THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Fishery Products)
DEGREE

Fishery Products
FIELD

Fishery Products
DEPARTMENT

TITLE: Encapsulation of Wasabi Flavor and Its Application in Canned Tuna Spread

NAME: Miss Phutthiya Ratanasiriwat

THIS THESIS HAS BEEN ACCEPTED BY

_____ **THESIS ADVISOR**
(Associate Professor Wanchai Worawattanamateekul, Ph.D.)

_____ **THESIS CO-ADVISOR**
(Miss Wanwimol Klaypradit, Ph.D.)

_____ **THESIS CO-ADVISOR**
(Assistant Professor Utai Klinkesorn, Ph.D.)

_____ **THESIS CO-ADVISOR**
(Assistant Professor Suched Samuhasaneetoo, Ph.D.)

_____ **DEPARTMENT HEAD**
(Assistant Professor Jiraporn Runglerdkriangkrai, Ph.D.)

APPROVED BY THE GRADUATE SCHOOL ON _____

_____ **DEAN**
(Associate Professor Gunjana Theeragool, D.Agr.)

THESIS

ENCAPSULATION OF WASABI FLAVOR AND ITS APPLICATION IN CANNED TUNA SPREAD

PHUTTHIYA RATANASIRIWAT

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Fishery Products)
Graduate School, Kasetsart University
2012

Phutthiya Ratanasiriwat 2012: Encapsulation of Wasabi Flavor and Its Application in Canned Tuna Spread. Doctor of Philosophy (Fishery Products), Major Field: Fishery Products, Department of Fishery Products. Thesis Advisor: Associate Professor Wanchai Worawattanamateekul, Ph.D. 106 pages.

Ally Isothiocyanate (AITC), a sulphur compound mainly responsible for the pungent flavor of wasabi, is used as a major flavoring agent in foods. However, absence of its flavor characteristic after high pressure and temperature processing is of great concern for the canned food industry. The objective of this study was to apply encapsulation technology with wasabi to protect AITC loss during the canning process. The microencapsulated wasabi flavor produced from spray drying was investigated with respect to the effects of various kinds of wall materials (HICAP^{TM}100, blending of HICAP^{TM}100 with maltodextrin, CAPSUL or whey protein concentrate) on physical and chemical properties. Results demonstrated that the emulsion stability and surface AITC of microencapsulated wasabi flavor prepared from HICAP^{TM}100, blending of HICAP^{TM}100 with maltodextrin or CAPSUL showed higher stability than the microencapsulated wasabi flavor prepared from blending of HICAP^{TM}100 with whey protein concentrate. However, there were no difference in the emulsion viscosity, moisture content, water activity, color value and the microstructure of all microencapsulated wasabi flavor. The encapsulated wasabi flavor prepared from HICAP^{TM}100, blending of HICAP^{TM}100 with maltodextrin or CAPSUL at various flavoring agent concentrations (10, 15 or 20% w/w) were determined for physicochemical properties. All encapsulated treatments were stored at various relative humidity (RH) levels (11, 33 and 52 %RH) and intervally evaluated for encapsulation efficiency (EE) during 60 days of storage. Microcapsules of 20% wasabi flavor derived from the mixture of HICAP^{TM}100 with maltodextrin and HICAP^{TM}100 with CAPSUL demonstrated excellent properties including low moisture content, acceptable flowing properties, surface appearance and EE. The release rate of encapsulated flavor increased as the RH increased for most encapsulated treatments. A difference-from-control test was conducted to evaluate the magnitude of wasabi flavor retention added in the canned tuna spread. The intensity of wasabi flavor retention in the canned tuna spread with encapsulated flavor agents added was higher than that of the samples without flavor encapsulation.

_____ _____ ____/____/____

Student's signature Thesis Advisor's signature

ACKNOWLEDGEMENTS

I would like to express my deepest and sincere gratitude to my advisor, Associate Professor Dr. Wanchai Worawattanamateekul of the Department of Fishery Products, Faculty of Fisheries, Kasetsart University, for his kindness, valuable suggestion.

I also wish to express my profound gratitude to my co-advisor, Dr. Wanwimol Klaypradit of the Department of Fishery Products, Faculty of Fisheries, Kasetsart University, for her kindness, guidance and assistance in reading, correcting and criticizing the manuscript.

I am also grateful to my examining committee, Dr. Maruj Limpawattana of the Food Science and Technology, Faculty of Science, Siam University; Assistant Professor Dr. Utai Klinkesorn of the Department of Food Science and Technology, Faculty of Ago-Industry, Kasetsart University and Assistant Professor Dr. Suched Samuhasaneetoo of the Department of Food Technology, Faculty of Engineering and Industrial Technology, Silpakorn University, for their kindness, comment and helpful suggestion.

Finally, I am grateful to my family and my dear friends who encouraged, gave great understanding and helped in various ways.

This project was supported by the Kasetsart University Research and Development Institute (KURDI); and the Center for Advanced Studies for Agriculture and Food, Institute for Advanced Studies, Kasetsart University.

Phutthiya Ratanasiriwat

September 2012

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

ENCAPSULATION OF WASABI FLAVOR AND ITS APPLICATION IN CANNED TUNA SPREAD

INTRODUCTION

Thailand is one of the world's most important exporters of seafood products, especially canned tuna. In 2010, total Thai exports of tuna were 500,000 Tons, which represented a 20% increase when compared to 2005 (Thai Frozen Food Association, 2011). In addition, the value of export tuna products also increase to USD 4,850 million in 2010 (Thai Frozen Food Association, 2011). In order to maintain Thailand's position as an exporter leader, it is necessary for the food industry to take steps to improve the product quality and also introduce new products into the market. The addition of wasabi flavor in canned tuna spread is a new product development that can satisfy customer demand and increase market share.

Wasabi, known as Japanese horseradish (*Wasabi japonica* Matsum) is used primarily as a main condiment for Japanese cuisine. The major naturally occurring sulfur compound of wasabi is 2-propenyl isothiocyanate, commonly referred to as allyl isothiocyanate (AITC) comprising 90% of the total volatiles (Depree *et al*., 1999; Li *et al*. 2007; Sultana and Savage, 2008). When plant tissues are mechanically disrupted, isothiocyanates are formed by the action of myrosinase on glucosinolates and responsible for the hot taste and pungent odor of wasabi (Depree *et al*., 1999; Sultana *et al*., 2003b). Due to its pungency characteristic and ability to increase palatability, wasabi possesses a widespread appeal in culinary meals including as wasabi flavor incorporated in processed foods. As a consequence, it offers flavor diversity for products that meet consumers' choices. However, in the presence of water at both room temperature and higher, AITC undergoes gradual degradation with more rapid decomposition at 37°C to other compounds presenting a garlic-like odor (Depree *et al*., 1999; Li *et al*., 2007). Under high temperature and pressure as well as alkaline conditions the decomposition of AITC is accelerated and thus refrain the use of wasabi flavor as a flavoring ingredient for thermal processed foods. The use of encapsulation process is therefore applied to tackle this problem.

Microencapsulation is a process by which small particles of sensitive core ingredients are coated within a wall material to form microcapsules in order to protect against adverse environment, promote stability, prevent the loss and control release of core ingredients (Shahidi and Han, 1993; Jafari *et al.*, 2008). One of the most used techniques employed in food industry is spray-dried microencapsulation, which involves transformation of emulsion containing ingredients into a dry powder. Spray-dried microencapsulation has been favorably used in the processing of savory flavors having hygroscopic and thermoplastic properties due to its operational flexibility (Youngs, 1986). The incorporation of volatile flavor compounds into dry powders remains of current interest for food industry as to improve the stability and control release of food flavors during processing or final food products (Baranauskiene *et al*., 2006; Gharsallaoui *et al*., 2011; Nadeem *et al*., 2011).

The common wall materials used to encapsulate flavors for spray drying are maltodextrin, gum arabic and modified starches, each of which has pros and cons regarding to specific properties, cost and availability and encapsulation efficiency (Jackson and Lee, 1991; Wilson and Shah, 2007; Spada *et al*., 2012). Although, maltodextrins are favored due to their good ability of bulking, film forming and binding with flavor but caking of microcapsules under high temperature storage is yet concerned (Drusch *et al.*, 2006). Gum arabic provides good volatile retention similarly to modified starches whereas the latter lack of emulsifying characteristics. The wax maize starch chemically modified with n-octenyl succinic anhydride (OSA) aimed at conferring functional characteristics compared to those of gum arabic has been investigated for flavor encapsulation (Krisnan *et al*., 2005; Soottitantawat *et al.*, 2005b; Rocha *et al.*, 2012). These researches indicated its potential to better retain and control release of core ingredients. Moreover, the reports on combination of these wall materials have been shown to improve emulsification properties and offer a better protection when appropriate blends were used (Krishnan *et al*., 2005; Sansone *et al*., 2011; Spada *et al*., 2012). In addition, the release of microencapsulated flavors from matrices may also increasingly occur with increasing relative humidity during storage (Rosenberg *et al*., 1990). The release behavior of the AITC incorporated in different types of cyclodextrins under various relative humidity (RH) storage and also its

stability has been recently reported (Li *et al.* 2007; Zhang *et al*. 2007; Zhang *et al*. 2011).

OBJECTIVES

The objectives of this study were:

1. To study the effect of different types of wall materials used for wasabi flavor encapsulation by spray drying on physical and chemical properties.

2. To study the effect of relative humidity on the release characteristics of encapsulated wasabi flavor.

3. To study the application of encapsulated wasabi flavor to canned tuna spread.

LITERATURE REVIEW

Flavors

Flavor is the most important in consumer acceptance and influences further consumption of foods, which stimulating taste or aroma receptors to produce an integrated psychological (Lindsay, 1996; Guichard, 2006; Madene *et al*., 2006). Generally, the flavor has evolved to a usage that implies an overall integrated perception of sensory (smell, taste, sight, feeling and sound) at the time of consumption food. The ability of specialized cells of the olfactory epithelium of the nasal cavity is detecting trace amounts of volatile odorants account for the nearly unlimited variations in intensity and quality of odors and flavors. Taste buds located on the tongue and back of the oral cavity enable humans to sense sweetness, sourness, saltiness and bitterness and these sensations contribute to the taste component of flavor. Nonspecific or trigeminal neural responses also provide important contributions to flavor perception through detection of pungency, cooling, umami, or delicious attribute and other chemical induced sensations that are incompletely understood (Lindsay, 1996).

Stability of flavor in different foods has been of increasing interest because of its relationship with the quality and acceptability of foods, but it is difficult to control. Manufacturing and storage processes, packaging materials and ingredients in foods often cause modifications in overall flavor by reducing aroma compound intensity or producing off-flavor components. Some carbohydrates are more stable, which are water-soluble and some are more stable in lipid-based coating. Many factors linked to aroma affect the overall quality of the food; examples are physicochemical properties, volatile aroma molecules concentration and interaction with food components. The degradation or loss during processing and storage of aroma is to limit, it is beneficial to encapsulate volatile ingredient prior to use in foods or beverages (Madene *et al*., 2006).

The loss of flavors during the processing or storage of foods is a very common occurrence in the food industry. Flavors are very volatile, react with other components and are susceptible to heat and moisture. Because flavor is a desirable characteristic of food, it is necessary to find methods that allow flavors to be retained in food for a longer of time period. Microencapsulation and controlled release offer a method for protecting flavor compounds (Pothakamury and Barbosa-Canovas, 1995).

Wasabi

Wasabi (*Wasabia japonica* Matsum. sometime referred to as *Eutrema wasabia* Matsum.), called Japannese horseradish, is also a member of the Brassicaceae or Cruciferae family of vegetables. It was first cultivated in Japan, but is now being grown in other countries such as Taiwan and China and its production in New Zealand is increasing. In Japan, wasabi is grown in two traditional ways, upland or soil grown, and water grown in specially built flooded beds. (Chadwick *et al*., 1993). Figure 1 shows a drawing of *Wasabia japonica* Matsum. Although, the mature wasabi plants produce thickened rhizomes, which are used extensively for its characteristic flavor (the hot taste with a pungent smell) in Japanese cuisine and is considered one of the staple condiments in the Japanese diet, such as traditional raw fish and noodle dishes, as well as more modern foods (Sultana *et al*., 2003a). The characteristic odor and taste of wasabi comes from volatile elements which are liberated when tissues are damaged such as the rhizomes are grated during preparation of the wasabi sauce (Depree *et al*., 1999).

Figure 1 *Wasabia japonica* Matsum. The mature plant has a distinctive thickened stem, or rhizome, connected to the heart-shaped leaves by long, thin petioles.

Source: Sultana and Savage (2008)

Flavors compounds found in wasabi

The main pungent component of wasabi comes from isothiocyanates (ITCs), the volatile sulfur compounds, which are liberated from the precursor glucosinolates (GSLs) when plant tissues become damaged (Sultana *et al*., 2003b). The development of flavor proceeds as outlined in Figure 2. GSLs are a group of glucosides, stored within the cell vacuoles of all Crucifereae plants and are biosynthesized from protein amino acid (Fahey *et al*., 2001).

When plant tissues are mechanically disrupted or injured, myrosinase is release from the cell wall, and in the presence of adequate moisture it rapidly hydrolyses the GSLs to yield glucose and an aglucone (Fenwick *et al*., 1983). Under neutral pH the aglucone are formed to an isothiocyanate. However, the weakly acidic

pH or in the presence of Fe^{2+} ions or endogenouse nitrile factor, a nitrile and elemental sulphur may be formed instead. However, once formed ITCs are more stable under acidic condition (Depree, 1999).

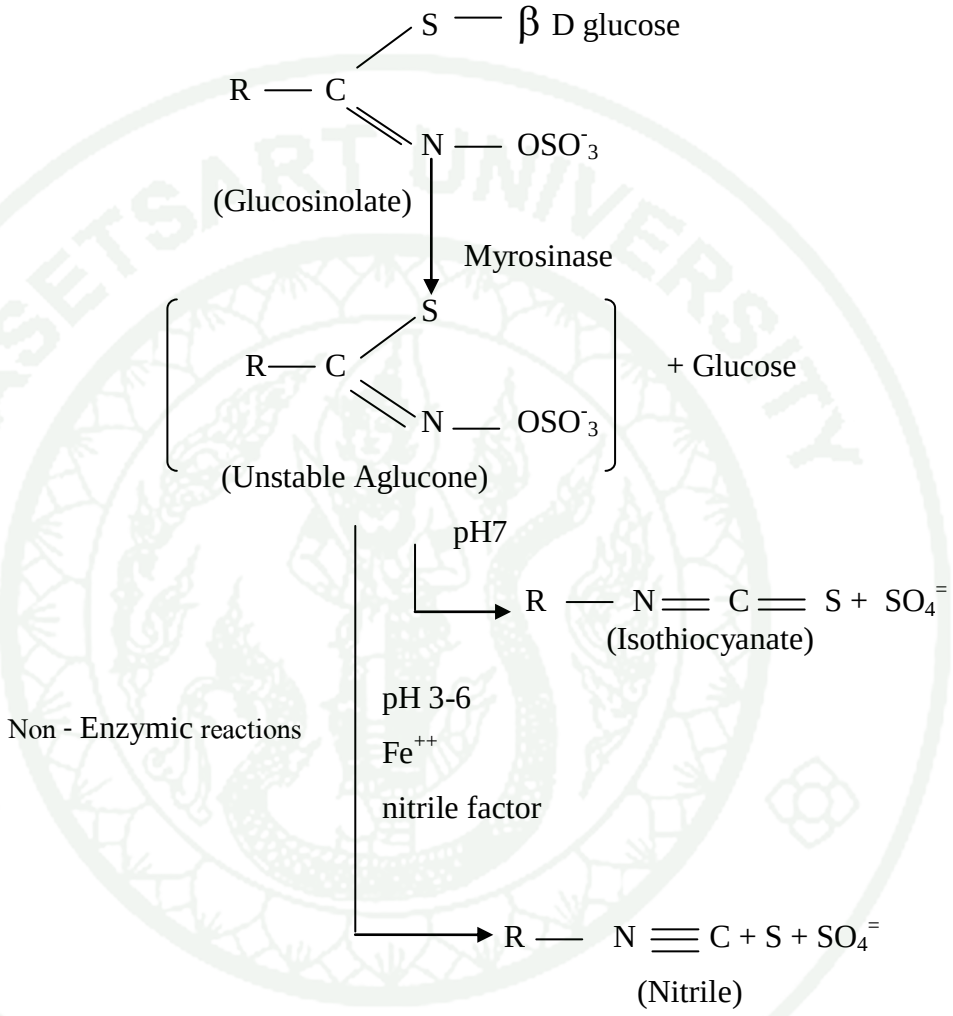


Figure 2 Conversion of glucosinolates to isothiocyanates and nitriles.

Source: Modified from Depree *et al*. (1999)

The major pungent compound of wasabi is 2-propenyl isothiocyanate, commonly referred to as allyl isothiocyanate (AITC, CH_2=$CHCH_2N$=C=S) (Table 1).

Table 1 Comparison of flavor compounds from the ether extracts of wasabi and horseradish

Isothiocyanates (ITCs)	Wasabi root	Horseradish root
Allyl ITC	111	96.60
n-Butyl ITC	1.74	0.42
3-Butenyl ITC	1.83	0.81
4-Pentenyl ITC	3.90	0.10
5-Hexenyl ITC	1.02	0.18
2-Phenylethyl ITC	-	22.50
5-Methylthiopentyl ITC	0.48	-
6-Methylthiohexyl ITC	1.89	-
7-Methylthioheptyl ITC	1.44	-
5-Methylsulphinylpentyl ITC	2.17	0.81
6-Methylsuphinylhexyl ITC	7.80	0.90
7-Methylsulphinylheptyl ITC	1.41	0.78

Source: Modified from Etoh *et al*. (1990)

Note Concentrations are expressed as mg/100g fresh weight.

AITC is unstable and is gradually decomposed to other compounds having a garlic like odor in the presence of water at both room temperature with more rapid decomposition at 37 °C and higher. High temperature and pressure and also alkaline conditions accelerate the decomposition of AITC. Therefore, its lack of stability after high pressure and temperature processing makes it very difficult for it to be used in food components and it is of great concern to the canned food industry. This problem can be overcome though the encapsulation process (Depree *et al*., 1999; Li *et al*., 2007).

Microencapsulation

Microencapsulation is defined as a process in which core material (liquid droplets, solid particles or gas compounds) are embedded in a homogeneous or heterogeneous matrix, or entrapped into thin films (wall material) of a food grade-microencapsulating agent (Gharsallaoui *et al.*, 2007). It has been successfully used in the food industry to protect sensitive core materials from environmental parameters (oxygen, light and temperature), to promote handling ability, to control release of the core material and to mask taste (Versic, 1988).

Morphology of microcapsules

Most microcapsules are small sphere with diameters comprised between 0.2-5000 μm. However, many of microcapsules bear little resemblance to their simple spheres. In fact, both the size and shape of formed micro-particles depend on the materials and method used to prepare them (King, 1995; Gharsallaoui *et al*, 2007).

A schematic illustration of different types of particles can be obtained (Figure 3): simple sphere surrounded by a coating of uniform thickness; particle containing an irregular shape core; several core particles embedded in a continuous matrix of wall material; several distinct cores within the same capsule and multi-walled microcapsules (Jackson and Lee, 1991; Shahidi and Han, 1993; Gibb *et al*., 1999)

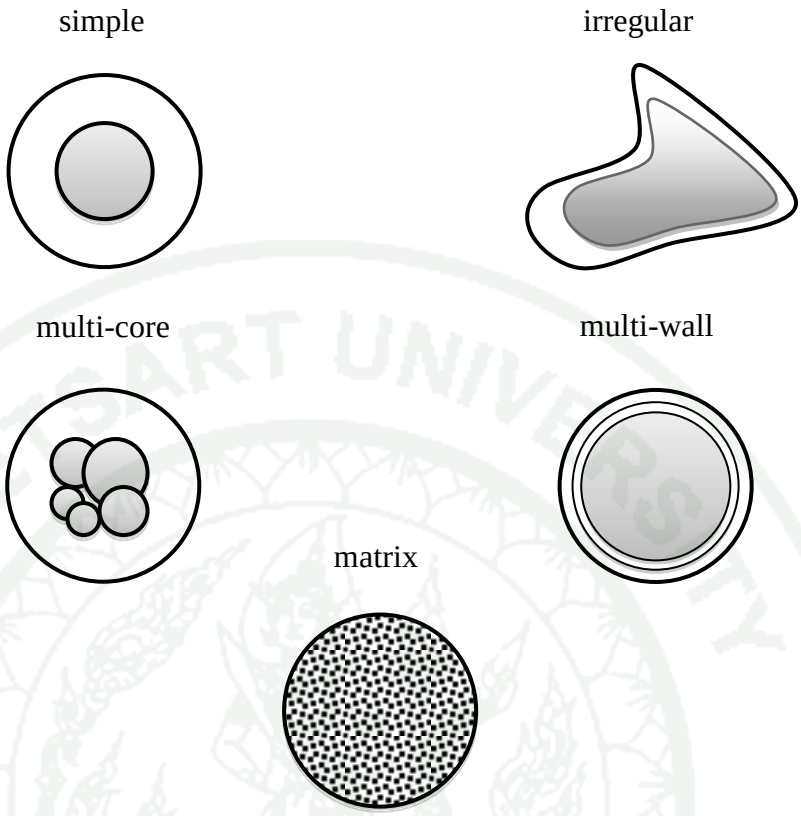


Figure 3 Morphology of microcapsule

Source: Modified from Gharsallaoui *et al*. (2007)

The food industry applies encapsulation for a number of reasons:

1. Release the core reactively with environmental
2. Decrease the transfer rate of the core material to the outside environment
3. To promote easier handling
4. To control the release of the core material under desirable condition
5. To mask the core taste
6. Dilute the core material when it should be used in only very small amounts

Wall Materials

The food industry has core materials for encapsulation such as flavors, antimicrobial agents, antioxidants, enzyme, nutraceutical and therapeutic active, vitamin, mineral, colors, acid, alkalis, buffers, sweetener, nutrients, cross-linking agent, yeast, chemical leavening agents, etc. The objective is to entrap a sensitive ingredient in a capsule or wall material for prevent contact ingredient from the environment (oxygen, light or heat). An ideal wall materials should exhibit the following characteristics (Pegg and Shahidi, 1999; Madene *et al*., 2006; Gharsallaoui *et al*, 2007):

1. The wall materials must have no reactivity with the core materials to be encapsulated both during processing and on prolonged storage.
2. Good rheologygical propertes: low viscosity at high concentration and easy work ability during encapsulation.
3. The ability to completely release the solvent or other materials used during the process of encapsulation under drying or other desolventization condition.
4. Solubility in solvents acceptable in the food industry (e.g., water, ethanol).
5. Give the maximum protection of the active material against the external factors (e.g., oxygen, heat, light, humidity).
6. Good emulsion stabilization properties; the ability to disperse or emulsify the active material and stabilize the emulsion produced.
7. Effective redispersion behaviour in order to release the core materials under specific conditions.
8. The wall materials must be film forming, non hygroscopic, flexible, tasteless and stable.
9. Inexpensive and food grade status.

For encapsulation of the flavor compounds, the wall materials should be basically of film forming that is cohesive with the core material, can be selected from many natural or synthetic polymers, depending on the material to be coated and the characteristic desired in the final microcapsules. Characteristics of the main wall materials used for flavor encapsulation are presented in Table 2.

In fact, no single coating material can meet all of the criteria, in practice either coating materials are employed in combination or modifiers are added. Some commonly used coating materials reported in Table 3 (Pegg and Shahidi, 1999).

Table 2 Characteristics of the wall materials used for encapsulating flavors

Wall materials	Characteristics
Maltodextrin (DE < 20)	Film forming
Corn syrup solid (DE > 20)	Film forming, reductability
Modified starch	Very good emulsifier
Gum Arabic, Gelatin	Emulsifier, film forming
Modified cellulose	Film forming
Cyclodextrin	Encapsulant, emulsifier
Lecithin	Emulsifier
Whey protein	Good emulsifier
Hydrogenated fat	Barrier to oxygen and water

Source: Modifed from Madene *et al*. (2006)

Table 3 Coating materials for encapsulation of food ingredients

Type	Coating materials
Carbohydrate	starch, maltodextrins, core syrup solid, dextran, modified starch, sucrose, cyclodextrins
Cellulose	carboxymethylcellulose, methylcellulose, ethycellulose, nitrocellulose, acetylcellulose, cellulose acetate-phthalate, cellulose acetate-butylate-phthalate
Gum	gum acacia, agar, sodium alginate, carrageenan
Lipid	wax, paraffin, beeswax, tristearic acid, diacylglycerols, monoacylglycerols, oils, fats, hardened oils
Protein	gluten, casein, gelatin, albumin, hemoglobin, peptides, whey protein

Source: Modified from Pegg and Shahidi (1999)

Type of the wall material used for encapsulating flavors

The choice of a wall material for microencapsulation of food ingredients is often achieved with biopolymers of various sources. (Pegg and Shahidi, 1999; Madene *et al*., 2006; Gharsallaoui *et al.*, 2007; Lakkis, 2007)

1. Protein

Proteins are natural macromolecules composed of linear chains of amino acids. The possible sequences and frequency of 20 existing amino acids, more precisely 19 amino acids and one amino acid, in the chain give rise to their enormous variety. Proteins have an important role in all living organisms with a wide range of functions. Large amounts of proteins are directly used as food. The potential use for non-traditional applications is in the process of being extended (Wandrey *et al*., 2010). The important functional properties of protein allow them to be good coating

for the microencapsulation of food ingredients. In addition, proteins possess high binding properties for the flavor compounds. The most commonly used proteins for encapsulating food ingredients are milk or whey protein, gelatin, soy protein, zein and gluten. The food proteins has functional properties for microcapsule forming wall material, because of their different chemical groups, amphiphilic properties that offer physicochemical and functional properties required to encapsulate hydrophobic core materials, ability to self-associate and interact with a variety of different types of substances, large molecular weight, and molecular chain flexibility, these proteins have excellent functional properties such as solubility, viscosity, emulsification, and film forming properties and would be capable of being used in encapsulation. The formation of emulsion, the protein molecules becomes rapidly adsorbed at the newly formed oil-water interface. The resulting steric stabilizing layer immediately protects the oil droplets against re-coalescence and thereafter provides physical stability to the emulsion during processing and storage (Madene *et al*., 2006; Gharsallaoi *et al*, 2007)

2. Carbohydrates

Carbohydrates are the most commonly used coating material in flavor encapsulation process. The ability of carbohydrates such as starches, chitosan, maltodextrins with different dextrose equivalence, sucrose, glucose, ethycellulose and cellulose acetate to bind flavor is complemented by their diversity, low cost, and widespread use in foods and choice for encapsulation. In addition, the carbohydrates are considered as good encapsulating agents because they exhibit low viscosity at high solid contents and good solubility but most of them lack the emulsion properties and generally associated with other encapsulating materials such as natural gums or protein. (Madene *et al*., 2006; Gharsallaoi *et al*, 2007)

3. Natural gums

Gums are used in microencapsulation for both their film forming and emulsion stabilization properties. Food gums and thickener are hydrocolloids and obtained from a variety of sources such as seaweed extracts (alginates, carrageenan and agar), tree exudate (gum arabic, gum ghatti, gum karaya and gum tragacanth),

others are products of microbial biosynthesis and still others are produced by chemical modification of natural polysaccharides. These compound are long-chain polymers that dissolve or disperse in water to give a thickening or viscosity-building effect. Gums are generally used as texturing ingredients, but their secondary effects include encapsulation, stabilization of emulsions, suspension of particulates, control of crystallization, and inhibition of syneresis. (Pegg and Shahidi, 1999; Madene *et al*., 2006, Gharsallaoi *et al.*, 2007)

4. Lipid

A property of lipid is their common insolubility in water because they are hydrophobic. Lipid composed molecules and substances of large diversity and structural variety such as beeswax, candelilla and carnauba waxes, acetoacylglycerols, lecithin and liposomes. These are widely distributed in nature. (Pegg and Shahidi, 1999; Wandrey *et al*., 2010)

The choice of wall materials depends on a number of factors including: expected product objectives and requirements; nature of the core material; the process of encapsulation, economics and whether the coating material is approved by the Food and Drug Administration (US) or European Food Safety Authority (Europe) (Madene *et al*., 2006)

Maltodextrin

The Food and Drug Administration (FDA) defines maltodextrin $(C_6H_{12}O_5)_nH_2O$, are no sweet nutritive polysaccharides that consist of D-glucose units linked primarily by α-(1$\rightarrow$4) linked D-glucose units and that have a reducing sugar content or “dextrose equivalence” (DE) less than 20. Maltodextrins are prepared as white powder or concentrated solutions by partially hydrolysis corn flour with acid or enzymes. If the DE is equals or higher 20, they are referred as corn syrup solids. (Kenyon, 1995; Pegg and Shahidi, 1999)

The general designations of maltodextrins are 5, 10, 15 and 18 DE, while commercial corn syrup solid have 20, 25, 36 and 42 DE. Products with a DE greater than 42 cannot be easily dried and hence are sold only as syrups. Because maltodextrins and corn syrup solids are so closely related to one another in terms of their physical and chemical properties as well as their applicability to food ingredient encapsulation (Pegg and Shahidi, 1999). The characteristic of maltodextrins and corn syrup solids can be used to quantify the relative amounts of shorter chain polymers found in a particular DE product. Maltodextrins and corn syrup solids are made up of polymers of anhydroglucose units having varied chain lengths rather than one particular polymer size. The average molecular weight decreases when the DE of a maltodextrins increase as well as even at low DE, it is much smaller than the original starch: 5 DE-3600; 10 DE-1800; 15 DE-1200; 20 DE-900. (Kenyon and Anderson, 1988)

This relative molecular size difference between starch and the hydrolysis sugars gives maltodextrin and corn syrup solids their valuable functional properties for the flavor industry.

1. Functional properties of maltodextrin

1.1 Emulsion stabilization

Emulsion stability and particle size are critical for the production of encapsulated flavor oils. Maltodextrins and corn syrup solid do not have good emulsifying capabilities (lipophilic/hydrophilic properties). They are made up of glucose units, but the average chain length is also too small to stabilize normal levels of citrus oils or other oil carried flavors by viscosity. For these reasons, maltodextrins are usually combined with other true emulsifying matrix materials such as gum arabic or specially modified starches to achieve the necessary emulsion stability. The amount of the emulsifying agent necessary will vary depending on its ability to emulsify, the level of flavor oil to be encapsulated, the production system used, and the desired stability when the encapsulated flavor oil is used.

1.2 Film forming properties

The encapsulation of flavor oil, the quality of the end product is affected by both how quickly the matrix material forms a film or selective membrane around the flavoring agent, and by the quality of the matrix film and its ability to protect the flavoring agent.

1.3 Hygroscopicity

Maltodextrins and corn syrup solids are greatly nonhygroscopic. Therefore, flavor dried with these products are free-flowing powders. Hygroscopicity does increase with higher DE such as maltodextrins 5 DE and 10 DE was soft lumps at 70% and 50 % relative humidity, respectively.

1.4 Viscosity

Viscosity and solubility are the most important characteristics of encapsulation matrix ingredient. The increase in solid to the dryer at a constant solid / flavor ratio can greatly increase the economic efficiency of an operation. General, processing systems have a maximum viscosity at which they can operate. The low viscosity of maltodextrin and corn syrup solid is shown in Figure 4. The high DE of maltodextrin is decreased viscosity of these products. Maltodextrins and corn syrup solids also exhibit Newtonian viscosity, decreasing in viscosity as they are heated. Maximum solids levels for encapsulation may also be reached at the solubility limits of the ingredients. Above these limits, the level of flavor compound may be out of ratio with the active matrix polymers, so the retention of flavor decrease. Insoluble materials may also affect the quality of encapsulating film by interfering with the continuous film matrix. Maltodextrins and corn syrup solids will demonstrate good solubility in the following solids range: 5 DE – 30-45 %; 10 DE – 45-55 %; 15 DE – 50-65 %; 20 DE – 60-75 %.

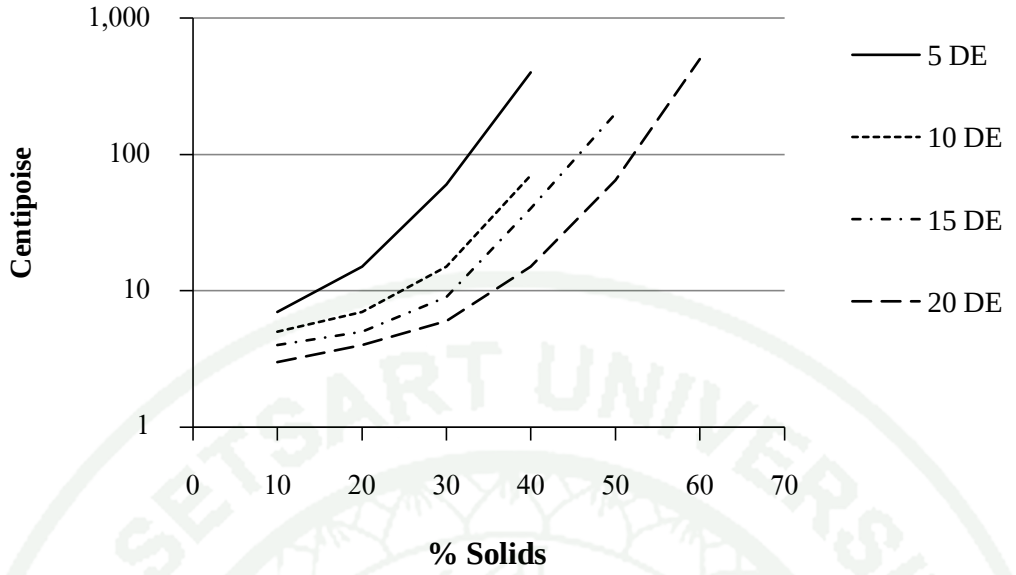


Figure 4 Viscosity of maltodextrin and corn syrup solids solution at varied percent solids

Source: Modified from Kenyon and Anderson (1988)

1.5 Flavor release

Encapsulated flavor find used throughout the food industry. One major example would be beverage dry mixes. Maltodextrins and corn syrup solids have excellent cold-water solubility, so their use in encapsulated flavor will provide a rapid release of flavors used in beverage applications. Maltodextrins and low DE corn syrup solids also have very little flavor or sweetness of their own, form clear solutions, and virtually disappear once in an application.

1.6 Low cost - reliable supply

Maltodextrins and corn syrup solids carry a much lower cost than all other encapsulating matrix ingredients. They are produced both in the US and in foreign countries by several suppliers, so they are readily available. When used alone

or a combination of encapsulation matrix ingredients, maltodextrins and corn syrup solids are an effective part of the encapsulating system (Kenyon and Anderson, 1988).

Modified Starch

In generally, the food processors prefer starches with better behavioral characteristics than provided by native starches. Starch presents an interesting situation with regard to flavor binding because the amylose fraction forms helical structures, starch can entrap flavor molecules, thereby producing very stable complexes. However, starch is hydrophilic and hydrolysates derived from it afford virtually no emulsification properties to the compound being encapsulated. The lack of emulsification properties of native starch creates two significant problems. The first problem is poor flavor retention. The fineness of the emulsion has a strong influence on determining the extent of flavor retention during drying. The second problem is the stability of the flavor emulsion once reconstituted in the final product. If the carrier provides no emulsification to the flavor, then the flavor rapidly separates from the product and forms a ring at the top. Thus, for a compound to function as an emulsifier, it must contain both lipophilic and hydrophilic groups. The properties of starches can be improved by modification; starches can be modified chemically to change their functional characteristics. The U.S Food and Drug Administration (FDA) have approved the reaction of starch with octenyl succinic anhydride (OSA) to form a modified starch containing both hydrophobic and hydrophilic group. The FDA has aggregation a maximum level of octeny succinic acid anhydride for treatment on the starch at 3%, which corresponds to a degree of substitution, usually in the range of 0.02, results in a product that is vastly different from that of the native starch. The addition of lipophilic moieties along the starch polymer permits the formation of emulsions with tight alignment of the polymer around an oil droplet. This stabilization is extremely important for encapsulation of flavor oil products. This treatment can be conducted on a wide variety of starch bases, acid hydrolyzed starches and dextrins. The structure of the starch octenyl succinate is show in Figure 5.

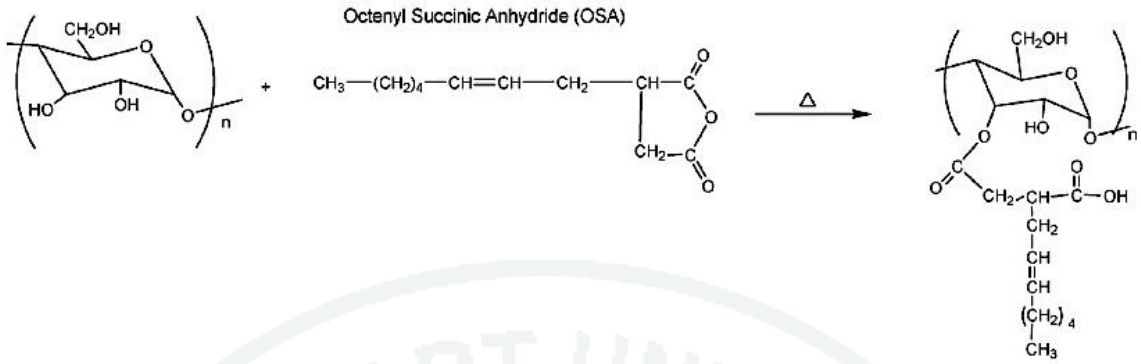


Figure 5 Molecular structure of the starch octenyl succinate

Sorce: Shogren and Biresaw (2007)

The emulsification properties of lipophilic starches as well as the oil retention in the spray-dried powder are reported to be equal to or greater than of gum acacia. Modified starch also excels in promoting emulsion stability. One means of doing so is to produce small particle size droplets. Solution of gum acacia produced an average emulsion droplet size of about 3 μm, and modified starch gave droplets of less than 2 μm. The emulsions made with modified starch were physically more stable than those made with the standard gum acacia. (Trubiano and Lacourse, 1988; Bemiller and Whistler, 1996; Pegg and Shahidi, 1999).

The modified starches (HICAP™ 100 and CAPSUL) involve the addition of lipophilic component aimed at conferring emulsifying properties. Both of HICAP™ 100 and CAPSUL, which were derived from waxy maize base, were modified with OSA for the encapsulation of flavors, vitamins and spices. HICAP™ 100 is blended with high-dextrose equivalent (DE) corn syrup solids with the final of 32-37 DE and designed for the high load encapsulation agent. HICAP™ 100 is its ability to form very stable oil-in-water emulsion. The fine particle size of the emulsion results in reduced losses during spray drying. The lower viscosity of HICAP™ 100 permits the preparation of emulsions at higher solid. These can be spray dried at faster rates because less water has to be evaporated. This trait also results in lower energy cost. CAPSUL is a starch modification gives the material excellent film forming ability and

excellent capacity for retaining volatiles during atomization in a spray dryer and storage (Krishnan *et al*., 2005; Soottitantawat *et al*., 2005; Shaikh *et al*., 2006; Rocha *et al*., 2011).

Whey Protein

Whey proteins and caseins are the two major fractions in bovine milk contains, in addition to water, lactose, fat and other minor component about 3.0 - 3.6 %w/w. Whey is a by-product of cheese or casein production and has several commercial uses. Whey proteins primary include α-lactalbumin, β-lactoglobolin, immonoglobinlins and serum albumin but also numerous minor protein. The four principal protein of the highly heterogeneous whey protein fraction are summarized in Table 4. The α-lactalbumin is a calcium metalloprotein with four intramolecular disulfide cross-links, for which genetic variants exist. β-Lactoglobolin has two intramolecular disulfide cross-links and one free SH group. Many genetic variants are known which vary in their degree of glycosylation.

Table 4 Whey protein fraction and some characteristics typical for bovine milk

Factor	Whey proteins			
	α-lactalbumin	β-lactoglobolin	Immonoglobinlins	Serum albumin
Concentration (%w/w)	0.07-0.15	0.2-0.4	0.06-0.1	0.01-0.04
Isoionic point	4.2-4.5	5.2	-	5.3
Molar mass ($x10^{-4}$ g/mol)	1.4	1.8	15-90	6.6

Source: Modified from Wandrey *et al*. (2010)

Whey protein is globular protein, soluble in their native forms in the ionic environment of milk, almost independent of pH. But they become insoluble at their isoelectric point (pH about 5) at very low ionic strength. In contrast to caseins, whey

proteins denature at temperatures above 70 °C and become insoluble. Then they form thermally irreversible gels of different quality. The solution of non-denatured whey protein is much less viscous than caseinate solution. They exhibit Newtonian flow at concentrations in the range of 4-12 %w/w but become pseudoplastic in the range of 18-29 %w/w concentrations. As already mentioned for caseins, milk proteins exhibit good surface-active properties, films formed when thermally induced disulfide cross-linking takes place, are excellent gas barriers, while the incorporation of lipids reduces the water vapor permeability. The source of whey protein remains after removal of fat and casiens from milk. The following products are isolated: whey powder of different quality (demineralized, delactosed and demineralized-delactosed), whey protein concentrate (WPC), whey protein isolate (WPI), lactalbumin and individual whey protein fraction. WPC contain a low level of fat and typically 35-80 %w/w protein. During the isolate of WPI, the removal of fat and lactose is intended. The protein content becomes > 90 %w/w. β-lactoglobolin is the most important whey protein.

Whey protein demonstrates the functional properties that are desired for wall material. Application using whey protein as microencapsulating agents were first proposed in the early 1990s. Since then, several research groups have studied the encapsulation behavior of different WPC and WPI, either alone or in combination with other wall material. In the international market, whey proteins are available as WPI (95-96 %protein) or whey protein concentrate (WPC-50, WPC-70) powder.

Whey protein isolates have been shown to provide a good barrier against oxidation for microencapsulated orange oil and they provide an effective basis for microencapsulation of flavor by spray drying. Whey proteins in combination with carbohydrate have been as carrier material in encapsulation of volatile components. In such system, whey protein served as an emulsifying and film-forming agent while the carbohydrates (maltodextrin or corn syrup solid) acted the matrix-forming material. β-Lactoglobolin is the most important whey protein and possesses interesting emulsifying and foaming properties and is widely used in the food industry (Madene *et al*., 2006; Vega and Roos, 2006; Wandrey *et al*., 2010).

Microencapsulation Techniques

Many encapsulation techniques are base on making first droplets of the active (in gas, liquid or powder form) and these droplets are subsequently surrounded by carrier material in a gas or liquid phase via different chemical processes or mechanical processes (Figure 6) (Madene *et al*., 2006; Zuidam and Shimoni, 2010).

Flavor encapsulation is accomplished by a variety of method. The two major industrial processes are spray drying and extrusion. Major applications of the different encapsulation techniques are shown in Table 5. (Madene *et al*., 2006; Zuidam and Shimoni, 2010).

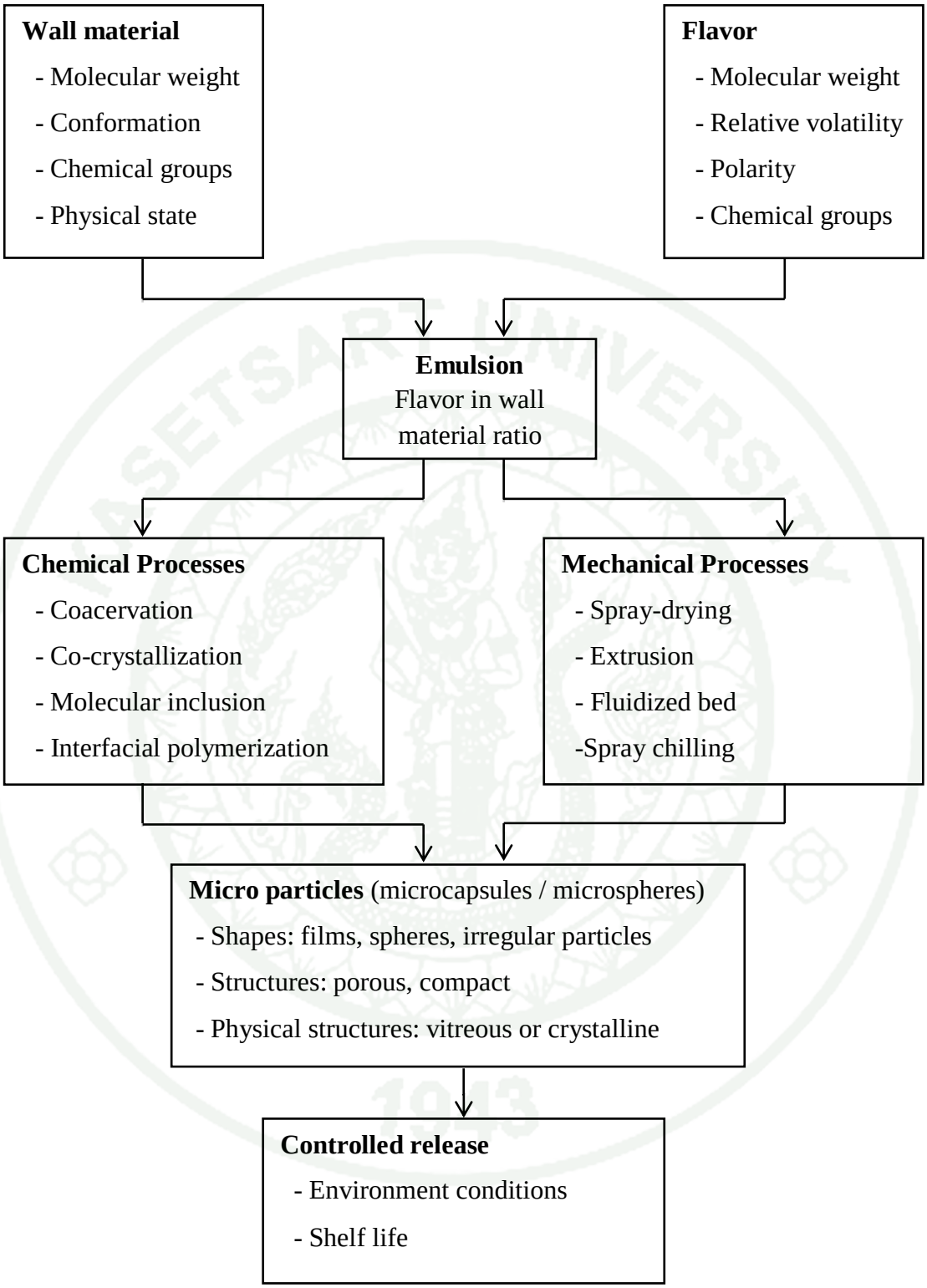


Figure 6 Different processes of encapsulation of flavor compounds

Source: Modified from Madene *et al*. (2006)

Table 5 Application of common microencapsulation of flavor compound

Encapsulation methods	Steps of process	Encapsulated form	Area of application
Coacervation	1. Prepare o/w emulsion with lipophilic active in oil phase 2. Mix under turbulent conditions 3. Induce three immiscible phases 4. Cool 5. Crosslink (optionally)	Paste, powder, capsule	Chewing gum, toothpaste, baked foods
Spray drying	1. Disperse or dissolve active in aqueous coating solution 2. Atomize 3. Dehydrate	Powder	Confectionery, milk powder, instant desserts, food flavors, instant beverages.
Fluid bed drying	1. Fluidize active powder 2. Spray coating 3. Dehydrate or cool	Powder/ granule	Prepared dishes, confectionery
Spray cooling / chilling	1. Disperse or dissolved active in heated lipid solution 2. Atomize 3. Cool	Powder	Prepared dishes, ices
Extrusion	1. Melt the coating 2. Disperse or dissolve active in the coating 3. Extrude with twin-screw extruder 4. Cool	Powder, granule	Instant beverages, confectionery, teas
Molecular inclusion	1. Mix carrier, active and water together 2. Incubate and dry if necessary	Powder	Confectionery, instant drinks, extruded snack

Source: Modified from Madene *et al*. (2006); Zuidam and Shimoni (2010)

This review focuses on the most commonly used encapsulation method applied to flavor compound that is spray drying because which is readily available and widely used in large scale to produce encapsulated flavor. The main advantages of spray drying are the ability to handle labile material because of the short contact time in dryer, in addition, the production costs are lower than most other methods of encapsulation. Compared to freeze drying, the cost of spray drying method is 30-50 times cheaper (Pegg and Shahidi, 1999; Madene *et al*., 2006; Gharsallaoui *et al*., 2007; Bansode *et al*., 2010).

Spray drying

Spray drying is a unit operation, which a liquid product is atomized in a hot gas current to instantaneously obtain a powder. This process is widely used in the industry for the production of dry flavoring agent. According to the initial nature of the sprayed liquid are solution, suspension or emulsion. The particles size obtained is a very fine powder (10-50 μm) or large size particles (2-3 mm), with a large size distribution due to variety of droplet sizes in the spray. The most influential parameter is the geometry of nozzle and the initial solution viscosity (Gharsallaoui *et al*., 2007; Munin and Edwards-Lévy, 2011). The process is conducted in a spray dryer such as the one shown in Figure 7 and involves the following basic steps.

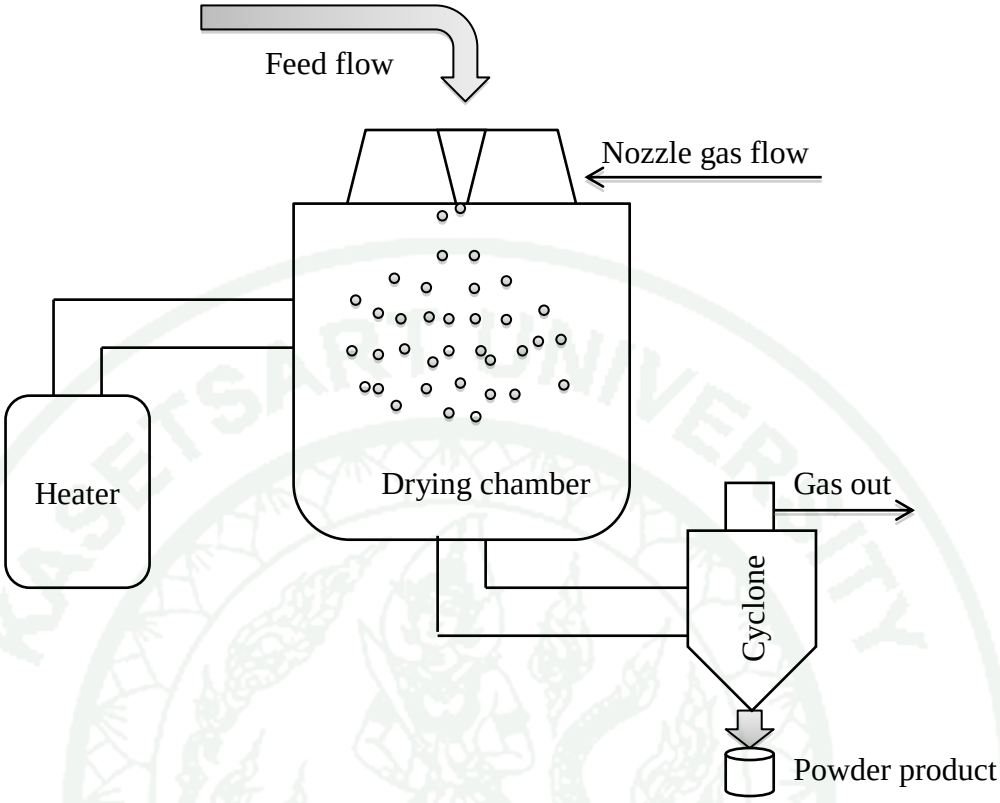


Figure 7 Spray drying operation consisting of a drying chamber with an atomizer, heater and cyclone for product recovery

Source: Modified from Munin and Edwards- Lévy (2011)

1. Preparation of the emulsion

The first stage of spray drying an encapsulated food ingredient is the formation of a fine and stable emulsion of the core material in the wall solution. The selection of a suitable wall material or encapsulating agent should have functionality profile that are optimal for spray drying such as enough emulsifying properties, be a good film forming characteristics, high solubility in water and have low viscosity at high solid content (<500 cps at ≥45% solid contents). A food grade hydrocolloid such as a whey protein, gum arabic, maltodextrin and hydrophobically modified starch (such as octyl-substituted starches) is common used as wall material (Pegg and Shahidi, 1999; Gouin, 2004; Madene *et al.*, 2006).

2. Homogenization of the emulsion

Prior to spray drying, the emulsion to be atomized is prepared by homogenize in order to produce small droplets of flavor within the encapsulating solution. The dispersion must be heated and homogenized, with or without the addition of an emulsifier depending on the emulsifying properties of the wall materials because some of them have themselves interfacial activities (Pegg and Shahidi, 1999; Gharsallaoui et al., 2007). In the spray drying process, the initial emulsion droplets are in the order of diameter 1-100 μm because the production of a finer emulsion increases the retention of flavor during atomizes process. Before the spray-drying step, the formed emulsion must be stable over a certain period of time, oil droplets should be rather small and viscosity should be low enough to prevent air inclusion in the particle. Emulsion viscosity and particle size distribution have significant effect on microencapsulation by spray drying. High viscosities interfere with the atomization process and lead to the formation of elongated and large droplet that adversely affects the drying rate (Pegg and Shahidi, 1999; Gharsallaoui *et al*., 2007).

3. Atomization of the mass in the drying chamber

The liquid formulation containing a wall material and the core material in a solvent is atomized into droplets via either a nozzle using compressed gas to atomize the liquid feed, or a rotary atomizer using a wheel rotating at high speed. After that, a hot air stream is brought into the drying chamber and the evaporation of the solvent. As the solvent rapidly evaporates from the droplet, a particle forms and falls to the bottom of the chamber. The powder is recovered from the exhaust air using a cyclone or a bag filter (Gharsallaoui *et al*., 2007; Munin and Edwards-Lévy, 2011). During drying of process a film at the droplet surface and the concentration of core material in drying droplet increases. Finally, a porous of dry particle is formed (Zuidam and Shimoni, 2010).

The main limitation of the spray drying technique in microencapsulation is the limited number of wall materials available and that must have a good solubility in

water. The disadvantage of spray drying that should be considered is that it produces a fine microcapsules powder which needs further processing such as agglomeration (Gharsallaoui *et al*., 2007). In addition, a spray drying is that some low boiling point aromatics can be lost during spray drying and the core material may also be on the surface of the capsule, this would encourage oxidation and possible flavor changes of the encapsulated product (Madene *et al*., 2006).

Controlled flavor release

Encapsulation allows reactive ingredients to be separated from their environment until their release is desired. Although separation is indeed the objective of encapsulation, release mechanisms of the core material must be considered as well. In fact, when designing a custom encapsulated ingredient, one must determine the desired release mechanism and a method for quality measurement. A well-controlled release of core material is a very important property of microcapsules. For example, a substance in formulated food may be release upon consumption but prevented from diffusing throughout the product during processing operations (e.g., flavors, nutrients) (Pegg and Shahidi, 1999).

Controlled release is a method by which one or more active agent or ingredients are made available at a desired site and time and at a specific rate (Pothakamury and Barbosa-Cánovas, 1995). The advantage of controlled release are: the active ingredients are release at controlled rates over prolonged periods of time; loss of ingredients during processing and cooking can be avoided or reduced; reactive or incompatible components can be separated (Madene *et al*., 2006)

The mechanism of release for the capsule may be based on solvent effects, diffusion, degradation or particle fracture. A variety of release mechanism that is proposed for microcapsules summarized below.

1. Release of flavor by diffusion

Diffusion is important in food because it is the dominate mechanism in controlled release from encapsulation matrices (Madene *et al*., 2006). This mechanism acts to limit the release of core material from its location in the capsule to the surface of the particle by controlling diffusion of the active compounds. The bulk of the capsule material its self may control release (matrix controlled release) or a membrane may be added to the added to the capsule for controlling release (membrane controlled release) (Whorton, 1995). The vapor pressure of a volatile substance on each side of the matrix is the major driving force influencing diffusion (Gibbs *et al*., 1999a). The steps in the release of a flavor compound from matrix system are: diffusion of the active agent to the surface of the matrix: partition of the active component between the matrix and the elusion medium (i.e. the surrounding food) and transport away from the matrix surface (Pothakamury and Barbosa-Cánovas, 1995).

2. Release of flavor by degradation

The release of an active agent from a matrix type delivery system may be controlled by diffusion, erosion or a combination of both. In addition, erosion controlled processes may involve heterogeneous or homogeneous erosion. Heterogeneous erosion occurs when degradation is confined to a thin lager at the surface of the delivery system, whereas homogenous erosion is a result of degradation occurring at a uniform rate throughout the polymer matrix (Madene *et al*., 2006). The type of erosion depends on the hydrophobicity and morphology of the polymer. Heterogeneous erosion is more common with hydrophobic polymer, whereas homogeneous erosion is common with hydrophilic polymer. Heterogeneous erosion is more desirable because it can lead to a constant release rate that is independent of the chemical and physical properties of the active agent. The release rate can be varied by varying the active agent loading while maintaining the integrity because erosion is limited to the surface (Pothakamuy and Barbosa-Canovas, 1995). Erosion of the coat due to pH and enzymatic hydrolysis causes core release with certain coat material like glyceryl mono stearate, beeswax and steryl alcohol (Bansode *et al*., 2010).

3. Release of flavor by swelling

The active ingredient is release when the food material comes in contact with a solvent and resulting in swelling of the microcapsule. In systems controlled by swelling of the active agent dissolved or dispersed in a polymeric matrix is unable to diffuse to any significant extant within the matrix. When the matrix polymer is placed in a thermodynamically compatible medium, the polymer swells because of absorption of fluid from the medium. The active agent in the swollen part of the matrix then diffuses out (Madene *et al*., 2006). The swelling membrane consists of three zones. Adjacent to the solvent surface is the completely swollen gel; next is a fairly thin swelling zone in which the polymer chains are relaxing; and finally, there is a layer of un-swollen, completely dehydrated rigid polymer matrix in the glassy state. The swelling zone moves into the membrane at a uniform rate, and solvent gain in the polymer increases with time. The rate of relaxation of the polymer chains from the glassy state to the gel state is the slowest step in the sorption process (Pothakamury and Barbosa-Canovas, 1995). The degree of swelling is controlled by water absorption or presence of solvents such as glycerine or propylene glycerol (Gibbs *et al*., 1999b).

4. Release of flavor by melting

The integrity of the coating can be destroyed by thermal means. This mechanism of release involves the melting of the protective coating on the capsule wall to release the active material (Pegg and Shahidi, 1999). This method of release is readily accomplished in the food industry since there are numerous materials that can be melted and that are approved for use (lipid, modifies lipid or waxes) (Risch and Reinecious, 1988). In such application, the coated particles are at temperatures well below the melting point of the coating, then heated above this temperature during preparation or cooking (Madene *et al*., 2006).

MATERIALS AND METHODS

Materials

1. Wall Materials

1.1 Wasabi flavoring agent was purchased from V. Mane Fils Co., Ltd. (Thailand).

1.2 Modified starches (HICAP™ 100 and CAPSUL) were supplied by National Starch and Chemical Co., Ltd. (Thailand).

1.3 Maltodextrin with DE 5 was obtained from Berli Jucker Specialties Co., Ltd. (Thailand).

1.4 Whey protein concentrate (protein, 80.0% dry basis; fat, max. 8.5%; ash, max. 4.5% and moisture, max. 6.0%) were obtained from Vicchi Enterprise Co., Ltd. (Thailand).

2. Reagents

2.1 Allyl isothiocyanate (AITC), with 98% GC purity was purchased from Fluka Chem. Co. (Germany).

2.2 Methyl isothiocyanate, analytical grade was purchased from Dr. Ehrenstorfer GmbH. (Germany).

2.3 n-hexane, analytical grade was purchased from LAB-SCAN Ltd. (Ireland).

3. General Equipment

3.1 Homogenizer, Ultra-Turrax T-25 (IKA® Fisher, UK)

3.2 HV Homogenizer, S 25 N – 25 F (IKA® Fisher, UK)

3.3 Spray dryer, PDS-01 (Hemraj, India)

4. Equipment for Analysis

4.1 SPME fiber assembly polydimethylsiloxane/divinylbenzene (PDMS/DVB) (Supelco, Bellefonte, PA) with manual fiber holder

4.2 Gas chromatography, GC-17A (Shimadzu, Japan)

4.3 Mastersizer 2000 (Malvern Instruments Ltd., UK)

4.4 Scanning electron microscope, JSM 5410 LV (JEOL, Japan)

4.5 Chroma meter, Spectrophotometer CM-3500d (Minolta, Japan)

4.6 Spectrophotometer UV-1700 (Shimadzu, Japan)

4.7 Viscometer, DV-III Ultra (Brookfield, USA)

4.8 Hot air oven, UNB400 (Memmert, Germany)

4.9 Water activity (a_w) meter, Thermoconstanter TH200 (Novasina, Switzerland)

Methods

1. Effect of types wall material and core material proportion on properties of emulsion, encapsulated wasabi flavor and wasabi flavor retention

Maltodextrin (MD), Modified starches (HICAP^{TM} 100; H and CAPSUL; C) and whey protein concentrate (WPC) were used as wall materials. These wall material systems were H, blending of H with C (mass ratio = 1:1), blending of H with MD (1:1) and blending of H with WPC (1:1). Wasabi flavor at various concentrations (10, 15 or 20% w/w of total solids) was dispersed in wall material systems.

1.1 Preparation of emulsions containing wasabi flavor

The wall material solution was prepared by blending and rehydrating the wall material powder in distilled water and equilibrated at room temperature overnight. The total concentration of dissolved solid in the wall material solutions was 30% w/w on a wet basis. The emulsions were prepared by homogenizing the wasabi

flavor with the wall material solution using a homogenizer (IKA® Fisher, UK) at a rotational speed of 10,000 rpm for 30 min.

1.1.1 Emulsion stability

The stability of emulsions were measured by monitored at room temperature (modified from Klinkesorn *et al*., 2005). The emulsion samples (10 mL) obtained from homogenizer was transferred into test tubes and then stored at room temperature. The emulsion stability was determined by visual observation of the height of total emulsion in the tube (HE) and the height of the serum layer formed at the bottom of tube (HS). The emulsion stability was measured every hour for 24 hour. Emulsion stability was reported as a "creaming index" = 100 x (HS/HE). The measurements were performed in triplicate.

1.1.2. Emulsion Viscosity

The viscosities of the emulsions were measured by Brookfield viscometer (Brookfield, USA) with spindle No 1. The samples were placed in the temperature controlled measurement vessel and allowed to equilibrate to the required temperature (25 °C). The measurements were performed in triplicate.

1.2 Spray drying

The emulsion was fed through a spray dryer (Hemraj, India), equipped with a two-fluid nozzle atomizer with diameter 0.5 mm at flow rate 40 ± 3 mL/min. The operational conditions of the spray drying were: inlet air temperature, 190 ± 10 °C; outlet air temperature, 100 ± 10 °C and nozzle air pressure was 4 ± 0.08 MPa. The encapsulated wasabi flavor was collected in screwed cap amber glass bottles, stored at room temperature until analysis.

1.2.1 Moisture contents and water activity

The encapsulated wasabi flavor (approximately 2 g) was placed in aluminum can and dried in a hot air oven (Memmert, Germany) at 105 °C until reach constant weight. The percentage loss in weight was reported as the moisture content.

$$\% \text{ Moisture} = \left[\frac{W_1 - W_2}{W_1} \right] \times 100$$

Where: W1 = weight of sample before drying

W2 = weigh of sample after drying

Measurement of water activity was carried out using a water activity meter (Novasina, Switzerland). The moisture content was conducted in triplicate.

1.2.2 Color

The color analyses were carried out using a chroma meter (Minolta, Japan) and color value expressed in L* (darkness/whiteness), a* (greenness/redness) and b* (blueness/yellowness). The encapsulated wasabi flavor (3 g) was filled into a sample holder of the instrument (the height of the powder in the sample holder was about 0.5 cm). Measure three samples or more and record the average value.

1.2.3 Surface AITC

The surface AITC was determined by a washing method according to Li *et al.* (2007). AITC was extracted by mixing 0.10 g of the encapsulated wasabi flavor with 10 mL of hexane and gently shaken for 20 min at room temperature. The solvent phase containing AITC was filtered with filter paper Whatman No. 1. The residue was washed for 10 min with 10 mL of hexane and procedure was repeated once. The supernatant was collect. The content of AITC in hexane was determined

using ultraviolet spectrophotometer at 248 nm. The concentration of AITC was assessed by plotting the calibration curve of AITC standards.

1.2.4 Total AITC

Total AITC in the encapsulated wasabi flavor was measured according to a solvent (hexane) extraction method described by Li *et al.* (2007). The encapsulated powder (0.01g) was weighed into a 50 mL flask and mixed with 5 mL of distilled water and 7 mL of hexane. The AITC was extracted by condensation process; the flask was connected with upright glass condenser cooled by tap water. Then the mixture was heated in a water bath at 85 °C for 20 min. After the first extraction was finished, the flask was cooled to room temperature and the inner wall of the condenser was washed with 2-3 mL of hexane in order to collect the maximum amount of AITC. After that, the upper hexane containing AITC was separated by pipette. Finally, the content of AITC was measured by the UV absorbance at 248 nm was recorded by a spectrophotometer. The concentration of AITC of the extracts was assessed by plotting the calibration curve of AITC standards.

1.2.5 Encapsulation efficiency

Encapsulation efficiency (EE) was calculated according to the equation (Li *et al.* 2007):

$$EE\ (\%) = \frac{\text{Total AITC} - \text{Surface AITC}}{\text{The theoretical maximum include AITC}} \times 100$$

1.2.6 Morphological Characterization

A scanning electron microscope (JEOL, Japan) was used to investigate the outer structural changes in the encapsulated wasabi flavor. The encapsulated powders were placed on SEM stubs using two-sided adhesive tape and the stubs were coated with gold. The coated samples were then analyzed using the SEM operated at 15 kV.

The H, blending of H with C (mass ratio = 1:1), blending of H with MD (1:1) were the most efficacies on properties of emulsion and encapsulated wasabi flavor. These were selected for further study.

2. Effect of relative humidity on the release characteristics of encapsulated wasabi flavor

Three different wall material systems were prepared: 1) H, 2) blending of H with C (mass ratio = 1:1) and 3) blending of H with MD (1:1). The wasabi flavor was added at 10, 15 or 20% (w/w) of total solids.

2.1 Preparation of emulsions containing wasabi flavor

The emulsions were prepared as described in section 1.1. The emulsion particle size distribution of the liquid emulsions was measured after homogenization using a laser scattering particle size analyzer (Malvern Instruments Ltd., UK). Distilled water was used as a dispersant. The average particle diameter, $D_{3,2}$, and $D_{4,3}$ were determined as the surface area and volume mean diameter, respectively. Each sample was analyzed in triplicate and the data were presented as averages.

2.2 Preparation of microcapsules by spray drying

The preparations of microencapsulated wasabi flavor powders were prepared as described in section 1.2. The flowing properties of powders were evaluated using Carr's Index (Car) and the Hausner Ratio (HR), which were calculated using equations 1 and 2, respectively:

$$\text{Car} = \frac{\rho_T - \rho_B}{\rho_T} \times 100 \qquad (1)$$

$$\text{HR} = \frac{\rho_T}{\rho_B} \qquad (2)$$

Where ρ_B and ρ_T are the bulk and tapped densities, respectively, determined in a 25 mL glass graduated cylinder as described by Quispe-Condori *et al.* (2011). The flowing properties were measured in triplicate.

2.3 Release characteristics of wasabi flavor from encapsulated powder

The release characteristics of flavor during storage were investigated by determining the release of AITC at constant temperature and relative humidity (%RH). Samples of the encapsulated powder were placed in desiccators at 11, 33 and 52% RH, which were controlled by a saturated salt solution of LiCl, $MgCl_2$ and $Mg(NO_3)_2$, respectively. All samples were stored at 35 °C and samples were taken at days 0, 10, 20, 40 and 60 for evaluation.

2.3.1 Moisture content and water activity

Moisture content and water activity were determine as described in section 1.2.1.

2.3.2 Color

The color analyses were determined as described in section 1.2.2.

2.3.3 Determination of Surface AITC

The surface AITC was determine as described in section 1.2.3 by a washing method according to Li *et al.* (2007).

2.3.4 Determination of Total AITC

Total AITC in the encapsulated wasabi flavor was determine as described in section 1.2.4.

2.3.5 Encapsulation Efficiency

Encapsulation efficiency (EE) was calculated as described in section 1.2.5.

2.3.6 Morphological characterization

A scanning electron microscope was used to investigate the outer structural changes in the encapsulated powder; samples were taken at days 0 and 60 for evaluation. The encapsulated powders were placed on SEM stubs using two-sided adhesive tape and the stubs were coated with gold. The coated samples were then analyzed using the SEM operated at 15 kV.

The encapsulated powder derived from blending of H with C (mass ratio = 1:1) and blending of H with MD (1:1) at 20 % w/w wasabi flavor were the most efficacies on physicochemical properties and wasabi flavor retention. These were chosen for use as ingredients in tuna spread.

3. Application of encapsulated wasabi flavor as food ingredient in canned tuna spread

3.1 Preparation of canned tuna spread

Canned tuna spread was the model system chosen for the microencapsulation application. The preparation of tuna spread consisted of mixing of various ingredients (47% mayonnaise, 26% minced water chestnut and 7% chopped onions) and then adding 20% tuna (w/w).

The wasabi flavor was added by mixing it into the tuna spread. The contents of the encapsulated wasabi flavor and wasabi flavor without encapsulation as an ingredient in tuna spread samples are shown in Table 6. The encapsulated powder derived from the blending of H with C and H with MD at 20% w/w wasabi flavor were chosen for use as ingredients in tuna spread.

Table 6 Contents of wasabi flavor (with or without encapsulation) added in 100 g of tuna spread as flavoring ingredients

Treatment	Content of encapsulated wasabi flavor (% w/w)		
	Without encapsulation	Wall material : H with C	Wall material : H with MD
T1 (control)	-	-	-
T2	5	-	-
T3	-	5	-
T4	-	-	5
T5	7	-	-
T6	-	7	-
T7	-	-	7
T8	9	-	-
T9	-	9	-
T10	-	-	9

The tuna spread with or without encapsulated wasabi flavor was weighted (85 g) and transferred to separate enamel-coated cans (211x109). The cans were vacuum-sealed and sterilized in a horizontal steam-heated retort at 121 °C for 35 min and then allowed to cool for 15 min.

3.2 Analysis of sensory evaluation in canned tuna spread

The retaining wasabi flavor in the canned tuna spread after 60 days of storage was evaluated by the modified difference-from-control test (Meilgaard *et al*., 2007). A panel consisting of 36 untrained panellists (28 females and 8 males with age range between 21 and 32 yr) was recruited from the graduate students and staffs of the Department of Fishery Products, Kasetsart University based on their health, availability and frequent tuna consumption and acquaintance to wasabi. Panellists

were briefly instructed to familiarize themselves with the test protocol and strength of wasabi flavor by the experimenters.

Sensory evaluation was conducted in individual booths under controlled fluorescent lighting, temperature and humidity (500 LUX, 25 °C, 55% RH, respectively). A balanced incomplete block (BIB) design (Plan 11.11, t = 9, k = 4, r = 8, b = 18, λ= 3, E = 0.84, Type II) was used (Cocharan and Cox, 1957) because the panellists would experience lingering effect as the number of wasabi flavor tuna spread increase. Consequently, four of nine canned tuna spreads (only those with wasabi flavor added, T2-T10) were individually spread on unsalted plain crackers and randomly served to each panel on a white plastic dish coded with a three-digit random number together with the control sample (T1). Panellists were asked to rate the intensity of the difference between each sample and the control using a numerical scale ranging from less wasabi-flavored (value = -5) to more wasabi-flavored (value = 5) where 0 means no difference.

4. Analysis of allyl isothiocyanate in wasabi flavoring agent, microencapsulated wasabi flavor and canned tuna spread

The sample for analysis of AITC by GC-FID were wasabi flavoring agent, microencapsulated wasabi flavor giving the highest of encapsulation efficiency during storage (section 2) and canned tuna spread giving the highest of sensory score (section 3). Wasabi flavoring agent and microencapsulated wasabi flavors was steam-heated at 121 °C for 35 min and then allowed to cool for 15 min.

4.1 Preparation of sample for allyl isothiocyanate analysis

Sampling of the headspace was done by a solid phase microextraction (SPME; Supelco, Bellefonte, PA). A fused silica fiber, coated with a polydimethylsiloxane/divinylbenzene (PDMS/DVB) was chosen to collect the volatile component of the selected samples. The sample (10 g) was placed into 40 mL glass vial and allows to volatile in the gas and liquid phase to come to equilibrium at 40 °C

(approximately 15 min). The needle was inserted through the septum and the fiber was exposed to the vial headspace for 30 min then fully retract the interior fiber. The SPME device was removed from the bottle and insert the SPME needle into the injector of gas chromatography (Shimadzu, Japan).

4.2 Analytical condition

All volatile samples from the wasabi flavor extraction step were analyzed by gas chromatography GC-17A series, which was equipped with a flame ionization detector (FID) with a ZB-WAX (polyethylene glycol, 30 m x 0.25 mm ID, 1 μm film thickness), nitrogen as carrier gas, using a split mode. The oven temperature program was 100 °C for 2 min, then increased at 10 °C /min to 145 °C for 5 min, followed by 10 °C /min to 230 °C for 5 min. The injector and detector temperature were 160 °C (2 min) and 240 °C, respectively. Quantification of ally isothiocyanate was performed using methyl isothiocyanate as an internal standard.

5. Statistical Analysis

Data obtained from physicochemical parameters were subjected to analysis of variance (ANOVA). The mean values from triplicate determinations were tested for significance at the $p< 0.05$ level using Duncan's multiple range test.

Sensory analysis was conducted using ANOVA based on a balanced incomplete block (BIB) design and the sample means were tested for significance at the $p < 0.05$ using Dunnett's test for multiple comparisons.

Places and Duration of Experiments

1. Places

The experiments were conducted at Department of Fishery Products, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand.

2. Duration

The experiments were carried out from March 2009 to August 2011.

RESULTS AND DISCUSSION

1. Effect of types wall materials and core material proportion on properties of emulsion, encapsulated wasabi flavor and wasabi flavor retention

1.1 Emulsion stability

A preliminary study was made to determine the physical stability of emulsions prepared with the various wall materials. Emulsions stability were observed during 24 hour after preparation of emulsion according to regard of a phase separation. The creaming indexes of H, blending of H with C or MD were no significant differences ($p>0.05$). However, the blending of H with WPC had significantly higher creaming index ($p<0.05$) than H, blending of H with C or MD. The results indicated that the emulsions of H, blending of H with C or MD were 0 % which its showed excellent stability (Table 7). There was no surface oil for the emulsions prepared containing all three different oil concentration after 24 hour of storage at 25 °C (Figure 8 (a-i)). The amount of wall material employed in the emulsification of the wasabi flavoring agent was in excess of that needed for fully coating the droplets and preventing their coalescence and flocculation (Kim *et al*., 1996; Beristain *et al*., 2001). According to Figure 8 (j-l) the emulsion containing 10-20% of wasabi flavoring agent with H + WPC were least stable to creaming, which droplet separation in the upwards of the tube after 1 hour of preparation. All other emulsions were considered kinetically stable, showing no phase separation after 24 hour. Kim *et al*. (1996) evaluated orange oil emulsions prepared with 10, 20 and 30 % w/w on a total solids basis of whey protein isolate (WPI). The emulsions were indicated that WPI stabilized emulsions had intermediate stabilities. WPI functioned well for emulsions that contained ≤ 20% orange oil on a solid basis while WPI stabilized emulsions were least stable to creaming for emulsions that contained 30% orange oil.

Table 7 Creaming index (%) for emulsion containing of wasabi flavor at various concentrations (10, 15 or 20 %w/w) in H, blending of H with C, MD or WPC

No.	Treatment		Creaming index (%)
	Content of wasabi flavor (%)	Type of wall material	
1	10	H	0.00 ± 0.00^{a}
2	15	H	0.00 ± 0.00^{a}
3	20	H	0.00 ± 0.00^{a}
4	10	H + C	0.00 ± 0.00^{a}
5	15	H + C	0.00 ± 0.00^{a}
6	20	H + C	0.00 ± 0.00^{a}
7	10	H + MD	0.00 ± 0.00^{a}
8	15	H + MD	0.00 ± 0.00^{a}
9	20	H + MD	0.00 ± 0.00^{a}
10	10	H + WPC	39.22 ± 0.62^{d}
11	15	H + WPC	37.70 ± 0.45^{c}
12	20	H + WPC	36.76 ± 0.63^{b}

Note Value are given as mean ± SD from triplicate determinations.
The different letters in the same column indicate the significant differences ($p<0.05$).

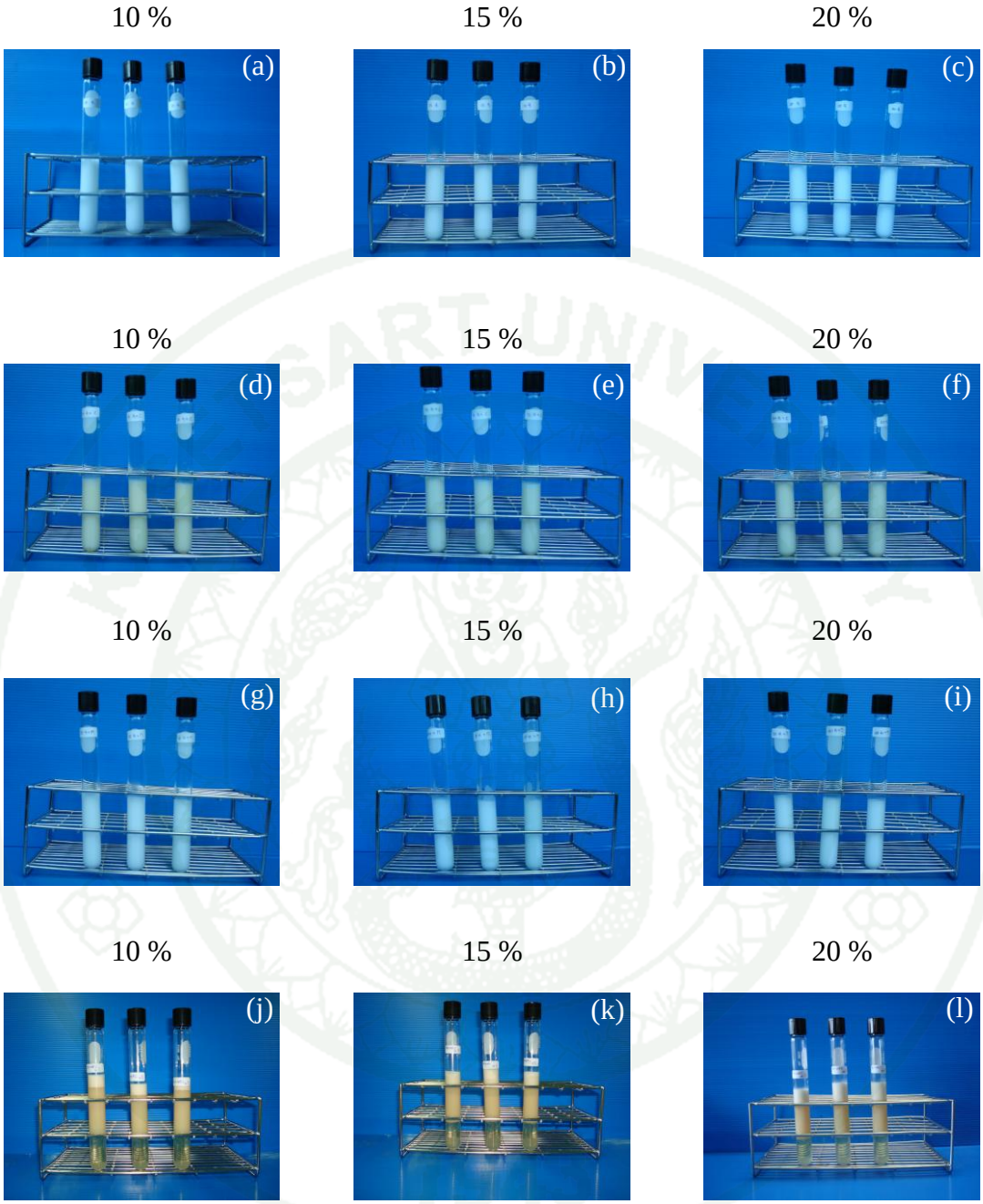


Figure 8 Image of emulsion stability for emulsion containing of 10, 15 and 20 % w/w wasabi flavoring agent in H (a-c), blending of H with C (d-f), MD (g-i) or WPC (j-l)

1.2 Emulsion viscosity

Viscosity of emulsion is important, since this parameter affect the size of microencapsulated particles and the thickness of their walls (Kim *et al*., 1996). The viscosity of emulsions varied from 88.16 to 125.88 Cp and significant difference was detected between samples based on types of wall material. Results showed that viscosity of emulsion prepared from blending of H with C was higher than H, blending of H with MD or WPC at the same wasabi oil concentration (Table 8). The increase in oil concentrations resulted in lower viscosities, which can be related to the lower amount of wall material present in the emulsions formed with higher oil concentration (for the same total solid content), resulting in a slightly less pronounced thickening effect and thus in less viscous emulsion (Tonon *et al*., 2011; Frascareli *et al*., 2012). According to Turchiuli *et al*. (2012), the viscosity of the emulsion decreased when the oil content was increased in agreement with the evolution of the viscosity of the continuous phase, which was found to be the governing factor affecting flow properties of sunflower oil emulsions containing maltodextrin. Similar behavior was observed by Hogan *et al*. (2001), which studied the emulsification and microencapsulation properties of sodium caseinate/carbohydrates blends, using soy oil as core material. The authors observed that viscosity was lower for higher ratios of oil/wall material and that the increase of total solid content (10-40%) led to the increase of viscosity.

Table 8 Viscosity for emulsion containing of wasabi flavor at various concentrations (10, 15 or 20 % w/w) in H, blending of H with C, MD or WPC

No.	Treatment		Viscosity (Cp)
	Content of wasabi flavor (%)	Type of wall material	
1	10	H	111.33 ± 9.86^{d}
2	15	H	105.99 ± 3.60^{d}
3	20	H	102.23 ± 9.34^{bcd}
4	10	H + C	125.88 ± 4.75^{e}
5	15	H + C	110.77 ± 2.85^{d}
6	20	H + C	104.35 ± 1.83^{cd}
7	10	H + MD	95.70 ± 2.82^{abc}
8	15	H + MD	90.28 ± 1.39^{a}
9	20	H + MD	88.16 ± 8.71^{a}
10	10	H + WPC	94.78 ± 1.39^{abc}
11	15	H + WPC	93.56 ± 1.85^{ab}
12	20	H + WPC	91.95 ± 6.34^{a}

Note Value are given as mean ± SD from triplicate determinations.
The different letters in the same column indicate the significant differences ($p<0.05$).

1.3 Moisture content

The characteristics of the dry microcapsules depend on the physical and chemical properties of the feed, the dryer design, and the operating conditions (Quispe-Condari *et al*., 2011). In this study, the operating conditions of temperature and flow rate in the spray dryer were kept constant. The microencapsulated wasabi flavor produced from wasabi flavor at various concentrations (10, 15 or 20 %w/w) in H, blending of H with C, MD or WPC showed significantly difference ($p<0.05$) (Table 9). Moisture contents of spray dried powder varied from 2.63-4.75 %. Powder

moisture contents were found to be in the targeted rang of < 5% as it is suggested to be in the range of 3 and 4% for dried powder in the food industry (Klaypradit and Haung, 2008) and have a good stability during storage.

The moisture content decreased with an increase in wasabi oil at the same wall concentration. These different could be attributed to the structure of granules and viscosity of biopolymer and increased oil content reduce water activity. Higher apparent a viscosity, the water was limited the diffusion through the viscous network. (Loksuwan, 2007; Quisp-Condari, 2011).

Table 9 Moisture content and water activity of wasabi flavor microcapsules prepared from wasabi flavor at various concentrations (10, 15 or 20 %w/w) in H, blending of H with C, MD or WPC

No.	Treatment		Moisture content (%)	Water activity
	Content of wasabi flavor (%)	Type of wall material		
1	10	H	3.92 ± 0.00^{j}	0.28 ± 0.00^{f}
2	15	H	2.91 ± 0.01^{b}	0.24 ± 0.00^{bc}
3	20	H	2.63 ± 0.02^{a}	0.23 ± 0.02^{ab}
4	10	H + C	3.81 ± 0.01^{i}	0.29 ± 0.00^{f}
5	15	H + C	3.77 ± 0.04^{h}	0.27 ± 0.01^{e}
6	20	H + C	3.64 ± 0.01^{f}	0.22 ± 0.00^{a}
7	10	H + MD	3.73 ± 0.03^{g}	0.26 ± 0.00^{d}
8	15	H + MD	3.55 ± 0.01^{e}	0.25 ± 0.00^{cd}
9	20	H + MD	3.35 ± 0.01^{d}	0.25 ± 0.01^{cd}
10	10	H + WPC	4.75 ± 0.01^{l}	0.32 ± 0.00^{g}
11	15	H + WPC	3.96 ± 0.01^{k}	0.28 ± 0.00^{e}
12	20	H + WPC	3.13 ± 0.01^{c}	0.24 ± 0.00^{c}

Note Value are given as mean ± SD from triplicate determinations.
The different letters in the same column indicate the significant differences ($p<0.05$).

1.4 Water activity

Water activity (a_w) is an important index for spray-dried powder because it can greatly affect the shelf life of the powder produced. It is defined as the ratio of vapor pressure of water in a food system to vapor pressure of pure water at the same temperature. Water activity is different from moisture content as it measures the availability of free water in a food system that is responsible for any biochemical reactions, whereas the moisture content represents the water composition in a food system. High water activity indicates more free water available for biochemical reactions and hence, shorter shelf life. Generally, food with $a_w < 0.6$ is considered as microbiologically stable and if there is any spoilage occur, it is induced by chemical reactions rather than by micro-organism (Quek *et al*., 2007). According to the mentioned theory, the concept of water activity has been used as a reliable assessment for lipid oxidation, non-enzymatic as well as enzymatic activities in food (Rahman and Labuza, 1999). These is indication of an optimum water activity for product shelf life, which corresponds to the monolayer region. For typical dry products, the monolayer region corresponds to a water activity from 0.2 to 0.3 (Anker and Reineccius, 1988). From the results (Table 9), the water activities of microencapsulated wasabi flavor prepared from wasabi flavor at various concentrations (10, 15 or 20 % w/w) in H, blending of H with C, MD or WPC varied from 0.22-0.32 and were significantly difference ($p<0.05$). This mean that, the powders produced were relatively stable microbiological. The data also showed that the water activity decreased with higher concentration of wasabi flavoring agent increased oil content reduced water activity (Loksuwan, 2007).

1.5 Color value

The results of the color value for microencapsulated wasabi flavor prepared from H, blending of H with C, MD or WPC are as shown in Table 10. The L* value measure the lightness of the sample, a* measures the redness (+) and greenness (-) while b* measures the yellowness (+) and blueness (-). Statistical analysis (Table 10) showed that there were significantly differences ($p<0.05$) between the color (L*, a*, b* value) responses of microencapsulated wasabi flavor prepared

from H, blending of H with C, MD or WPC. The L* of microencapsulated wasabi flavor make from blending of H with C or WPC was smaller (less light), the a* value was low positive (less green) and the b* was higher (more yellow) than the microencapsulated wasabi flavor prepared from H and blending of H with MD. This can be related with the original color of C and WPC that is yellow color. It has been reported that the color of encapsulated tuna oil prepared from chitosan and MD was more whitish when compared to the powder that made from chitosan and whey protein isolate that probably due to whey protein isolate itself has a yellow color (Klaypradit and Huang, 2008). It was found that when wasabi flavoring agent increased, the L* and a* value tend to decrease whereas the increasing of b* value. This can be explained by dilution of wasabi flavoring agent with increasing the core concentration.

Table 10 L*, a* and b* of wasabi flavor microcapsules prepared from wasabi flavor at various concentrations (10, 15 or 20 %w/w) in H, blending of H with C, MD or WPC

No.	Treatment		L*	a*	b*
	Content of wasabi flavor (%)	Type of wall material			
1	10	H	94.33 ± 0.02^{i}	-0.91 ± 0.01^{d}	5.05 ± 0.01^{b}
2	15	H	93.90 ± 0.03^{g}	-1.09 ± 0.03^{b}	6.10 ± 0.02^{d}
3	20	H	93.68 ± 0.01^{f}	-1.19 ± 0.02^{a}	7.18 ± 0.01^{f}
4	10	H + C	89.99 ± 0.03^{c}	0.21 ± 0.02^{f}	12.43 ± 0.01^{i}
5	15	H + C	89.75 ± 0.03^{b}	0.23 ± 0.01^{f}	12.31 ± 0.03^{h}
6	20	H + C	90.69 ± 0.01^{e}	0.22 ± 0.01^{f}	12.21 ± 0.03^{g}
7	10	H + MD	94.49 ± 0.02^{j}	-0.81 ± 0.02^{e}	4.74 ± 0.02^{a}
8	15	H + MD	94.05 ± 0.01^{h}	-1.00 ± 0.02^{c}	5.10 ± 0.02^{c}
9	20	H + MD	94.32 ± 0.04^{i}	-1.18 ± 0.05^{a}	6.21 ± 0.01^{e}
10	10	H + WPC	89.73 ± 0.01^{b}	0.51 ± 0.02^{h}	15.31 ± 0.02^{k}
11	15	H + WPC	88.95 ± 0.01^{a}	0.48 ± 0.01^{h}	15.74 ± 0.03^{m}
12	20	H + WPC	90.07 ± 0.04^{d}	0.39 ± 0.01^{g}	14.84 ± 0.01^{j}

Note Value are given as mean ± SD from triplicate determinations.
The different letters in the same column indicate the significant differences ($p<0.05$).

1.6 Surface allyl isothiocyanate, total allyl isothiocyanate and encapsulation efficiency

The content of Allyl isothiocyanate (AITC) in this study was determined by using UV colorimetry instead of the GC method used in many previous studies. Compared with GC analysis, the UV spectrophotometer is much simpler, fast, and easy to operate, which is suitable to the determination of AITC (Li *et al.,* 2007). AITC possesses ultraviolet absorption due to its unsaturated bond in the molecule. The used

AITC (in hexane) solution in this study showed a maximum absorbance at 248 nm by UV scanning analysis.

Since the surface oil can be easily oxidized to form off-flavor compounds, the amount of surface oil in the spray-dried powder is quite important for stable storage of the microencapsulated powder (Soottitantawat *et al*., 2003; Baranausleiene *et al*., 2006; Anwer and Kunz., 2011; Quispe-Condari *et al*., 2011). The surface AITC of microencapsules at various wasabi flavoring agent contents and various H and blending of H with C, MD or WPC were examined. The microencapsulated wasabi flavor was prepared at a constant total solid content of 30 % w/w. According to Table 11, the surface AITC of microencapsulated wasabi flavor prepared from wasabi flavor at various concentrations (10, 15 or 20 %w/w) in H and blending of H with C, wasabi flavor at various concentrations (10 or 15 %w/w) in blending of H with MD was no significantly differences ($p>0.05$). Nevertheless, the microencapsulated wasabi flavor prepared from wasabi flavor at 20 %w/w in blending of H with MD and microencapsulated wasabi flavor prepared from wasabi flavor at various concentrations (10, 15 or 20 %w/w) in blending of H with WPC was significantly differences ($p<0.05$). From the result, the surface AITC of microencapsulated wasabi flavor increasing when the mass ratio of wasabi flavor to wall materials increased especially with blending of H with WPC. This indicates that the surface AITC depended on the type of wall material. As for the effect of the type of wall material on the surface AITC, blending of H with WPC showed higher surface AITC content than those other wall materials. That might was explained the emulsifying properties of each type of wall materials (Soottitantawat *et al*., 2005a). These results demonstrate the importance of having sufficient quantities of the coating material to encapsulate the oil rather than having a portion of the oil left on the surface (Quispe-Condari *et al*., 2011).

The effect of increasing initial concentration of wasabi flavor from 10 – 20 % (w/w), probably causes an increase in the concentration of oil in the wall and outer surface of the capsule during crust formation and more oil is last. Such losses are enhanced by internal circulation streams inside the drying liquid during droplet formation in spray drying and the expansion of droplets followed by crust formation

in the dry skin, leading to evaporation of the interior of the particle (Beristain *et al*., 2001).

The encapsulation efficiency (EE) reflects the real amount of core material that is encapsulated inside the wall matrix (Anwer and Kunz, 2011). The successful encapsulation of oils should result in an encapsulated powder with minimum surface oil content on the particles and maximum retention of the core material inside the particles (Jafari *et al*., 2008; Rocha *et al*., 2011). The results showed that the encapsulation efficiency varied from 22-42 % and was significantly difference ($p<0.05$) influenced by wasabi flavor concentration (Table 11). Oil concentration was the factor that most affected encapsulation efficiency, showing a positive effect on this response. The higher the oil concentration, the higher the encapsulation was the higher the amount of total oil (Table 11). Table 11 shows that the EE increased, as indicated by the volatile retention, when the powder had a higher total oil concentration (113-115 mg AITC/100 g sample). Generally, the products of spray-dried powder are prepared with solids to flavor ratio of 4 to 1 (Reineccius, 1988; Rish, 1995). This ratio has been reported for wall material such as gum arabic and other carbohydrate derivatives (Bhandari *et al*., 1992).

Table 11 Surface allyl isothiocyanate, total allyl isothiocyanate and encapsulation efficiency of wasabi flavor microcapsules prepared from wasabi flavor at various concentrations (10, 15 or 20 %w/w) in H, blending of H with C, MD or WPC

No.	Treatment		Surface allyl isothiocyanate (mg AITC/100 g sample)	Total allyl isothiocyanate (mg AITC/100 g sample)	Encapsulation efficiency (%)
	Content of wasabi flavor (%)	Type of wall material			
1	10	H	0.42 ± 0.05^{a}	47.38 ± 1.05^{b}	32.85 ± 1.71^{b}
2	15	H	0.54 ± 0.09^{a}	88.12 ± 0.78^{g}	42.46 ± 1.63^{c}
3	20	H	0.66 ± 0.14^{a}	115.70 ± 0.65^{h}	43.36 ± 1.20^{c}
4	10	H + C	0.66 ± 0.19^{a}	51.61 ± 0.56^{c}	32.53 ± 1.44^{b}
5	15	H + C	0.75 ± 0.19^{a}	70.02 ± 1.99^{e}	33.01 ± 1.01^{b}
6	20	H + C	0.66 ± 0.14^{a}	113.58 ± 2.07^{h}	42.49 ± 1.61^{c}
7	10	H + MD	0.57 ± 0.23^{a}	50.82 ± 1.97^{c}	$33.\ 90 \pm 2.12^{b}$
8	15	H + MD	0.57 ± 0.11^{a}	82.68 ± 2.20^{f}	40.47 ± 1.42^{c}
9	20	H + MD	1.16 ± 0.18^{b}	113.12 ± 2.07^{h}	42.85 ± 1.77^{c}
10	10	H + WPC	1.90 ± 0.24^{c}	39.48 ± 1.31^{a}	$25.\ 53 \pm 2.62^{a}$
11	15	H + WPC	1.75 ± 0.14^{c}	52.44 ± 1.45^{c}	24.58 ± 2.52^{a}
12	20	H + WPC	5.51 ± 0.61^{d}	64.56 ± 1.55^{d}	$23.\ 39 \pm 1.51^{a}$

Note Value are given as mean ± SD from triplicate determinations.
The different letters in the same column indicate the significant differences ($p<0.05$).

1.7 Morphological characterization of wasabi flavor microcapsule

Microencapsulated wasabi flavor prepared from H, blending of H with C, MD or WPC were observed for granular structure using SEM (Figure 9). The microencapsulated wasabi flavor produced from wasabi flavor at various concentrations (10, 15 or 20 %w/w) in H (Figure 9-a, 9-b, 9-c), blending of H with C (Figure 9-d, 9-e, 9-f), blending of H with MD (Figure 9-g, 9-h, 9-i) and blending of H with WPC (Figure 9-j, 9-k, 9-l) had similar appearance of microstructure. The image obtained the SEM result (Figure 9, a-l) showed that microencapsulated wasabi flavor were irregularly spherical shaped particles and various size, which is typical of materials produced by spray drying (Tonon *et al*., 2008; Ortiz *et al*., 2009; Frascareli *et al*., 2012). Some of particles with smooth surface were also found but most of particle showed a rounded external surface with a continuous wall and no apparent fissures cracks or interruptions, which is important to provide lower gas permeability, better protection and core retention. Moreover, surfaces were concave and shriveled, which is typical of microcapsules produced by spray drying (Tonon *et al*., 2008; Rocha *et al*., 2011). It has been reported that both the shrinkages on the surface of the particles and the expansion in the size of the particles occur particularly with the addition of the carrier materials which decrease the evaporation rate of water from the system due to their water holding characteristics (Loksuwan, 2007; Nadeem *et al*., 2011). The rough surface as observed in the wasabi microcapsules might also be caused by the variation in the rate of evaporation. The low evaporation rate caused agglomeration of wet granules and resulted in a coarse morphology (Becher and Schlunder, 1998).

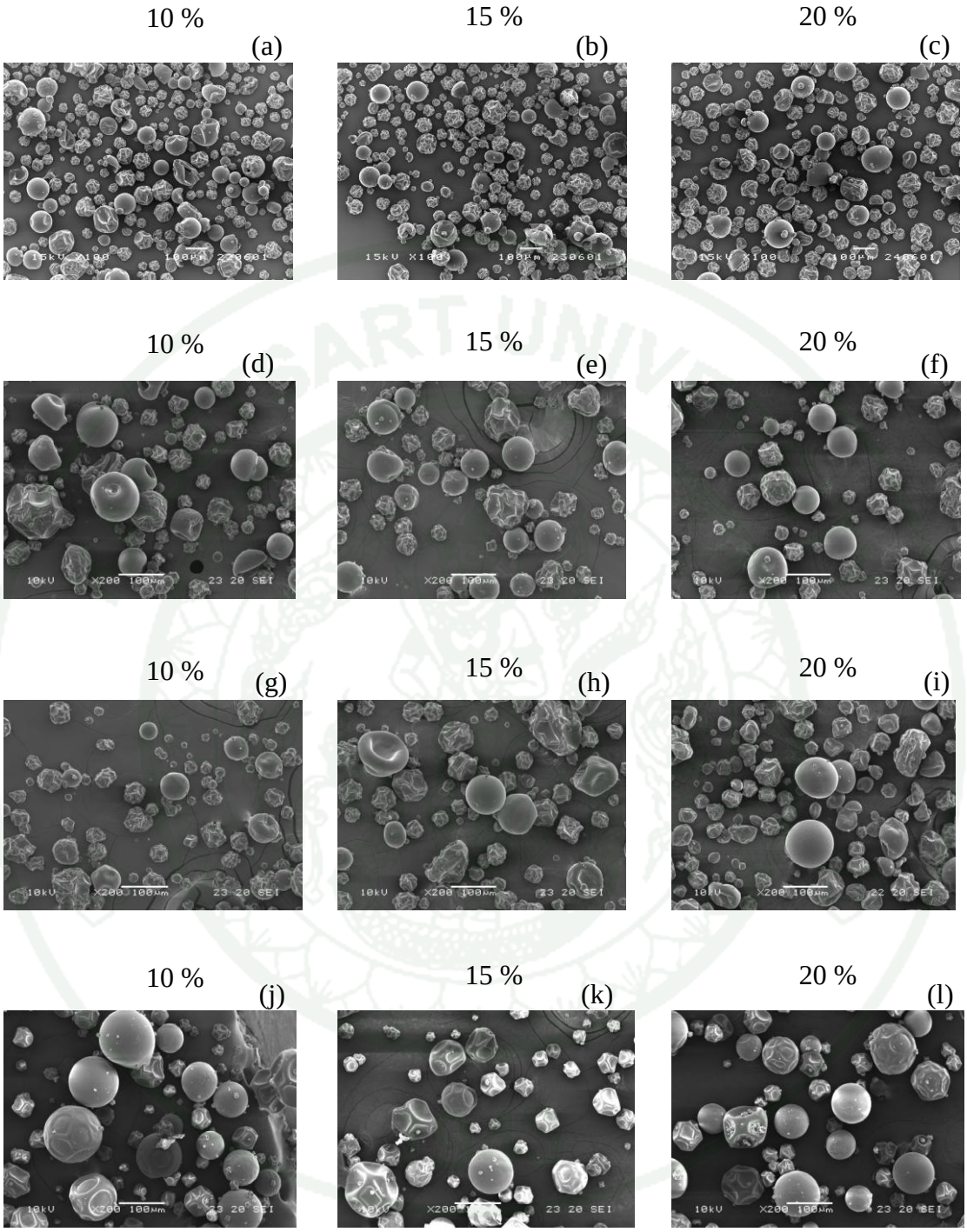


Figure 9 Microstructure of microencapsulated wasabi flavor containing of 10, 15 and 20 % w/w wasabi flavoring agent in H (a-c), blending of H with C (d-f), MD (g-i) or WPC (j-l)

It was found that the microencapsulated wasabi flavor prepared from wasabi flavor at various concentrations (10, 15 or 20 %w/w) in blending of H with WPC (Figure 9-j, 9-k, 9-l) had less surface dents and shrinkage than other wall material. In other words, incorporating WPC into emulsion composition had a profound influence on the structure and surface morphology of encapsulated powders, resulting in particles with smooth surface and less indentations. This suggested a slower rate of wall matrix solidification with WPC sample and possibly a higher elasticity of this wall system. These are in agreement with the results of Sheu and Rosenberg (1998) who found that increasing the proportion of whey protein isolate: maltodextrin from 1 : 19 to 3 : 1 lead to powder particles with minimum indentations.

From the result, blending of H with WPC was not exhibit satisfactorily. It was showed least stable of emulsion and higher surface AITC and lower encapsulation efficiency than the other wall materials. Therefore, blending of H with WPC was not used as the wall material for encapsulated wasabi flavor. However, H, blending of H with C or MD was the most effective wall materials for encapsulated wasabi flavor by spray drying.

2. Effect of storage conditions (relative humidity) on the release characteristics of encapsulated wasabi flavor

2.1 Average particle size diameter

The particle size distribution of the emulsions made from H, blending of H with C or MD containing various wasabi flavors is presented in Table 12. There was no significant difference ($p > 0.05$) in particle size between the emulsions made from various wasabi-flavored concentrations in H and blending of H with C, while that of emulsions made from various wasabi-flavored concentrations in blending of H with MD were significantly different ($p < 0.05$) and significantly larger size ($p < 0.05$) than those from the others. Although, the particle size of less than 2 μm in emulsions was reported to positively correspond to the encapsulation efficiency (Trubiano and Lacourse, 1988). Nonetheless, in our study, particle size of encapsulated wasabi flavor

(15 and 20% w/w) made from blending of H with MD slightly over 2 μm remained pronounce effects on encapsulation efficiency which might be party subject to the types of wall materials used. This could be additionally explained by both the modified starches having their emulsifying properties modified with n-octenyl succinyl anhydride (OSA; Soottitantawat *et al.,* 2005b). An emulsifier is a surface-active substance, which is capable of adsorbing to an oil-water interface and protecting the emulsion droplets from coalescence or flocculation (Tonon *et al.,* 2011). The major shortcomings of MD are its lack of emulsifying capacity and its marginal retention of volatile compounds (Krishnan *et al*., 2005). Thus, the increase in the MD content may have promoted higher droplet coalescence in the emulsions studied, resulting in the formation of larger droplets. Soottitantawat *et al.* (2005a) reported similar results by spray drying d-limonene emulsions with H and gum arabic with MD. They found that H had the lowest droplet size (about 0.6 μm) while the emulsions produced with gum arabic and a blend of gum arabic and MD had a droplet size of about 0.8 and 0.9 μm, respectively.

2.2 Flowing properties

The Carr index and Hausner ratio are parameters to assess powder flowability; the Carr index is an indication of the compressibility of a powder and the Hausner ratio is correlated to the flowability of a powder or granular material. According to the classification of powder flowability, the Carr index is related to the Hausner ratio. Values of the Carr index and Hausner ratio greater than 25 and 1.34, respectively, are both considered to be indicators of poor flowability, with values between 16 and 20, and 1.19 and 1.25, respectively, to be indicators of fair flowability, while values below 15 and 1.18, respectively, show good flowability (Turchiuli *et al.,* 2005).

The flowing properties of encapsulated wasabi flavors made from all types of wall materials used in the study showed acceptable flowing properties (Table 13). The flow property of the powder is dependent on the properties of the wall materials and the relative amount of wasabi flavor remaining unencapsulated on the surface of

the particles (Fuchs *et al.,* 2006). The encapsulated powder of this study showed better properties than the studies of Turchiuili *et al.* (2005), who reported that the microencapsulated oil made from a mixture of MD and gum acacia had poor flowability. Fuchs *et al.* (2006) also reported the spray-dried powder prepared from vegetable oil in a blend of MD with acacia gum had a Carr index of 44, which indicated extremely poor flowability. Generally, encapsulated powders with good flowability are convenient in handling and processing operation (Zhang *et al*., 2010.)

Table 12 Average particle diameter of emulsions made from wasabi flavor at various concentrations (10, 15 or 20 % w/w) in H, blending of H with C or MD

No.	Treatment		Average particle diameter	
	Content of wasabi flavor (%)	Type of wall material	$D_{3,2}$ (μm)	$D_{4,3}$ (μm)
1	10	H	0.271 ± 0.00^{a}	5.700 ± 0.47^{ab}
2	15	H	0.203 ± 0.01^{a}	5.060 ± 1.04^{ab}
3	20	H	0.223 ± 0.03^{a}	4.864 ± 0.87^{ab}
4	10	H + C	0.353 ± 0.04^{a}	5.415 ± 0.67^{ab}
5	15	H + C	0.233 ± 0.02^{a}	5.595 ± 1.02^{ab}
6	20	H + C	0.255 ± 0.01^{a}	4.571 ± 0.64^{a}
7	10	H + MD	1.845 ± 0.10^{b}	6.221 ± 0.66^{b}
8	15	H + MD	2.516 ± 0.19^{d}	9.661 ± 0.19^{c}
9	20	H + MD	2.313 ± 0.17^{c}	9.187 ± 1.33^{c}

Note Value are given as mean ± SD from triplicate determinations.

The different letters in the same column indicate the significant differences ($p<0.05$).

$D_{3,2}$ is the surface area mean diameter.

$D_{4,3}$ is the volume mean diameter.

Table 13 Flowing properties of emulsions made from wasabi flavor at various concentrations (10, 15 or 20 % w/w) in H, blending of H with C or MD

No.	Treatment		Carr' Index	Hausner ratio
	Content of wasabi flavor (%)	Type of wall material		
1	10	H	15.56 ± 0.96^{ab}	1.19 ± 0.01^{ab}
2	15	H	16.35 ± 2.34^{b}	1.20 ± 0.03^{b}
3	20	H	15.26 ± 0.46^{ab}	1.18 ± 0.01^{ab}
4	10	H + C	13.70 ± 2.25^{ab}	1.16 ± 0.03^{ab}
5	15	H + C	14.22 ± 2.97^{ab}	1.16 ± 0.04^{ab}
6	20	H + C	13.40 ± 2.83^{ab}	1.15 ± 0.04^{ab}
7	10	H + MD	12.01 ± 0.43^{a}	1.13 ± 0.01^{a}
8	15	H + MD	12.67 ± 2.70^{a}	1.15 ± 0.03^{ab}
9	20	H + MD	16.11 ± 0.96^{b}	1.19 ± 0.01^{b}

Note Value are given as mean ± SD from triplicate determinations.
The different letters in the same column indicate the significant differences ($p<0.05$).

2.3 Moisture content

The moisture content of the encapsulated samples is shown in Figure 10. The initial powder moisture content of encapsulated powders varied from 3.08 to 4.77%, which were generally within the targeted moisture range of dried powder in the food industry between 3 and 4% (Klaypradit and Huang 2008). The similar results of moisture content of many encapsulated powder products made by spray drying were also reported in previous study including encapsulated β-carotene, fish oil and flax oil showed 2.11-6.0%, 2.23-6.59% and 3.49-5.06 %, respectively (Loksuwan 2007; Anwar and Kunz 2011; Quisp-Condari *et al*., 2011).

The results in the present study showed that the moisture content of the spray-dried powder for all samples increased with increasing relative humidity. A possible reason for this result could be that when the encapsulated powder has moisture content lower than that of the surrounding air, the powders will adsorb ambient moisture causing the powder to have higher hygroscopicity so that consequently, the moisture content increases (Gharsallaoui *et al.,* 2011). In contrast, when powders were placed in a dry environment (11% RH), water diffused through the wall matrix resulting in lower moisture content than in powders placed in a wet environment (33% or 52% RH).

The microencapsulated wasabi flavor made from H tended to have significantly ($p < 0.05$) higher moisture content than the other wall materials. The H is the mixing of high-dextrose equivalent (DE) corn syrup solid with the final of 32-37 dextrose equivalent (DE) (Soottitantawat *et al*., 2005b), so that it has quite high content of low molecular weight sugars such as glucose, sucrose, fructose and organic acid (Quek *et al.,* 2007). Each of these materials has a low glass transition temperature (Tg) with values of 62, 5 and 31°C for sucrose, fructose and glucose, respectively (Bhandari and Howes, 1999) that results in a very hygroscopic product (Cano-Chauca *et al.,* 2005). Conversely, the microencapsulated wasabi flavor made from blending of H with MD tended to have lower moisture content than more did by other wall materials. This might have been caused by the maltodextrin used in this study having a low DE value (DE 5), which is the lowest for the low molecular weight sugars, thus tending to produce samples with low hygroscopicity. The results of Quek *et al.,* (2007) also support the present study, as they reported that maltodextrin could increase the total solid content in watermelon juice and reduce the moisture content of the product.

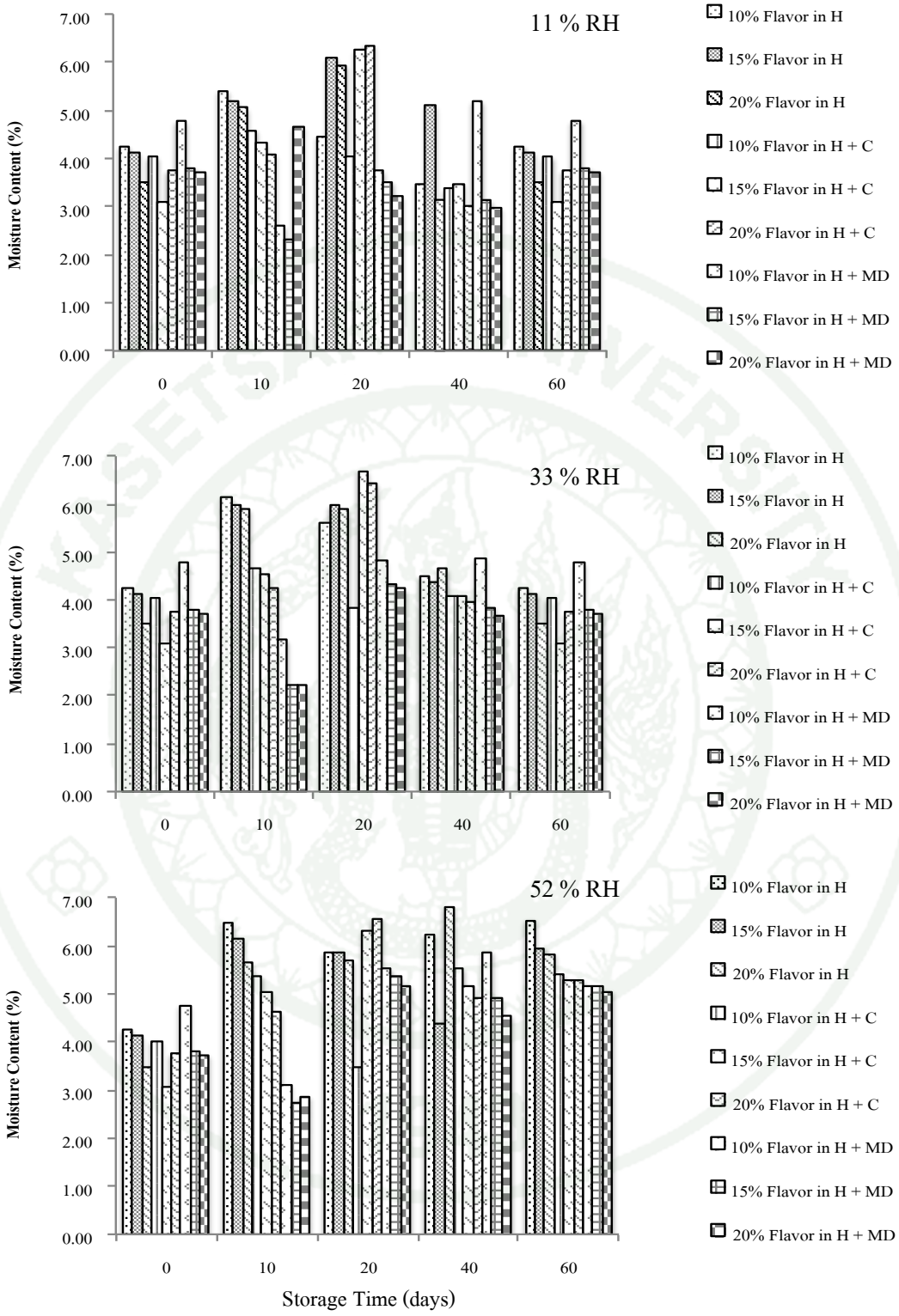


Figure 10 Changes in moisture content of microencapsulated wasabi flavor made from different wall materials with various concentration of wasabi flavor during storage in various relative humidity at 35 °C during 60 days of storage

2.4 Water activity

Measurements of a_w were performed after powder production and during the storage test. Due to only slight differences in each measurement, the results listed in Figure 11 are the average value of the three observations. The water activities of the microencapsulated wasabi flavor were in the range 0.25-0.45, 0.25-0.48 and 0.25-0.51 for the 11 %RH, 33 %RH and 52 %RH, respectively during 60 days of storage. The microencapsulated wasabi flavor prepared from H showed higher a_w (0.33-0.51) than blending of H with C (0.25-0.47) or MD (0.30-0.43) for 52 %RH during 60 days. This results depend on wall material property which was a lower a_w value along with lower moisture content. These differences could be attributed to the structure of granules of starch. The a_w value of microencapsulated wasabi flavor prepared from blending of H with C or MD was about 0.3, which very positive for stability since it represents less free water available for biochemical reactions and hence longer shelf life and the maximum a_w specification for most dried powder in the food industry (Klaypradit and Huang, 2008; Fritzen-Freire *et al*., 2012).

2.5 Color value

The color attributes of microencapsulated wasabi flavor prepared from wasabi flavor at various concentrations (10, 15 or 20 %w/w) in H, blending of H with C or MD is shown in Table 14, 15 and 16. Table 14 shows the change in L* (lightness) as a function of relative humidity during storage at 35 °C. The microencapsulated wasabi flavor prepared from H stored at low humidity (11 and 33 % RH) showed higher L* value than the microencapsulated wasabi flavor at high humidity (52 % RH) after 60 days of storage ($p<0.05$). The L* value decreased with increasing storage time in all samples. However, microencapsulated wasabi flavor prepared from wasabi flavor at various concentrations (10, 15 or 20 %w/w) in blending of H with C or MD did not change significant within 60 days of storage. For a* value, the microencapsulated wasabi flavor no difference were observed between the samples storage at 11, 33 and 52 % RH. The b* value of the microencapsulated wasabi flavor stored at low humidity (11 and 33 % RH) showed the lower b* value

than the microencapsulated wasabi flavor stored at high humidity (52 % RH) during 60 days of storage, especially the microencapsulated wasabi flavor prepared from H showed b* value increased with increasing storage time in (Table 16). This was probably due to the color of the microencapsulated wasabi flavor became little darker as storage time increased. One of the explanation for this phenomenon was starch contains sugar which could contribute to browning of the powder especially with non-enzymatic browning reactions (Quek *et al*., 2007).

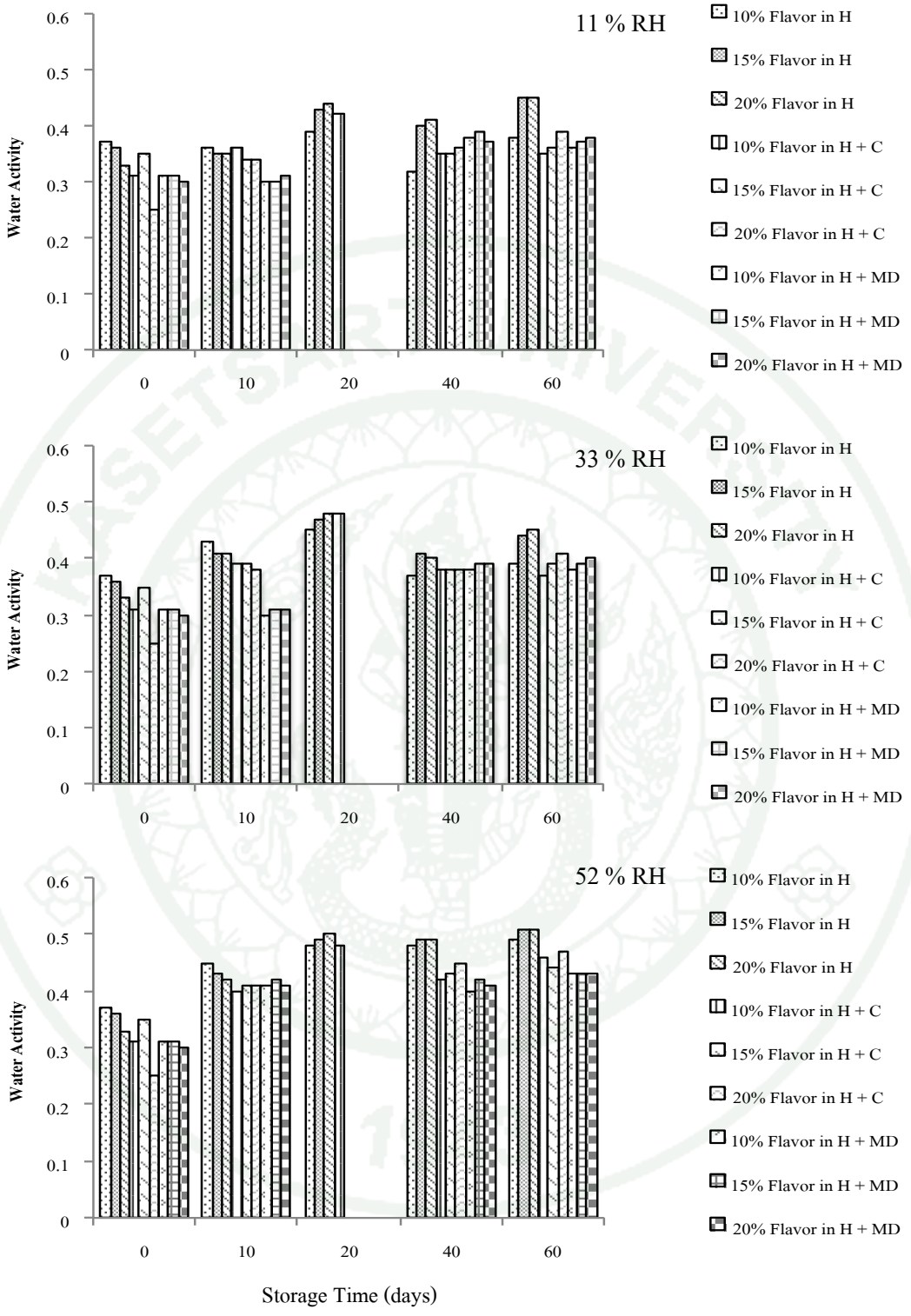


Figure 11 Changes in water activity of microencapsulated wasabi flavor made from different wall materials with various concentration of wasabi flavor during storage in various relative humidity at 35 °C during 60 days of storage

Table 14 Changes in L* of microencapsulated wasabi flavor during storage in various relative humidity at 35 °C during 60 days of storage

No	Treatments	% RH	Storage Time (Days)				
			0	10	20	40	60
1	10% Flavor in H	11	94.51^{i}	93.23^{h}	93.11^{n}	93.07^{l}	93.07^{k}
		33	94.51^{i}	93.04^{g}	92.99^{l}	92.77^{k}	93.00^{j}
		52	94.51^{i}	92.97^{f}	92.62^{j}	91.93^{j}	91.93^{i}
2	15% Flavor in H	11	94.37^{h}	94.17^{r}	93.03^{m}	94.18^{w}	94.18^{u}
		33	94.37^{h}	94.09^{pq}	93.15^{o}	94.03^{t}	93.92^{r}
		52	94.37^{h}	93.91^{n}	91.23^{h}	93.40^{p}	93.40^{o}
3	20% Flavor in H	11	94.07^{f}	94.00^{o}	93.05^{m}	94.08^{u}	94.08^{t}
		33	94.07^{f}	94.05^{p}	92.74^{k}	93.68^{r}	93.69^{q}
		52	94.07^{f}	93.72^{m}	91.50^{i}	93.13^{m}	93.13^{l}
4	10% Flavor in H + C	11	89.35^{a}	89.49^{a}	89.52^{b}	89.42^{c}	89.42^{c}
		33	89.35^{a}	89.47^{a}	89.43^{a}	89.30^{a}	89.18^{a}
		52	89.35^{a}	89.49^{a}	89.43^{a}	89.36^{b}	89.36^{b}
5	15% Flavor in H + C	11	90.36^{c}	90.44^{d}	90.56^{g}	90.26^{h}	90.26^{g}
		33	90.36^{c}	90.38^{c}	90.39^{f}	90.03^{g}	90.31^{h}
		52	90.36^{c}	90.38^{c}	90.29^{d}	89.95^{f}	89.95^{f}
6	20% Flavor in H + C	11	90.07^{b}	90.53^{e}	90.16^{c}	90.32^{i}	90.32^{h}
		33	90.07^{b}	90.29^{b}	90.32^{e}	89.86^{e}	89.80^{e}
		52	90.07^{b}	90.46^{d}	90.17^{c}	89.73^{d}	89.73^{d}
7	10% Flavor in H + MD	11	94.25^{g}	94.10^{q}	94.02^{w}	94.29^{x}	94.29^{w}
		33	94.25^{g}	94.19^{r}	94.12^{x}	94.15^{v}	94.23^{v}
		52	94.25^{g}	93.97^{o}	94.20^{y}	93.99^{s}	93.99^{s}
8	15% Flavor in H + MD	11	92.64^{d}	93.61^{l}	93.79^{v}	94.08^{u}	94.08^{t}
		33	92.64^{d}	93.35^{i}	93.04^{m}	93.36^{o}	93.50^{p}
		52	92.64^{d}	93.51^{k}	93.76^{u}	93.48^{q}	93.48^{p}
9	20% Flavor in H + MD	11	93.49^{e}	94.07^{pq}	93.55^{t}	93.39^{p}	93.39^{o}
		33	93.49^{e}	93.90^{n}	93.39^{s}	93.37^{o}	93.35^{n}
		52	93.49^{e}	93.41^{j}	93.33^{r}	93.16^{n}	93.16^{m}

Note Value are given as mean ± SD from triplicate determinations.

The different letters in the same column indicate the significant differences ($p<0.05$).

Table 15 Changes in a* of microencapsulated wasabi flavor during storage in various relative humidity at 35 °C during 60 days of storage

No	Treatments	% RH	Storage Time (Days)				
			0	10	20	40	60
1	10% Flavor in H	11	-0.19^{a}	-0.24^{ab}	-0.21^{ab}	-0.24^{a}	-0.23^{a}
		33	-0.19^{a}	-0.22^{ab}	-0.17^{cde}	-0.15^{cde}	-0.12^{cd}
		52	-0.19^{a}	-0.16^{def}	-0.12^{h}	-0.11^{fg}	-0.11^{de}
2	15% Flavor in H	11	-0.22^{a}	-0.25^{a}	-0.23^{a}	-0.21^{b}	-0.24^{a}
		33	-0.22^{a}	-0.22^{ab}	-0.16^{de}	-0.12^{fg}	-0.11^{de}
		52	-0.22^{a}	-0.21^{bc}	-0.19^{bc}	-0.06^{i}	-0.09^{defg}
3	20% Flavor in H	11	-0.09^{a}	-0.15^{ef}	-0.19^{bc}	-0.18^{c}	-0.16^{b}
		33	-0.09^{a}	-0.11^{g}	-0.12^{h}	-0.05^{i}	-0.07^{fg}
		52	-0.09^{a}	-0.16^{def}	-0.17^{cde}	-0.01^{j}	-0.06^{g}
4	10% Flavor in H + C	11	0.52^{b}	0.52^{m}	0.51^{l}	0.51^{m}	0.59^{lm}
		33	0.52^{b}	0.52^{m}	0.54^{m}	0.61^{n}	0.61^{m}
		52	0.52^{b}	0.49^{kl}	0.57^{n}	0.58^{n}	0.57^{kl}
5	15% Flavor in H + C	11	0.38^{b}	0.41^{i}	0.36^{i}	0.41^{k}	0.37^{h}
		33	0.38^{b}	0.43^{ij}	0.47^{k}	0.49^{lm}	0.47^{j}
		52	0.38^{b}	0.37^{h}	0.40^{j}	0.47^{l}	0.45^{ij}
6	20% Flavor in H + C	11	0.52^{b}	0.46^{j}	0.47^{k}	0.43^{k}	0.43^{i}
		33	0.52^{b}	0.49^{k}	0.50^{l}	0.61^{n}	0.54^{k}
		52	0.52^{b}	0.41^{i}	0.47^{k}	0.59^{n}	0.55^{k}
7	10% Flavor in H + MD	11	-0.18^{a}	-0.14^{f}	-0.15^{efg}	-0.14^{def}	-0.15^{bc}
		33	-0.18^{a}	-0.10^{g}	-0.17^{cde}	-0.06^{hi}	-0.15^{bc}
		52	-0.18^{a}	-0.14^{f}	-0.17^{cde}	-0.09^{gh}	-0.10^{def}
8	15% Flavor in H + MD	11	-0.13^{a}	-0.11^{g}	-0.15^{efg}	-0.15^{cde}	-0.15^{bc}
		33	-0.13^{a}	-0.16^{ef}	-0.16^{ef}	-0.11^{fg}	-0.16^{b}
		52	-0.13^{a}	-0.10^{g}	-0.17^{cde}	-0.07^{hi}	-0.08^{efg}
9	20% Flavor in H + MD	11	-0.21^{a}	-0.18^{de}	-0.19^{bc}	-0.16^{cd}	-0.16^{b}
		33	-0.21^{a}	-0.18^{de}	-0.13^{fgh}	-0.16^{cd}	-0.10^{def}
		52	-0.21^{a}	-0.19^{cd}	-0.13^{fgh}	-0.13^{ef}	-0.15^{bc}

Note Value are given as mean ± SD from triplicate determinations.

The different letters in the same column indicate the significant differences ($p<0.05$).

Table 16 Changes in b* of microencapsulated wasabi flavor during storage in various relative humidity at 35 °C during 60 days of storage

No	Treatment	% RH	Storage Time (Days)				
			0	10	20	40	60
1	10% Flavor in H	11	3.56^{d}	3.58^{i}	3.56^{f}	3.69^{g}	3.69^{h}
		33	3.56^{d}	3.69^{j}	3.84^{h}	4.10^{i}	4.10^{k}
		52	3.56^{d}	3.86^{l}	4.07^{i}	4.61^{l}	4.57^{n}
2	15% Flavor in H	11	3.65^{e}	3.41^{h}	4.28^{j}	3.67^{g}	3.67^{h}
		33	3.65^{e}	3.57^{i}	4.37^{k}	4.08^{i}	4.13^{k}
		52	3.65^{e}	3.89^{m}	4.38^{k}	4.75^{m}	4.63^{o}
3	20% Flavor in H	11	3.98^{f}	4.03^{n}	4.56^{m}	3.92^{h}	3.92^{j}
		33	3.98^{f}	3.77^{k}	4.50^{l}	4.21^{j}	4.33^{m}
		52	3.98^{f}	4.00^{n}	4.94^{n}	4.57^{k}	4.27^{l}
4	10% Flavor in H + C	11	13.99^{i}	13.61^{t}	13.52^{t}	13.57^{s}	13.57^{t}
		33	13.99^{i}	13.89^{u}	13.90^{u}	13.85^{r}	14.44^{v}
		52	13.99^{i}	13.97^{v}	13.96^{v}	13.77^{u}	14.53^{w}
5	15% Flavor in H + C	11	12.95^{g}	12.77^{o}	12.67^{o}	12.81^{o}	12.81^{q}
		33	12.95^{g}	12.89^{q}	12.91^{p}	12.98^{p}	13.21^{r}
		52	12.95^{g}	12.93^{r}	13.33^{r}	13.35^{r}	13.50^{s}
6	20% Flavor in H + C	11	13.33^{h}	12.81^{p}	13.23^{q}	12.49^{n}	12.49^{p}
		33	13.33^{h}	13.13^{s}	12.94^{p}	13.15^{q}	13.56^{t}
		52	13.33^{h}	12.90^{qr}	13.48^{s}	13.66^{t}	13.82^{u}
7	10% Flavor in H + MD	11	3.40^{c}	3.26^{f}	3.20^{c}	3.35^{d}	3.35^{d}
		33	3.40^{c}	3.42^{h}	3.69^{g}	3.46^{f}	3.56^{g}
		52	3.40^{c}	3.37^{g}	3.66^{g}	3.69^{g}	3.71^{i}
8	15% Flavor in H + MD	11	2.82^{a}	3.02^{c}	3.11^{b}	3.12^{b}	3.12^{b}
		33	2.82^{a}	2.96^{a}	3.19^{c}	3.14^{b}	3.11^{b}
		52	2.82^{a}	3.11^{e}	3.50^{e}	3.38^{d}	3.51^{f}
9	20% Flavor in H + MD	11	3.04^{b}	3.40^{h}	3.07^{a}	2.99^{a}	2.99^{a}
		33	3.04^{b}	2.99^{b}	3.09^{ab}	3.28^{c}	3.31^{c}
		52	3.04^{b}	3.09^{d}	3.25^{d}	3.42^{e}	3.46^{e}

Note Value are given as mean ± SD from triplicate determinations.

The different letters in the same column indicate the significant differences ($p<0.05$).

2.6 Release characteristic of wasabi flavor from the encapsulated powder

The encapsulation efficiency (EE) is important parameter for encapsulated powder, which is defined as the amount of core material encapsulated inside the powder particles. For the present study (Figure 12-14), the relative humidity had an effect on the EE of AITC; it tended to decrease with increasing relative humidity, especially where only H was used as the wall material and stored at 52% RH (Figure 14-a). The result might be explained by the capsule structure of H being damaged by water uptake because H had adsorbed ambient water so that the release of the entrapped flavoring materials was closely related to the adsorption of water in the wall materials and hydration of the powder occurred. The water then penetrated to the surface wall of the spray-dried particle, after which a crack appeared near the surface of the particle resulting in the subsequent release of the flavor (Yoshii *et al.*, 2001) and a decrease in the EE of AITC. However, the wasabi flavor released more slowly from the blend of H with C and H with MD compared to only using H as the wall material (Figure 14 (b-c)). This implies that C and MD have benefit to support H in preventing flavor release during storage at various relative humidity, especially at high relative humidity. The result was similar to the study of Soottitantawat *et al*., (2005b) who reported that the *l*-menthol was released more slowly from C compared to H at the high humidity condition (74-83 %RH). At the higher RH, the C used as the wall material showed a lower water uptake property than the H and gum arabic resulting the lower release rate of *l*-menthol from C.

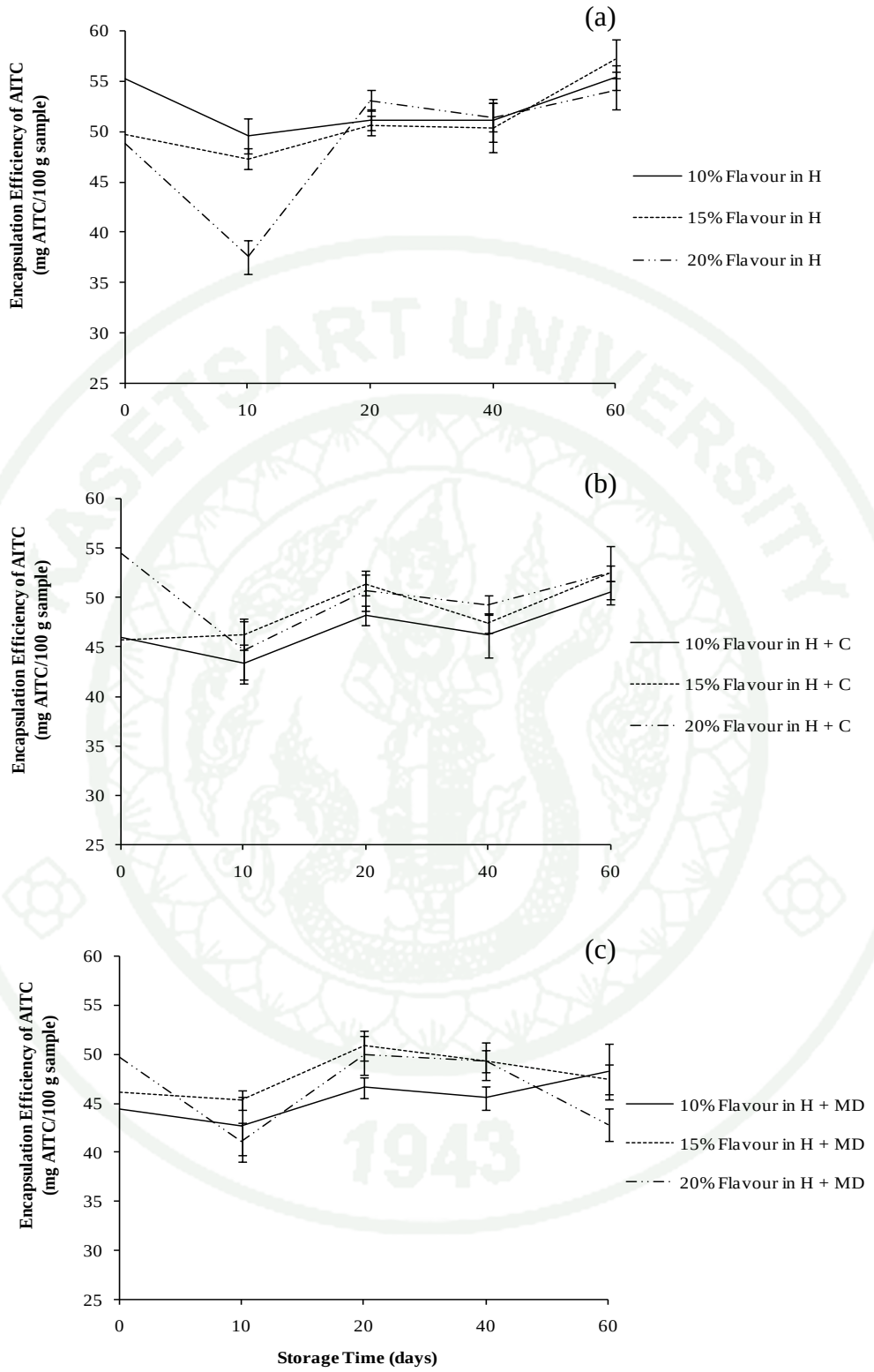


Figure 12 Changes of encapsulation efficiency of AITC in encapsulated powders stored at 11 % RH

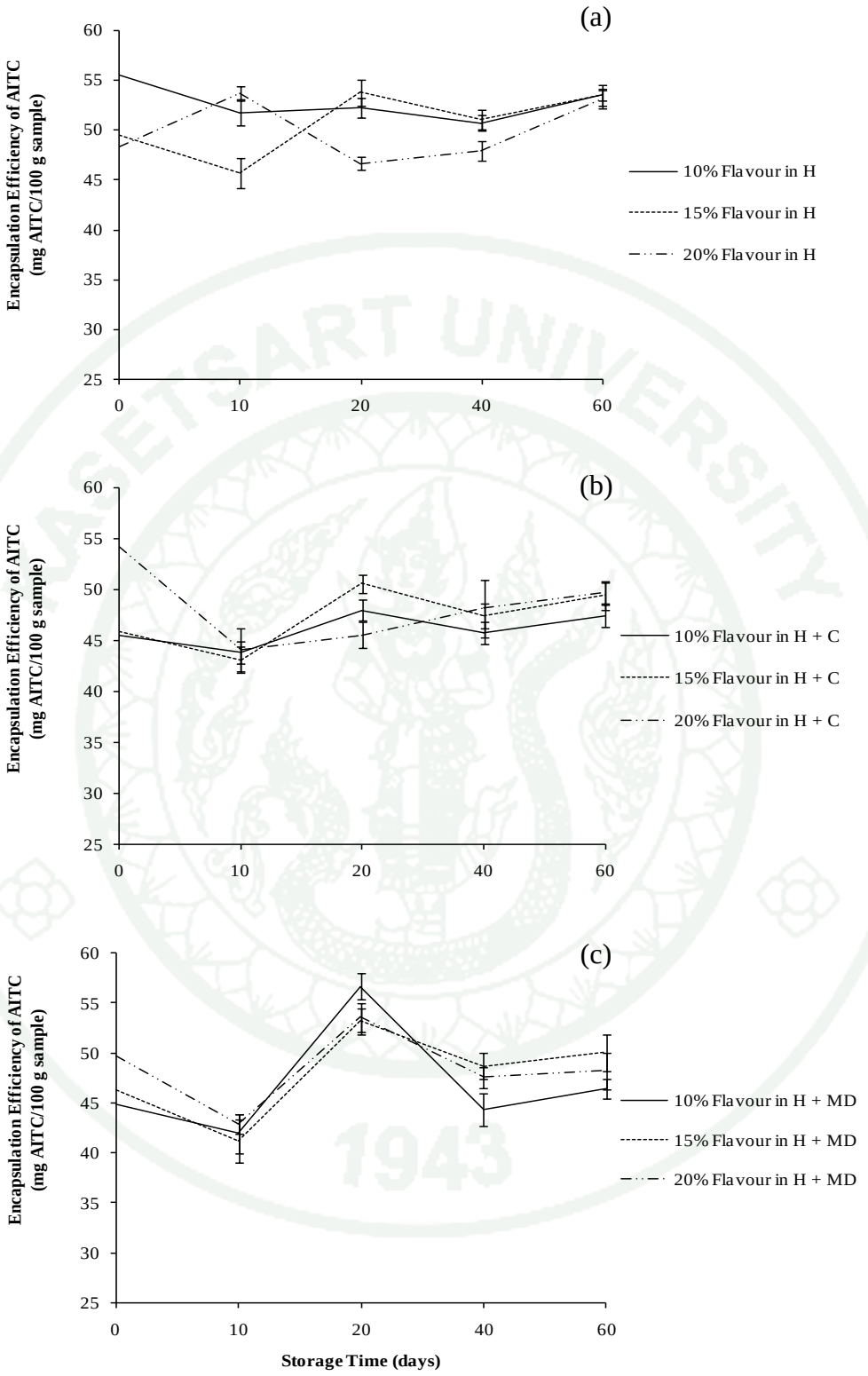


Figure 13 Changes of encapsulation efficiency of AITC in encapsulated powders stored at 33 %RH

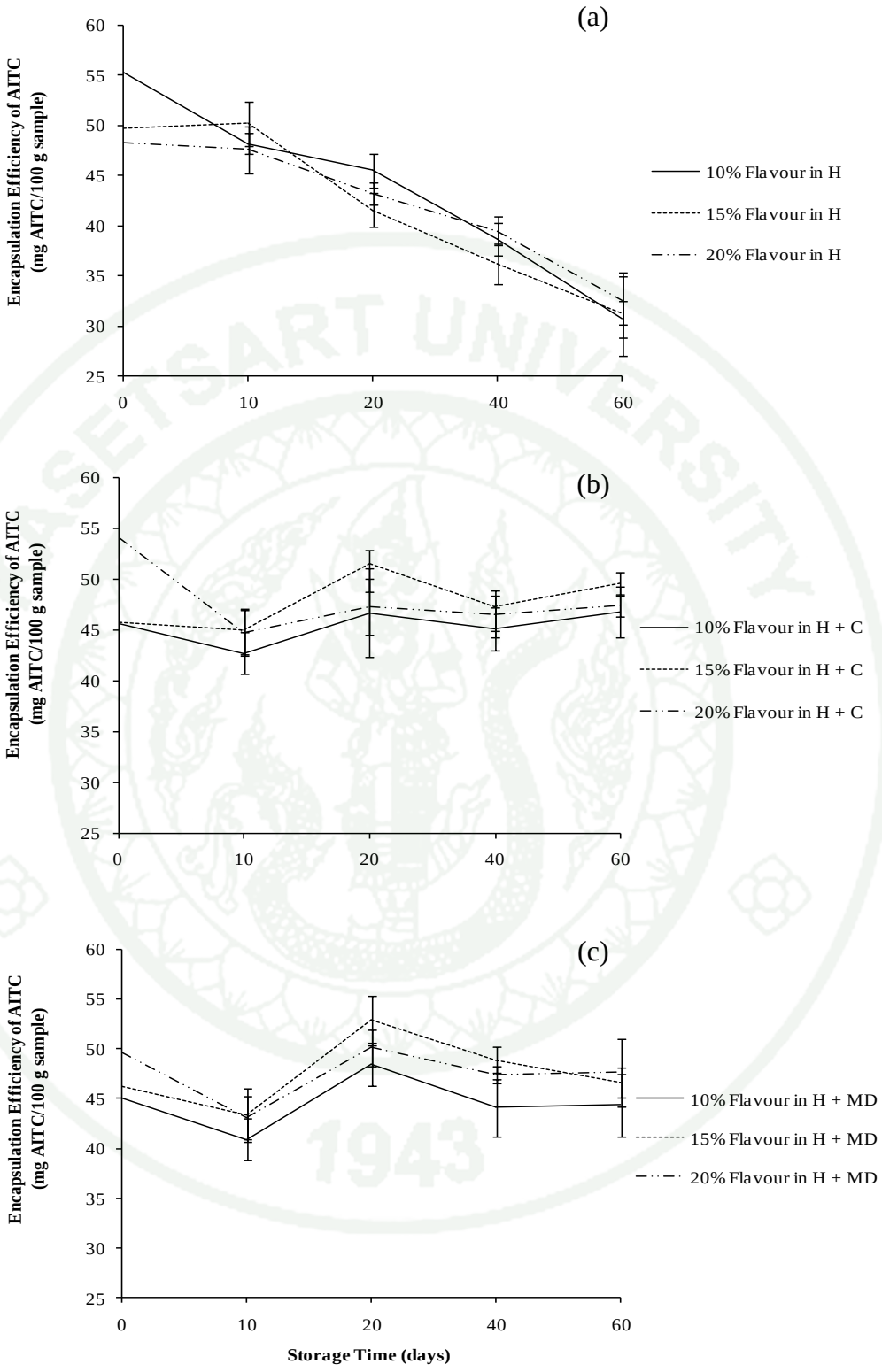
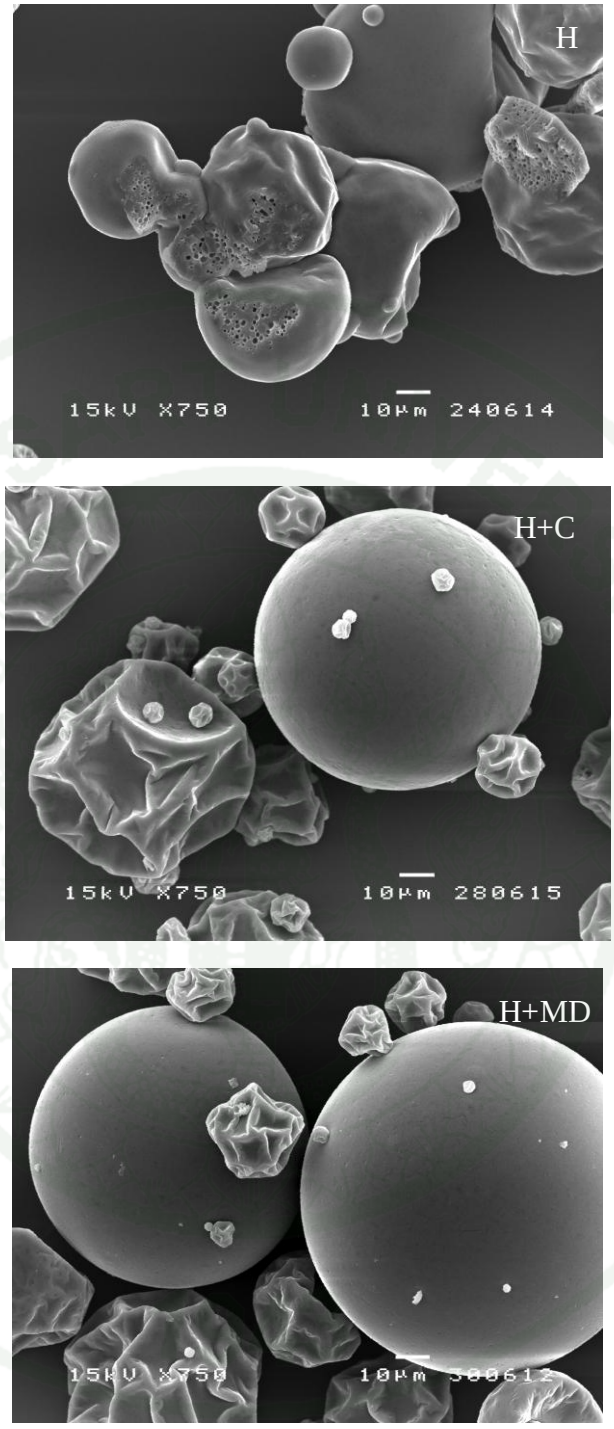


Figure 14 Changes of encapsulation efficiency of AITC in encapsulated powders stored at 52 % RH

2.7 Microstructures of wasabi flavor microcapsules

Microcapsules prepared by spray drying of wasabi flavor (20% w/w) using H, and using a blending of H with C or MD as wall materials after day 60 of storage at the high levels of relative humidity (52% RH) were observed by SEM. The SEM results showed that spray-dried particles of blending of H with C (Figure 15-b) or MD (Figure 15-c) retained their original shape are irregularly spherical-shaped particles with many signs of shrinkage and dents on the surface in general. It has been reported that both shrinkage on the surface of the particles and the expansion in the size of the particles occur particularly with the addition of wall material that decrease the evaporation rate of water from the system due to their water-holding characteristics (Nadeem *et al.*, 2011). Particles showing a rounded external surface with a continuous wall and no apparent fissures or cracks can have lower permeability to flavors, and produce better protection and core retention of flavor (Tonon *et al*., 2008). This result was similar to the study by Rocha *et al.* (2012), who used lycopene encapsulation that showed external surfaces with no fissures, cracks or interruptions, which was considered an essential characteristic to ensure lower flavor permeability, better protection and lycopene retention.



(a)

(b)

(c)

Figure 15 Outer structural characteristics of encapsulated powders made from different wall material; (a) H only, (b) H+C, (C) H+MD of 20% (w/w) wasabi flavor at day 60 of storage under 52 % RH

However, the microcapsules of wasabi flavor using only H (Figure 15-a) as wall material seemed to still be in a glassy to rubbery state, which might also have been a result of the unique polymer structure of H with a DE of 32-37 (Soottitantawat *et al.,* 2005b). This implies that the H contains a high amount of low molecular weight sugar with a low Tg, resulting in an increase in molecular mobility and a decrease in viscosity, which may result in structural changes such as stickiness and collapse. Carolina *et al*., (2007) reported a spray-dried encapsulated flavor (strawberry and orange) at 75% RH was in a rubbery state (Tg was well below room temperature) and collapse was observed with samples appearing as hard and sticky masses with released flavor oil throughout the dense matrix. In addition, outer structural changes of encapsulated d-limonene (a blend of gum arabic with MD as wall material) stored at 50 °C were noted after the relative humidity had increased up to around 75% for one day and the powders began to be rehydrate (Soottitantawat *et al.,* 2004).

3. Application of encapsulated wasabi flavor as food ingredient in canned tuna spread

3.1 Sensory evaluation

The mean difference for wasabi-flavored note retaining in canned tuna spread after 60 days of storage are shown in Table 17. Generally, the intensity of wasabi flavor perceived by panelists corresponds to the amount of AITC constituent. However, after thermal processing, canned tuna spreads with non-encapsulated wasabi flavor added (T2, T5 and T8) were not different from the controls in terms of lacking wasabi-flavor perception. Similarly, the wasabi flavor of canned tuna spreads with encapsulated wasabi flavor added (T3, T4, and T6) was not perceptible. Results from canned tuna spread with encapsulated wasabi flavor added (T7, T9, and T10) indicated significance difference in wasabi-flavor retention as compared to the controls. Even though there was no significant difference in terms of wasabi flavor retention as perceived by the panelists among these three treatments, there was a tendency for wasabi-flavor retention of treatment 7 to be lower than those of the two other treatments (T9 and T10). It should be noted that the wall materials in Treatment

9 and 10 were different but similarly able to significantly retain wasabi flavor to some extent, suggesting that they can be used interchangeably.

Table 17 Mean different intensities of wasabi flavor retention perceived by untrained panelists (n=36) in canned tuna spread

Treatment[a]		Mean Difference intensities[b]	Significance[c]
Sample	Control		
T2	T1	0.44[a]	0.882
T3	T1	0.81[a]	0.279
T4	T1	0.75[a]	0.364
T5	T1	-0.12[a]	1.000
T6	T1	1.12[a]	0.052
T7	T1	1.75[b]	0.000*
T8	T1	0.56[a]	0.684
T9	T1	2.44[b]	0.000*
T10	T1	3.00[b]	0.000*

Note [a] For the meanings of T1-T10, refer to Table 6.

[b] Values with different superscript capital letters within a column are significantly different at $p < 0.05$.

[c] Differences between sample and control within a row are significantly different at $p < 0.05$.

Also the concentration of wasabi flavor at 9% (w/w) seemed to be the minimum concentration for recognized detection. Since AITC is freely soluble in organic solvents but only slightly soluble in water, so that in an emulsion such as mayonnaise, which was used in the present study as the main ingredient for the tuna spread, the flavor molecules tended to be partitioned between the oil and aqueous phase depending on their relative solubility (Depree and Savage, 2001). Therefore, the

results of sensory evaluation strongly support the present study that the wasabi flavor encapsulation process could be used to protect wasabi flavor lost in emulsion foods subjected to a high pressure and temperature process.

4. Analysis of Allyl isothiocyanate in wasabi flavoring agent, microencapsulated wasabi flavor and canned tuna spread

Allyl isothiocyanate is the main component of wasabi and exhibit the most pungent odor (Masuda *et al*., 1996). It has been shown that AITC is unstable under high temperature (Chen and Ho, 1998). When wasabi oil is encapsulation in the different materials it is important to know the changes of AITC during spray drying and especially under thermal processing. For this purpose wasabi oil, spray dried encapsulated and canned tuna spread were analyzed by GC-FID. AITC were the major constituents in wasabi flavoring agent used as initial material for encapsulation.

The composition of wasabi flavoring agent and 20 % wasabi flavoring agent in H+C and H+MD matrixes was quite similar (Figure 16 (a-c)). However, the content of AITC in encapsulated powder was lower than in wasabi flavoring agent (Table 18). The result could be explained by the losses of AITC during encapsulation, not entrapped in the capsules and consequently not protected from evaporation (Baranauskiene *et al*., 2006). The changes obtained during processing were significant.

From the results, the content of AITC of wasabi flavoring agent through thermal processing decreased significantly compared to their content in the non-thermal processing (Table 18). Figure 17 shows that the AITC recovered from sample through thermal processing presents a similar profile when compared to the non-thermal treatment.

The canned tuna spread added 9 % of ingredients of wasabi flavoring agent, 20 % wasabi flavoring agent in H+C and H+MD showed significant differences ($p<0.05$). Canned tuna spread added of 20 % wasabi flavoring agent in H+MD showed the highest concentration of the AITC content compared with 20 % wasabi flavoring

agent in H+C. However, all sample has concentration of AITC more than that the odor threshold that is 0.046 μg AITC/g sample (Masuda *et al*., 1996).

Table 18 Amounts of allyl isothiocyanate (AITC) in wasabi flavoring agent, microencapsulated powder and canned tuna spread with and without sterilization

Treatments	Head space concentration of AITC (μg AITC/g Sample)	
	Non-sterilized	sterilized
Wasabi flavoring agent	340.486 ± 5.00^{b}	302.745 ± 8.67^{a}
Encapsulated powder		
- 20% wasabi flavor in H+C	209.366 ± 2.26^{a}	209.299 ± 5.15^{a}
- 20% wasabi flavor in H+MD	208.448 ± 5.91^{a}	198.391 ± 2.96^{a}
Canned tuna spread (9% of ingredients)		
- wasabi flavoring agent	311.126 ± 8.09^{Aa}	51.211 ± 3.90^{Ab}
- 20% wasabi flavor in H+C	208.364 ± 7.30^{Ba}	76.822 ± 4.79^{Bb}
- 20% wasabi flavor in H+MD	209.001 ± 1.17^{Ba}	100.51 ± 2.73^{Cb}

Note Value are given as mean ± SD from triplicate determinations.

The different lower letters in the same row indicate the significant differences ($p<0.05$).

The different capital letters in the same column indicate the significant differences ($p<0.05$).

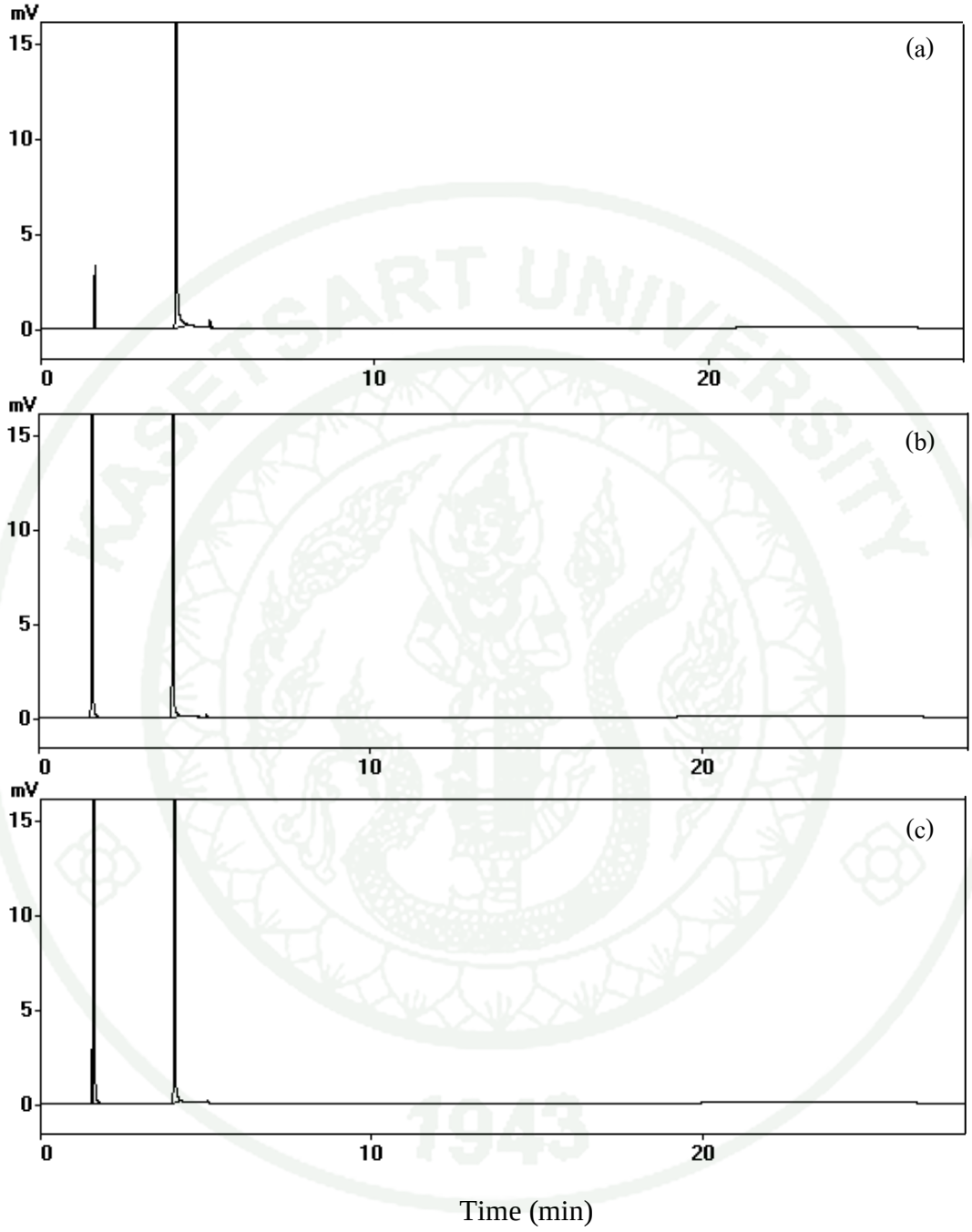


Figure 16 GC-FID chromatogram of allyl isothiocyanate detected in wasabi flavoring agent (a), encapsulated wasabi flavor in blending of H+C (b) and H+MD (c) under non-sterilized condition

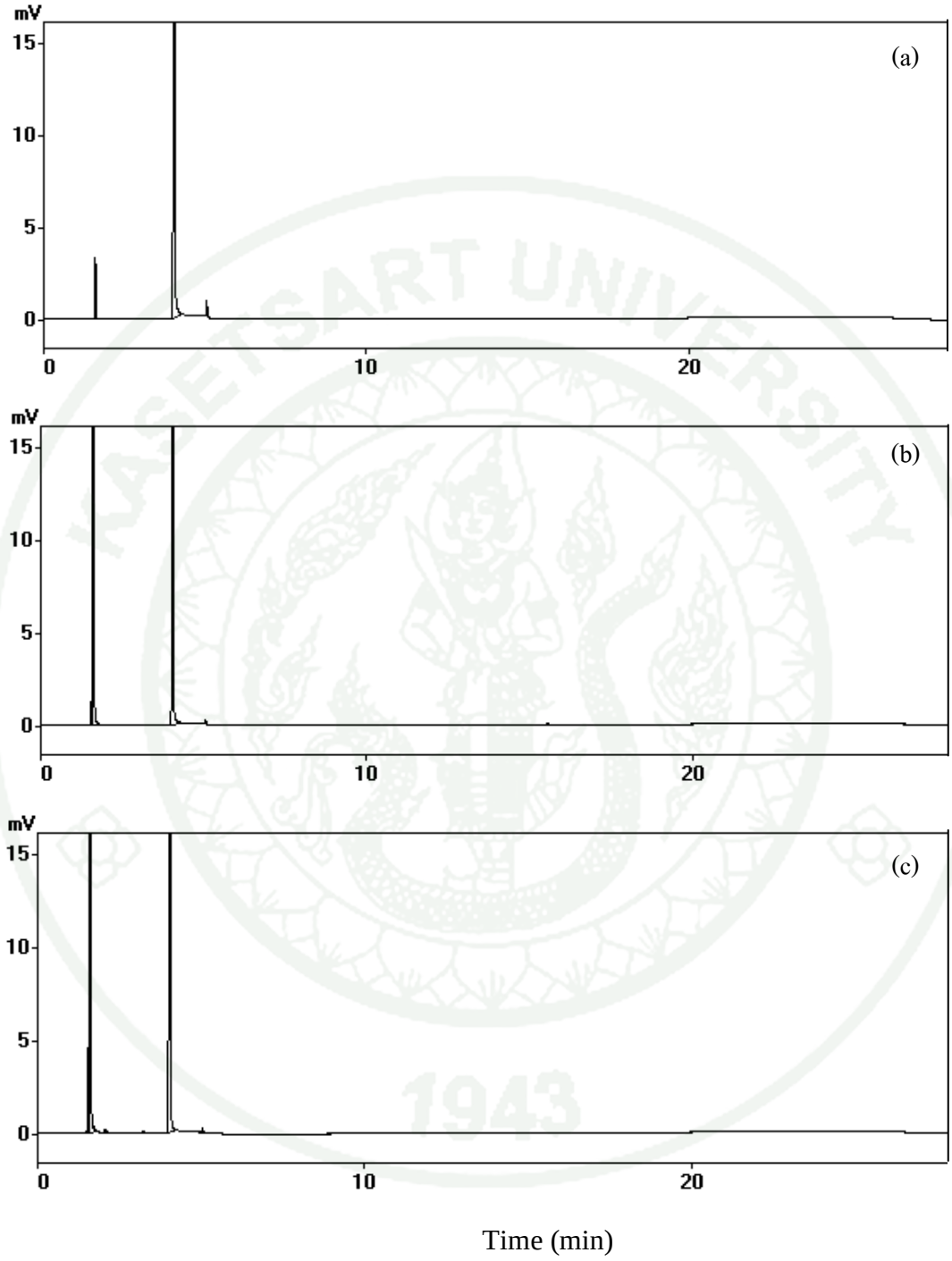


Figure 17 GC-FID chromatogram of allyl isothiocyanate detected in wasabi flavoring agent (a), encapsulated wasabi flavor in blending of H+C (b) and H+MD (c) under sterilized condition

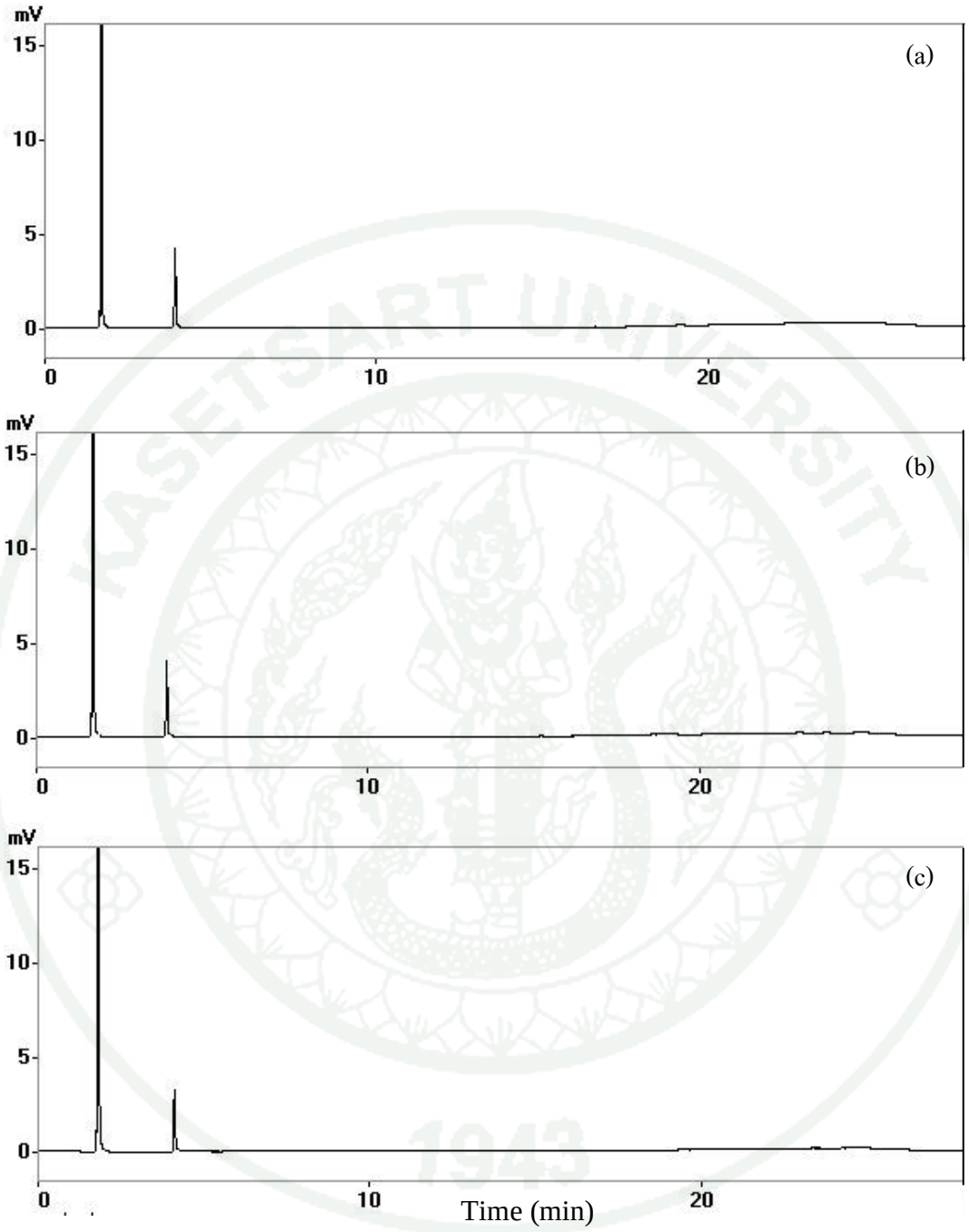


Figure 18 GC-FID chromatogram of allyl isothiocyanate detected in wasabi flavoring agent (a), encapsulated wasabi flavor in blending of H+C (b) and H+MD (c) in tuna spread under non- sterilized condition

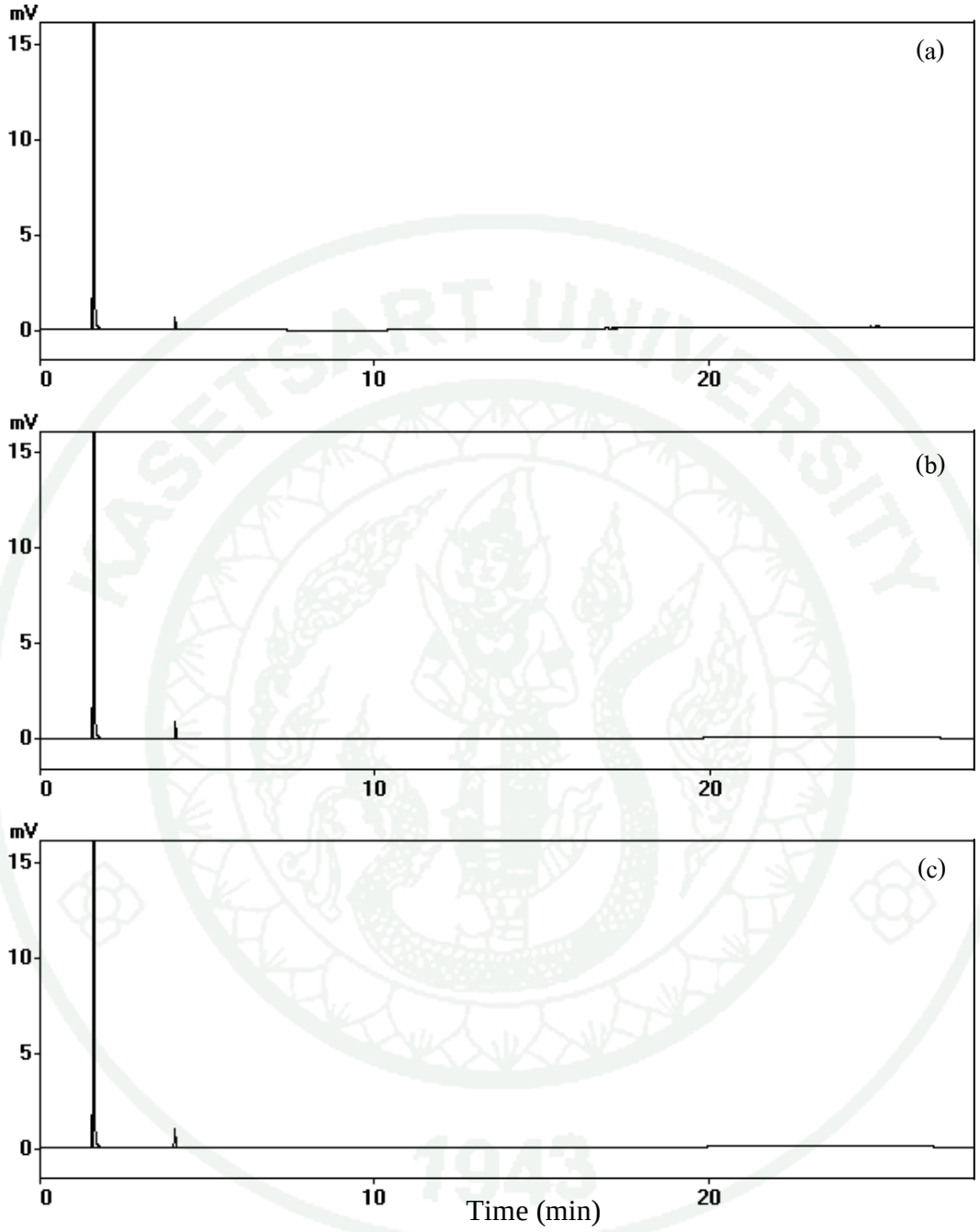


Figure 19 GC-FID chromatogram of allyl isothiocyanate detected in wasabi flavoring agent (a), encapsulated wasabi flavor in blending of H+C (b) and H+MD (c) in sterilized canned tuna spread

CONCLUSION

Samples with 20% (w/w) wasabi flavour in a blend of H with C and a blend of H with MD used as wall materials were chosen for addition to canned tuna spread according to their excellent properties of encapsulation efficiency and good protection of AITC during 60 days of storage at various levels of RH. Sensory evaluation using a difference-from-control test revealed that the retention of wasabi flavour in canned tuna spread containing encapsulated wasabi flavour was higher than that in samples containing wasabi flavour without encapsulation. In particular, adding 9% (w/w) of encapsulated wasabi flavour in the blends of H with MD as wall materials was found to pronounce the highest amount of retained wasabi flavour. The findings showed that the encapsulated wasabi flavour produced under the studied conditions had potential to be used in canned foods to protect degradation of AITC under the thermal process.

LITERATURE CITED

Anker, M. H. and G. A. Reineccius. 1988. Encapsulated orange oil: influence of spray-dryer air temperatures on retention and shelf life, pp. 78-86. *In* S. J. Risch and G. A. Reineccius, eds. **Flavour Encapsulation**. American Chemical Society, Washington, DC.

Anwar, S.H. and B. Kunz. 2011. The influence of drying method on the stabilization of fish oil microencapsules: comparison of spray granulation, spray drying, and freeze drying. **J. Food Eng**. 105: 367-378.

Bansode, S. S., S. K. Banarjee, D. D. Gaikwad, S. L. Jadhav and R. M. Thorat. 2010. Microencapsulation: a review. **Int. J. Pharma. Sci. Rev. Res**. 1: 38-43.

Baranauskiene, R., P. R. Venskutonis, K. Dewettinck and R. Verhé. 2006. Properties of oregano (*Origanum vulgare* L.), citronella (*Cymbopogon nardus* G.) and marjoram (*Majorana hortensis* L.) flavors encapsulated into milk protein-based matrices. **Food Res. Int**. 39: 413-425.

Becher, R. D. and E. U. Schlunder. 1998. Fluidized bed granulation-the importance of a drying zone for the particle growth mechanism. **Chem. Eng. Process**. 37: 1-6.

Bemiller, J. N. and R. L. Whistler. 1996. Carbohydrates, pp. 157-224. *In* O. R. Fennema, ed. **Food chemistry**. Marcel Dekker, New York.

Beristain, C. I., H. S. Garcia and E. J. Vernon-Carter. 2001. Spray-dried encapsulation of cardamom (*Elettaria cardamomum*) essential oil with Mesquite (*Prosopis juliflora*) gum. **Lebensm. Wiss. u.-Technol**. 34: 398-401.

Bhandari, B. R., H. M. J. Dumoulin, H. M. J. Richard, I. Noleau and A. M. Lebert. 1992. Flavor encapsulation by spray drying: application to citral and linalyl acetate. **J. Food Sci**. 57: 217-221.

Bhandari, B.R. and T. Howes. 1999. Implication of glass transition for the drying and stability of dried foods. **J. Food Eng**. 40: 71-79.

Cano-chauca, M., P. C. Stringheta, A. M. Ramos and J. Cal-vidal. 2005. Effect of the carriers on the microstructure of mango powder obtained by spray drying and its functional characterization. **Innov. Food Sci. Emerg. Technol**. 6: 420-428.

Carolina, B.C., S. Carolina, M. C. Zamora and C. Jorge. 2007. Glass transition temperatures and some physical and sensory changes in storage spray-dried encapsulated flavors. **LWT-Food Sci. Tech**. 40: 1792-1797.

Chadwick, C. I., T. A. Lumpkin and L. R. Elberson. 1993. The botany, used, and production of *Wasabia japonica* (Miq) (Cruciferae) Mastsum. **Econ. Bot**. 47: 113-135.

Chen, C. W. and C. T. Ho. 1998. Thermal degradation of allyl isothiocyanate in aqueous solution. **J. Agric. Food. Chem**. 46: 220-223.

Cochran, W.G. and G. M. Cox. 1957. **Experimental Design**, pp. 439-469. John Wiley and Sons, New York.

Depree, J.A. and G. P. Savage. 2001. Physical and flavor stability of mayonnaise. **Trends Food Sci. Tech**. 12: 157-163.

Depree, J.A., T. M. Howard and G. P. Savage. 1999. Flavour and pharmaceutical properties of the volatile sulphur compounds of wasabi (*Wasabia japonica*). **Food Res. Int**. 31: 329-337.

Drusch, S., Y. Serfert, A. V. Heuvel and K. Schwartz. 2006. Physicochemical characterization and oxidative stability of fish oil encapsulated in an amorphous matrix containing trehalose. **Food Res. Int**. 39: 807-815.

Etoh, H., A. Nishimura, R. Takasawa, R. Yagi, K. Saito, K. Sakata, I. Kishima and K. Ina. 1990. ω - methylsulphinylalkyl isothiocyanates in wasabi, *Wasabia japonica* Matsum. **Agric. Bio. Chem**. 54: 1587-1589.

Fahey, J. W., A. T. Zalcamann and P. Talalay. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates amoung planis. **Phytochemistry** 56: 5-51.

Fenwick, R., R. K. Heaney and W. J. Mullin. 1983. Glucosinolates and their breakdown products in food and food plants. **Crit. Rev. Food Sci**. 18: 123-201.

Frascareli, E. C., V. M. Silva, R. V. Tonon and M. D. Hubinger. 2012. Effect of process conditions on the microencapsulation of coffee oil by spray drying. **Food Bioprod. Process**. 90: 413-424.

Fuchs, M., C. Turchiuli, M. Bohin, M. E. Cuvelier, C. Ordonnaud. M. N. Peyrat-Maillard and E. Dumoulin. 2006. Encapsulation of oil in powder using spray drying and fluidized bed agglomeration. **J. Food Eng**. 75: 27-35.

Gharsallaoui, A., G. Roudaut, L. Beney, O. Chambin, A. Voilley and R. Saurel. 2011. Properties of spray-dried food flavours microencapsulated with two-layered membranes: roles of interfacial interactions and water. **Food Chem**. 132: 1713-1720.

Gharsallaoui, A., G. Roudaut, O. Chambin, A. Voilley and R. Saurel. 2007. Application of spray-drying in microencapsulation of food ingredients: an overview. **Food Res. Int**. 40: 1107-1121.

Gibb, B. F., S. Kermasha, I. Alli and C. N. Mulligan. 1999. Encapsulation in the food industry. **Int. J. Food Sci. Nutri**. 50: 213-224.

Gibbs, B. F., S. Kermasha, I. Alli and C. N. Mulligran. 1999a. Pressure and heat-induced gelation of mixed beta-lactoglobulin/polysaccharide solution: scanning electron microscopy of gel. **Food Hydrocolloid**. 13: 339-351.

Gibbs, B. F., S. Kermasha, I. Alli and C. N. Mulligran. 1999b. Encapsulation in the food industry. **Int. J. Food Sci. Nutrit**. 50: 213-224.

Gouin, S. 2004. Microencapsulation: industrial appraisal of existing technologies and trends. **Trends Food Sci. Tech**. 15: 330-347.

Guichard, E. 2006. Flavour retention and release from protein solutions. **J. Bio. Tech. Adv**. 24: 226-229.

Hogan, S. A., B. F. McNamee, E. D. O'Riordan and M. O'Sullivan. 2001. Microencapsulation properties of sodium caseinate. **J. Agric. Food Chem**. 49: 1943-1938.

Jackson, L.S. and K. Lee. 1991. Microencapsulation and food industry. **LWT-Food Sci. Tech**. 24: 89-297.

Jafari, S.M., E. Assadpoor, B. Bhandari and Y. He. 2008. Nano particle encapsulation of fish oil by spray drying. **Food Res. Int**. 41: 172-183.

Kenyon, M. M. 1995. Modified starch, maltodextrin, and corn syrup solids as wall materials for food encapsulation, pp. 42-50. *In* S. J. Risch and G. A.Reineccius, eds. **Encapsulation and Controlled Release of Food Ingredients**. American Chemical Society, Washington, DC.

Kenyon, M. M. and R. J. Anderson. 1988. Maltodextrins and low-dextrose-equivalence corn syrup solid: production and technology for the flavor industry, pp. 7-11. *In* S. J. Risch and G. A. Reineccius, eds. **Flavor Encapsulation**. American Chemical Society, Washington, DC.

Kim, y.d., C. V. Morr and T. W. Schenz. 1996. Microencapsulation properties of gum arabic and several food proteins: liquid orange oil emulsion particles. **J. Agric. Food Chem**. 44: 1308-1313.

King, A. H. 1995. Encapsulation of food ingredients: an review of available technology, focusing on hydrocolloids, pp. 26-39. *In* S. J. Risch and G. A. Reineccius, eds. **Encapsulation and Controlled Release of Food Ingredients**. American Chemical Society, Washington, DC.

Klaypradit, W. and Y. W. Huang. 2008. Fish oil encapsulation with chitosan using ultrasonic atomizer. **LWT-Food Sci. Tech**. 41: 1133-1139.

Klinkesorn, U., P. Sophanodora, P. Chinachoti, E. A. Decker and D. J. McClements. 2005. Encapsulation of emulsified tuna oil in two-layered interfacial membranes prepared using electrostatic layer-by-layer deposition. **Food Hydrocolloid**. 19: 1044-1053.

Krishnan, S., R. Bhosale and R. S. Singhal. 2005. Microencapsulation of cardamom oleoresin: evaluation of blends of gum arabic, maltodextrin and a modified starch as wall materials. **Carbohyd. Polym**. 61: 95-102.

Lakkis, J. M. 2007. Introduction, pp. 1-11. *In* J. M. Lakkis, ed. **Encapsulation and Controlled Release Technologies in Food Systems**. Blackwell Publishing, Iowa.

Laohasongkram, K., T. Mahamaktudsanee and S. Chaiwanichsiri. 2011. Microencapsulation of macadamia oil by spray drying. **Procedia Food Sci**. 1: 1660-1665.

Li, X., Z. Jin and J. Wang. 2007. Complexation of allyl isothiocyanate by α- and β -cyclodextrin and its controlled release characteristics. **Food Chem**. 103: 461-466.

Lindsay, R. C. 1996. Flavors, pp. 724-765. *In* O. R. Fennema, ed. **Food Chemistry**. Marcel Dekker, New York.

Loksuwan, J. 2007. Characteristics of microencapsulation β-caratene formed by spray drying with modified tapioca starch, native tapioca starch and maltodextrin. **Food Hydrocolloid**. 21: 928-935.

Madene, A., M. Jacquot, J. Scher and S. Desobry. 2006. Flavour encapsulation and controlled release-a review. **Int. J. Food Sci. and Tech**. 41: 1-21.

Masuda, H., Y. Harada, K. Tanaka, M. Nakajima and H. Tabeta. 1996. Characteristic odorants of wasabi (*Wasabia japonica* Mutum), Japanese horseradish, in comparison with those of horseradish (*Armoracia rusticana*), pp. 67-78. *In* G. R. Takeoka, R. Teranishi, P. J. Williams and A. Kobayashi, eds. **Biotechnology for Improved Foods and Flavours**. American Chemical Society, Washington, DC.

Meilgaard, M., G. V. Civille and B. T. Carr. 2007. **Sensory Evaluation Technique**, 4th ed. pp. 92-100. CRC Press, Boca Raton.

Munin, A. and F. Edwards- Lévy. 2011. Encapsulation of natural polyphenolic compounds; a review. **J. Pharm**. 3: 793-829.

Nadeem, H.S., M. Torun and F. Özdemir. 2011. Spray drying of the mountain tea (*Sideritis stricta*) water extract by using different hydrocolloid wall materials. **LWT-Food Sci. Tech**. 44: 1626-1635.

Ortiz, S. E. M., A. Mauri, E. S. Monterrey-Quintero, M. A. Trindade, A. S. Santana and C. S. Favaro-Trindade. 2009. Production and properties of casein hydrolysate microencapsulated by spray drying with soybean protein isolate. **LWT-Food Sci. Tech**. 42: 919-923.

Pegg, R. B. and Shahidi, F. 1999. Encapsulation and controlled release in food preservation, pp. 611-667. *In* M. S. Rahman, ed. **Handbook of Food Preservation.** Marcel Dekker, New York.

Pothakamury, U. R and G. V. Barbosa-Cánovas. 1995. Fundamental aspects of controlled release in foods. **Trends Food Sci. Tech**. 6: 397-406.

Quek, S.Y., N. K. Chok and P. Swedlind. 2007. The physicochemical properties of spray-dried watermelon powders. **Chem. Eng. Process**. 46: 386-392.

Quispe-Condori, S., M. D. A. Saldana and F. Temelli. 2011. Microencapsulation of flax oil with zein using spray and freeze drying. **LWT-Food Sci. Tech**. 44: 1880-1887.

Rahman, M. S. and T. P. Labuza. 1999. Water activity and food preservation, pp. 339-382. *In* M. S. Rahman, ed. **Handbook of Food Preservation**. Marcel Dekker, New York.

Reineccius, G. A. 1988. Spray drying of food flavors, pp. 55-66. *In* S. J. Risch and G. A. Reineccius, eds. **Flavour Encapsulation**. American Chemical Society, Washington, DC.

Risch, S. J. 1995. Encapsulation: overview of used and techniques, pp. 2-7. *In* S. J. Risch and G. A. Reineccius, eds. **Encapsulation and Controlled Release of Food Ingredients**. American Chemical Society, Washington, DC.

Rocha, G.A., C. S. Fávaro-Trindate and C. R. F. Grosso. 2012. Microencapsulation of lycopene by spray drying: characterization, stability and application of microcapsules. **Food Bioprod. Process**. 90: 37-42.

Rosenberg, M., I. J. Kopelman and Y. Talmon. 1990. Factors affecting retention in spray-drying microencapsulation of volatile materials. **J. Agr. Food Chem**. 38: 1288-1294.

Sansone, F., T. Mencherini, P. Picerno, M. Amore, R. P. Aquino and M. R. Lauro. 2011. Maltodextrin/pectin microparticles by spray drying as wall material for nutraceutical extracts. **J. Food Eng**. 105: 468-476.

Shahidi, F. and X. Q. Han. 1993. Encapsulation of food ingredients. **Crit. Rev. Food Sci**. 33: 501-547.

Shaifh, J., R. Bhosale and R. Singhal. 2006. Microencapsulation of black pepper oleoresin. **Food Chem**. 94: 105-110.

Sheu, T. Y. and M. Rosenberg. 1998. Microstructure of microcapsules consisting of whey protein and carbohydrate. **J. Food Sci**. 63: 491-494.

Shogren, R. and G. Biresaw. 2007. Surface properties of water soluble maltodextrin, starch acetates and starch acetates/alkenylsuccinates. **Colloid. Surface. A.** 298: 170-176.

Soottitantawat, A., F. Bigeard, H. Yoshii, T. Furuta, M. Ohkawara and P. Linko. 2005a. Influence of emulsion and powder size on the stability of encapsulated D-limonene by spray drying. **Innov. Food Sci. Emerg. Technol**. 6: 107-114.

Soottitantawat, A., H. Yoshii, T. Furuta, M. Ohkawara and P. Linko. 2003. Microencapsulation by spray drying: influence of emulsion size on the retention of volatile compounds. **J. Food Sci**. 68: 2256-2262.

Soottitantawat, A., H. Yoshii, T. Furuta, M. Ohkawara, P. Forssell, R. Partanene, K. Poutanen and P. Linko. 2004. Effect of water activity on the release characteristics and oxidative stability of D-limonene encapsulated by spray drying. **J. Agr. Food Chem**. 52: 1269-1276.

Soottitantawat, A., K. Takayama, K. Okamura, D. Muranaka, H. Yoshii, T. Furuta, M. Ohkawara and P. Linko. 2005b. Microencapsulation of l-menthol by spray drying and its release characteristics. **Innov. Food Sci. Emerg. Technol**. 6: 163-170.

Spada, J.C., L. D. F. Marczak, I. C. Tessaro and C. P. Z. Norena. 2012. Microencapsulation of β-carotene using, native pinho starch, modified pinhão starch and gelatin by freeze-drying. **Int. J. Food Sci. Technol**. 4: 186-194.

Sultana, T. and G. P. Savage. 2008. Wasabi-Japanese Horseradish. **Bangladesh Journal of Scientific & Industrial Research** 43: 433-448.

Sultana, T., D. L. Mcneil, N. G. Porter and G. P. Savage. 2003a. Investigation of isothiocyanate yield from flowering and non-flowering tissues of wasabi grown in a flooded system. **Journal of Food Composition and Analysis** 16: 637-646.

Sultana, T., N. G. Porter, G. P. Savage and D. L. Mcneil. 2003b. Comparison of isothiocyanate yield from wasabi rhizome tissues grown in soil or water. **J. Agric. Food Chem**. 51: 3589-3591.

Thai Frozen Food Association. 2011. Crisis of canned tuna industry: an issue to keep an eye on. **TFFA Newsletter** January-March: 26-29.

Tonon, R.V., C. Brabet and M. D. Hubinger. 2008. Influence of process conditions on the physicochemical properties of acai (*Euterpe oleraceae* Mart.) powder produced by spray drying. **J. Food Eng**. 88: 411-418.

Tonon, R.V., C. R. F. Grosso and M. D. Hubinger. 2011. Influence of emulsion composition and inlet air temperature on the microencapsulation of flaxseed oil by spray drying. **Food Res. Int**. 44: 282-289.

Trubiano, P. C. and N. L. Lacourse. 1988. Emulsion-stabilizing starches: use in flavor encapsulation, pp. 45-54. *In* S. J. Risch and G. A. Reineccius, eds. **Flavor Encapsulation**. American Chemical Society, Washington, DC.

Turchiuli, C., M. Fuchs, M. Bohin, M. E. Cuvelier, C. Ordonnaud, M. N. Peyrat-Maillard and E. Dumoulin. 2005. Oil encapsulation by spray drying and fluidized bed agglomeration. **Innov. Food Sci. Emerg. Technol**. 6: 29-35.

Vega, C. and Y. H. Roos. 2006. Invited review: spray-dried dairy and dairy-like emulsions-compositional considerations. **J. Dairy Sci**. 89: 383-401.

Versic, R. J. 1988. Flavor encapsulation: an overview, pp. 1-6. *In* S. J. Risch and G. A. Reineccius, eds. **Flavour Encapsulation**. American Chemical Society, Washington, DC.

Wandrey, C., A. Bartkowiak and S. E. Harding. 2010. Materials for Encapsulation, pp. 31-100. *In* N. J. Zuidam and V. A. Nedovié, eds. **Encapsulation Technologies for Active Food Ingredients and Food Processing**. Springer, London.

Whorton, C. 1995. Factor influencing volatile release from encapsulation matrices, pp. 134-142. *In* S. J. Risch and G. A. Reineccius, eds. **Encapsulation and Controlled Release of Food Ingredients**. American Chemical Society, Washington, DC.

Wilson, N. and N. P. Shan. 2007. Review paper: microencapsulation of vitamins. **ASEAN Food Journal** 14: 1-14.

Yoshii, H., A. Soottitantawat, X. D. Liu, T. Atarashi, T. Furuta, S. Aishima, M. Ohgawara and P. Linko. 2001. Flavor release from spray-dried maltodextrin/gum arabic or soy matrices as a function of storage relative humidity. **Innov. Food Sci. Emerg. Technol**. 2: 55-61.

Youngs, R.A. 1986. Spray drying encapsulation-Today's view. **Food Flavor Ingredients Processing and Packaging** January: 31-36.

Zhang, Q.F., Z. Y. Jiang and R. Li. 2007. Complexation of allyl isothiocyanate with β–cycrodextrin and its derivatives and molecular microcapsule of allyl isothiocyanate in β–cycrodextrin. **Eur. Food Res. Technol**. 225: 407-413.

Zhang, Z., D. Law and G. Lian. 2010. Characterization method of encapsulates. pp.101-125. *In* N. J. Zuidam and V. A. Nedovic, eds. **Encapsulation Technologies for Active Food Ingredients and Food Processing**. Springer, New York.

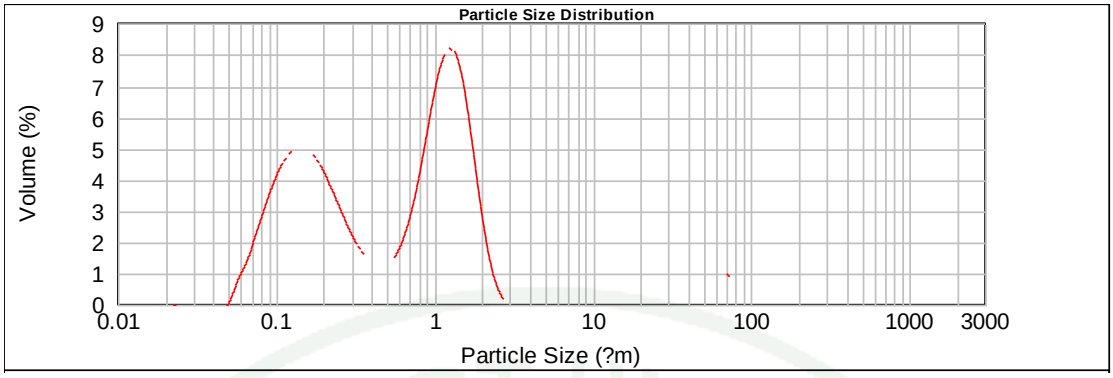
Zhang, Z.Q., C. H. Pan and D. Chung. 2011. Tannic acid cross-linked gelatin-gum Arabic coacervate microspheres for sustained release of allyl isothiocyanate: Characterization and in vitro release study. **Food Res. Int**. 44: 1000-1007.

Zuidam, N. J. and E. Shimoni. 2010. Overview of microencapsulates for use in food products or processes and methods to make them, pp. 3-30. *In* N. J. Zuidam and V. A. Nedovié, eds. **Encapsulation Technologies for Active Food Ingredients and Food Processing**. Springer, London.

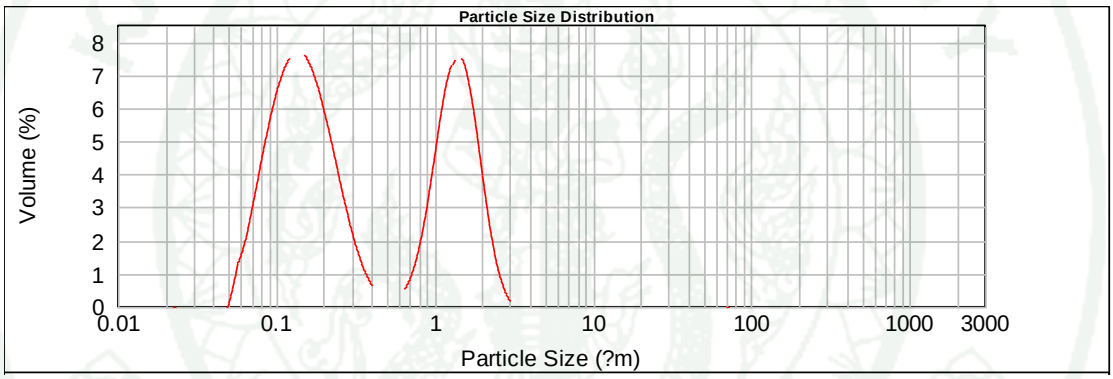
APPENDICES

Appendix A

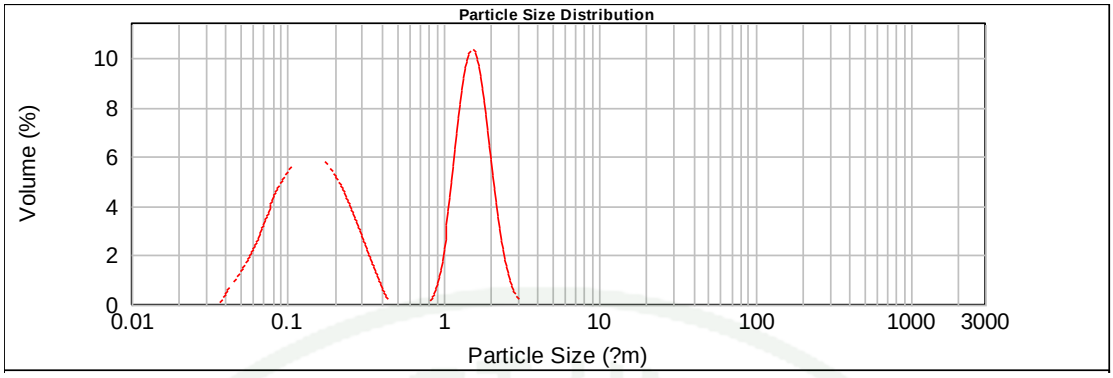
Particle Size Distribution of Emulsion



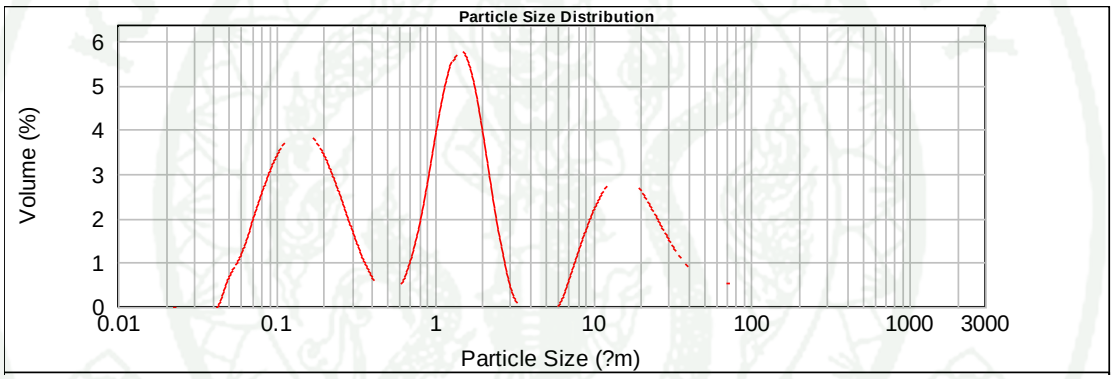
Appendix Figure 1 Particle size distribution of emulsion droplet prepared from 10 % wasabi flavor in H



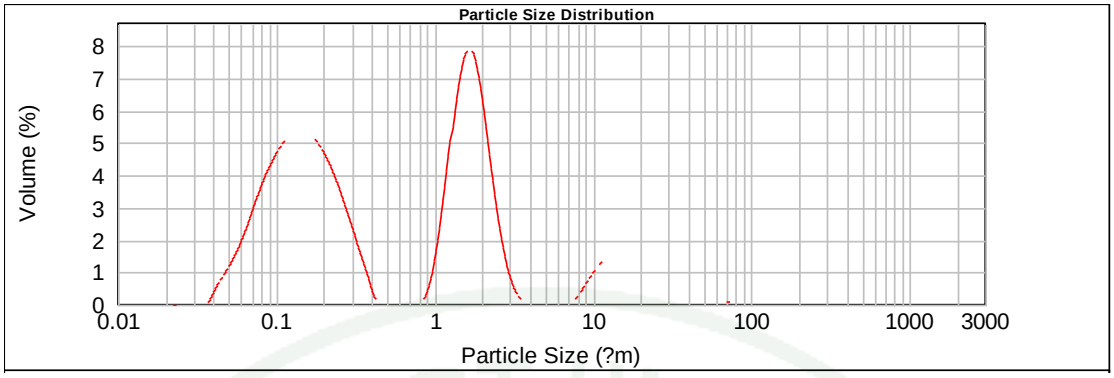
Appendix Figure 2 Particle size distribution of emulsion droplet prepared from 15 % wasabi flavor in H



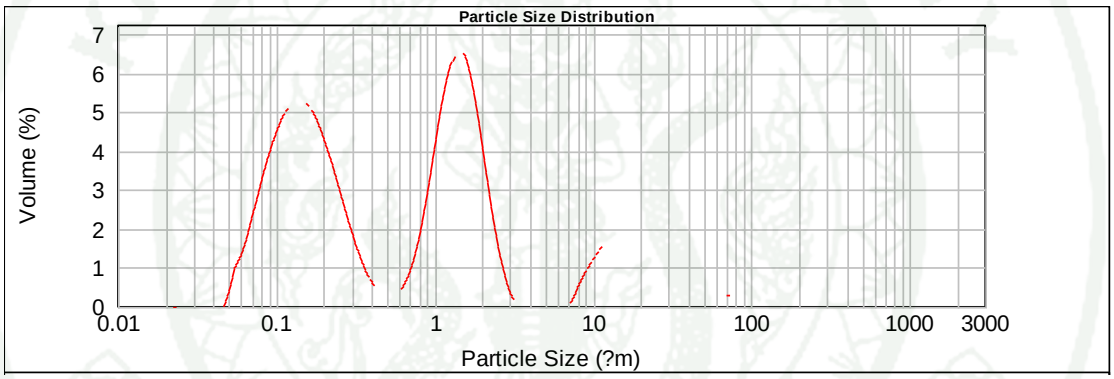
Appendix Figure 3 Particle size distribution of emulsion droplet prepared from 20 % wasabi flavor in H



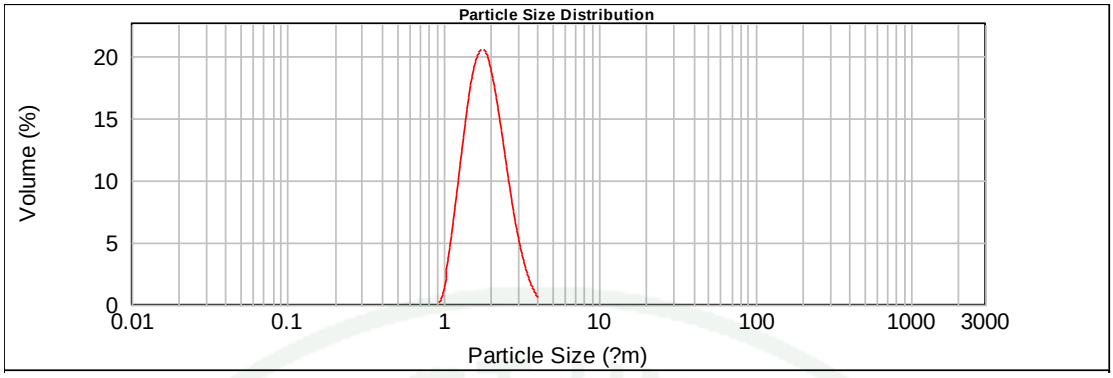
Appendix Figure 4 Particle size distribution of emulsion droplet prepared from 10 % wasabi flavor in blending of H with C



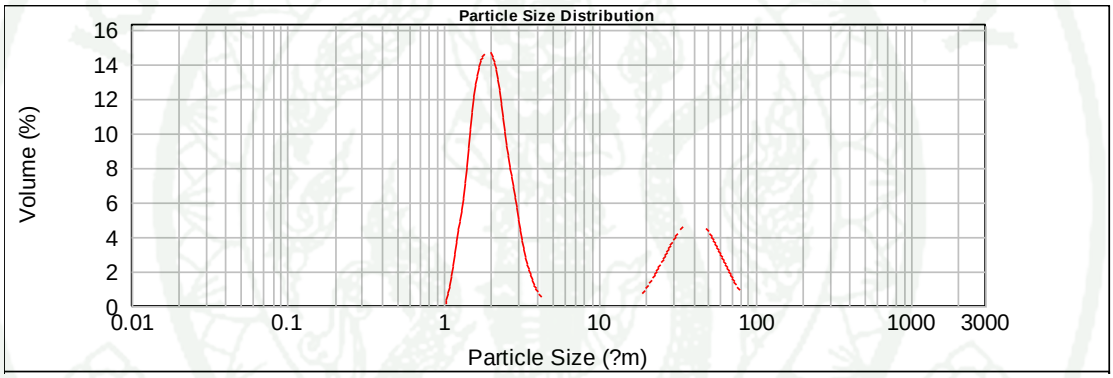
Appendix Figure 5 Particle size distribution of emulsion droplet prepared from 15 % wasabi flavor in blending of H with C



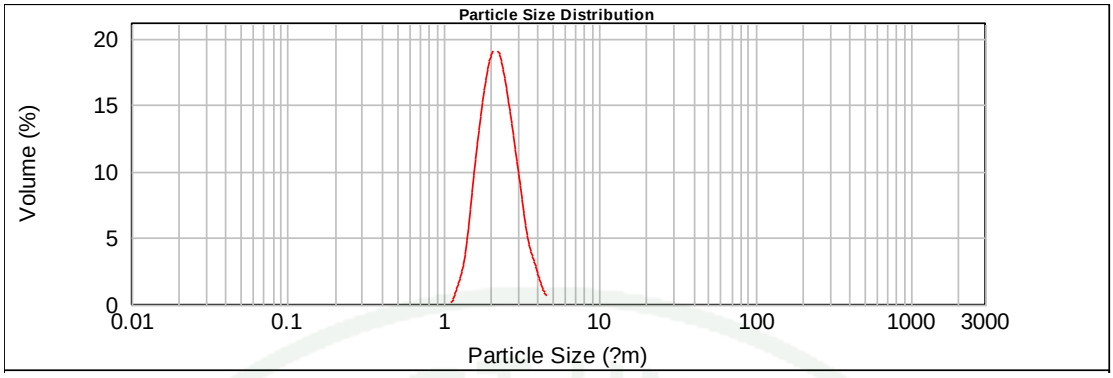
Appendix Figure 6 Particle size distribution of emulsion droplet prepared from 20 % wasabi flavor in blending of H with C



Appendix Figure 7 Particle size distribution of emulsion droplet prepared from 10 % wasabi flavor in blending of H with MD



Appendix Figure 8 Particle size distribution of emulsion droplet prepared from 15 % wasabi flavor in blending of H with MD



Appendix Figure 9 Particle size distribution of emulsion droplet prepared from 20 % wasabi flavor in blending of H with MD

Appendix B

Questionnaire; Different from control test

ชุดที่.....

แบบสอบถาม

Difference – from – control test

วันที่.....

ชนิดตัวอย่าง.....ชื่อผู้ทดสอบ.....

คำแนะนำ : กรุณาทดสอบตัวอย่าง “C” ก่อนเพื่อทำความคุ้นเคยกับคุณลักษณะด้านกลิ่นรสของวาซาบิในทูน่าสเปรดซึ่งกำหนดให้เท่ากับ “0” บนสเกล แล้วทดสอบตัวอย่างที่มีรหัส จากนั้นบอกความแตกต่างว่ามากกว่า (+) หรือน้อยกว่า (-) ตัวอย่าง “control” โดยขีด “X” ตรงช่องสเกล

รหัสตัวอย่าง	ลักษณะ	-5	-4	-3	-2	-1	0	1	2	3	4	5
C	กลิ่นรสของวาซาบิ						X					
.....	กลิ่นรสของวาซาบิ											
.....	กลิ่นรสของวาซาบิ											
.....	กลิ่นรสของวาซาบิ											
.....	กลิ่นรสของวาซาบิ											

ข้อเสนอแนะ

.....

.....

ขอบคุณที่ให้ความร่วมมือ

CURRICULUM VITAE

NAME Miss Phutthiya Ratanasiriwat

BIRTH DATE December 16, 1977

BIRTH PLACE Ubon Ratchathani, Thailand

EDUCATION

YEAR	INSTITUTION	DEGREE
2000	Rajamangla Institute of Technology	B. Sc. (Food Science and Technology)
2003	Prince of Songkla Univ.	M. Sc. (Food Technology)

POSITION Lecturer

WORK PLACE Department of Food Science and Technology, Faculty of Science and Technology, Nakhon Pathom Rajabhat University

SCHOLASHIP Nakhon Pathom Rajabhat University, Education scholarship. 2007-2012

The Center for Advanced Studies for Agriculture and Food, Institute for Advanced Studies, Kasetsart University. 2009-2010