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THESIS

ENCAPSULATION OF TOCOPHERYL ACETATE USING HYDROLYZED AND HEAT MOISTURE TREATMENT RICE STARCH

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High amylose rice starch was modified using a controlled pyrodextrinization process under conditions designed to avoid gelatinization of starch. Rice starch was hydrolyzed with 5% HCl and 5% citric acid solution at 130 °C for 1, 2 and 3 hours (H1, H2, H3). Hydrolyzed starch was hydro-thermal treated (HMT) at 25% mc 115°C for 1 h. Pasting properties, swelling power, solubility, crystallinity and *in vitro* starch digestibility were investigated. Hydrolyzed-HMT rice starch was then used as wall material for tocopheryl acetate encapsulation by spray drying. The encapsulation efficiency, microstructure, *in vitro* releasing property under simulated gastric (SGF), simulated intestinal fluid (SIF) and *in vivo* blood glucose responses were measured. Results show a decrease in rapid digestible starch (RDS) and slow digestible starch (SDS) but an increase in resistant starch (RS). Blood glucose responses in Wistar rat feed with hydrolyzed-HMT rice starch were much lower than native starch. The X-ray diffraction pattern was remain unchanged. Encapsulation efficiency ranked from high to low as H1 > H2 > H3. The encapsulated microcapsule showed a better resistance to *in vitro* digestibility and control release property in SGF and SIF. Hydrolyzed-HMT rice starch with plasticizers gave lower protection and % core material release either as a wall material or protect against environment. H1 showed the highest % release of tocopheryl acetate under SGF and SIF even after one year storage.

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INTRODUCTION

Encapsulation is extensively used in food industry for shelf-life extension, either for preventing reactions with components in the food system or controlling releasing property to the target system. The principle of this technique is a process to entrap one substance within another. Substances that is entrapped, called "core materials" such as starch, maltodextrin, chitosan. Substances that are used for entrapping core materials called "coating materials" (Jafari *et al.,* 2008). Criterion for an encapsulation wall material by spray drying was emulsion properties, retention of the core material and shelf life. Carbohydrates are commonly used in this process such as starch, gum arabic and cyclodextrins. Hydrolyzed starches produced by hydrolyzing starch with acid and/or enzyme are less viscous at high concentration and has excellent protection (Jafari *et al.,* 2008). But hydrolyzed starch has poor retention behavior as it lacked emulsifying properties. So the combination of wall materials such as a blending of gum, maltodextrin, and modified starch are used to improve this problem (Jafari *et al.,* 2008).

Properties of rice starch could be improved by methods such as heat-moisture treatment, annealing, acid treatment or crosslinking. In this study, acid hydrolyzed of high amylose rice starch followed by HMT was used as a process to modify rice starch properties for encapsulation. Hydrolyzed-HMT was proposed as a modified pyrodextrinization process as HMT was conducted in a condition that was higher than glass transition temperature but below gelatinization temperature. This resulted in a more intact starch granule which still exhibited its typical properties such as swelling, pasting and enzymatic digestibility properties. Acid hydrolysis altogether with HMT was aimed to increase more digestibility resistant starch which could pass through gut system. Hydrolyzed starch in a certain degree produce higher rapid digestible starch (RDS) which could not resist gastric environment. HMT caused starch to be slowly digestible which mostly go down to small and large

intestine. Manipulation of the hydrolyzed HMT starch could help designing appropriate amount of RDS, SDS and RS which could help controlling the target release of bioactive compound according to its digestibility in human gut system.

OBJECTIVES

1. To study the effect of acid hydrolysis followed by HMT on enzymatic digestion starch and its effect on tocopheryl acetate releasing in gut model *in vivo* and *in vitro*.

2. To study the effect of hydrolyzed HMT rice starch as wall material on encapsulation properties and its shelf life.

3. To study the effect of plasticizer addition on encapsulation properties and shelf life.

LITERATURE REVIEW

1. Heat Moisture Treatment (HMT)

Rice starch exhibits limited applications due to low shear stress resistance, thermal decomposition, high retrogradation and syneresis, poor process ability and solubility in common organic solvents. Therefore, the properties of rice starch should be modified for specific application (Neelam e*t al.*, 2012).

Heat moisture treatment refers to a method that treat starch at restricted moisture levels (22 – 27%) and temperature above the onset temperature of gelatinization. HMT altered the structure and physicochemical properties of starch. HMT could decrease amylose leaching, granular swelling and peak viscosity but increase thermal stability, gelatinization temperatures and susceptibility towards amylase and acid hydrolysis. These changes were attributed to factors such as amylose content, interactions between starch chains, arrangement of amylose chains within the amorphous domains and amylose lipid complexes (Neelam e*t al.*, 2012). HMT promoted the interaction of polymer chains by disrupting the crystalline structure and dissociating the double helical structure in the amorphous region, followed by the rearrangement of the disrupted crystals. This disruption facilitated enzymatic access to the sites of interaction between the amylose chains during the rearrangement of the polymer chains (Zavareze and Dias, 2011). Moreover, HMT did not change the size, shape and surface characteristics of rice starch granules (Khunae *et al.*, 2007).

HMT could decrease the amylose leaching which has been attributed to the additional interaction between amylose-amylose and/or amylose-amylopectin chains and increasing in the amount of amylose-lipid complexes chains. During HMT, thermal energy imparted to amylose chain and mobility increased thereby facilitating interaction (Chung *et al*., 2009). Moreover, HMT also could decrease the swelling power and solubility of starches. Starch solubility results from the leaching of amylose, which dissociates from and diffuses out of granules during swelling. This leaching represents a transition from order to disorder within the starch granules that occurs when starch is heated with water (Zavareze

and Dias, 2011). The reduction in swelling power and solubility following HMT has been attributed to the internal rearrangement of the starch granules between starch chains. This caused further interactions between starch and functional groups such as the formation of more ordered double helical amylopectin side chain clusters. The more order could increase crystallinity, strengthened intermolecular bonds, the formation of amylose-lipid complexes (Olayinka *et al.*, 2008; Chung *et al.*, 2009; Varatharajan *et al.*, 2010; Zavareze *et al*., 2010, Zavareze and Dias, 2011; Sun *et al.*, 2014).

HMT significantly altered pasting profile of starches. Pasting temperature was increased which indicated that HMT tended to increase crystalline regions. The reduction in pasting viscosity happened because of the swelling restriction as ever mentioned. Breakdown and setback of HMT starches decreased which indicated shear stability of HMT starches. Retrogradation was reported to decrease as a result of the decreasing in amylose leaching (Zavareze *et al*., 2010; Zavareze and Dias, 2011; Sun *et al.*, 2014).

The increasing of onset temperature, peak temperature and conclusion temperature of gelatinization in HMT starch were occurred due to the formation of amylose-amylose and amylose-lipid complexes (Sun *et al.*, 2014). HMT shifted onset temperature of gelatinization (T_o) which has been attributed to structure changes within the starch granules. The interaction reduced mobility of amylopectin chains causes lowering in swelling power, therefore, high temperature was needed to gelatinize HMT starch. HMT stabilizes amorphous region so the crystallite melting temperature of HMT starches were higher.

HMT did not alter size or shape of potato, taro, yam, cassava and rice starch granules (Zavareze and Dias, 2011). The effect of HMT on crystallinity depended on the source of starch and treatment conditions. HMT changed in the X-ray diffraction pattern of potato and yam starches from B to A type. However, not all temperature and moisture conditions could induce a change in crystallinity pattern. HMT often results in the transformation of the less thermodynamically stable B polymorphic structure (with hexagonal packing of double helices and about 36 water molecules inside each cell) into a more stable monoclinic structure of A- type polymorphs (with about 6 water molecules inside the helices) (Zavareze and Dias, 2011). The relative crystallinity and the ratio of short-range molecular order to

amorphous of HMT treated rice starches decreased with increasing moisture level of the treatment which can be attributed to a loss of crystalline order during treatment. A weak peak at 20° (2θ) represented as V-amylose-lipid complexes crystalline was clearly exhibit in HMT high amylose rice starches (Khunae *et al.,* 2007). Moreover, HMT may provide the formation of new crystallites or recrystallization and perfection of small crystalline regions of the starch granule. During HMT, partial gelatinization and double helical movement were occurred which disrupted starch crystallites and/or changed crystallite orientation. The changes of crystallinity depend on moisture levels of HMT. Sorghum starch HMT at 20% moisture content has higher %crystallinity than native sorghum starch (Sun *et al.,* 2014). But % crystallinity of normal corn and potato starches was reduced by HMT (Lim *et al.,* 2001).

The enzymatic susceptibility of starch is influenced by several factors including, the ratio of amylose to amylopectin, the crystalline structure, and the particles size. HMT induced changes probably occur in the amorphous regions of the starch granules, which are more accessible to hydrolysis. These amorphous areas are more rapidly degraded by α-amylases, therefore, the structural rearrangement of starch caused by HMT could facilitate enzymatic accessibility to the amorphous areas (Zavareze and Dias, 2011; Sankhon *et al.,* 2013). HMT could increase the rapidly digestible starch (RDS) and resistant starch (RS) and decreased slowly digestible starch (SDS) contents of starches due to the disruption of double helices and/or the crystallite reorientation. The increase in resistant starch on HMT reflects more starch chain interaction (Chung *et al.,* 2009). HMT could be used as a process to increase RS content without disruption of granular structure. HMT of higher amylose pea and lentil starches obtained more RS than corn starch (Zavareze and Dias, 2011).

The changes in the extent of acid hydrolysis altogether with HMT can be explained through the following factors: (1) crystallite disruption: mild acid hydrolysis could increase amorphous regions available (2) interactions between starch chains: hydrolysis reduces chain flexibility, hence, hindering the conformational change and (3) the disruption of double helices in the amorphous region: hydrolysis helps glycosidic oxygen to be more accessible to protonation (Zavareze and Dias, 2011).

2. Acid Treatment

In acid modification, acid acts on the surface of the starch granule first before it gradually enters the inner region. Acid attacks mostly the amorphous regions in the granule. Acid modification changed the physicochemical properties of starch without destroying its granule structure (Neelam e*t al.*, 2012). Waxy rice starches were treated with 2.2 M HCl at 30°C for 1-45 days. The gelatinization transition temperatures and the breadth of the gelatinization endotherm of acid treated waxy rice starches increased. Acid treated starches melted at a higher temperature and its transition temperature range was broader (Hoover, 2000).

During hydrolysis, first, the hydronium ion (H_3O^+) carries out an electrophilic attack on the oxygen atom of α (1→4) glycosidic bond (figure 1A). In the next step, the electrons in one of the C-O bonds move onto the oxygen atom (figure 1B) to generate an unstable, high energy carbocation intermediate (figure 1C). The carbocation intermediate subsequently reacts with water (figure 1D) and leading to regeneration of a hydroxyl group (figure 1E). Thereby, the amorphous regions of the starch granule are more susceptible to acid hydrolysis than the crystalline regions (Hoover, 2000).

The difference in the rate and extent of starches hydrolysis attributed to the differences in granular size, extent of starch chain interactions (within the amorphous and the crystalline regions of the granule), and starch composition (extent of phosphorylation, amylose content, and lipid complexed amylose chains) (Hoover, 2000). Sulphuric acid treated potato starch showed unimodal granule size distribution but bimodal granule size distribution was found in acid treated rice and maize starches.

Acid treated corn starches showed lower pasting viscosity (Wang et al., 2003). Likewise, acid treated corn, potato and rice starches showed higher final viscosity due to re-association and formation of network structure (Wang et al., 2001). Hydrochloric acid treated rice starch showed higher gelatinization temperature due to reordering of the crystalline structure after acid hydrolysis (Thirathumthavorn and Charoenrein, 2005).

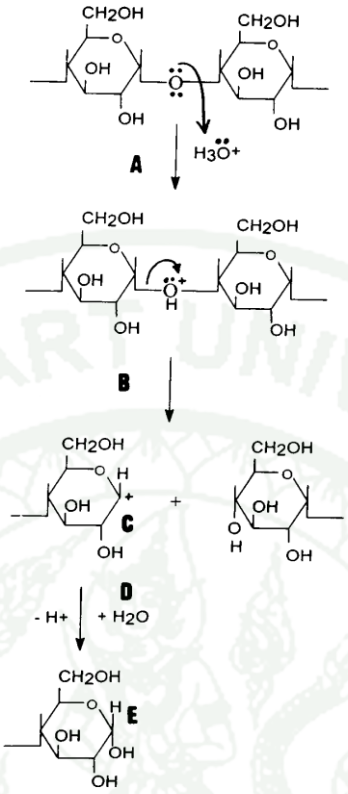


Figure 1 Mechanism of acid hydrolysis of starch

Source: Hoover (2000)

Acid treated corn starches showed slightly lower in onset and peak temperature but acid treated rice starch had higher peak temperature due to the internal structure of the rice starch was converted to a heat-stable structure (Wang *et al.,* 2003). The gelatinization endotherm of citric acid treated rice starch was broader due to starch was composed with various chains length (Shin *et al.,* 2009). Sulphuric acid treated rice, maize and potato starches also showed higher peak gelatinization temperature due to the reduction of the amorphous regions and higher proportion of the crystalline region which increased peak temperature (Palma-Rodriguez *et al.,* 2012).

X-ray diffraction patterns of acid treated corn starches still remained an A-type. The degree of crystallinity of acid treated corn starches increased slightly with increase in acid concentration due to the cleavage of starch chains in amorphous regions that allowed

reordering of the chain and provided more crystalline structure (Wang *et al.,* 2003). Acid treated rice starch showed A-type pattern with additional peaks at 7.5°, 13° and 20° or V-type pattern from the complexation of amylose and lipids. The crystallinity of acid treated rice starch was higher according to the repolymerization during the heating under acidic condition. The amorphous regions of starch disappeared therefore crystallinity of acid treated rice starch increased (Shin *et al.,* 2009). Acid treated corn and rice starches showed slightly sharper peaks at 20°(2θ) as well as the slightly increasing in crystallinity of acid treated corn starches which described by the reordering of cleavage starch chains (Wang *et al.,* 2001). The X-ray pattern of sulphuric acid treated rice, maize and potato starches remained unchanged with the increase in acid concentration. Microstructure of acid treated starch showed disrupted, associated structures and irregularly-shaped particles (Shin *et al.,* 2009). The erosion and exo-corrosion on the surface of starch granules were occurred during the acid treatment. Sulphuric acid treated rice and maize starches had some pores on granule surfaces or exo-corrosion (Palma-Rodriguez *et al.,* 2012).

3. Pyrodextrinization

Dextrins (pyrodextrins or roasted starch) are made by heating dry starch with or without acid. Hydrochloric acid is favoured, but sulfuric and orthophosphoric acids are also used. Dextrins are more completely hydrolyzed products than fluidity starches. Dextrins have been used in the food industry as sweeteners, additives, binders and encapsulating agents (Campechano-Carrera *et al.*, 2007; BeMiller and Whistler, 2009). Yellow dextrins are used as carriers for food flavourings, spices and colorants. Yellow corn dextrin is used for the encapsulation of water insoluble flavorings instead of gum Arabic. A white dextrin, which is treated at low temperatures and relatively short reaction periods, is used as a fat replacer.

The chemical conversion or dextrinization can be described start from hydrolysis, transglucosidation and repolymerization. Glycosidic linkage (α-1,4 and α-1,6) of starch is hydrolyzed during predrying step. In dextrinization, long chain of amylose and amylopectin are randomly hydrolyzed to short chain. The initial stage of hydrolysis is called white dextrin stage. Short chain of amylose and amylopectin which was hydrolyzed by acid are

polymerized and rearranged as brush like structure. Glucose is repolymerized at high temperature and low pH and conversion to yellow dextrins (figure 2) (Kenedy and Fischer, 1984).

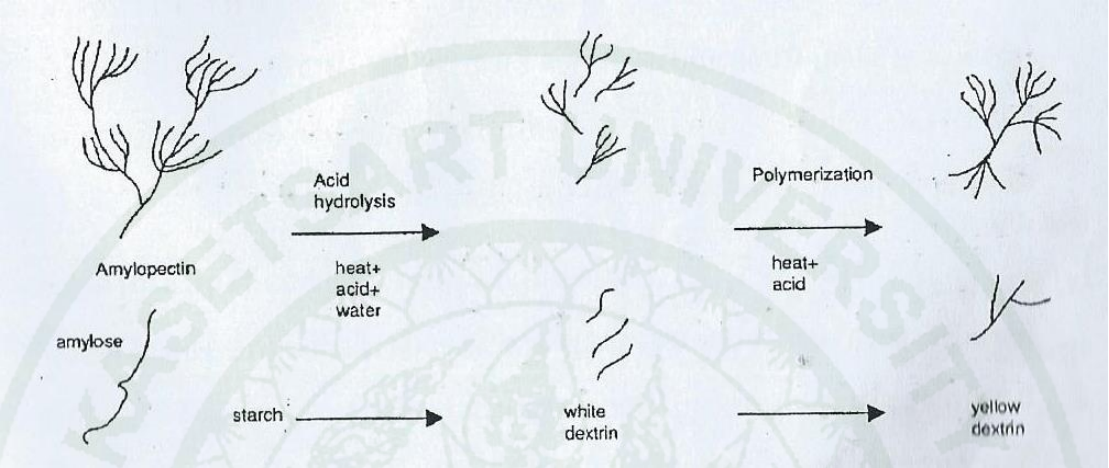


Figure 2 Mechanism of dextrinization of starch

Source: Swinkels (1985)

The production of pyrodextrin has four steps as followings (Kenedy and Fischer, 1984).

3.1 Pretreatment

Dried starch (MC 5% or greater) is sprayed with diluted acid solution or other chemical reagents that can donate hydrogen atom such as hydrochloric acid solution or ammonium carbonate buffer solution. The amount of acid addition depends on the type of pyrodextrin, degree of hydrolysis, moisture content, type of starch/flour and equipment (Kenedy and Fischer, 1984).

3.2 Heating or pyroconverting

Pretreated starch is heated at temperature ranging from 100-200°C (or greater) for 2-3 minutes or up until many hours. Regularly, white dextrin is produced at low temperature and short time but yellow dextrin needs higher temperature and longer time (Kenedy and Fischer, 1984).

3.3 Predrying

During heating (pyroconverting), moisture in starch may affect hydrolysis of starch especially at low pH, therefore, predrying step could help lowering hydrolysis. Vacuum drying is commonly use in predrying in order to eliminate moisture at low temperature hence decrease hydrolysis and offer high quality dextrin (Kenedy and Fischer, 1984).

3.4 Cooling

The cooling step is used for stop the dextrinization reaction. The final moisture content of dextrins is very low around 5 – 12% (Kenedy and Fischer, 1984).

Pyrodextrinized Lima bean (*Phaseolus* lunatus) and Cowpea (*Vigna unguiculata)* starches exhibited high solubility, low viscosity, swelling power and available starch (Campechano-Carrera *et al.*, 2006). Corn starch was pyrodextrinized with 0.1% HCl for 180 min at 130°C. Water solubility of pyrodextrinized corn starch was increased which increase accessibility and/or affinity of the bacterial enzymes to pyrodextrins. High solubility of the pyrodextrins was related to the reduction in the molecular weight of pyrodextrins. The reducing sugar content increased indicated that a measurable hydrolysis of glycosidic linkages had occurred. Moreover the enzyme resistance increased which has been attributed to the changes of structure during heating, depolymerization, transglucosidation and repolymerization. This pyrodextrinization process could be use produce soluble dietary fiber (Kapuśniak and Jane, 2007). Available starches of pyrodextrinized cassava, cocoyam, lentil, maize, sagu and sorghum starches were decreased as pyrodextrinization promoted

generation of non-digestible fractions (Laurentin *et al.,* 2003). Pyrodextrinization could increase the proportion of RDS because it was susceptible to enzymatic hydrolysis. Swelling power of pyrodextrinized starch was lower which attributed to the partial changes in the amorphous regions of starch structure as ever mentioned. Starch was partially lost the ability to hold or absorbed water. On the other hand, the solubility of pyrodextrinized starch increased due to the increase in low molecular weight linear fractions with hydroxyl groups that facilitated solubilization in hot water (Sankhon *et al.,* 2013; Bai *et al.,* 2014).

Jochym *et al.* (2012) tried to produce new enzymatic digestion RS by heating and acid hydrolyzing potato starch with the combination of HCl and citric acid or tartaric acid. Sample with tartaric acid was more chemically modified than citric acid. Organic acid concentration had a significant influenced on the chemical structure of dextrins and its enzymatic digestibility. At low concentration of organic acid (citric acid), small molecules were repolymerized after the initial hydrolysis, leading to larger and highly branched molecules. Higher concentrations of organic acid (tartaric acid) produced highly chemically modified molecules which increased the content of RS type 4.

Modification of starch using acid hydrolysis and heat-moisture treatment could manipulate enzymatic resistant digestion properties of starches. Acid hydrolysis could increase enzymatic resistant digestion and heat-moisture treatment could promoted the interaction of polymer chains by disrupting the crystalline structure which made it more freedom leading to changes of properties as ever mentioned and was interested by this study.

4. Plasticization

Plasticizer defines as a compound that gives flexibility, reducing stiffness and permitting easier process. Moreover, it is a compound that imparts a desirable degree of flexibility over a broad range of temperatures and lowers the brittle point. There are two main groups of plasticizers which are internal plasticizers and external plasticizers. Internal plasticizers are a part of the polymer molecule which copolymerizes into the polymer structure. They influence polymer structure which is less ordered more difficult for the

polymer chains to fit closely together. External plasticizers are more important, since they provide useful combinations of properties and allow the manufacturer various choices of formulation flexibility (Edmund and Herman, 1965).

In food, plasticization could decrease glass transition temperature. Plasticizer in foods is mainly water, but other small solutes may also act as plasticizers. Branching in polysaccharides may work as internal plasticization, inducing a small decreased in Tg when compared to linear chains. Cross-linking is known to strongly raise the glass transition temperature in starch (Meste e*t al.*, 2002).

4.1 Factors opposing plasticization

4.1.1 Intermolecular forces

It is the principal function of the plasticizer to interfere itself between the polymer chains. The main barrier to this try is the attractive forces between the polymer molecules, which depends on the chemical and physical structure of polymer. The following describes the various possible intermolecular forces. Dispersion forces exist between all polar or nonpolar molecules which resulted from the attraction between atoms, arising from interaction between small dipoles, induced in one atom by those formed by the nucleus and electrons of the other atom. Induction forces arise when a molecule with a permanent dipole caused by a polar group, induces a dipole in a neighboring molecule. This effect is particularly strong with aromatics because of high polarizability. Dipole-dipole interactions occur between two molecules which contain polar group. Hydrogen bonds occur with molecules containing OH or NH groups such as water, acids, amines, polyamides, polyvinyl alcohol, cellulose (Edmund and Herman, 1965).

4.1.2 Crystallinity

Polymer chains which possess a regular structure are able to crystallize under suitable conditions. This means that the chain molecules change from a coiled and disordered state to a tightly folded aligned and ordered state. Since a material consisting of ordered chains, a plasticizer molecule will have much more difficulty in penetrating into the

crystalline regions, where there are a minimum of free space between the polymer chains (Edmund and Herman, 1965).

4.2 Plasticizer requirements

4.2.1 Solvent power

The plasticizers should have a high degree of solvent power for the polymer. Especially crystalline polymer, only a solvent type of plasticizer will be able to penetrate into the ordered and disordered regions, whereas a non-solvent plasticizer (softener) will only be able to enter the amorphous region. So the high solvent power plasticizers could penetrate better and be used easier (Edmund and Herman, 1965).

4.2.2 Compatibility

The plasticizer should be compatible with the polymer system over both the processing and the use temperature ranges and it is desirable that substances or conditions such as water, oil, oxygen or sunlight should not disturb the compatibility balance. Factors affecting compatibility are the molecular weight and structure of the plasticizer. Similar chemical structure (polar, shape, size) between polymer and plasticizer could be good compatibility such as camphor and cellulose nitrate polymer system (Edmund and Herman, 1965).

4.2.3 Efficiency

Plasticizer efficiency is used to relate a desirable modification of the properties of a give product to the amount of plasticizer required to achieve this effect. The efficiency of various plasticizers in “plasticizing” a give polymer may be expressed in terms of depression of the glass transition temperature by a given mole or volume fraction of plasticizer. Therefore, there is no absolute value for the efficiency of a certain plasticizer and the relative efficiency of different plasticizers will depend on which polymer property is used to measure plasticizer efficiency (Edmund and Herman, 1965).

4.2.4 Permanence

The permanence of plasticizer depends on the size of plasticizer molecule and its diffusion rate in the polymer. The larger plasticizer molecule exhibited the lower vapour pressure, or volatility, therefore the greater its performance. Other factors, such as polarity and hydrogen bonding will also affect the vapour pressure of the plasticizer. The rate of diffusion of the plasticizer molecules within the polymer matrix will also determine plasticizer permanence. Unfortunately, a high diffusion rate provides greater plasticizer efficiency, it results in low plasticizer permanence (Edmund and Herman, 1965).

4.3 Plasticizer efficiency as a function of plasticizer structure

Concentration, molecular weight, polar group, shape, branching, and internal mobility (flexibility) of plasticizer may affect the relationship between plasticizer action and plasticizer structure. Concentration of plasticizer is directly proportional to the lowering of glass transition temperature in polymer systems. The depression of glass transition temperature of plasticizer increases with higher molecular weight. Extended plasticizer molecules such as aliphatic chains with a high degree of flexibility, usually lower glass transition temperature much more than bulky plasticizer molecules. When comparing the branched and linear plasticizer molecules, the linear molecules are more efficient in lowering glass transition temperature than the branched molecules. Moreover, the internal mobility plays an important role in determining plasticizer efficiency (Edmund and Herman, 1965). The effective plasticizers should have a high degree of solvent power, high diffusion rate with small molecule.

Many plasticizers have been used for improving film properties, thermoplastic which glycerol and sorbitol were a commonly used. Glycerol is a simple polyol compound. It is a colorless, odorless, viscous liquid. The molecular formula of glycerol is $C_3H_8O_3$ and molecular mass is 92.09 g/mol. Structure of glycerol is shown in figure 3a. Glycerol has three hydroxyl groups that are responsible for its solubility in water and hygroscopic. Sorbitol (glucitol) is a sugar alcohol which obtains by reduction of glucose by changing the

aldehyde group to hydroxyl group. The molecular formula of sorbitol is $C_6H_{14}O_6$ and molecular mass is 182.17 g/mol. Structure of sorbitol is shown in figure 3b.

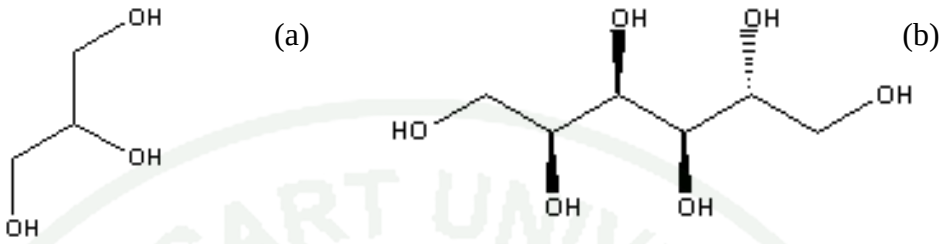


Figure 3 Structure of glycerol (a) and sorbitol (b)

Source: Lewis (2004)

Plasticization is the changing of thermal and mechanical properties of polymer by plasticizing effect (Edmund and Herman, 1965). Many plasticizers such as glycerol, sorbitol, xylitol, fructose, glucose, and glycol are used in many processes which water shows a strong plasticizing effect. Mechanisms of plasticizers in starch films are described by the water holding property of plasticizers which provide free volume and interact with starch polymer chains by hydrogen bonds (Zhang and Han, 2006). Molecular weight, structure and polar groups affect to plasticizers efficiency which low molecular weight, polar groups on the aliphatic molecule could show high efficiency of plasticization (Edmund and Herman, 1985). Many works study on the different efficiency between glycerol and sorbitol on film formation which both of them are in polyols group and aliphatic chain with hydroxyl groups. Glycerol has lower molecular weight and hydroxyl groups comparing with sorbitol. Glycerol film provided more solubility, moisture content, hygroscopic, water permeation, oxygen permeation than sorbitol films due to its hydrophilicity and gas permeability. Hydrophilic plasticizers could interact with polymer chains and held a definite of amount water, thereby chains mobility was allowed and flexibility of film increase (García *et al.*, 2000; Laohakunjit and Noomhorm, 2004; Mehyar and Han, 2004; Mali *et al.*, 2005; Zhang and Han, 2006; Müller *et al.*, 2008). Moreover, glycerol and sorbitol (polyols) were used to produce wheat gluten/plasticizer mixtures by using in the ratio of gluten to polyol of 10:0, 10:1, 10:2, and 10:3. The presence of glycerol or sorbitol had a significant

plasticizing effect on gluten. This plasticizing effect could be attributed to their low molecular weight and hydroxyl groups leading to the formation of polymer-plasticizer interactions to the detriment of polymer-polymer interactions (Pouplin *et al.*, 1999).

Glycerol and sorbitol were used as plasticized starch-based coating in strawberry. Strawberries coated with formulations containing plasticizer were homogeneous and covered the whole surface of the fruit. Coatings without plasticizer were brittle and some cracks were observed which may attributed to the addition of plasticizer could provide the flexibility to plasticized starch-based coating film. Glycerol films provided higher water vapor permeability compared to sorbitol ones can be attributed to glycerol has smaller size, lower molecular weight, and higher compatibility with amylose matrix, which favored plasticizer migration, facilitating amylose mobility and giving a looser structure. On the other hand, plasticizer addition could decrease the water vapor permeability of starch-based coating due to starch-based coating without plasticizers provided some cracks and brittle. So the plasticizer content was a very important factor by added plasticizer content higher than optimum content, plasticizers would be migrated to the surface of film and provided a sticky film. Due to plasticizer addition decreased the intermolecular attractions and the increased of polymer chain mobility. (García *et al.*, 1998). The three levels (0, 20 and 40g/100g of starch) of mixture of glycerol and sorbitol (1:1) were used in cassava starch film. Plasticizers acted as a mobility enhancer due to their low molecular weight leaded a large increased in molecular mobility of amorphous and partial crystalline which increased free volume of the system. The hydrophilicity of plasticizer and its concentration were found to be important factors in determining the moisture affinity of cassava starch films. Glycerol films adsorbed faster and more water during its storage comparing to sorbitol films (Mali *et al.*, 2005). Plasticized (glycerol or xylitol) low amylose starch films were used to study their moisture migration behavior. Xylitol plasticized starch film was found to be a more effective plasticizer compared to glycerol plasticized starch film. This can be attributed to the relatively larger molecular size of xylitol, combined with its propensity to form a stronger hydrogen bond with starch molecules. The xylitol plasticized films had consistently higher moisture migration fluxes and effective moisture diffusivity values. Glycerol plasticized films had lower moisture migration rates and lower moisture diffusivity values. When the glycerol and xylitol were used in combination, a synergistic enhancement in

plasticization was observed. On the other hand, at lower plasticizer contents (10 wt%) the combined use of these plasticizer exhibited anti-plasticization behavior resulting in low moisture migration rates and lower moisture diffusivity values. The anti-plasticization behavior has been attributed to the strong hygroscopicity and hydrophilic nature of glycerol, which tightly held the water molecules rather than facilitating their molecular diffusion (Adhikari *et al.,* 2010) However, glycerol was a better plasticizer than xylitol at lower water activity but at higher concentrations, xylitol could be able to impart overall better plasticization ability. Both plasticizers also showed typical anti-plasticization behaviour at lower water contents (Chaudhary *et al.*, 2011).

5. Encapsulation

Nowadays encapsulation is a very extensive technique for extend the shelf-life, either by protecting them against oxidation or by preventing reactions with components in the food system. There are three main methods for encapsulation (1) physical methods such as spray drying, spray cooling and chilling, fluidized bed coating, extrusion, freeze drying and co-crystallizaition; (2) chemical methods such as molecular inclusion and interfacial polymerization; (3) physicochemical methods such as coacervation, organic phase separation and liposome entrapment.

Encapsulation is the technique by which one material or a mixture of materials is coated with or entrapped within another material or system. The coated material is called active or core material, and the coating material is called shell, wall material, carrier or encapsulant. Two main structures are single-core and multiple-core microcapsules. The first one which has high core loading is typically produced by complex coacervation, fluidized bed drying, droplet co-extrusion, and molecular inclusion. The multiple core capsules which are produced principally by spray drying, has core material dispersed throughout the shell with hollow center which resulted from the expansion of particles during the drying stages (Jafari e*t al*,. 2008).

5.1 Encapsulation by spray drying

Spray drying is the most commonly used encapsulation technique in the food industry. The process of spray drying is economical and flexible, equipment is readily available, and produces powder particles of good quality (Jafari e*t al.*, 2008). A successful spray drying encapsulation depends on completing high retention of the core materials. Minimum amounts of the surface oil on the powder particles for both volatiles and non – volatiles during the process and storage was required. The properties of wall and core materials and the prepared emulsion along with the drying process conditions will influence the efficiency and retention of core materials (Jafari e*t al.*, 2008). For flavor and oil encapsulation, the ideal wall materials should have emulsifying properties, be a good film former, have low viscosity at high solids levels, exhibit low hygroscopicity, release when reconstituted in a finished product, low in cost, bland taste, stable supply, and give a good protection to the encapsulated flavour and oil. No any wall material that obtained these properties so a combination of plasticizers has been used (Jafari e*t al.*, 2008).

Wall material must be rehydrated (sometimes with heating) in water. This is particularly important for surface-active biopolymers to exhibit their emulsifying capabilities during emulsion formation. When the wall material has been hydrated, the core material must be added to make a coarse emulsion, usually via high-speed mixing or high-shear emulsification by colloid mills. Then, final emulsion will be prepared by other emulsification methods including high pressure homogenization, e.g., micro-fluidization. The infeed emulsion will be pumped to the drying chamber of spray drier. The rapid evaporation of water from these droplets during surface film solidification keeps the core temperature below 100°C even if using the high temperatures (>150°C) in the process. Spray-dried encapsulated powders regularly have a very small particle size (generally less than 10 mm) with a multiple-core structure (Jafari e*t al.*, 2008).

5.2 Encapsulation efficiency

There are at least four group of criteria that can influenced encapsulation efficiency (a) properties of wall materials (b) characteristics of core materials (c)

specifications of the infeed emulsion and (d) conditions of the spray drying (Jafari e*t al.*, 2008).

5.2.1 Properties of the wall materials

For spray drying, the choice of wall material is critical as it will influence emulsion properties, retention of the volatiles during the process and shelf – life of the encapsulated powder after drying. The major wall materials used for spray drying are carbohydrates including modified and hydrolyzed starches, cellulose derivatives, gums and cyclo-dextrins; proteins including whey proteins, casein, and gelatine; and new emerging biopolymers such as products of Maillard reaction (Jafari e*t al.*, 2008).

(a) Carbohydrates

Hydrolyzed starches (maltodextrin, corn syrup solid) and modified starches are depolymerized ingredients produced by hydrolyzing starch with acid and/or enzymes which could be used for spray drying encapsulation due to their solubility, low viscosity and ease of drying conditions (Ré, 1998). These wall materials offer the advantage of inexpensive, bland in flavour, low viscosity at high solids content, and excellent protection to encapsulated core materials such as orange oil, milk fat, soy oil and fish oil. The dextrose equivalent (DE) of hydrolyzed starch is directly influenced the degree of protection, higher DE systems are less permeable to oxygen and result in powders with higher encapsulation efficiencies. However, hydrolyzed starches are lack of emulsifying properties and poor retention of flavour during spray drying. Therefore, it is desirable to use them in combination with a surface – active biopolymer such as esterified modified starches, gum Arabic, or milk proteins (Jafari e*t al.*, 2008.)

Gum arabic has been the most popular and common ingredient for spray drying encapsulation of oil and flavour, since it has emulsifying properties and provides excellent volatile retention during the drying process, but its high cost, limited availability, and the impurities. It is a polymer consisting primarily D-glucuronic acid, L-rhamnose, D-galactose and L-arabinose with 5% protein (Ré, 1998). Many researchers have

tried to use a blend of gum Arabic with other wall materials and/or to replace gum arabic completely. For example, a combination of gum arabic and maltodextrin was reported to be effective for the encapsulation of cardamom oil, citral and linalyl acetate, citrus oil, soy oil, rice flavour, fatty acid, pine flavour, and bixin. Maltodextrin can successfully replace a part of gum Arabic as wall material (Jafari e*t al.*, 2008). Kanakdande *et al.*, (2007) also used the combination of gum arabic, maltodextrin and modified starch (Hicap®100) as wall materials for encapsulation of cumin oleoresin. They indicated that gum arabic / maltodextrin / modified starch (4/6 : 1/6 : 1/6) blend exhibited more efficient than the other blends, and even better than gum arabic itself.

Cyclo-dextrin has been used in spray drying encapsulation of oil and flavour. They are cyclic molecules containing six (alpha-), seven (beta-) or eight (gamma-) glucose monomers that are produced from starch. These monomers are connected to each other, giving a ring structure that is relatively rigid and have a hollow cavity with the ability to encapsulate other molecules. Some researchers have tried to apply novel biopolymers in spray drying encapsulation of food flavour and oil such as alginate, chitosan, soluble soy polysaccharide, sucrose, flour, product of Maillard reaction, and modified cellulose. This studies open new areas of research and need more works to be done (Jafari e*t al.*, 2008).

(b) Proteins

Functional properties of proteins including solubility, film formation, the ability to interact with water, emulsification and stabilization of emulsion droplets, exhibit many of the desirable characteristics for a wall material. Gelatine is commonly used in spray drying encapsulation. However, other proteins, particularly soy proteins, and milk proteins such as whey protein concentrate, skimmed milk powder, and caseinate have also been used for spray drying encapsulation of flavour and oil. These proteins change their structure during emulsification through unfolding and adsorption at the oil-water interface. They offer significantly stable emulsions, which are critical for encapsulation purpose, by forming resistant multi-layer around oil droplets and also with the help of repulsive forces. Investigations have proven that proteins are good wall material for

encapsulating anhydrous milk fat, orange oil, soy bean oil, caraway essential oil, fish oil and fatty acids, and oregano and marjoram flavour. Researchers are also investigating the combination of protein with different carbohydrates as wall materials. For example, a blend of whey proteins with maltodextrin and corn syrup solids and lactose, soy protein with maltodextrin, sodium caseinate with lactose and carbohydrates blends, and WPI or SMP with maltodextrin (Jafari e*t al.*, 2008). Combinations of maltodextrin and soy protein as well as maltodextrin and soy lecithin were also evaluated which carbohydrate (maltodextrin) acted as a matrix forming material and lipid (soy lecithin) or protein (soy protein) acted as an emulsifying agent. These combinations could improve the amount of encapsulated core by improving emulsion stability before drying (Ré, 1998).

5.2.2 Properties of the core material

The loss of some volatile including flavour during spray drying encapsulation is unavoidable. Other than properties of the used wall material, the core material will also affect the retention during the process. Molecular weight and vapour pressure of the flavour compounds have an influence on their retention during spray drying (Jafari e*t al.*, 2008).

Molecular weight is the primary factor influence diffusion rate. The increasing of molecular size generally results in slower diffusion rate. The molecules will take more time to reach the atomized droplet surface during drying especially at initial stages, and % retention will increase. When diffusion effectively stop at low moisture content, the droplet surface becomes impermeable to large flavour molecules more quickly during drying. Both of these factors support the retention of larger molecular weight volatiles (Jafari e*t al.*, 2008). Thereby, molecular weight is directly related to the diffusion of molecules which the high volatile and high solubility of flavors might encourage a higher loss of flavor during spray drying (Soottitantawat *et al.*, 2003).

Relative volatility plays a secondary role in determining flavour retention because of its influence in controlling flavour loss until the droplet surface becomes semi-permable. Volatility reflects the ability of a compound to reach the gaseous

phase and can be evaluated by measuring the vapour pressure of the pure compound. Relative volatility of a compound is calculated with respect to water (Jafari e*t al.*, 2008).

The retention of volatiles also depends on their polarity. This could be explained by the greater solubility of polar compounds in water. As the water solubility of the volatile increases, the volatile losses increase due to the ability of the water fraction to diffuse through the selective membrane, even at late stages of the drying process (Jafari e*t al.*, 2008).

It should be noted that individual volatiles can be retained at different rates during spray drying encapsulation. The retention of aroma compounds with various functional groups is in the order of acids < aldehydes < esters ≤ ketones ≤ alcohols with acids having the minimum retention. Therefore, it can be seen that retention of volatiles depends on their molecular weight, relative volatility, polarity, and type. These different parameters act on the capacity of the volatile to diffuse through the droplet surface and on its ability to form small pools. The small molecules are volatize easily and more solubility in water than the larger. Besides factors such as volatility, solubility and diffusivity of the volatile compound through the droplet, another factor is the possible interactions between the volatiles and the wall material. This may involve physical or physicochemical interactions including formation of insoluble complexes, and molecular association of the wall material with the volatile through hydrogen bonds. These interactions can have an effect on the formation of the interfacial film at the interface of O/W, which stabilizes the emulsion and may affect the retention indirectly (Jafari e*t al.*, 2008).

Using the highest possible core concentration that provides high core retention in the microcapsule is important to increase the yield and was more economic. There is an optimum core concentration that can be encapsulated efficiently. Higher oil loads generally result in poorer retention or lower encapsulation efficiency and higher surface oil content of the powder due to insufficient wall material to produce a sufficiently strong structural matrix and thinner layers of wall material between encapsulated oil droplets (Rosenberg *et al.*, 1990; Ré, 1998; Jafari e*t al.*, 2008; Xie *et al.*, 2010; Garcia *et al.*, 2012).

5.2.3 Role of the initial emulsion

The key steps in spray drying encapsulation of oil and flavour are preparation of the in-feed emulsion. This emulsion plays an important role in defining the retention of volatiles and surface oil content of the final encapsulated powder. The significant parameters to consider are total solids concentration, viscosity, stability, droplet size, and emulsification method (Jafari e*t al.*, 2008).

(a) Total solids content of the emulsion

High solids content of the prepared emulsion could increase the retention principally by reducing the required time to form a semi-permeable membrane at the surface of the drying particle. Also higher total solids leads to the increase of emulsion viscosity, preventing the circulation movement inside the droplets and thereby, resulting in a rapid skin formation (Jafari e*t al.*, 2008).

(b) Emulsion stability

The encapsulation efficiency of oil and flavour is influenced by the stability of initial emulsion. The stable initial emulsion offers a higher the encapsulation efficiency (Danviriyakul *et al.,* 2002). On the other hand, the unstable initial emulsion was broken inside the droplet, resulting in losing of flavour during drying (Jafari e*t al.*, 2008).

Increasing of the initial emulsion viscosity should help volatile retention because of internal circulation in the droplets reduction and rapid semi-permeable membrane formation, whereas decreasing of the initial emulsion viscosity could delay formation of semipermeable surface and loss of volatile (Ré, 1998). An increase in the solids concentration of initial emulsion is favourable up to optimum viscosity. Some works claimed the optimum viscosity that was easy to atomize and reasonably formed spherical particles such as the optimum emulsion viscosity of alginate concentration ranging from 125 to 250 mPa·s. Emulsion viscosity plays an important role in determining volatiles retention, due to its large influence of controlling volatile losses until the droplet surface becomes

semi-permeable. An increasing of the emulsion viscosity up to an optimum viscosity will suppress the internal circulations and waving of droplets, and will put the selective diffusion into action earlier, thus improves flavour and oil retention. However, the increasing of viscosity could decrease the flavour and oil retention, due to a larger exposure during atomization, the slow formation of discrete droplets during atomization, and difficulties in droplet formation. At higher viscosities, in-feed emulsion is shown that a more viscous feed will produce larger droplet sizes, difficulties to form droplets, and irregular particles (oval, cylindrical and stringy) will be produced (Jafari e*t al.*, 2008).

(c) Emulsion size

The advantage of producing small emulsion droplets (fine emulsion) is higher stability, which is critical during spray drying. The emulsion size may also affect the characteristics of the final encapsulated powder including the surface oil and total oil content of the microcapsules. Smaller emulsion size exhibited a higher retention and lower unencapsulated oil (surface oil) (Soottitantawat *et al.*, 2005; Jafari e*t al.*, 2008; Garcia *et al.*, 2012). Therefore, a fine emulsion may contribute to keep the core in to a product for a longer period of time and higher resistance to oxidation in the product (Ré, 1998). The larger emulsion droplets would be sheared into smaller droplets because of the large velocity gradient and the turbulence in the thin liquid film on the surface of the rotating atomizer. Some of the sheared droplets were then broke down and evaporated during atomization. These results explain the greater loss of flavor from the larger emulsion droplets during spray drying which leading to decreasing encapsulation efficiency (Danviriyakul *et al.*, 2002; Soottitantawat *et al.*, 2003).

Soottitantawat *et al.*, (2005) also studied the effect of emulsion size on the stability of encapsulated of D-limonene by spray drying. Gum Arabic, maltodextrin, and modified starch (Hicap ® 100) were used as wall material and found that optimal size of flavor in the powder should be recommended for both of the high retention during spray drying and its stability during storage as well as the ability to control the release of flavor.

Danviriyakul *et al.*, (2002) studied the emulsion stability of milk fat on the spray dried milk fat. Varying oil droplet size of milk fat (average diameter 0.5, 1.0 and 1.2 μm) has an effect on surface fat by increasing oil droplet size, the surface oil increased. The bigger oil droplet size of milk fat emulsion was not stable as the one with 0.5 μm average droplet diameter.

Therefore, it could be possible to improve the oil and flavour retention by more efficient covering of fine oil droplets inside the wall material and minimum effect of atomization and spray drying on emulsion droplets. However, more works need to be done in this area to find the exact mechanism of the influence of emulsion size and powder particle size on encapsulation efficiency of oil and flavour during spray drying (Jafari e*t al.*, 2008).

(d) Emulsification method

Homogenization is a common method used to produce a finer emulsion. Homogenization pressure influenced the oil retention of encapsulation process which could improve oil retention of microcapsule (Garcia *et al.*, 2012). Many researchers used the combination of emulsification method to improve the emulsion stability which expected to produce a product with better emulsion stability resulted in an extended shelf life, and higher oil or flavour load. For example, use of ultrasound could increase emulsion quality for the wall material which has low emulsifying properties and low viscosity such as maltodextrin (Jafari e*t al.*, 2008).

5.2.4 Conditions of the spray drying

Encapsulation efficiency could be maximized by the proper choice of spray drying parameters including inlet and outlet drying air temperature, infeed temperature, atomization type and conditions, drying air flow and humidity, and powder particle size (Jafari e*t al.*, 2008).

(a) Powder particle size

Particle size of the encapsulated powder is primarily determined by the physical properties of the emulsion (such as viscosity and solids concentration). The choosing of operational parameters for atomization, such as the rotational speed and wheel diameter (in the case of centrifugal wheel atomization), the orifice size and pressure (in the case of nozzle atomization) are important. A high pressure and small orifice result in smaller particles. Nozzle atomization produces substantially bigger particles than centrifugal wheel atomization, and the type of atomization was evidently more important in determining particle size distribution than dryer temperatures. Particle size can also be influenced by the operating temperatures. A high inlet air temperature and low difference between inlet and outlet air temperatures will produce slightly larger particles than slow drying. Fast drying could primarily sets up the particle structure and does not allow then to shrink during drying. In-feed solids have a similar effect if they are high in solids which cannot shrink as much (Jafari e*t al.*, 2008).

The influence of powder particle size on encapsulation efficiency of food flavour and oil still is not clear. Several studies have reported that larger particle sizes could improve flavour retention and lower surface oil contents during spray drying. On the other hand, some studies reported that the particle size is not effect on oils and flavours retention if solids content of in-feed is high enough (Jafari e*t al.*, 2008). Soottitantawat *et al.*, (2005) showed that powder particle size was not an only factor effect on flavour retention, but should considered other parameters such as emulsion size as well. The larger powder size leads to higher stability and lower release of encapsulated flavour, if the initial emulsion size is small.

Although the role of particle size is not clear, it is often desirable to produce large particles to facilitate rehydration. Small particles tend to disperse very poorly, especially in cold water, and instead from lumps on the liquid surface. Large particles can be obtained through appropriate choice of spray dryer operating conditions, or using of agglomeration techniques. Agglomeration of spray dried encapsulated powders by fludized bed processing can improve the flowability and wettability of powders because of the

increasing of particle size. Some of the encapsulation parameters could also be changed, such as reduction in surface oil content of the powders due to stripping effect of fluidized bed agglomeration (Jafari e*t al.*, 2008).

(b) Atomization type

As mentioned before, significant losses of volatiles occur during early stage of drying, especially in the atomization step. During this time, the emulsion is sprayed into very turbulent air, forming a thin sheet (high surface area) with substantial mixing, all promoting volatile losses. Thus, it is necessary to optimize the atomization process for maximum volatile retention. In the case of pressure nozzles, it is shown that a higher pressure promotes volatile retention. Due to reducing the length of the emulsion sheet emitted from the nozzle atomizer before break – up into spherical droplets, thereby reducing the length of time that the liquid is in a sheet (high loss rate period). High pressures provide a greater momentum to the atomized droplets, thereby drawing more hot air into the spray stream, so more rapid drying and quicker formation of the selective film around the drying droplet. Another parameter is the spray angle of the nozzle that can affect volatile retention. A wide spray pattern (without wetting the dryer walls) is recommended, since this will increase the atomized droplet contact with drying air, thereby increasing of the drying rate. For centrifugal atomization, higher wheel speed would promote volatile retention for similar reasons. Recently work showed that using centrifugal atomization for producing of powders produced had much higher surface oil contents than nozzle counterparts. Thus, the type of the atomization process and the associated dryer geometry can influence the encapsulation efficiency of food oil and flavour, an effect indirectly related to the powder particle size (Jafari e*t al.*, 2008).

(c) In-feed temperature

Cooling the feed (30% coffee solids extract) before drying clearly improved the coffee flavour of the final spray dried powder. The increase in the feed viscosity would affect internal circulations of the droplets and size altogether with the vapour pressure and diffusibility of the flavour compounds (Jafari e*t al.*, 2008).

(d) Air flows and humidity in the spray dryer

The mixing of air and atomized emulsion exhibited the better in the volatiles retention, due to a more rapid heat and mass transfer associated with the drying process. For example, a wide spray pattern with nozzle atomization or higher pressures will improve air / product mixing, thereby promoting the drying rate. Lower dryer air humidity can also promote rapid drying and better flavour retention, but dehumidifying the inlet air regularly is expensive, and is rarely performed during spray drying encapsulation (Jafari e*t al.*, 2008).

(e) Inlet air temperature

High air inlet temperature (160 - 220°C) leads to a rapid formation of the semi-permeable membrane on the droplet surface, giving optimum flavour retention, thereby increased volatiles retention (Ré, 1998). However, high inlet air temperature could cause heat damage to the dry product, or "ballooning" and excessive bubble growth and surface imperfections which increase volatiles loss during spray drying. Ballooning occurs when steam is formed in the interior of the drying droplet. Due to quite high inlet air temperatures, cause the droplet to puff or balloon, thereby producing a thin walled hollow particle. This particle will not retain core materials as well as its non-ballooned counterpart (Jafari e*t al.*, 2008). Danviriyakul *et al.*, (2002) varied spray drying conditions of spray dried milk fat by setting inlet temperature at 160, 180, and 210°C. Result showed that inlet air temperature affect powder particle diameter profiles but not surface fat. Moreover, the increasing of the spray drying inlet temperature resulted in an increase in the powder particle size distribution. However, when the inlet air temperature is high, the crust formation is formed quickly and the flavour or oil cannot evaporate easily from the surface. Thus, the increasing of inlet air temperature can promote flavour and oil retention (Rosenberg *et al.*, 1990; Jafari e*t al.*, 2008).

(f) Outlet air temperature

The influence of outlet air temperature on the encapsulation efficiency of flavour and oil is also unclear. For example, retention of small soluble volatiles such as diacetyl improved with increasing outlet air temperatures, probably due to a lower relative humidity at higher outlet air temperature (at a fixed inlet air temperature), which resulted in more rapid drying and better flavour retention. In contrast, some research found that the increasing in air outlet temperature resulted in a poor volatile retention and higher surface oil. This was hypothesized by "ballooning" effect, where particles may develop cracks, even split and release the trapped volatiles (Jafari e*t al.*, 2008). Danviriyakul *et al.,* (2002) found that surface oil content of milk fat encapsulated powder was not affected by outlet temperature.

5.3 Capsule Morphology

The outer surface of spherical structure of microcapsule was free of crack but presence of dent which were probably formed by shrinkage of the droplets during the early stage of the drying process. The formation of the central void was related to the expansion of the capsule (Ré, 1998). Other researchers found that the external surface of microcapsules showed concavities but no visible holes and fractures found in the outer surfaces (Xie *et al.,* 2010; Gracia *et al.,* 2012).

5.4 Release Mechanism

Some microencapsulated materials are made from controlled release of the encapsulant during processing, storage. Food additives which may benefit controlled release are preservative, redox agent, colour, sweetener and enzyme. The common method used for control-release in food included temperature and moisture release for hydrophilic encapsulant, and thermal release for fat capsules. Other release methods include pH control, addition of surfactant, enzymatic release, ultrasonic, grinding and photo release (Wilson and Shah, 2007).

The influence factors for releasing mechanism in encapsulation included the diffusion of volatiles from matrixes (wall materials), geometry of particle and degradation/dissolution of matrixes. There are four types of releasing mechanism as following.

5.4.1 Diffusion control release

The vapour pressure of volatile or flavour determines the diffusion control release. Volatile or flavour will diffuse to the surface of capsule and emit from the capsule. Moreover, the concentration of volatile or flavour in matrix also determines the diffusion rate of the volatile or flavour (Madene *et al.,* 2006).

5.4.2 Pressure activated release

Pressure activated release need fast rate releasing such as releasing of perfume, chewing gum, candy. This type of releasing need completely release after pushing. Recently, the extrusion of cereal products applies pressure activated release for releasing encapsulated material during extrusion process (Madene *et al.,* 2006).

5.4.3 Swelling control release

When the polymeric matrix mixes with water, swelling of polymeric matrix will occur. After adding flavour, it will dissolve and disperse in water. The polymeric matrix will absorb water into the matrix and flavour cannot emit from the matrix, thus using swelling property to protect core materials. For releasing flavour from matrix by swelling controlled, polymeric matrix interacts with water and the matrix swells. After swelling of matrix, the porous surface will occur on the surface allow diffuse or emit of core material (Madene *et al.,* 2006).

5.4.4 Solvent active release

Solvent active release is applied in dry beverage or dry cake mix. This releasing type require high solubility rate and emit flavour or the active ingredient is emitted by the swelling of coating material (Madene *et al.*, 2006).

Yoo *et al.* (2006) studied the microencapsulation of α-tocopherol using sodium alginate and its controlled release properties. The main objectives are optimizing microencapsulation condition and controlling the releasing of α-tocopherol to the desirable nutrient delivery system. Sodium alginate (coating material) could protect core material under strong acidic environment and rapidly released under mild alkali condition. Once the microcapsule reach small intestine, it should initially release the core material rapidly, and keep the releasing rate to maintain the effective concentration of core material as the absorption rate decreases. The *in vitro* α-tocopherol releasing property was used for evaluating the releasing of α-tocopherol in simulated digestive tract in human body.

As mentioned above, encapsulation can improve the product quality by protecting core materials from the outside environment and preventing the reaction with other components in food system. Thus, encapsulation can prolong shelf life of products and help controlling of the core material releasing to the desirable nutrient delivery system.

MATERIALS AND METHODS

1. Materials

1.1 Chainat1 white rice grain (Rice Department, Ministry of Agriculture, Thailand)

1.2 Gum arabic (food grade, Jumbo Trading Co., Ltd, Bangkok, Thailand)

1.3 Maltodextrin (DE 10, food grade, Siam Victory Chemicals Co., Ltd, Bangkok, Thailand)

1.4 Polyoxyethylene sorbitan monooleate (Tween80, food grade, Siam Victory Chemicals Co., Ltd, Bangkok, Thailand)

1.5 Glycerol (food grade, Siam Victory Chemicals Co., Ltd, Bangkok, Thailand)

1.6 Sorbitol (food grade, Siam Victory Chemicals Co., Ltd, Bangkok, Thailand)

1.7 Tocopheryl acetate (food grade, Siam Victory Chemicals Co., Ltd, Bangkok, Thailand)

1.8 Sodium hydroxide (NaOH, analytical grade, TTK Science Co., Ltd, Bangkok, Thailand)

1.9 Hydrochloric acid (HCl, analytical grade, TTK Science Co., Ltd, Bangkok, Thailand)

1.10 Citric acid ($C_6H_8O_7$, analytical grade, TTK Science Co., Ltd, Bangkok, Thailand)

1.11 Ethanol 95% (C_2H_6O, analytical grade, TTK Science Co., Ltd, Bangkok)

1.12 Pancreatin from porcine pancreas (Sigma-Aldrich Co. LLC, Taiwan)

1.13 Amyloglucosidase (EC 3.2.1.3, Sigma-Aldrich Co. LLC, Taiwan)

1.14 Invertase (EC 3.2.1.26, Sigma-Aldrich Co. LLC, Taiwan)

1.15 Sodium acetate (CH_3COONa, analytical grade, TTK Science Co., Ltd, Bangkok, Thailand)

1.16 Acetic acid (CH_3COOH, analytical grade, TTK Science Co., Ltd, Bangkok, Thailand)

1.17 Glucose assay kit (GAGO-20, Sigma-Aldrich Co. LLC, Taiwan)

1.18 Hexane (C_6H_{14}, analytical grade, Fortune Scientific Co., Ltd, Bangkok, Thailand)

1.19 Iso-propanol (C_3H_8O, analytical grade, Fortune Scientific Co., Ltd, Bangkok, Thailand)

1.20 Anhydrous sodium sulphate (Na_2SO_4, analytical grade, TTK Science Co., Ltd, Bangkok, Thailand)

1.21 Sodium chloride (NaCl, analytical grade, Chemical Express Co., Ltd, Samutprakarn, Thailand)

1.22 Sodium dihydrogen phosphate (NaH_2PO_4, analytical grade, Chemical Express Co., Ltd, Samutprakarn, Thailand)

1.23 Sodium hydrogen phosphate (Na_2HPO4, analytical grade, Chemical Express Co., Ltd, Samutprakarn, Thailand)

1.24 Methanol (CH_4O, HPLC grade, Fortune Scientific Co., Ltd, Bangkok, Thailand)

1.25 Dichloromethane (CH_2Cl_2, HPLC grade, Fortune Scientific Co., Ltd, Bangkok)

1.26 Tocopheryl acetate (Sigma-Aldrich Co. LLC, Taiwan)

2. Equipments

2.1 Double-disk stone mill (locally made in Thailand)

2.2 Hammer miller (SR 300, Retsch, Haan, Germany)

2.3 Test sieve 100 mesh, 200 mesh (Retsch, Germany)

2.4 Overhead stirrer (IKA RW20 Digital, Malaysia)

2.5 Refrigerated centrifuge (Himac, CR20B2, Hitachi, Japan)

2.6 Hot air oven (ED115, Binder, Germany)

2.7 Tray dryer (Reliance Tech-Service Co., Ltd, Thailand)

2.8 High pressure homogenizer (15MR-8TA, APV Gaulin, Inc., Wimington, MA, USA)

2.9 Spray dryer (GEA Niro A/S, Denmark)

2.10 Rapid visco analyzer (RVA) (Newport Scientific, Australia)

2.11 Microplate reader (Anthos 2010, Biochrom, UK)

2.12 Moisture balance (MS70, A&D, USA)

2.13 Scanning Electron Microscopy (SEM) (Hitachi S-4300, Japan)

2.14 Reverse phase high performance liquid chromatography (RP-HPLC) (RP-18 GP 250-4.6, Mightysil, Kanto Chemical Co.,inc., Japan) with 295 nm UV-detector (Hitachi L-7420, Japan)

2.15 Blood glucose meter with blood glucose test strip (ACCU-CHEK, Performa, USA)

2.16 X-ray diffractometer (D8 Advance, Bruker AXS GmbH, Germany)

2.17 Vacuum Rotary evaporator (Rotavapor, Büchi RE111, Switzerland)

Methods

1. Experiment preparation

1.1 Preparation of rice starch

High-amylose rice flour (Chainat 1 variety) was obtained from paddy rice supplied by the Chainat Rice Seed Center (Rice Department, Ministry of Agriculture, Thailand). Paddies were dehusked and milled to 90 degree of milling. Polished rice grains were steeped in water for 4 h and then wet-milled using a double-disk stone mill (locally made in Thailand). Rice slurry was centrifuged using a basket centrifuge. The rice cake was then dried in a tray dryer at 40± 5 ^{o}C overnight until moisture content reached 10–14%. Rice flour of 100 mesh particle size was obtained using a rotor mill (SR 300, Retsch, Haan, Germany). Sample was packed in sealed plastic bags, and kept in refrigerator (Cham and Suwannaporn, 2010).

1.2 Pyrodextrinization process

Hydrolyzed rice starch was prepared by spraying rice starch with 5% (by weight) hydrochloric acid followed by 5% (by weight) citric acid solution respectively to obtain a final acid concentration of 0.1% of starch. The hydrolysis with hydrochloric acid considerably dominated chemical modification with citric acid thus low molecular weight fraction increase and hydroxyl groups of rice starch was esterified (modified from Jochym *et al.*, 2012). Sample was mixed well and then dried in an oven at 50°C until moisture content reached 10–14%. A differential scanning calorimeter (DSC) (Star® System; Mettler Toledo AG, Greifensee, Switzerland) was used for glass transition (Tg) determination and calorimetric measurements. DSC was previously calibrated using indium and zinc standards.

The hydrolyzed starch sample (25% mc) was heated from 25 to 95 ^{o}C at 1 ^{o}C/min increasing rate. Sample was cooled down immediately to 5 ^{o}C at 20 ^{o}C/min rate. It was then reheated at 1 ^{o}C/min. Tg appeared as the peak point on the first derivative curve in a thermogram. The temperature at the midpoint of the change in slope was taken as Tg (Cham and Suwannaporn, 2010).

The pyrodextrinization process was modified in this study to avoid gelatinization of starch. Starch was hydrolyzed by acid followed by HMT. Sample (5% mc) was heated in an oven at 130°C for 1 hr (H1), 2 hr (H2) and 3 hr (H3). Hydrolyzed rice starch was washed with 95% ethanol until it reached pH 7 and milled to a particle size of 100 mesh. Hydrolyzed rice starch was adjusted to a moisture content of approximately 25%, equilibrated in desiccators until stable moisture content was obtained. Sample was then put in a closed container to keep its moisture and then HMT, by heating to a temperature higher than Tg but lower than gelatinization temperature (115°C) for 1 hour.

1.3 Tocopheryl acetate microencapsulation by spray drying

Hydrolyzed-HMT rice starch was used as carrier or encapsulation wall material for spray drying. The ratio of gum arabic : hydrolyzed-HMT rice starch : maltodextrin (DE 10) was 4/6:1/6:1/6 (modified from Kanakdande *et al.*, 2007) (Table 1). The lower DE maltodextrin was more suitable encapsulating agent due to less stick to walls of the drying chamber, better solubility and lower hygroscopicity and moisture content of the samples (Man *et al.*, 1999). The dry mix was suspended in distilled water at 30% (w/v) at temperature 10-12°C for 12 hours. Ten percent tocopheryl acetate (Sigma Aldrich Co.,Ltd, Thailand) and 0.2 % Tween 80 (Siam Victory Chemicals, Thailand) were mixed in an overhead stirrer to form an oil base. Load of core material was calculated as percent dry weight of carrier. Solution was mixed using a two stage high pressure homogenizer (15MR-8TA, APV Gaulin, Inc., Wilmington, MA, USA) at 5,000 psi to obtain a stable emulsion (modified from Kanakdande, *et al.*, 2007). Slurry was immediately spray dried at 160±5°C inlet temperature and 90±5°C outlet temperature. Microcapsules were collected from the cyclone and put in a sealed, airtight pouch. These pouches were stored in a desiccator for the next study. Plasticizers which are Glycerol and Sorbitol were added into the wall material

mixture to investigate its effect on microcapsules (modified from Kanakdande *et al.*, 2007) (Table 1). Hydrolyzed-HMT with plasticizer microcapsules were collected and kept in sealed and airtight pouches.

Table 1 Formula of different blends for microencapsulation

Sample	Components					
	Gum arabic	Maltodextrin	Rice starch	Plasticizer	Tween 80	Tocopheryl acetate (% by carrier)
Hydrolyzed-HMT						
Native	-	-	6/6	-	0.2%	10
H1	4/6	1/6	1/6	-	0.2%	10
H2	4/6	1/6	1/6	-	0.2%	10
H3	4/6	1/6	1/6	-	0.2%	10
Hydrolyzed-HMT with plasticizer						
MD+G	3/6	2/6	-	1/6	0.2%	10
H1+G	3/6	1/6	1/6	1/6	0.2%	10
H2+G	3/6	1/6	1/6	1/6	0.2%	10
H3+G	3/6	1/6	1/6	1/6	0.2%	10
MD+S	3/6	2/6	-	1/6	0.2%	10
H1+S	3/6	1/6	1/6	1/6	0.2%	10
H2+S	3/6	1/6	1/6	1/6	0.2%	10
H3+S	3/6	1/6	1/6	1/6	0.2%	10

Note : MD = Maltodextrin, G = Glycerol, S = Sorbitol

2. Determination of hydrolyzed-HMT rice starch properties

2.1 Pasting properties

Pasting properties of all starch samples were measured using Rapid visco analyzer (Newport Scientific, Australia). Starch (3g, 14% wb) was mixed with 25 g of distilled water in a metal RVA canister. Heating pattern was follow rice starch profile (AACC Method 61-02, 2000).

2.2 Swelling power and solubility

Rice starch sample (0.5 g) was mixed with 15 mL distilled water. The suspension was heated at 55, 65, 75 and 85°C for 30 min. The gelatinized sample was cooled down and centrifuged at 2200 g for 15 min. The supernatant was dried at 100°C and weighed to quantify solubility. The sediment was weighed to quantify swelling power. Calculations were shown as followings (Paipong and Naivikul, 2005).

(i) Swelling power = weight of sediment / weight of dried starch
(ii) % Solubility = (weight of dried solid in supernatant / weight of dried starch) x 100

2.3 *In vitro* starch digestibility

Porcine pancreatin (0.45 g) (EC 232.468.9; 30000 BPU/g, Sigma Aldrich Co.,Ltd, Thailand) was dispersed in water (4 ml) and centrifuged at 1500g for 10 min. The supernatant (2.7 mL) was transferred to a beaker. Then, 0.3 mL of diluted amyloglucosidase (EC 3.2.1.3; 400 AGU/mL, Sigma Aldrich Co., Ltd, Thailand) (0.32 mL of amyloglucosidase diluted to 0.4 mL with distilled water) and 0.2 mL distilled water were added to the solution. This enzyme solution was freshly prepared for each digestion (modified from Regand *et al.*, 2011).

Hydrolyzed-HMT rice starch (100 mg) was weighed into glass tubes in triplicate. Five glass beads (4 mm diameter) were added into each tube. 0.05 M HCl (2 ml)

and pepsin (EC 3.4.23.1, Sigma Aldrich Co., Ltd, Thailand) (10 mg) were added to the tubes and incubated at 37°C with continuous shaking (200 strokes/min) for exactly 30 min. Then, 0.5 M sodium acetate buffer pH 5.2 (4 mL) was added into each tube at 1 min intervals followed by a previously prepared enzyme solution (1 ml). The test tubes were then put in a shaking water bath at 37°C. Aliquots (0.1 mL) were then taken at 20 and 120 min intervals and mixed with 50% ethanol (1 mL). The solutions were centrifuged at 2000 rpm for 10 min and the hydrolyzed glucose content of the supernatants was measured by glucose oxidase–peroxidase assay (Regand *et al.*, 2011). Starch classifications based on the rate of hydrolysis were: rapidly digestible starch (RDS; starch which was digested within 20 min), slowly digestibly starch (SDS; starch which was digested between 20 and 120 min) and resistant starch (RS; starch which was undigested after 120 min). The amount of these three fractions was divided by the total amount of starch in the sample and expressed as percent of total starch (%RDS, %SDS and %RS) (Regand *et al.*, 2011).

2.4 Blood glucose response (*In vivo*)

At the beginning of the study, adult male Wistar rats (age 54 days) and weighed ~ 260 g each were housed individually in a temperature controlled (22±1°C) cage with a 12 h-12 h light-dark cycle. Samples of all tocopheryl acetate microcapsule were mixed with double distilled water and were fed to the rats at 2.5 mg/g rat weigh using a syringe. Eight Wistar rats were feed per sample. The blood glucose content (mg/dL) was measured by blood glucose meter with blood glucose test strips (ACCU-CHEK, Performa, USA) at 0, 15, 30, 45, 60, 90 and 120 min after feeding. Blood glucose content (mmol/L) was calculated as follows.

(i) Blood glucose content (mmol/L) = Blood glucose content (mg/dL) / 18.018

2.5 X-ray diffractions of hydrolyzed-HMT rice starch

X-ray patterns of hydrolyzed-HMT rice starches were determined using an X-ray diffractometer (D8 Advance, Bruker AXS GmbH, Germany) The operation scanning

conditions ranged from 5° to 30°, 30 kV target value, 30 mA and 1°/min scanning speed (Zavareze *et al.*, 2010).

3. Determination of encapsulation properties

3.1 Encapsulation efficiency

The amount of surface oil was determined gravimetrically by adding hexane (15 ml) to encapsulated samples (2.5 g). The mixture was mixed using a vortex mixer for 2 min and centrifuged at 8000 rpm for 20 min. The supernatant was filtered with filter paper and washed twice with hexane. The filtrate was evaporated using a rotary evaporator (Rotavapor, Büchi RE111, Switzerland) in a stirring water bath and dried at 105°C (Baik *et al.*, 2004).

The amount of total oil was determined gravimetrically by adding acetate buffer pH 3 (2 ml) in encapsulated samples (0.5 g). The mixture was mixed with vortex mixer for 1 min, and then extracted with hexane and isopropanol (3:1). The mixture was put in a shaking water bath (160 rpm) for 15 min and centrifuged at 8000 rpm for 15 min. The aqueous phase was taken and re-extracted with solvent. Samples were filtered using filter paper and sodium sulfate. The filtrate was evaporated using a rotary evaporator in a stirring water bath and dried at 105°C. Encapsulation efficiency (%EE) was calculated as follows (Baik *et al.*, 2004).

(i) %EE = (Total oil content – Surface oil content / Total oil content) x 100

3.2 Tocopheryl acetate releasing determination

Determination of tocopheryl acetate release was done under a simulated human digestive tract. 0.05 M HCl (pH 1.2) mixed with 0.2% NaCl was used as simulated gastric fluid (SGF) and 0.05 M phosphate buffer (pH 7.4) was used as simulated intestinal fluid (SIF) (Yoo *et al.*, 2006). Encapsulated samples (4 g) were soaked in SGF (20 ml) and SIF (20 ml) for 120 min at 37°C (body temperature). Aliquots (2 ml) were taken from the

medium at 30 min intervals for up to 120 min, extracted with *n*-hexane (5 ml) and evaporated using a vacuum evaporator. The remnant was dissolved in 1 ml mobile phase (methanol: dichloromethane, 85:15), then filtered through a 0.22-μm nylon filter directly into a vial. It was immediately kept in ice until analysis. Tocopheryl acetate was measured by reverse phase high performance liquid chromatography (RP-HPLC) (RP-18 GP 250-4.6, Mightysil, Kanto Chemical Co., Inc., Japan) with a 295 nm UV-detector (Hitachi L-7420, Japan). The mobile phase was methanol / dichloromethane (85:15 v/v) at a flow rate of 1 ml/min. Sample (200 μl) was injected into a RP-HPLC (5 μm) equipped with a 295 nm UV-detector and pump (Hitachi L-2130, Japan). The amount of tocopheryl acetate was calculated by standard samples (tocopheryl acetate) (Sigma Aldrich Co., ltd, Thailand). An automatic integrator (Hitachi L-2200, Japan) was used to calculate peak areas (modified from Kornsteiner *et al.*, 2006).

3.3 Microstructure

Encapsulated samples were weighed and soaked in HCl solution (pH 2.0) for 120 min. Samples were taken from the medium at 30 min intervals for up to 120 min and were centrifuged at 3000g for 10 min. The precipitate was dried at 50°C for 1 day. Microstructures of hydrolyzed dried samples were observed by scanning electron microscope (SEM) (Hitachi S-4300, Japan). Dried samples were mounted on double specimen stubs with double sided adhesive carbon tape. It was then coated with gold and examined at 15 kV.

3.4 Shelf life

All microencapsulated samples were put in sealed polyethylene bags and keep in a desiccator that was exposed to fluorescent light at room temperature for one year. Samples were then taken out to determine the releasing of tocopheryl acetate as mentioned in 3.2.

4. Statistical analysis

Data is reported as the average value of triplicate measurements. Difference analysis between means of data was carried out using ANOVA and Duncan's multiple range tests at $p \leq 0.05$ (SPSS program version 19.0 for Windows).

RESULTS AND DISCUSSIONS

1. Hydrolyzed-HMT rice starch properties

1.1 Pasting properties, swelling power and solubility

Pasting properties, swelling power and solubility of hydrolyzed-HMT of high amylose rice starch at different acid hydrolysis times were determined compared to native rice starch. Both acid hydrolysis and HMT had an effect on lowering pasting parameters. All hydrolyzed-HMT rice starches showed lowered pasting parameters except for pasting temperature (Table 2). Mild acid hydrolysis at strict water content first attacked the amorphous regions, mainly on the surface of the starch granule. The increase in hydrolysis time had no significant effect on these parameters. Mild acid modification manipulated physicochemical properties of starch without destroying its granule structure. The result agreed well with Wang et al. (2003) and Thirathumthavorn and Charoenrein (2005) that acid treatment could lowering pasting viscosity and higher gelatinization temperature in corn and rice starches due to the re-association and formation of network structure.

Table 2 Pasting properties of hydrolyzed-HMT starch

Pasting Properties (cP)	Hydrolyzing time (hour)			
	Native	1 (H1)	2 (H2)	3 (H3)
Peak viscosity	3194.00±9.90^{A}	306.00±5.66^{B}	336.50±10.61^{C}	316.00±5.66^{BC}
Trough	2695.50±3.53^{A}	157.50±10.61^{B}	136.00+7.07^{C}	98.50±2.12^{D}
Breakdown	498.50±13.44^{A}	148.50±16.26^{C}	200.50±3.53^{B}	217.50±7.78^{B}
Final viscosity	4241.00±25.46^{A}	284.00±11.31^{B}	266.50±12.02^{B}	212.00±1.41^{C}
Setback	1545.50±28.99^{A}	126.50±0.71^{A}	130.50±4.95^{B}	100.00±18.38^{B}
Peak time (min)	6.56±0.50^{A}	5.76±0.50^{B}	5.40±0.00^{C}	5.04±0.50^{D}
Pasting temp. (°C)	64.05±1.13^{A}	63.28±1.03^{A}	64.48±0.53^{A}	63.30±0.00^{A}

$^{A-D}$ Different letter superscripts in the same row indicate statistical difference (*p<0.05).

HMT promoted the formation of more ordered structure hence reduce swelling power of starch which effect on the decrease in all pasting properties. The increase in internal rearrangement of starch granule, causes more interactions between starch chain, the formation of ordered double helical amylopectin side chain clusters and the formation of amylose-lipid complexes (Zavareze and Dias, 2011). Swelling power and solubility of hydrolyzed-HMT starches were shown in table 3. Hydrolyzed-HMT starches increased solubility and decreased swelling power due to the increase in low molecular weight starch with more hydroxyl groups (Laurentin *et al.*, 2003; Campechano-Carrera *et al.*, 2007; Kapuśniak and Jane, 2007).

Table 3 Swelling power and solubility of hydrolyzed-HMT starch

Temperature (°C)	Hydrolyzing time (hour)			
	Native	1 (H1)	2 (H2)	3 (H3)
Swelling power (g/g)				
55	2.09 ± 0.08^{B}	2.53 ± 0.06^{A}	2.47 ± 0.05^{A}	2.57 ± 0.03^{A}
65	2.29 ± 0.06^{B}	2.62 ± 0.04^{A}	2.57 ± 0.08^{A}	2.64 ± 0.05^{A}
75	7.50 ± 0.21^{A}	5.32 ± 0.12^{C}	5.82 ± 0.14^{B}	5.84 ± 0.05^{B}
85	10.01 ± 0.20^{A}	6.48 ± 0.06^{C}	6.72 ± 0.06^{B}	6.82 ± 0.02^{B}
Solubility (g/100g)				
55	0.20 ± 0.02^{C}	1.70 ± 0.19^{B}	1.62 ± 0.18^{B}	2.04 ± 0.14^{A}
65	0.19 ± 0.09^{C}	5.64 ± 0.05^{A}	4.74 ± 0.16^{B}	5.00 ± 0.23^{B}
75	1.08 ± 0.19^{C}	8.44 ± 0.54^{B}	10.24 ± 1.73^{A}	11.72 ± 0.03^{A}
85	3.18 ± 0.17^{C}	17.14 ± 1.61^{B}	18.01 ± 0.44^{AB}	19.58 ± 0.34^{AB}

$^{A-D}$ Different letter superscripts in the same row indicate statistical difference (*p<0.05).

Swelling power of hydrolyzed-HMT starch was not much different at low temperature (55-65°C). But more strict swelling was found at higher temperature swelling (75-85°C). Acid hydrolysis caused cold water swelling and drastically increased in starch solubility. Solubility of pyrodextrinization of Lima bean (Campechano-Carrera *et al.*, 2007) and normal corn starch (Kapuśniak and Jane, 2007) was also reported to increase rapidly. These relatively low molecular fractions had better solubility than native starch which made

it an appropriate wall material for encapsulation. Better solubility caused more homogeneous slurry and better emulsified property. Orderly granule structure help resist shearing damage during spray dried under high pressure and high temperature. As a consequence, a better encapsulation efficiency and core material protection was expected.

1.2 Starch digestibility

1.2.1 *In vitro* starch digestibility

Starch digestibility was determined by rate of starch that was able to be digested in a human gastrointestinal tract model (*in vitro*). It was measuring as rapidly digestible starch (RDS; digested within 20 min), slowly digestible starch (SDS; digested between 20 and 120 min) and resistant starch (RS; undigested after 120 min). RDS, SDS, and RS content of hydrolyzed-HMT starch hydrolyzed for 1, 2 and 3 h are shown in table 4. Hydrolyzed-HMT rice starch was increased slightly in RDS as a result of a mild acid hydrolysis. HMT induced changes mostly in the amorphous regions, which are more susceptible to hydrolysis. This amorphous area was more rapidly degraded by α-amylase than the crystalline area. Therefore, the structural rearrangement of starch caused by HMT facilitates enzymatic accessibility in the amorphous area (Zavareze and Dias 2011). In addition, the solubility of pyrodextrinized starch increased as the increase in low molecular weight linear fractions with hydroxyl groups that also make it more susceptible to enzymatic hydrolysis (Sankhon *et al.*, 2013; Bai *et al.*, 2014).

SDS drastically decreased in proportion to the increase in RS content and was time dependent. The longer hydrolysis time the lowering of SDS and elevation amounts of RS (Table 4). SDS content was also reported to decrease after pyrodextrinization of African locust bean starch, along with an increase in RS (Sankhon, et al., 2013). With limited water hydrolysis, acid attacked mostly on the surface of starch granules and mostly in the amorphous region. Starch in the amorphous region was enzymatically hydrolyzed into RDS causing a higher percentage of crystalline starch left, that was then turn into RS after HMT. The result agreed well with Chung et al. (2009) and Zavareze and Dias (2011) that HMT could be used as a process to increase RS content without disruption of granular structure. HMT of higher amylose starches could obtain more RS.

Table 4 Enzymatic digestibility of hydrolyzed-HMT starch

Samples	Enzymatic digestible starch (%of total starch)		
	%RDS	%SDS	%RS
Native	41.83 ± 0.95 ^{B}	42.81 ± 0.89 ^{A}	15.36 ± 0.32 ^{C}
H1	42.07 ± 0.22 ^{B}	35.67 ± 1.18 ^{B}	22.26 ± 0.99 ^{B}
H2	45.45 ± 1.77 ^{A}	31.13 ± 0.81 ^{C}	23.42 ± 1.09 ^{B}
H3	43.35 ± 0.49 ^{B}	29.51 ± 0.79 ^{C}	27.14 ± 1.21 ^{A}

$^{A-C}$ Different letter superscripts in the same row indicate statistical difference (*p<0.05).

1.2.2 *In vivo* blood glucose response

Blood glucose content was measured by monitoring glucose content at 0, 15, 30, 45, 60, 90 and 120 min after consumption by Wistar rats. Native rice starch exhibited a much higher blood glucose response, especially at the first 30 min, and decrease sharply afterwards (Figure 4).

All hydrolyzed-HMT rice starches regulated blood glucose response quite steadily after 15 min. Blood glucose responded well with hydrolysis time. The slower blood glucose release indicates the longer core material protection by wall material within gastro-intestinal track. Good wall material should initially release the core material rapidly and keep the releasing rate to maintain effective concentration of core material as the absorption rate decreases (Yoo et al., 2006).

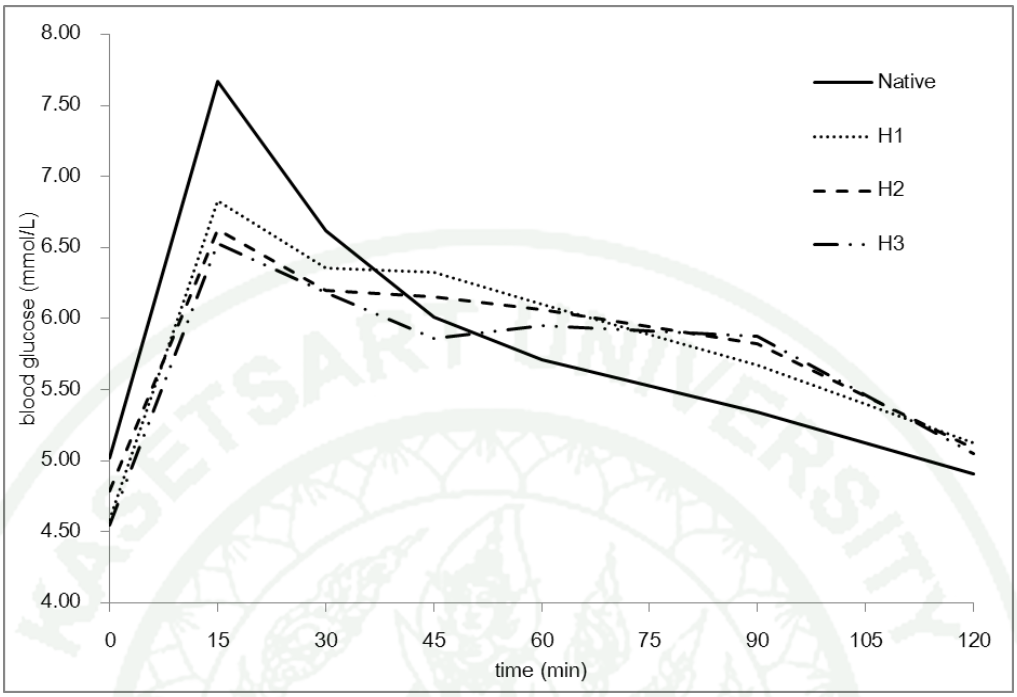


Figure 4 Blood glucose response in mice after consuming hydrolyzed-HMT starch

1.3 X-ray diffractions

X-ray diffractogram of native, H1, H2 and H3 starches are shown in figure 5. Diffractogram indicates an A-type crystalline pattern, which peaks appeared at 15°, 17°, 17.8° and 23° (2θ). Crystalline pattern of hydrolyzed-HMT starch remained unchanged due to A-type crystalline pattern is a stable monoclinic structure (Zavareze and Dias, 2011).

The effect of HMT on crystallinity depended on the source of starch and the treatment conditions. Taro, cassava, and cereal starches did not altered their X-ray diffraction pattern after HMT, (Zavareze et al., 2010; Khunae et al., 2007) however peaks at 17° and 17.8° (2θ) seemed to decrease with prolonged hydrolysis time.

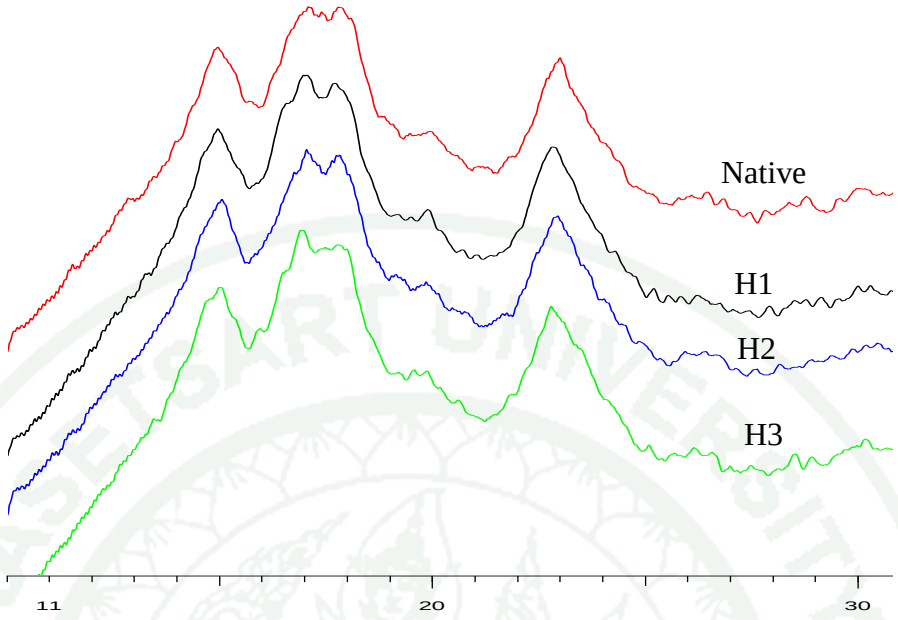


Figure 5 X – ray diffractograms of native starch and hydrolyzed-HMT starch

2. Hydrolyzed-HMT microcapsules properties

2.1 Encapsulation efficiency

Hydrolyzed-HMT rice starches were used as wall materials to encapsulate tocopheryl acetate by mixing the combinations of wall materials with homogenizer and encapsulated the tocopheryl acetate with spray drying process. The homogenization process could improve oil retention of microcapsule as small droplets emulsion could provide higher encapsulation efficiency (Danviriyakul *et al.*, 2002; Soottitantawat *et al.*, 2003; García *et al.*, 2012). Molecular weight is the primary factor influence diffusion rate. The increasing of molecular size generally results in slower diffusion rate. The molecules will take more time to reach the atomized droplet surface during drying especially at initial stages, and % retention will increase. When diffusions effectively stop at low moisture content, the droplet surface becomes impermeable to large flavour molecules more quickly during drying. Both of these factors support the retention of larger molecular weight volatiles (Jafari e*t al.*, 2008).

Encapsulation efficiency of encapsulated tocopheryl acetate with native, H1, H2 and H3 as wall materials was determined by % oil trapped inside the microcapsules. Percent entrapped oil is an indicator for tocopheryl acetate entrapment as it is soluble in oil. Percent encapsulation efficiency was determined by subtracting %surface oil (oil which was not encapsulated) from % total oil. Encapsulation efficiency of all hydrolyzed-HMT rice starches was much higher than native rice starch (Table 5).

Table 5 Encapsulation efficiency of hydrolyzed-HMT starch

Sample	Total oil (g/100g powder)	Surface oil (g/100g powder)	%Encapsulation efficiency
Native	2.42 ± 0.37^{A}	1.44 ± 0.20^{B}	39.18 ± 0.66^{A}
H1	2.76 ± 0.51^{A}	0.12 ± 0.05^{A}	95.30 ± 2.57^{B}
H2	3.84 ± 1.52^{A}	0.09 ± 0.04^{A}	97.34 ± 1.85^{B}
H3	2.87 ± 0.59^{A}	0.14 ± 0.02^{A}	94.80 ± 1.72^{B}

$^{A-B}$ Different letter superscripts in the same column indicate statistical difference ($*p<0.05$).

Higher content of surface oil indicates that core material was deposited on the surface of microcapsule, thus lowering % encapsulation efficiency. The ideal wall material should post emulsifying properties, high solubility and low viscosity at high solid levels (Jafari *et al.*, 2008). Hydrolyzed-HMT rice starch had high water solubility, promoting a more stable emulsion. Unstable emulsions were found to increase surface oil of spray dried milk powder (Danviriyakul *et al.*, 2002). Emulsion viscosity is an important factor for retention of core materials. Emulsion viscosity can suppress the internal circulation and waving of droplets, thus improving retention. Too high a viscosity caused too much exposure during atomization making droplet formation difficult and resulted in larger droplet sizes and decreased retention (Jafari *et al.*, 2008). The high orderly structure of hydrolyzed-HMT rice starch made it more resistant to high temperatures and shearing during spray drying. Moreover hydrolyzed-HMT rice starch exhibited low viscosity at high solid content (low swelling power). These properties of hydrolyzed-HMT rice starch made it an ideal carrier for encapsulation by spray drying.

2.2 Microstructure

Microstructures of hydrolyzed-HMT starches and microcapsules were shown in figure 6.

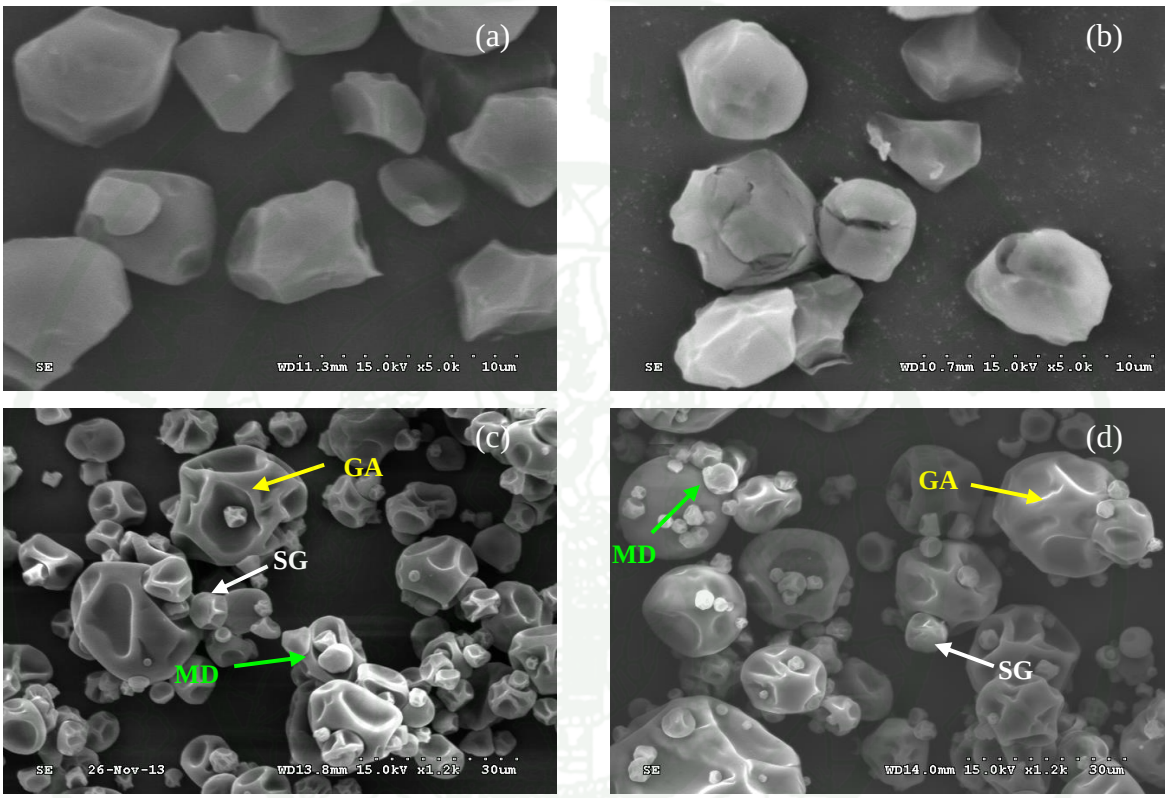


Figure 6 Microstructure of (a) HMT starch (b) hydrolyzed-HMT starch soaking in hydrochloric acid solution for 60 min
Microstructure of spray dried microcapsule using (c) native starch and (d) hydrolyzed-HMT rice starch as wall material

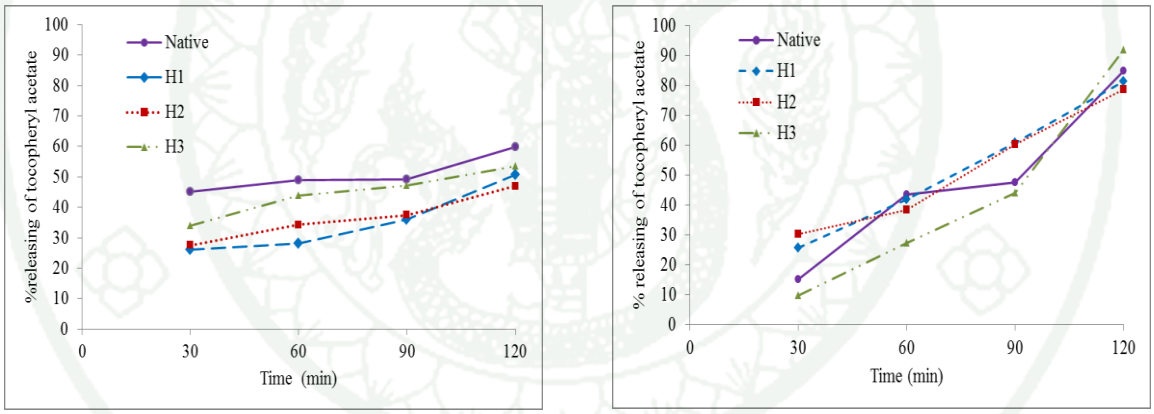
Note : gum arabic (GA), starch granule (SG) and maltodextrin (MD)

SEM of hydrolyzed-HMT starch granule soaked in acid for 1 h showed a disrupted surface by exo-corrosion from acid but still retained its granule structure (Figure 6b). Sulphuric acid treated rice and maize starches also had some pores in the granule surfaces during acid treatment (Palma-Rodriguez *et al.*, 2012). Under SEM, spray dried powder was seen as an agglomerated microcapsule to which the gum arabic (GA), starch granule (SG) and maltodextrin (MD) were evenly bound. GA size was around 20-30 μm,

SG size was around 2-8 μm and MD was a wide variety of particle size (Soottitantawat et al., 2005; Wani et al., 2012). Starch granule of samples using hydrolyzed-HMT as wall material was more swollen with less angular shape (Figure 6d) compared to native starch that formed a flat ball like shape (Figure 6c). The shrinkage of structure after spray drying indicates less resistance to shear, hence effects its encapsulation efficiency.

2.3 Tocopheryl acetate releasing in simulated human digestive tract (*in vitro*)

In SGF environment (0.05 M HCl, pH 1.2), % tocopheryl acetate release in all samples had a similar pattern; that a sharp increase within the first 30 min and reaching its plateau after that. However, hydrolyzed-HMT rice starch exhibited a lower % tocopheryl acetate release than native starch (Figure 7a).



(a) (b)

Figure 7 % Releasing of tocopheryl acetate of native, H1, H2, and H3 microcapsules in SGF (a) and SIF (b) at 0, 30, 60 and 120 min

The releasing of core material occurred via the digestion in digestive tract. Protonation in stomach digestive was occurred by proton dissociation of acid solution. Hydrochloric acid solution is a strongly acids completely dissociate in aqueous solution thereby protonation was occurred during the SGF soaking. These protons could hydrolyze and destroy wall material of microcapsule then tocopheryl acetate leached out. The more densely packed A-type structure could hinder the accessibility of H3O+ towards the α-1,4 and α-1,6 glycosidic bonds, thus decreasing the extent of acid susceptibility (Zavareze and

Dias, 2011). Percent of tocopheryl acetate released hydrolyzed-HMT is in the order of H3 > H2 >H1, as expected in shorter starch chains, in accordance with higher water solubility of H3> H2 >H1. In contrast, in SIF environment (0.05 M phosphate buffer, pH 7.4), % of tocopheryl acetate released from all samples had a similar pattern that continuous increase (Figure 7b). H3 showed the lowest release in the SIF environment. H1 which has high SDS, high encapsulation efficiency, and slow release in SGF but high release in SIF seemed to be the most appropriate wall material for spray drying.

3. Effect of plasticizer on microcapsule properties

3.1 Encapsulation efficiency

Plasticized wall material provided lower encapsulation efficiency than non-plasticized wall material (Table 6). Plasticizers could decrease total solid content of the blend system. Low solid content of the emulsion decreased the retention of core material as it required longer time to form a semi-permeable membrane of the dried particle surface. The higher solids content increased emulsion viscosity, resulted in a rapid skin formation (Jafari et al., 2008).

Plasticizer could increase the solubility because of its hydrophilic properties. Microcapsules with glycerol provided higher encapsulation efficiency than sorbitol. Hydrophilic plasticizer could interact with the polymer chains and promote the uniform suspension which supported the emulsion stability and encapsulation efficiency (Danviriyakul et al., 2002; Jafari et al., 2008). Glycerol has lower molecular weight comparing to sorbitol, thereby microcapsules with glycerol showed higher encapsulation efficiency.

Table 6 Encapsulation efficiency of encapsulated tocopheryl acetate using plasticized H1, H2 and H3 as a wall material

Samples	Total oil content (g/100g powder)	Surface oil content (g/100g powder)	(%) Encapsulation efficiency
H1	2.76 ± 0.51^{B}	0.12 ± 0.05^{EF}	95.30 ± 2.57^{BC}
H2	3.84 ± 1.52^{A}	0.09 ± 0.04^{F}	97.34 ± 1.85^{A}
H3	2.87 ± 0.59^{AB}	0.14 ± 0.02^{DEF}	94.80 ± 1.72^{AB}
MD+S (control)	2.81 ± 0.21^{AB}	0.23 ± 0.01^{BCDE}	91.57 ± 1.21^{BCD}
H1+S	2.46 ± 0.09^{B}	0.34 ± 0.05^{AB}	85.98 ± 2.52^{EF}
H2+S	2.60 ± 0.26^{B}	0.41 ± 0.03^{A}	84.23 ± 2.20^{F}
H3+S	2.76 ± 0.07^{B}	0.31 ± 0.11^{AB}	88.69 ± 3.83^{DE}
MD+G (control)	2.86 ± 0.21^{AB}	0.19 ± 0.03^{CDEF}	93.48 ± 0.83^{ABC}
H1+G	2.56 ± 0.14^{B}	0.27 ± 0.05^{BC}	89.36 ± 1.46^{CDE}
H2+G	2.78 ± 0.03^{B}	0.24 ± 009^{BCD}	91.35 ± 3.15^{BCD}
H3+G	2.91 ± 0.10^{AB}	0.27 ± 0.11^{BC}	90.89 ± 3.70^{BCD}

$^{A-F}$ Different letter superscripts in the same column indicate statistical difference (*p<0.05).

Note : MD = Maltodextrin, G = Glycerol, S = Sorbitol

3.2 Control releasing of tocopheryl acetate in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF)

Plasticizer could lower the tocopheryl acetate encapsulation protection as it promoted chain mobility and oxygen permeability (García *et al.*, 2000; Laohakunjit and Noomhorm, 2004) (Fig 8). The low molecular weight plasticizer has more effect on the degree of flexibility and also the aliphatic chain plasticizers (Edmund and Herman, 1965). The molecular weight of glycerol and sorbitol is 92.09 and 182.17 g/mol respectively. Thus glycerol plasticized wall materials exhibited higher tocopheryl acetate releasing both in SGF and SIF.

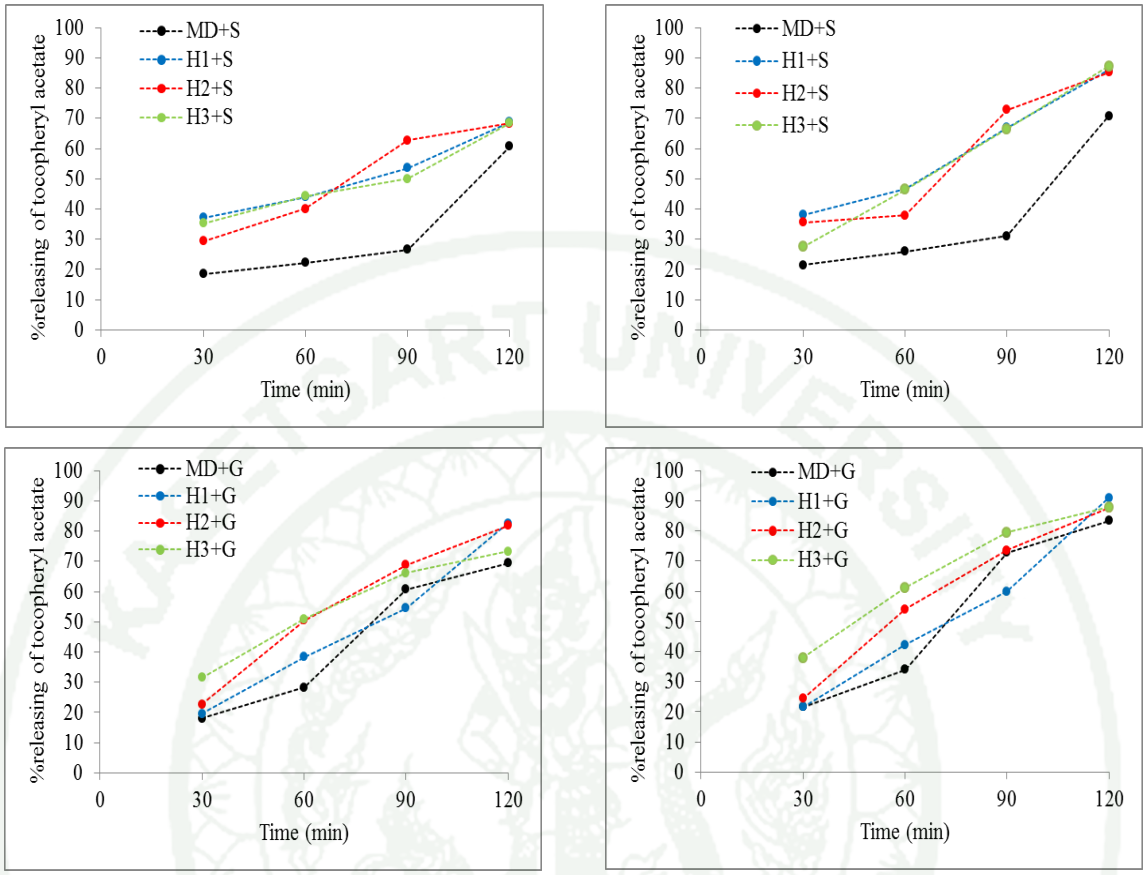


Figure 8 % Releasing of tocopheryl acetate of H1, H2, and H3 microcapsules with sorbitol and glycerol in SGF (a) and SIF (b) at 0, 30, 60 and 120 min

4. Effect of storage time on microcapsule properties

4.1 Percent release of tocopheryl acetate in Hydrolyzed-HMT microcapsules

After stored for 1 year, all samples showed significant lower in % releasing of tocopheryl acetate than freshly produced samples especially the native starch (figure 9). The higher surface oil content of native microcapsules had an effect on the loss of tocopheryl acetate by environmental factors. H_1 seemed to protect the core materials slightly higher than H_2 and H_3 respectively.

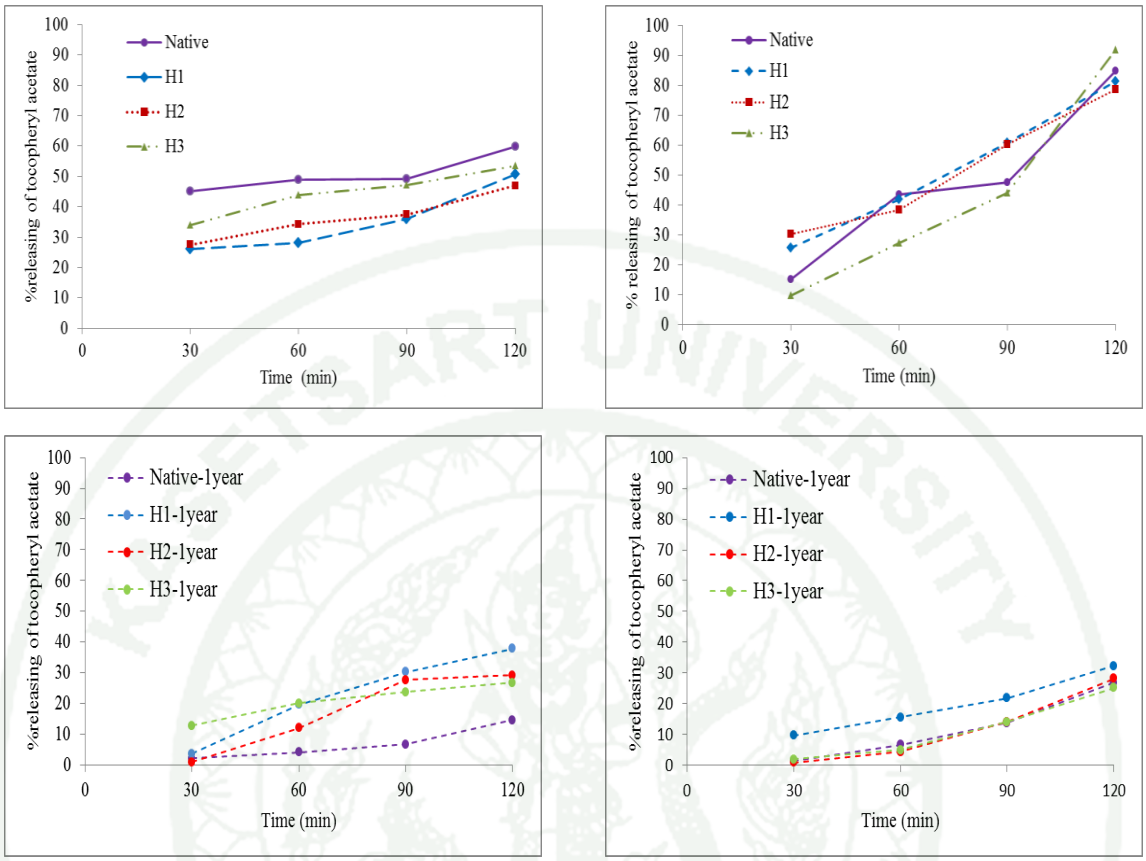


Figure 9 % Releasing of tocopheryl acetate of native, H1, H2, and H3 microcapsules in SGF(a) and SIF(b) at 0, 30, 60 and 120 min after 1 year storage

4.2 Percent release of tocopheryl acetate in Hydrolyzed-HMT microcapsules with plasticizer

All Hydrolyzed-HMT microcapsules with plasticizer exhibited a decrease in % tocopheryl acetate releasing in SGF and SIF (figure 10). The addition of plasticizers could decrease the protection of wall materials. Moreover plasticizers could decrease glass transition temperature of polymer by increase polymer mobility and and flexibility. Aliphatic plasticizer such as glycerol and sorbitol usually decrease glass transition temperature much more than bulky plasticizer molecules (Edmund and Herman, 1965). Decrease in glass transition temperature provided polymer more in a rubbery state which determined the strength of wall materials.

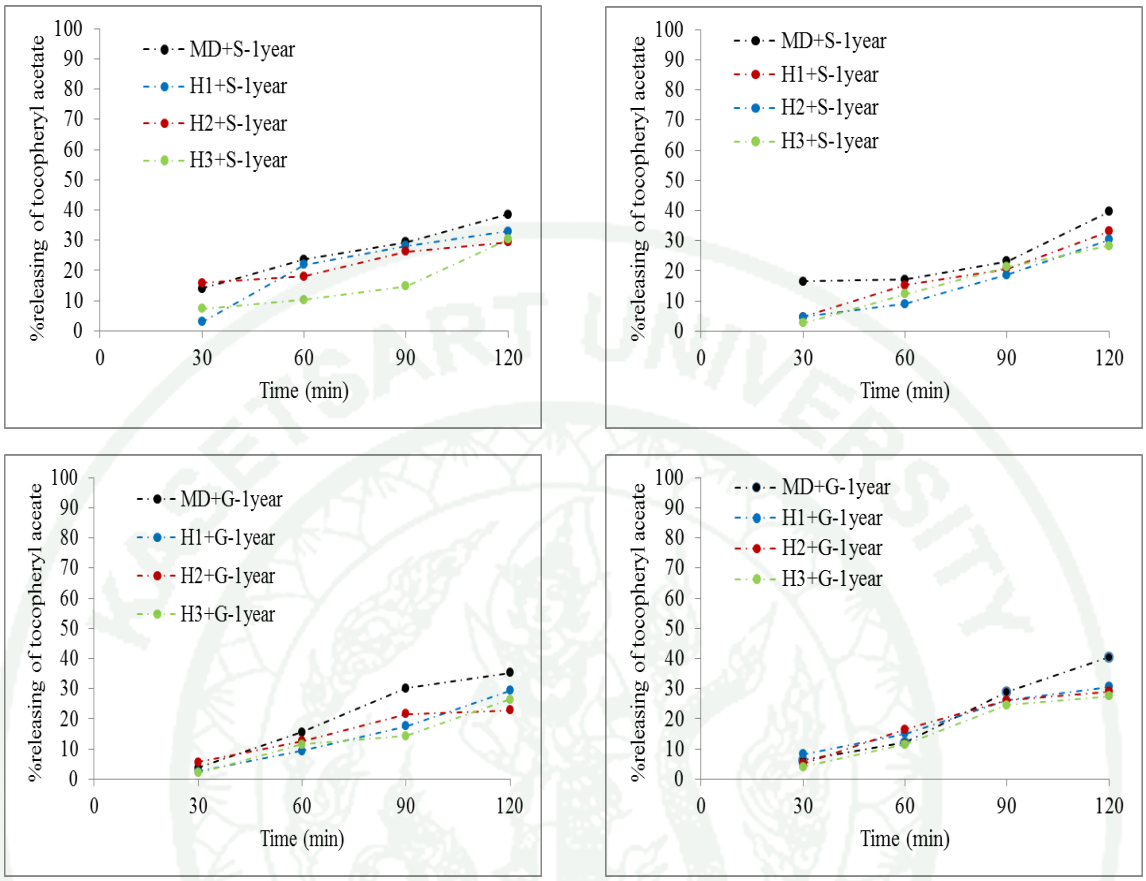


Figure 10 % Releasing of tocopheryl acetate of H1, H2, and H3 microcapsules with sorbitol and glycerol in SGF(a) and SIF(b) at 0, 30, 60 and 120 min after 1 year storage

CONCLUSION

Hydrolyzed-HMT rice starch can be used as wall material for encapsulation by spray drying. Its high solubility, cold water swelling and low viscosity at high concentration made it more emulsified. Moreover, it contained slow digestible starch fractions that are resistant to shear force giving it better protective ability under gastric pH and giving it high encapsulation efficiency. Hydrolyzed-HMT rice starch with plasticizers gave lower protection and % core material release either as a wall material or protect against environment. H1 showed the highest % release of tocopheryl acetate under SGF and SIF even after one year storage.

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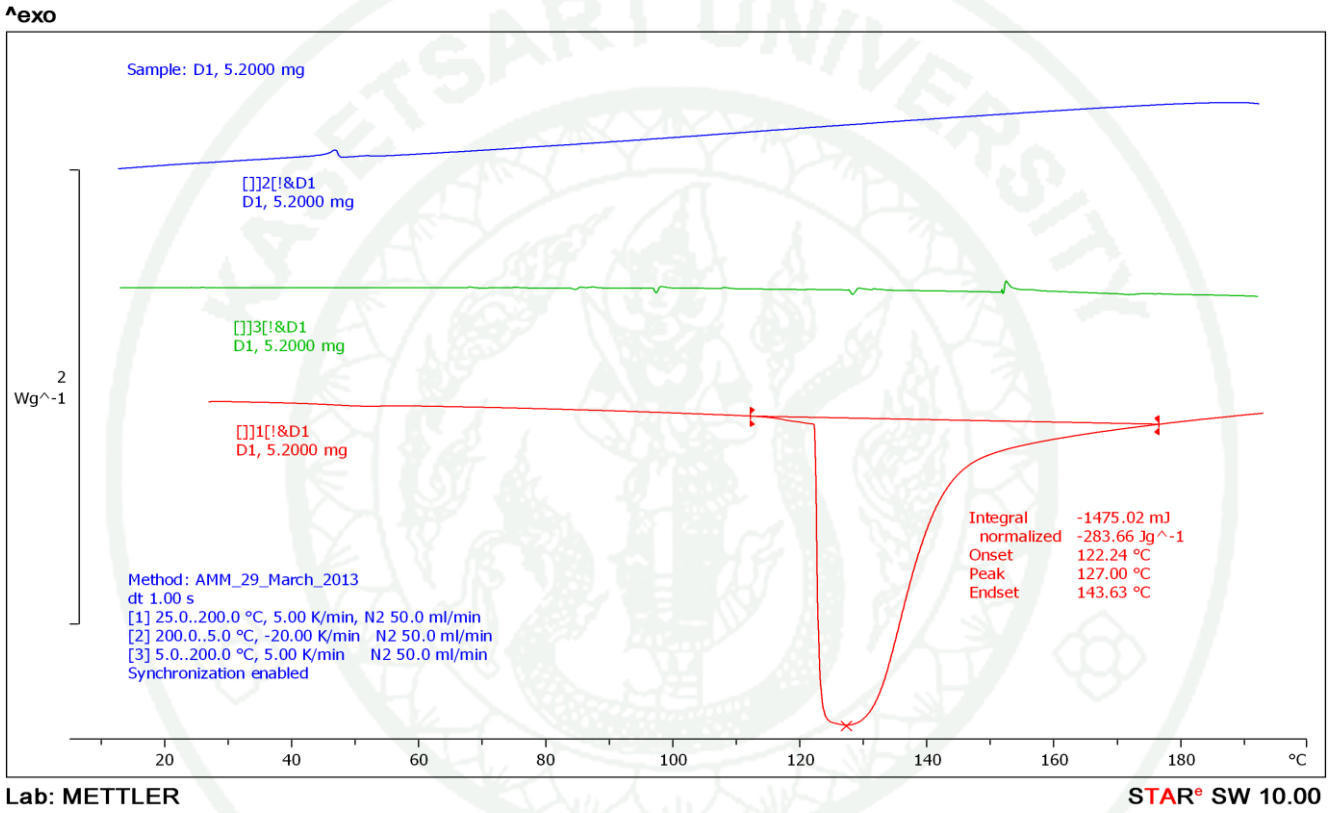
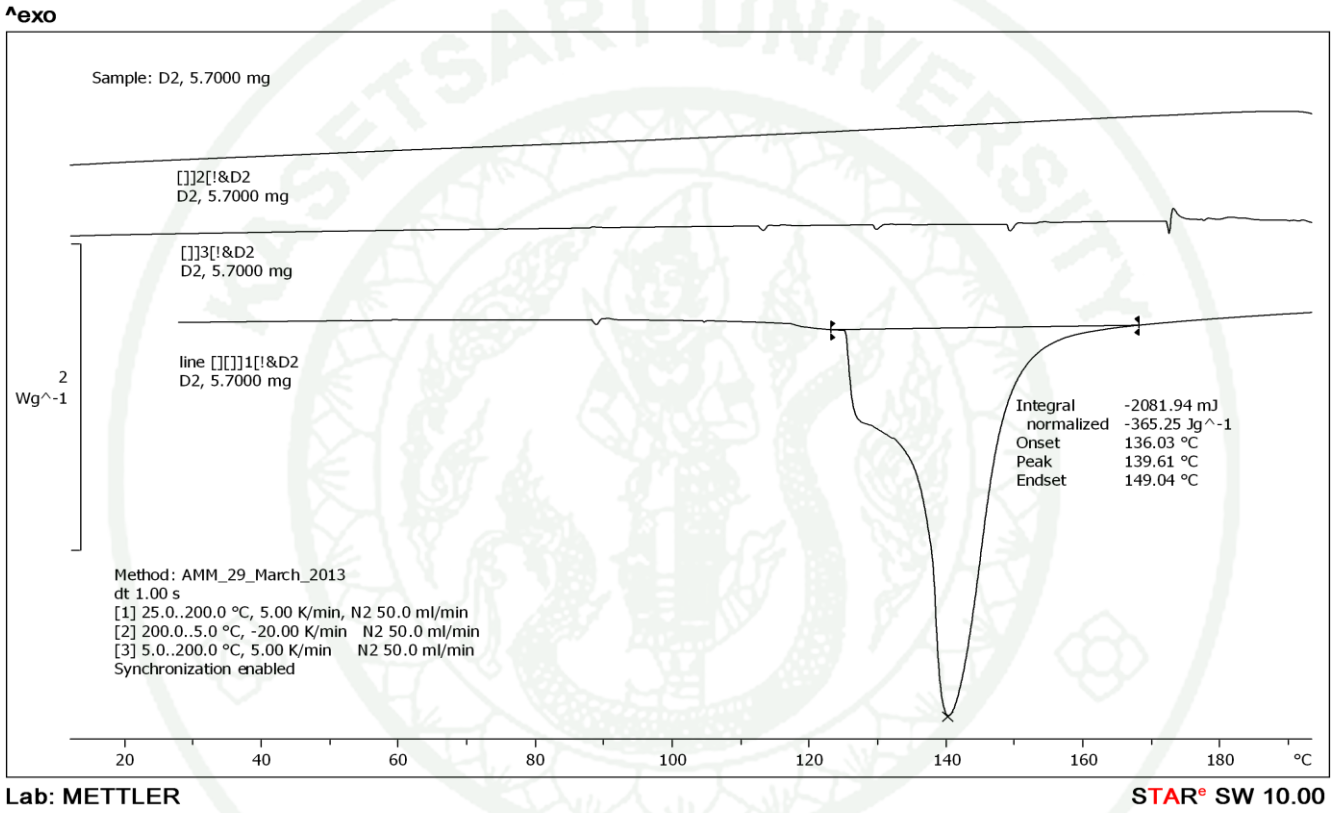
APPENDIX



Appendix Figure 1 Melting temperature (T_m) of hydrolyzed-HMT rice starches (H1)



Appendix Figure 2 Melting temperature (T_m) of hydrolyzed-HMT rice starch (H2)

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