highest peak of T1 was occurred at 400 – 500 nm in which highest peak should be at 200 – 300 nm. It was possible that this interference might come from the properties of nanoparticles which are colloidal structure. Due to properties of colloids, the colloidal nanoparticles caused light

scattering effect that alter the absorpbance and shift of lambda max in BSA nanoparticles from original of 220 nm to 400 – 500 nm in the measurement.

For T1C and TEC, the result confirmed the presence of curcumin in nanoparticle according to that of T1 and T1C which were clearly different. Moreover, with the increased amount of BSA and glutaraldehyde during the preparation for TEC, a peak at 400 – 500 nm increased. That confirmed the increasing of BSA and curcumin into the nanoparticles content.

Lastly, the lambda max of pure cucurmin solution was illustrated in Figure 5 which can be used as reference for comparison.

4.3 Encapsulation efficiency

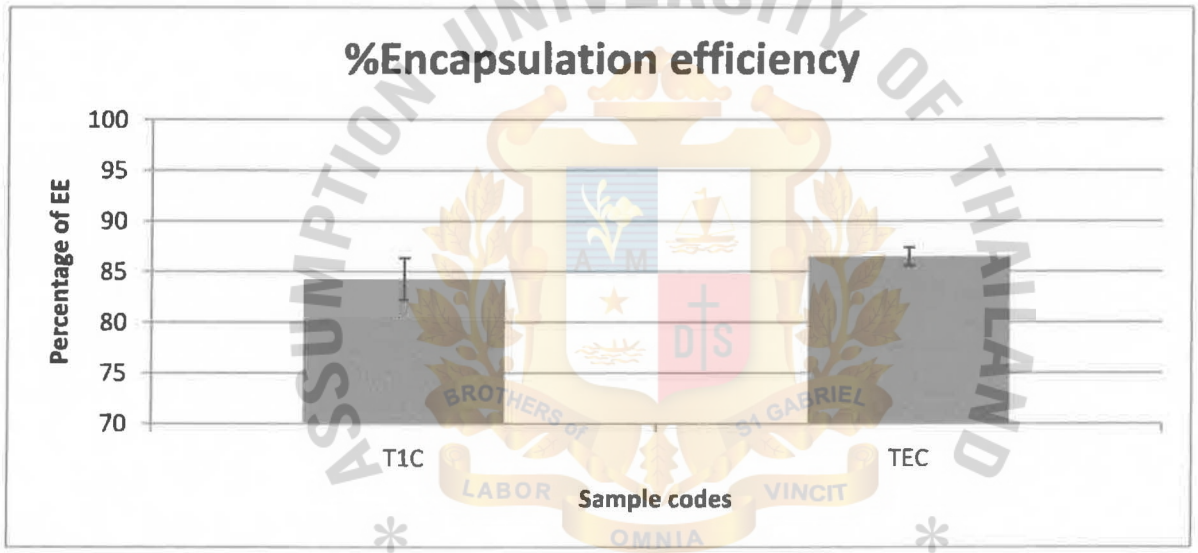

Figure 6 : Percentage of encapsulation efficiency

There was not a statistically significant difference between the input groups (p = 0.164).

From Figure 6, the encapsulation efficiency of curcumin in T1C and TEC nanoparticles were 84.29 ± 2.05 and 86.49 ± 0.92 respectively. Importantly, it was clear that the increasing amount of BSA and glutaraldehyde of TEC had slightly effect on the %encapsulation efficiency of curcumin. According to a preparation of TEC, amount of BSA and glutaraldehyde usage increased from 400 to 800 mg and 235 to 470 µl respectively.

Nevertheless, % encapsulation efficiency of T1C and TEC were not significantly different. Therefore, it can be concluded that increasing amount of BSA and glutaraldehyde during nanoparticles preparation was not a suitable way to enhance a percentage of curcumin

encapsulation efficiency. Possibly, % encapsulation efficiency of curcumin can be enhanced by improving preparation methods in other parameters.

4.4 Particle content determination

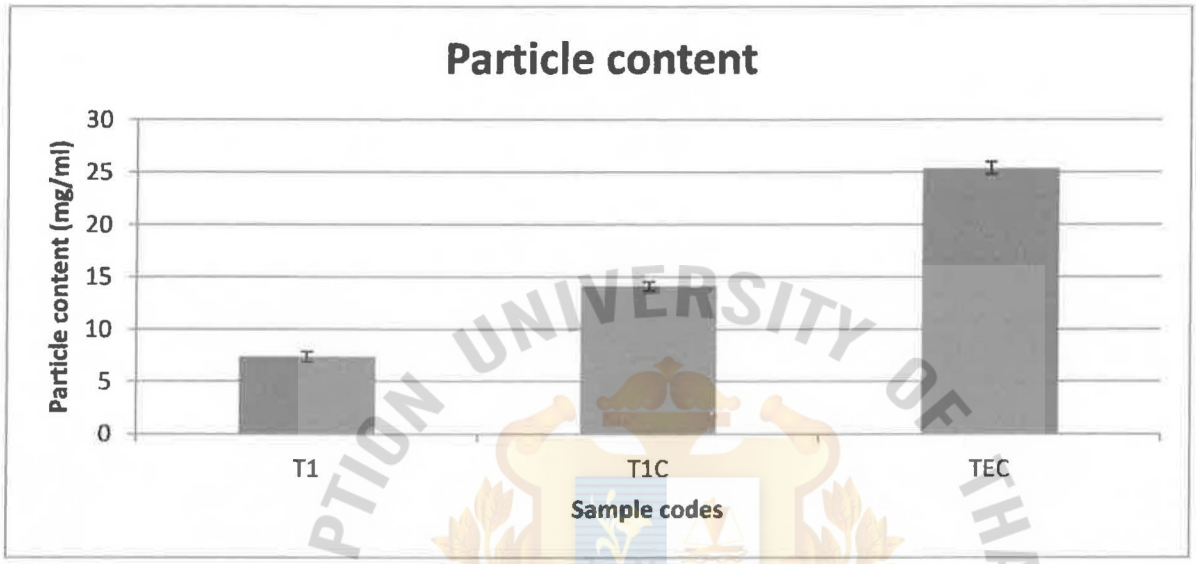

Figure 7 : Particle content of T1, T1C, and TEC

The differences in the mean values of the T1C and TEC treatment groups were two times greater than T1; there was a statistically significant difference among each treatment groups ($p = <0.001$).

From Figure 7, it was clear that particle content each sample was dramatically different. The result has shown that an addition of curcumin added up more content to overall particle content of nanoparticles. In general, curcumin molecules could be either encapsulated in nanoparticles T1C or adsorbed onto the surface of nanoparticles. Thus, particle content of T1 and T1C were significantly different.

Furthermore, particle content of T1C and TEC were also very contrast. However, according to the result from %encapsulation efficiency, %curcumin encapsulation of T1C and TEC were not significantly different. Therefore, the content added up for TEC was mostly come from the content of BSA, not the content from curcumin. To be simplified, due to the content of BSA, TEC was heavier than T1C, however, the amount of curcumin in both samples were not significantly different.

4.5 FT-IR

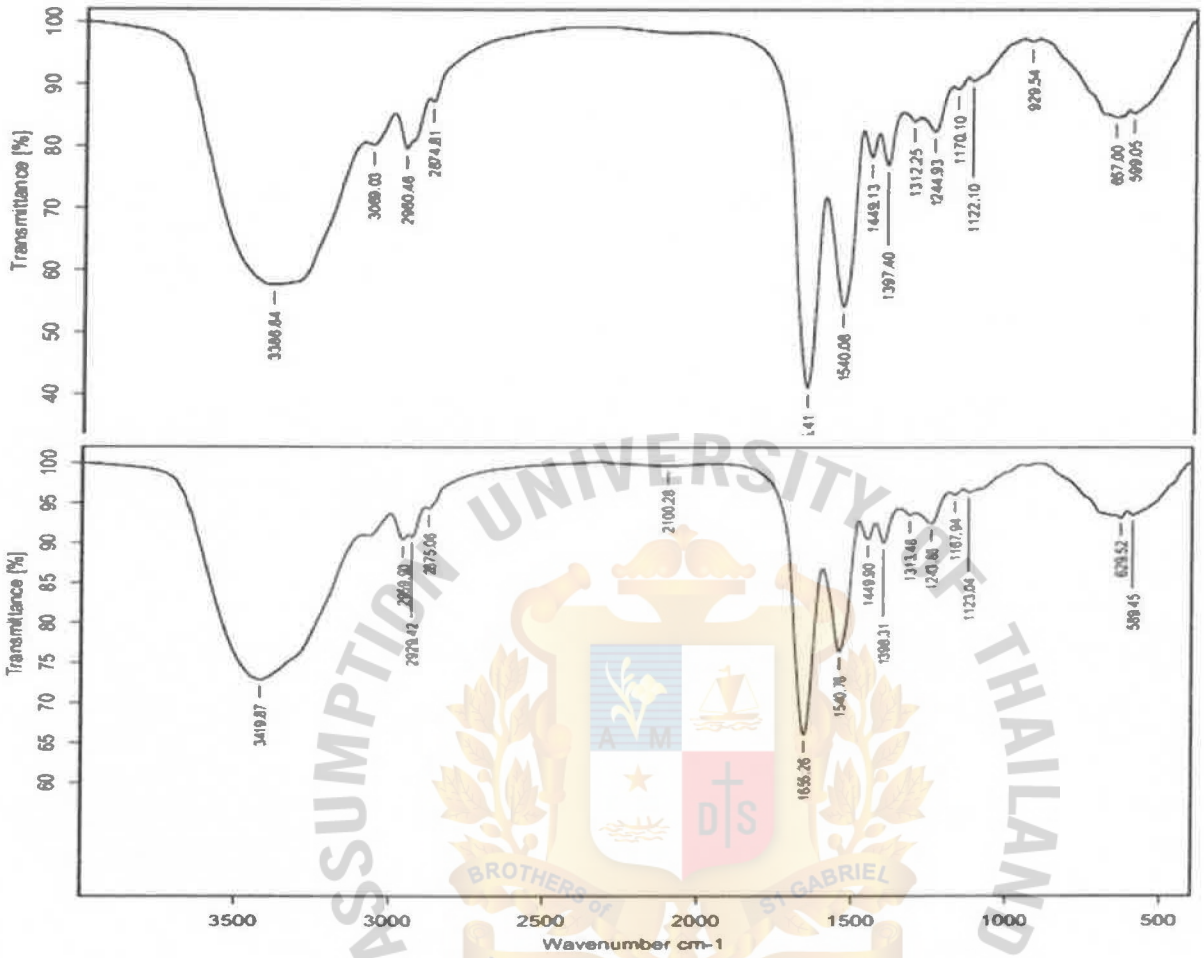

Figure 8 : Functional groups determination by FT-IR

From Figure 8, although their content are different; T1 without curcumin andT1C with curcumin, the results from FT-IR of T1 and T1C were very similar. It could relate to their functional groups. BSA and curcumin have pretty similar functional groups or maybe subset to each other. Therefore, the results from their FT-IR spectra have shown characteristic peaks at similar wavenumbers. Moreover, functional groups of these nanoparticles that can be clearly identified are O-H group (Alcohol) at 3200-3600 cm^{-1}, C=C group (Aromatic) at 1400-1600 cm-1, N-H group (amine) at 3300-3500 cm-1, C=O group (Carbonyl) at 1670-1820 cm-1, and N-H group (Amide) at 3100-3500 cm-1.

4.6 Freeze dry

In this study Freeze drying technique has been done in order to prepare samples for FTIR, SEM and TEM measurement and preserve the nanoparticles for further use. The freeze dry process was carried on 6 steps for 21 hours as described and provided solidified samples which is being shown in Figure 9;

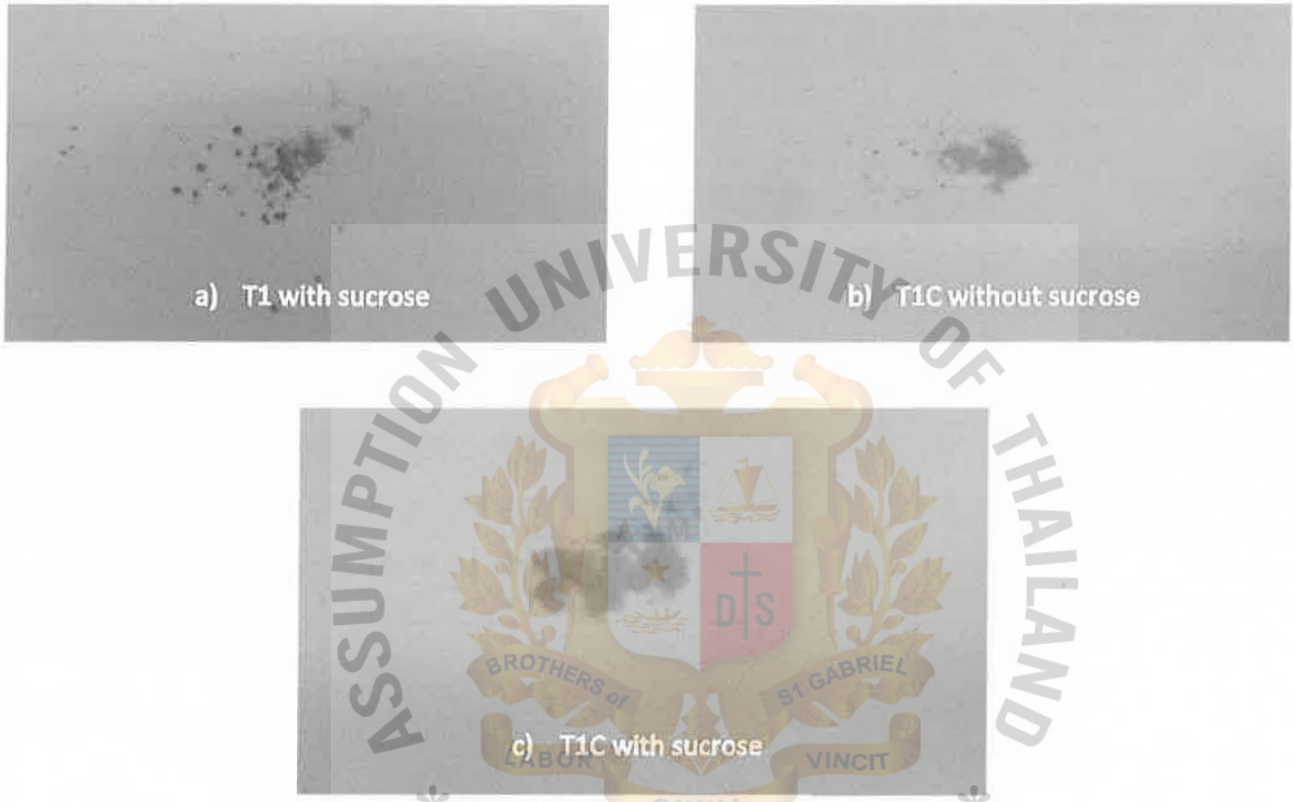

Figure 9 : Photographic images of freeze dried curcumin – BSA nanoparticles a) BSA nanoparticles after freeze drying (T1) with sucrose b) Curcumin - BSA nanoparticles after freeze drying (T1C) without sucrose c) Curcumin - BSA nanoparticle after freeze drying (T1C) with sucrose

After freeze dry, it was found that the freeze dried T1 with sucrose was not very fine powder and its solid had dark orange color. On the other hand, the freeze dried T1C without sucrose and T1C with sucrose were very fine, uniform, and had slightly dark yellow color. Additionally, the characteristics of T1C without sucrose and T1C with sucrose were similar.

4.6.1 Effect of freeze drying of BSA nanoparticle with or without sucrose

Freeze drying process dramatically affected the properties of nanoparticles as shown in Figure 10. From the results, it was clear that properties of nanoparticles before and after freeze drying were very different. For sample that did not undergo freeze drying process, its nanoparticles were spherical shape, linked together, and mostly uniform size. Nevertheless, characteristics of samples which have been freeze dried have changed compare to an original one. Their particles seem to break apart and no spherical nanoparticles found. Moreover, particles were formed like ice crystals with low resolution and did not suitable for observation of particles morphology. It is suggest that cold temperature might cause protein to change structure or lose its functions. Possibly, freeze dried BSA-curcumin nanoparticles can be obtained with the similar particle morphology if freeze drying process has been optimized solely for BSA-nanoparticles or other cryoprotectants should be used.

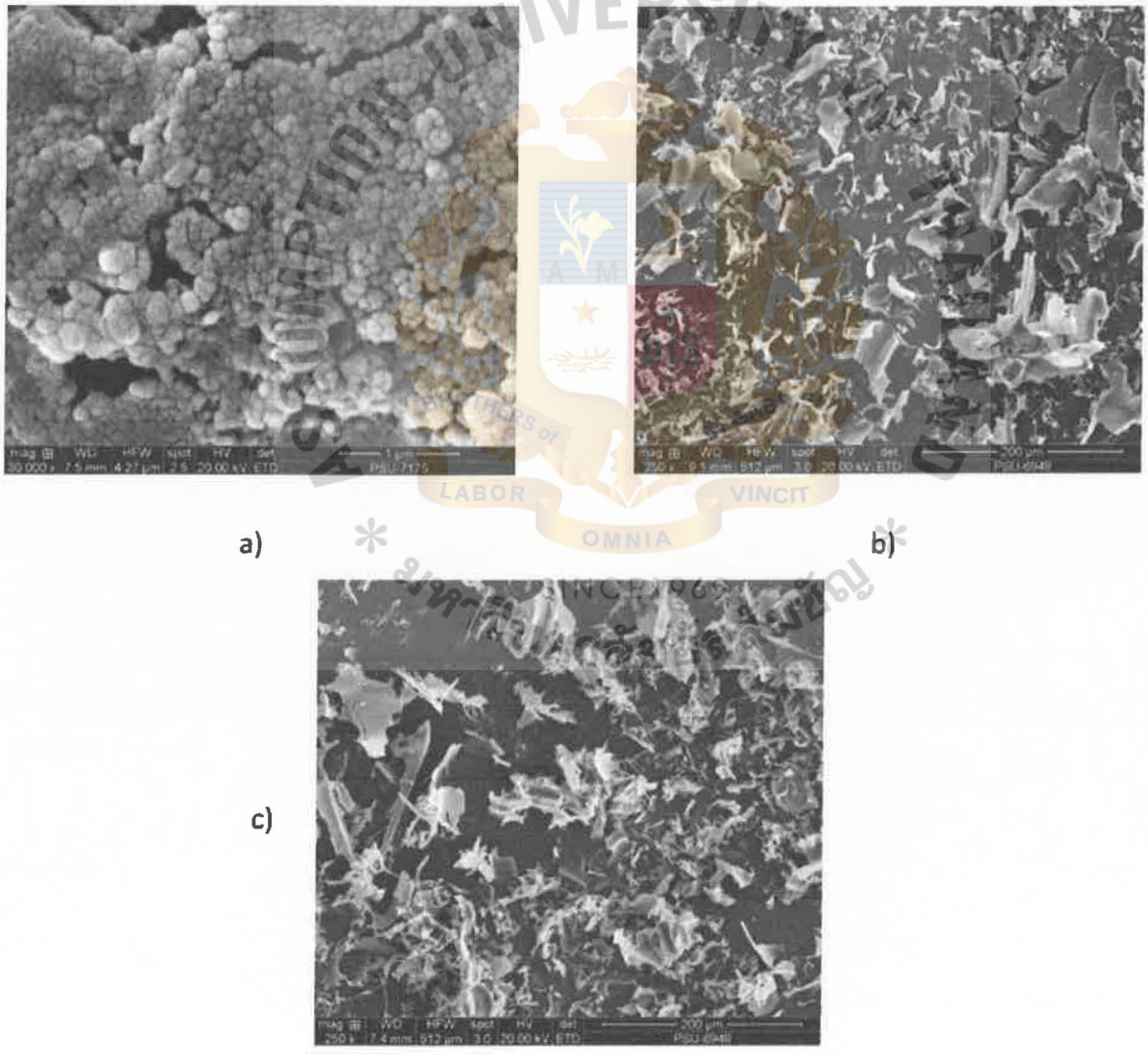

Figure 10 : Effect of freeze drying of BSA nanoparticle with or without sucrose a) BSA nanoparticle before freeze drying (T2) without sucrose b) BSA nanoparticle after freeze drying (T2) without sucrose c) BSA nanoparticle after freeze drying (T2) with sucrose

4.7 Particles morphology observation

Particle size, size distribution and morphology are important factors for nanoparticle systems that influence loading of bioactive agents, release mechanism and stability of nanoparticles. The morphology of the nanoparticles was monitored by scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

a)

b)

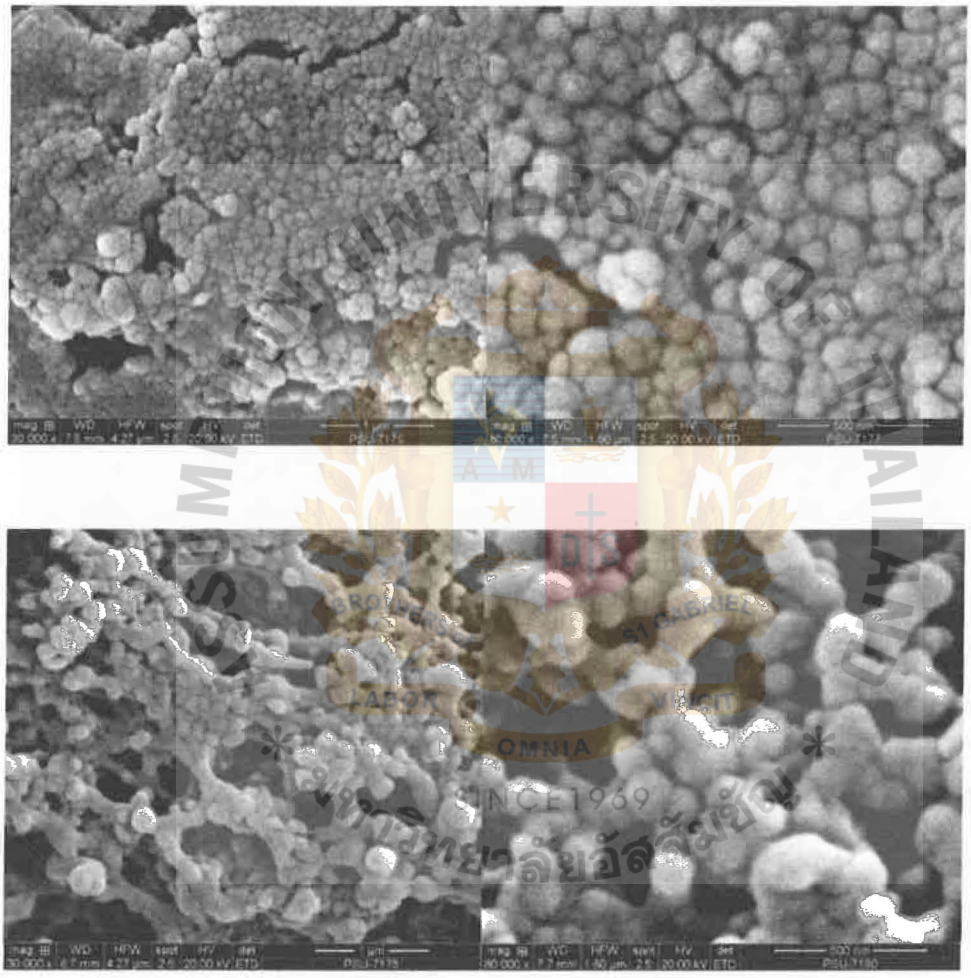

Figure 12 : Particles morphology by SEM a) T1 b) T1C

From Figure 11, the images clearly show that the nanoparticles were smooth and spherical shape in nature. However, the formation of T1 and T1C nanoparticles were noticeably different. The formation of T1 nanoparticles was mostly uniform and tight formation while the formation of T1C nanoparticles seemed to be spider web-like and moderately loose formation. It could be that the presence of curcumin in nanoparticles affected the formation of BSA-curcumin nanoparticles. Curcumin has either been encapsulated or adsorbed onto the surface of BSA nanoparticles. Thus, formation of BSA

4.9 % Antioxidant activity

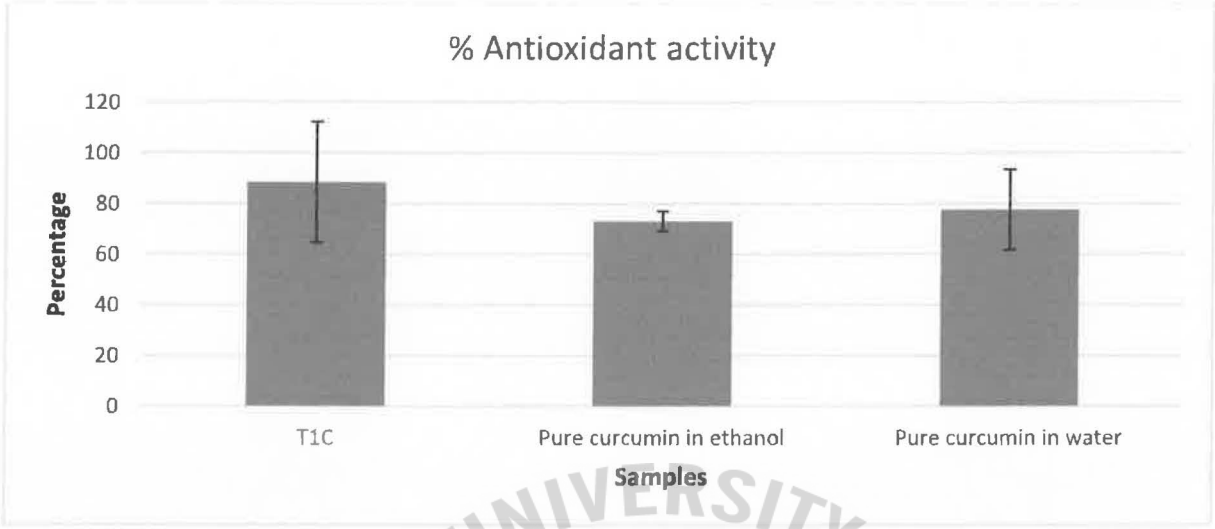

Figure 14 : % Antioxidant activity

There was not a statistically significant difference ($p = 0.547$)

DPPH is a stable free radical and is purple in color. In presence of antioxidants such as a hydrogen donor the odd electron of DDPPH is paired off, resulting in a decrease in absorbance and decolorization of solution. The extent of loss in color depends on the reducing ability of the antioxidant species.

Curcumin is known as effective antioxidant agent. However, its low bioavailability and poor solubility are its limitations. Therefore, curcumin was encapsulated in BSA nanoparticles in order to improve its bioavailability. From Figure 13, the result shows that % antioxidant activity of BSA-curcumin nanoparticles was higher than that of crude curcumin extract in water and ethanol (original form) 10% approximately. However, there was no significant difference between % antioxidant activity of BSA-curcumin nanoparticles and crude curcumin ($p>0.05$).

It can be implied that nanoparticles properties did not affect the % antioxidant activity of curcumin. In other words, curcumin does not lose antioxidant activity after being encapsulated in BSA nanoparticles and its antioxidant activity is not dramatically different from the original form.

Chapter 5

Conclusion

Nowadays, BSA nanoparticles are an interesting choice for delivery of therapeutic and bioactive agents to target organs in pharmaceutical industry. They have advantages over conventional carriers for example their self-reassembling capability, high conduction property, utilization diversity in many fields, reliability and sustainability, and small volume but large surface area.

In this study, BSA-curcumin nanoparticles (BCN) were successfully synthesized by a desolvation technique with % encapsulation efficiency of 84.3 ± 2.1%. Furthermore, encapsulation of curcumin into BSA nanoparticles resulted in an improvement of solubility and stability of curcumin. Moreover, the particle content of BCN was 14.1 ± 0.4 mg/ml. The particle size and morphology of BSA nanoparticles and BSA-curcumin nanoparticles were determined by SEM & TEM which were 212.7 ± 0.04 nm and 77.2 ± 0.02 nm respectively. Additionally, % antioxidant activity of BCN compared to crude curcumin extract in ethanol measured by DPPH assay showed 88.4 ± 23.9 % and 73.0 ± 3.9 % respectively.

All the above information suggests that curcumin loaded BSA nanoparticles can serve as potential sources for delivery of antioxidants such as curcumin and have the potential to be used as an efficient tool with therapeutic effect for a variety of applications in biomedical fields.

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Appendix

Appendix A: Raw data

Table 6 : Summary of samples preparation

Preparation									
Sample code	BSA (mg)	NaCl (ml)	pH adjusted	BSA solution (ml)	Ethanol (ml)	Curcumin in ethanol	8% GTA (µl)	pH	Incubation time (hrs.)
T1	400	8	8.41	2.5	8	-	235	7.57	24
T1C	400	8	8.42	2.5	8	10 mg/ml	235	7.49	24
TEC	800	8	8.35	2.5	8	10 mg/ml	470	7.77	24

- T1 : BSA nanoparticles
- T1C, TEC : BSA-curcumin nanoparticles

Figure 15 : Comparison of particle purification of T216 between one cycle, two cycles and three cycles of centrifugation (12000 rpm, 15 min)

Figure 16 : Comparison of T1 and T1C after purification

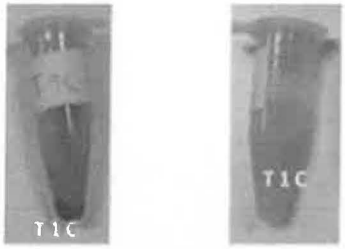

Figure 17 : Comparison of T1C after purification: before and after resuspension

Table 7 : % Encapsulation efficiency

Sample code	%EE
T1C	84.2889 ± 2.0470
TEC	86.4936 ± 0.9208

Table 8 : Particle content

Sample code	Particle content (mg/ml)
T216	7.3667 ± 0.4726
T1C	14.1 ± 0.4243
TEC	25.3667 ± 0.6028

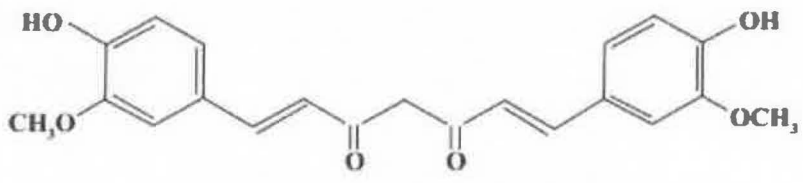

Figure 1: Chemical structure of Curcumin.

Figure 18 : Chemical Structure of curcumin

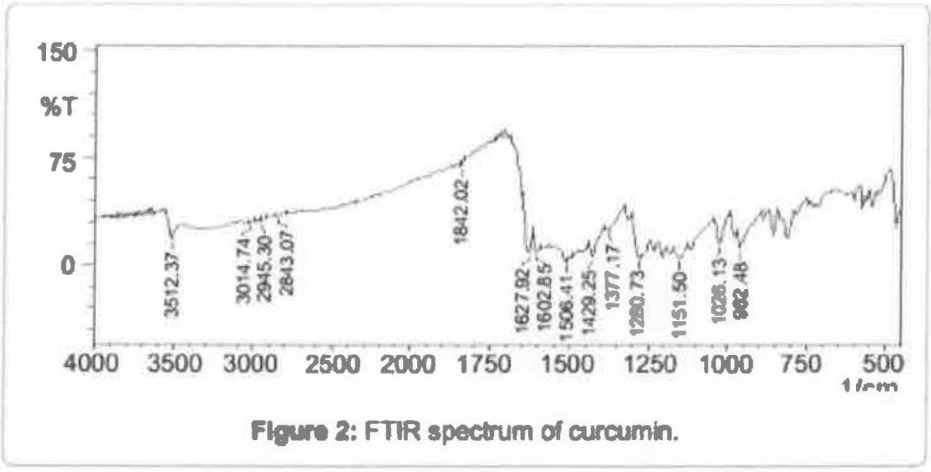

Figure 2: FTIR spectrum of curcumin.

Figure 19 : FT-IR spectrum of curcumin

Table 9 : Reference table for FT-IR

Characteristic IR Absorption Frequencies of Organic Functional Groups

Functional Group	Type of Vibration	Characteristic Absorptions (cm-1)	Intensity
Alcohol			
O-H	(stretch, H-bonded)	3200-3600	strong, broad
O-H	(stretch, free)	3500-3700	strong, sharp
C-O	(stretch)	1050-1150	strong
Alkane			
C-H	stretch	2850-3000	strong
-C-H	bending	1350-1480	variable
Alkene			
=C-H	stretch	3010-3100	medium
=C-H	bending	675-1000	strong

C=C	stretch	1620-1680	variable
Alkyl Halide			
C-F	stretch	1000-1400	strong
C-Cl	stretch	600-800	strong
C-Br	stretch	500-600	strong
C-I	stretch	500	strong
Alkyne			
C-H	stretch	3300	strong,sharp
-C≡C-	stretch	2100-2260	variable, not present in symmetrical alkynes
Amine			
N-H	stretch	3300-3500	medium (primary amines have two bands; secondary have one band, often very weak)
C-N	stretch	1080-1360	medium-weak
N-H	bending	1600	medium
Aromatic			
C-H	stretch	3000-3100	medium
C=C	stretch	1400-1600	medium-weak, multiple bands
Analysis of C-H out-of-plane bending can often distinguish substitution patterns			
Carbonyl			
C=O	stretch	1670-1820	strong
(conjugation moves absorptions to lower wave numbers)			
Ether			
C-O	stretch	1000-1300 (1070-1150)	strong
Nitrile			

CN	stretch	2210-2260	medium
Nitro			
N-O	stretch	1515-1560 & 1345-1385	strong, two bands

IR Absorption Frequencies of Functional Groups Containing a Carbonyl (C=O)

Functional Group	**Type of Vibration**	**Characteristic Absorptions (cm-1)**	**Intensity**
Carbonyl			
C=O	stretch	1670-1820	strong
(conjugation moves absorptions to lower wave numbers)			
Acid			
C=O	stretch	1700-1725	strong
O-H	stretch	2500-3300	strong, very broad
C-O	stretch	1210-1320	strong
Aldehyde			
C=O	stretch	1740-1720	strong
=C-H	stretch	2820-2850 & 2720-2750	medium, two peaks
Amide			
C=O	stretch	1640-1690	strong
N-H	stretch	3100-3500	unsubstituted have two bands
N-H	bending	1550-1640	
Anhydride			

C=O	stretch	1800-1830 & 1740-1775	two bands
Ester			
C=O	stretch	1735-1750	strong
C-O	stretch	1000-1300	two bands or more
Ketone			
acyclic	stretch	1705-1725	strong
cyclic	stretch	3-membered - 1850 4-membered - 1780 5-membered - 1745 6-membered - 1715 7-membered - 1705	strong
a,b-unsaturated	stretch	1665-1685	strong
aryl ketone	stretch	1680-1700	strong

Table 10 : Lyophilisation process

Step	Temperature (°C)	Time (hrs.)
Pretreatment	-20	1
Loading	-20	5
Freezing	5	3
Primary Drying	5	5
Secondary Drying	15	2
Backfill & Stoppering	15	5
Removal of Dried Product	30	-

Table 11 : % Antioxidant activity

Sample	%AA
T1C	88.4 ± 23.9
Pure curcumin in ethanol	73.0 ± 4.0
Pure curcumin in water	77.7 ± 15.9

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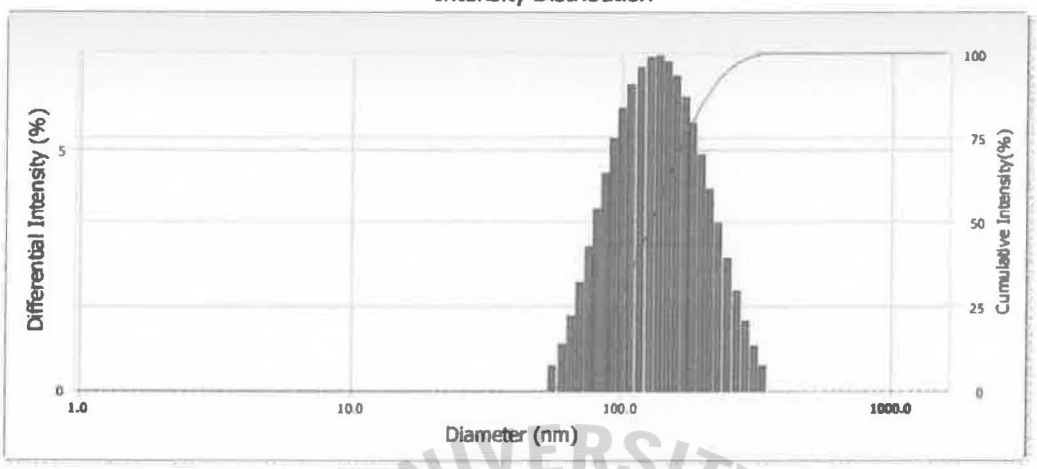
Intensity Distribution



Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	145.9	57.2
2	0.0	0.0
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	145.9	57.2
Residual :	2.246e-003	(O.K)

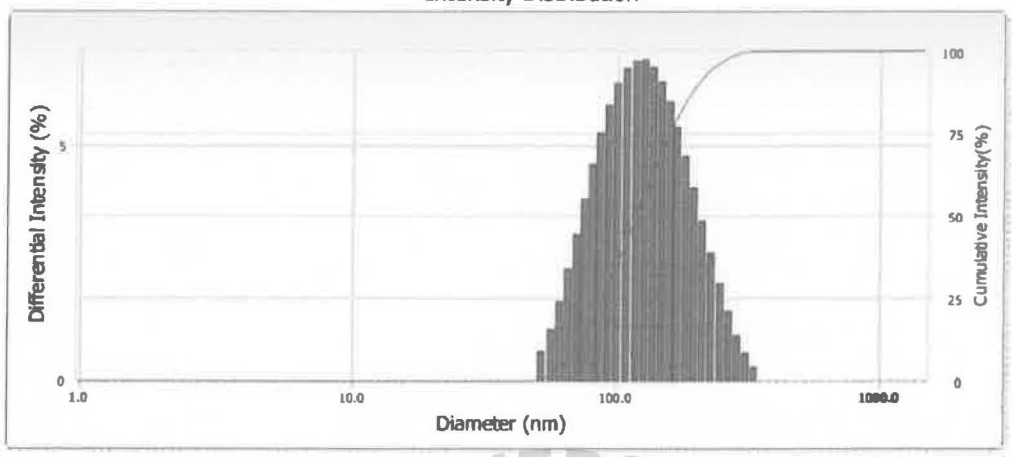
Cumulants Results

Diameter	(d)	: 127.1	(nm)
Polydispersity Index	(P.I.)	: 0.111	
Diffusion Const.	(D)	: 3.870e-008	(cm^2/sec)
Measurement Condition			
Temperature		: 25.0	(°C)
Diluent Name		: WATER	
Refractive Index		: 1.3328	
Viscosity		: 0.8878	(cP)
Scattering Intensity		: 73987	(cps)
Attenuator 1		: 0.024	(%)

Figure 20 : Size distribution determination by dynamic light scattering for T1

Version 6.01 / 4.01

Intensity Distribution



Distribution Results (Contin)			Cumulants Results			
Peak	Diameter (nm)	Std. Dev.	Diameter	(d)	: 120.8	(nm)
1	139.6	56.0	Polydispersity Index	(P.I.)	: 0.117	
2	0.0	0.0	Diffusion Const.	(D)	: 4.073e-008	(cm²/sec)
3	0.0	0.0	Measurement Condition			
4	0.0	0.0	Temperature		: 25.0	(°C)
5	0.0	0.0	Diluent Name		: WATER	
Average	139.6	56.0	Refractive Index		: 1.3328	
			Viscosity		: 0.8878	(cP)
Residual :	2.780e-003	(O.K)	Scattering Intensity		: 50299	(cps)
			Attenuator 1		: 0.024	(%)

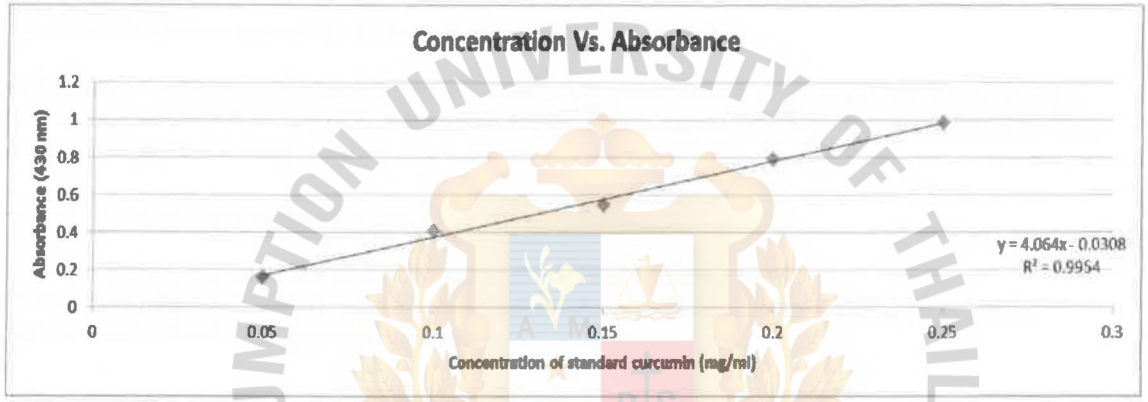
Figure 21 : Size distribution determination by dynamic light scattering for T1C

Appendix B: Standard curve of curcumin

Table 12 : Variation of curcumin concentration

Concentration of Curcumin (mg/ml)	Absorbance
0.05	0.164
0.1	0.404
0.15	0.548
0.2	0.792
0.25	0.986

**



Initial concentration of stock curcumin: 10 mg/ml

Figure 22 : Standard curve of curcumin

Table 14 : Statistical analysis for particles content determination using One way ANOVA and Multiple comparison (Duncan's Method)

One Way Analysis of Variance ¼ÄËÑÊº´Õ, ¡Ã¡®Ò¤Á 06, 2017, 15:43:47

Data source: Size SEM_TEM T2C in Ton BSA CUR.JNB

Group Name	N	Missing	Mean	Std Dev	SEM
Row 1	3	0	7.367	0.473	0.273
Row 2	3	0	14.100	0.424	0.245
Row 3	3	0	25.367	0.603	0.348

Source of Variation	DF	SS	MS	F	P
Between Groups	2	496.276	248.138	970.870	<0.001
Residual	6	1.533	0.256		
Total	8	497.809			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Duncan's Method) :

Comparisons for factor:

Comparison	Diff of Means	p	q	P	P<0.050
Row 3 vs. Row 1	18.000	3	61.669	--	Yes
Row 3 vs. Row 2	11.267	2	38.600	--	Yes
Row 2 vs. Row 1	6.733	2	23.069	--	Yes

Note: The P values for Dunnett's and Duncan's tests are currently unavailable except for reporting that the P's are greater or less than the critical values of .05 and .01.

ก.๔.๑ น้ำที่ใช้ล้างทำความสะอาดเครื่องมือ เครื่องจักร อุปกรณ์ และมือของผู้ทำ เป็นน้ำสะอาดและมีปริมาณ
เพียงพอ
ก.๔.๒ มีวิธีการป้องกันและกำจัดสัตว์นำเชื้อ แมลง และฝุ่นผงในบริเวณที่ทำตามความเหมาะสม
ก.๔.๓ มีวิธีการป้องกันไม่ให้สัตว์เลี้ยง เช่น สุนัข แมว เข้าไปในบริเวณที่ทำ
ก.๔.๔ มีการกำจัดขยะ สิ่งสกปรก และน้ำทิ้ง อย่างเหมาะสม เพื่อไม่ก่อให้เกิดการปนเปื้อนกลับลงสู่ผลิตภัณฑ์
มผช.๓๔๐/๒๕๕๔
ก.๔.๕ สารเคมีที่ใช้ล้างทำความสะอาด และใช้กำจัดสัตว์นำเชื้อและแมลง ใช้ในปริมาณที่เหมาะสม และ
เก็บแยกจากบริเวณที่ทำ เพื่อไม่ให้ปนเปื้อนลงสู่ผลิตภัณฑ์ได้
ก.๕ บุคลากรและสุขลักษณะของผู้ทำ
ก.๕.๑ ผู้ทำทุกคน ต้องมีสุขภาพดีทั้งร่างกายและจิตใจ รักษาความสะอาดส่วนบุคคลให้ดี เช่น สวมเสื้อผ้าที่
สะอาด มีผ้าคลุมผมเพื่อป้องกันไม่ให้เส้นผมหล่นลงในผลิตภัณฑ์ ไม่ไว้เล็บยาว ล้างมือให้สะอาด
ทุกครั้งก่อนปฏิบัติงาน หลังการใช้ห้องสุขา และเมื่อมือสกปรก
ก.๕.๒ ผู้ทำทุกคน ต้องไม่กระทำการใดๆ ที่ไม่ถูกสุขลักษณะในสถานที่ทำ เช่น รับประทานอาหาร สูบบุหรี่