II. LITERATURE REVIEW

2.1 THAI RICE CAKE AND PEA CAKE

Rice cake is indigenous food of many countries in Asia and Southeast Asia. Rice cake, in China, called migao, is famous for its soft-sticky texture and is usually served as a dessert. This cake is mainly prepared from rice flour and sticky rice flour (Ji et al., 2007). In Thailand, it is called kao ram phune (Figure 1) and is usually served with other ingredients such as vegetables, nut powder, spicy seasoning, etc. The vendors used to make it by soaking the rice grains overnight, wet milling, then boiling to gelatinize the starch.

In certain parts of Thailand, especially in the North, this type of cake is also made from pea (Pisum sativum) as presented in Figure 2. It is also usually served together with vegetables, nut powder, and spicy seasonings. The processing technique is a little bit different compared to rice cake. Pea grains are soaked overnight, then wet milled and filtered to separate coarse materials. The slurry then boiled to gelatinize the starch. After several minutes of cooling it forms a firm gel.

Figure 1. Rice cake

Figure 2. Pea cake
2.2 STARCH

Cereal grain stores energy in the form of starch. The amount of starch contained in cereal grain varies but is generally between 60 and 75% of the weight of the grain. Starch is found in plants in the form of granules (Hoseney, 1998). The cold-water-insoluble starch granule forms the major constituent of all cereal crops. Being a macromolecule composed entirely of α-D-glucose, this polysaccharide is readily assimilated in the human diet; in fact, a very high proportion of the world’s food energy intake is starch (Greenwood, 1976).

Starch from different cereals vary widely in their characteristics involve size, shape, chemical composition, and physical properties. Types of granule shape are large lenticular or lens-shaped (25-40 μm), small spherical 5-10 μm), round, elliptical, polyhedral, and polygonal. In the chemical composition, starch is composed essentially of glucose. It may contain a number of minor constituents which can and do affect the starch properties. Cereal starches contain low levels of fats, which is generally polar lipids in amount of between 0.5 and 1% (Hoseney, 1998). The presence of high amount of lipid in starch has unfavorable effects. The lipids repress the swelling and solubilization, also increase the pasting temperatures and reduce the water-binding ability. The formation of amylose-lipid complexes causes turbidity and precipitation in starch pastes and starch solutions. The oxidation of unsaturated lipids may cause the undesirable flavors in pregelatinized starch products. The other minor constituents are nitrogen substances (include proteins, peptides, amides, amino acids, nucleic acids, and enzymes), ash (corresponds partly with the amount of phospholipids in the starch granules), and phosphorus which occurs mainly as phospholipids (Collado and Corke, 2003).

One of the uniqueness of starch is that most starch granules are composed of a mixture of two polymers; an essentially linear polysaccharide called amylose, and a highly branched polysaccharide called amyllopectin. Most of starches contain about 25% amylose. While amylose is essentially a linear chain of (1→4)-linked α-D-glucopyranosyl units, many amylose molecules have a few α-D-(1→6) branches, perhaps 1 in 180-320 units, or 0.3-0.5% of the linkages. The branches in branched amylose molecules are either very long or very short, and the branch points are separated by large distances so that the physical properties of amylose molecules are essentially those of linear molecules. Amylose molecules have molecular weights of about 10^6. The axial → equatorial position coupling of the (1→4)-linked α-D-glucopyranosyl units in amylose chain gives the molecules a right-handed spiral or helical shape. The interior of the helix contains only hydrogen atoms and is lipophilic, while the hydroxyl groups are positioned on the exterior of the coil (BeMiller and Whistler, 1996). A helical conformation is common for amylose, and a double helix form when different helices pack together. An open channel in the center of a helix permits complexing with other molecular species, such as iodine, organic alcohols, or acids. Methods based on the iodine reaction remain a convenient means for estimating amylose content, giving accurate and reproducible results (Collado and Corke, 2003). The second polymer is amyllopectin, constituting about 75% of most common starches. It is a very large, very highly branched molecule, with branch-point linkages constituting 4-5% of the total linkages (BeMiller and Whistler, 1996). Like amylose, amyllopectin is composed of α-D-glucose linked primarily by α-(1→4) bonds. Amyllopectin is branched to a much greater extent than is amylose, with 4-5% of the glycosidic bonds being α-(1→6) bonds (Hoseney, 1998). Amyllopectin consists of a chain containing the only reducing end-group, called a C-chain, which has numerous branches, termed B-chains, to which one to several third-layer A-chains are attached. A-chains are unbranched. B-chains are branched with A-chains or other B-chains. The branches of amyllopectin molecule are clustered and occur as double helices. Molecular weights of from 10^7 to 5x10^8 make amyllopectin molecule among the largest molecules found in nature (BeMiller and Whistler, 1996).
2.2.1 Rice Starch

Rice starch exhibits typical characteristics compared to other starches (Appendix 1). Starch granules in rice endosperm grow as a single entity inside the cellular amyloplasts. Rice only contains compound granules in which many granules have developed within a single amyloplast. Compound granules having diameters up to 150 μm form as clusters containing between 20 and 60 individual granules (Champagne, 1996) which are the smallest known to exist in cereal grains (3 to 8 μm) as reported by Bao and Bergman (2004). There are some variations in starch granule size between different rice genotypes. Sodhi and Singh (2003) reported that a group of rice varieties grown in India had starch granules from 2.4-5.4 μm. The average starch size from some waxy rice ranged from 4.9 to 5.7 μm (Qi et al., 2003). Rice starch granules have a smooth surface but angular and polygonal shapes. The polygonal starch granules may be formed by compression of the starch granules during grain development (Hoseney, 1998).

2.2.2 Pea Starch

Being a legume starch, yellow pea (*Pisum sativum*) which is also known as field pea, garden pea, or smooth pea, exhibits a definitely different characteristic from rice which is grain starch, both in physical and chemical properties. It has a relatively large average granular size (28.82 μm) compared to other starches, with oval, round, spherical, elliptical, or irregular shapes. However, yellow pea starch has a closely range of gelatinization temperature (61.7-75.1 °C) compared to rice starch (61-80 °C), as reported by Collado and Corke (2003). Pea starch also contains higher amount of amylose (34.2-40.8 %) compared to any other type of starches. This typical characteristic of legume starch leads to less enzyme susceptibility (Singh et al., 2010).

2.3 ENZYMATIC DIGESTIBILITY OF STARCH

Such enzymes can hydrolyze the starch with the presence of water. The hydrolases are a large group of enzymes which have in common the involvement of water in formation of product. One of the important subgroups is carbohydrases. Amylases are carbohydrases that catalyse the hydrolysis of α-D-1,4-glycosidic linkages of starch and related oligo- and polysaccharides by the transfer of glycosyl residue (donor) to H₂O as the acceptor (Naz, 2002).

α-Amylase (EC. 3.2.1.1, 1,4-α-D-glucan glucanohydrolase) is an endo-enzyme that cleaves both amylose and amylopectin molecules internally, producing oligosaccharides. The larger oligosaccharides may be singly, doubly, or triply branched via (1→6) linkages, since α-amylase acts only on the (1→4) linkages of starch (BeMiller and Whistler, 1996). An α-1,4 linkage neighbouring an α-1,6 branching point in the substrate is resistant to attack by the enzyme (Naz, 2002). α-Amylase also does not attack double-helical starch polymer segments or polymer segments complexed with a polar lipid which is stabilized single helical segments (BeMiller and Whistler, 1996).

Glucoamylase (amyloglucosidase) is used commercially, in combination with an α-amylase, for producing D-glucose (dextrose) syrups and crystalline D-glucose. The enzyme acts upon fully gelatinized starch as an exo-enzyme, sequentially releasing single D-glucosyl units from the nonreducing ends of amylose and amylopectin molecules, even those joined through (1→6) bonds. Consequently, the enzyme can completely hydrolyze starch to D-glucose, but is used on starch that has been previously depolymerized with α-amylase to generate small fragments and more reducing ends (BeMiller and Whistler, 1996).
Various kinds of enzymes that catalyze hydrolysis of amylose and amylopectin molecules are obtained from a variety of sources, such as fungi, bacteria, cereals, and other plants, and in the case of α-amylase even from animal sources. Their efficiency, specificity, and optimum conditions of activity depend on the source (Collado and Corke, 2003). The node of enzymatic degradation of the granule also depends both on the enzyme source and the type of starch (Greenwood, 1976). Although the endo-enzyme implies random cleavage, numerous experiments have suggested that the enzyme action follows a definite pattern depending on the source of the enzyme (Naz, 2002).

Depending on their rate and extent of digestion, starch can be classified into three categories (Englyst et al., 1992); these include rapidly digested starch (RDS), slowly digested starch (SDS), and resistant starch (RS). The fraction of starch that is said to be RDS in vitro is defined as the amount of starch digested in the first 20 min of enzyme digestion, whereas SDS is defined as the starch that is digested after the RDS but in no longer than 120 min under standard conditions of substrate and enzyme concentration (Englyst et al., 1992). RDS and SDS are digestible starch, or that we call non-resistant starch (NRS). Total starch is the sum of non-resistant starch and resistant starch.

In the last 25 years the digestibility of foods has been classified by a number of metrics, the most popular of which is the glycemic index (Dona et al., 2010). However, aside from glycemic index (GI), resistant starch (RS) content has been established as an important measure to characterize starch digestibility (Frei et al., 2003). Resistant starch (RS) itself has been defined as the portion of starch that is not hydrolyzed by the enzymes in the small intestine and passes to the large intestine, or to be the total starch minus amount of glucose released within 120 min of in vitro digestion (Singh et al., 2010a).

The beneficial effects of resistant starch have received much attention. Resistant starch acts as a fermentation substrate in the colon, similar to non-starch carbohydrates, with positive implications for the prevention of food-borne diseases, such as colon cancer and hypolipidemia (Frei et al., 2003). According to Hu et al. (2004), RS is slowly absorbed in the small intestine resulting in decreased postprandial glucose and insulin responses. This behavior has significant implications for the use of RS in food formulations for persons with certain forms of diabetes. The slowed starch absorption also implies long-term benefits in controlling hyperlipidaemia. In the colon, RS increases fecal bulk, lowers colonic pH and the portion fermented by the intestinal microflora produces a range of short-chain fatty acids (SCFA), primarily acetate, propionate and butyrate. SCFA production has a positive impact on bowel health, including increased absorption of magnesium and calcium, epithelial proliferation, the balance of bacterial species, and bacterial metabolism of bile salts. Whether through its indirect effect on bile salts or through dilution effects, RS is thought to provide a degree of protection against bowel cancer.

2.3.1 Enzymatic Digestibility of Rice Starch

Many of researches have been carried out to investigate the digestibility of rice starch. The glycemic response of rice is known to be relatively high compared to other starchy foods (Frei et al., 2003). The same authors also reported the effect of cooking and storage to the in vitro starch digestibility and the glycemic index of six different rice cultivars from the Philippines. The results indicate substantial differences in the estimated glycemic index between rice cultivars. Values ranged between 68 and 109 for cooked rice and between 64 and 87 for stored rice containing retrograded starch. Storage under refrigeration also has been reported to slow the rate of rice starch digestion. The other result was that starch hydrolysis tended to be more rapid and more complete for waxy cultivars than for high amylose cultivars. Sasaki et al. (2009) reported the effect of physicochemical characteristics on the in vitro digestibility among waxy rice cultivars in the form of starch gels. The
authors suggested that the differences in amylopectin chain distribution reflect the stability, perfection, and extent of recrystallinity in the starch gel, inducing a difference in digestibility of the starch gel. Perez et al. (1991) reported that rice cultivars with similar amylose content varied in starch digestibility, the difference being associated with other properties, such as gelatinization temperature, cooking time, amylograph consistency, and volume expansion upon cooking. Processing techniques are also reported to impact the rate of rice starch digestion. Parboiling reportedly decreases rice starch rate of digestion (Tetens et al., 1997). Rashmi and Urooj (2003) found that the steaming of rice created more resistant starch than boiling or pressure cooking.

2.3.2 Enzymatic Digestibility of Pea Starch

Legume starch is generally less in digestibility compared to grain starch. It is mainly due to the composition between amylose and amylopectin. RS contents increased with increasing amylose content which means pea starch will be less in digestibility (Perera et al., 2010). This is also affected by morphological characteristics of pea starch which has a large granular average size. Parera et al. (2010) reported an apparent direct negative relationship between large size granules and starch digestibility. In other case, Lindeboom et al. (2004) reported that the small barley and wheat starch granules hydrolyze faster than the large granules. The presence of some non-starchy substances such as proteins over the granule surface may also limit the rate of enzymatic hydrolysis. Granule surface proteins and lipids can reduce surface accessibility by blocking the adsorption sites and therefore influences enzyme binding (Oates, 1997).

2.4. PHYSICOCHEMICAL PROPERTIES OF STARCH

2.4.1 Gelatinization

Starch granules are insoluble in cold water. They swell slightly but shrink back to their original size and consistency on drying. When heated in a water suspension to progressively higher temperature, very little happens until a certain critical temperature is reached. At that point starch granules begin to swell, simultaneously losing polarization crosses. This is termed gelatinization (Pomeranz, 1991). Other definition is the collapse (disruption) of the starch granule manifested in irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence, and starch solubilization. The point of initial gelatinization and the range over which it occurs is governed by starch concentration, method of observation, granular type, and heterogeneity within the granule population under observation (Collado and Corke, 2003).

The gelatinization temperature (GT) is always a temperature range. For a single starch granule in excess water, this temperature range might be 1 to 2 °C, whereas for the entire population the range might be 10 to 15 °C (Eliasson and Gudmundsson, 2006). Rice starch (20 %) has GT ranged from 60 to 77 °C while waxy rice starch at the same concentration ranged from 60 to 78 °C (Eliasson and Gudmundsson, 2006). After than a decade of study most agree that variation in rice GT is a result of differences in the proportion of amylopectin that is short versus long chains, thus degree of crystallinity is what is being measured as gelatinization temperature (Bao and Bergman, 2004).

Investigating gelatinization characteristics of starches is very important. Gelatinization is a physical process that is unique to starches and is responsible for its change properties during the preparation and processing of food (McWilliams, 2005; Collado and Corke, 2003). The heat energy required to completely gelatinize starch in rice is critical to the rice processor, who must optimize heat
input, cooking time, and temperature and, at the same time, minimize the cost of entire process (Bao and Bergman, 2004).

2.4.2 Pasting Properties

The terms gelatinization and pasting have often been applied to all changes that occur when starch is heated in water. However, gelatinization includes the early changes and pasting includes later changes. Pasting is the phenomenon following gelatinization in the dissolution of starch. It involves granular swelling, exudation of molecular components from the granule, and eventually total disruption of the granules (Collado and Corke, 2003). Simply stated, starch paste can be described as a two phase system composed of a dispersed phase of swollen granules and a continuous phase of leached amylose (Collado and Corke, 2003).

2.4.3 Instruments for Observing Gelatinization and Pasting Behavior of Starch

The gelatinization and pasting behavior can be recorded by using a Brabender Visco Amylograph, Rapid Visco Analyzer (RVA), or other viscometers which record the viscosity continuously as the temperature is increased, held constant for a time, and then decreased. However, RVA is likely to be preferred as it gives results in much shorter time (Bao and Bergman, 2004; Hoseney, 1998). At the initial step, the viscosity increases rapidly with the increase of temperature as the granule swells. The peak viscosity is reached when granules swelling have been balanced with the granules broken by stirring. With continued stirring, more granules rupture and fragment, causing a further decrease in viscosity. On cooling, some starch molecules partially re-associate to form a precipitate or gel, in which amylose molecules aggregate into a network, embedding remnants of starch granules (Bao and Bergman, 2004). Rice starch pasting parameters have been reported to be correlated with amylose content (Keeratipibul et al., 2008(386,770),(643,791)) but different in waxy rice compared to nonwaxy rice (Bao and Bergman, 2004).

2.4.4 Syneresis and Retrogradation

As the starch paste is cooled, the starch chains become less energetic and the hydrogen bonds become stronger, giving a firmer gel. As a gel ages or if it is frozen and thawed, the starch chains have a tendency to interact strongly with each other and thereby force water out of the system. The squeezing of water out of the gel is called syneresis (Hoseney, 1998). Longer storage gives rise to more interaction between the starch chains and eventually the formation of crystals. This process, called retrogradation is the crystallization of starch chains in the gel. The gel becomes more opaque as retrogradation progress. In addition, it becomes more rigid or rubbery, perhaps partially as a result of crystallization and partially just from interaction of the starch (Hoseney, 1998).

2.5 APPLICATION OF DIFFERENTIAL SCANNING CALORIMETRY (DSC) TO STARCH

Thermal analysis is broad term that encompasses numerous techniques that measure chemical or physical changes of a substance as a sample is subjected to a controlled temperature program over time. The most popular modern thermal analysis techniques are those that dynamically follow (a sequence
physicochemical changes that a substance undergoes during heating or cooling. One of them is differential scanning calorimetry or DSC (Schenz and Davis, 1998).

Differential scanning calorimetry (DSC) has been the most commonly used methods of thermal analysis in food science. This technique has come to dominate the studies of starch-related transitions (Eliasson, 2003). It measures the differential temperature or heat flow to or from a sample versus a reference material, and this is displayed as a function of temperature or time. This technique can differentiate between two types of thermal events: endothermic and exothermic (Schenz and Davis, 1998). Endotherms typically associated with the melting of mono-, di-, oligo-, and polysaccharides, denaturation of proteins (Kalentunc and Breslauer, 2003). Starch gelatinization is also an endothermic process with enthalpy values in the range of 10 to 20 J/g (Eliasson and Gudmundsson, 2006).

To gather, interpret, and calculate the proper onset ($T_o$), peak ($T_p$), conclusion temperatures ($T_c$) and heat transition, as well as the heat capacity of the sample, the instrument must be calibrated with well characterized standards, such as indium. Indium has a $\Delta H$ of fusion of 28.4 J/g, and melting point of 156.64 °C (Schenz and Davis, 1998).

Regarding to the sample size, it must be small to obtain a near-instantaneous response to the heat transfer and resulting high precision in the determination (Blond and Simatos, 1996). The usual sample size (6-12 mg) can be placed in either small (up to 20 mg) volatile or nonvolatile sample pans (usually sealed volatile sample pans or often called hermetic pans are used most commonly in food science work) or stainless steel capsule that can withstand high-pressure buildup inside them, such as that caused by the volatilization of water (Schenz and Davis, 1998).

Regarding to the heating rate, it should be slow enough to obtain distinct and reproducible peaks for each transition. For most applications, a rate of 10 °C/min suits well. However, in cases where precise temperature determinations are desired, slower rates (1-2 °C/min) must be used (Blond and Simatos, 1996).

Since DSC measures heat flow, larger sample and faster heating rates will give larger signal. However, too large sample and too fast heating rates also broaden transitions. In general, it is best to use the minimum sample size and slowest heating rate that is practicable to give the desired resolution of transitions and thermal data (Schenz and Davis, 1998).

This instrument will display the result of observation in the DSC thermogram. The start of the peak (where it deviates from the base line) corresponds to the start of birefringence loss. The area under curve is a measure of the energy (enthalpy, $\Delta H$) required for the transition from an ordered to a disordered state (i.e., for the crystalline area to melt). The end point of the lost of birefringence and the end of the peak are not quite the same, as there is a considerable lag in the DSC. However, in general, the two correlate well (Hoseney, 1998). One major controversy in the interpretation of DSC curves relates to the onset temperature, the meaning of the peak, and the determination of the baseline. Most researchers agree onset temperature is more significant than peak temperature, since peak temperature is greatly influenced by scan rate and sample size and does not always relate to a specific physical change. Although one may report peak temperatures to compare to other reports, onset temperatures should be used to interpret data (Schenz & Davis, 1998).

Ji et al. (2007) reported that two endotherms were observed by DSC studies of cake prepared from rice flour and sticky rice flour. The first transition with a peak temperature ($T_p$) at approximately 58.1 °C is characteristic of melting of retrograded amylopectin. The second endotherm observed by DSC above 100 °C is characteristic of melting of the amylase-lipid complex of starch.
APPLICATION OF SCANNING ELECTRON MICROSCOPY (SEM) TO STARCH

Scanning electron microscope (SEM) has been widely used in the area of research which is focused on starches, especially in examining granular morphology of starches. It gives satisfying information of starches granule size and shape among botanical sources which are necessary to be understood. In the best conditions, and with the most advanced equipment, the resolving power of the scanning electron microscope can reach 5 nm for biological matter (Blond and Simatos, 1996).

The morphological changes of starch during heating in excess water is also can be studied by SEM (Eliasson and Gudmundsson, 2006). Collado and Corke (2003) suggested that functional properties of the starches are related not only to their structure as polymers but also to the packing of polymers within the granules. Bao and Bergman (2004) also reported that the clarity of starch suspensions which is very important for many food applications varies among different rice cultivars and may be attributed to amylose content and granular size.

The mechanism of SEM in generating image reveals quite a bit of complexity, but it can be simplified (Hafner, 2007). Principally, the sample is bombarded by electrons energy. Under this bombardment, each point of the object spontaneously emits varied radiations, depending on its chemical nature, surface state and the conditions of the electron probe emission. This radiation is captured by appropriate detectors (Blond and Simatos, 1996). This instrument is composed by several supporting equipments such as a column containing a thermoelectronic emission system (electron gun), an electron probe focusing system (condenser and objective), and a scanning system (electromagnetic deflection) controlling the scan amplitude (Blond and Simatos, 1996).

One thing that must be concerned in SEM is sample preparation. Sample preparation for scanning electron microscopy depends on their hydration. The samples must be dehydrated before being placed under vacuum in the microscope column. If the moisture content does not exceed 16 % (e.g., cereal grains or dry products) they can be observed as they are. Contrarily, if their moisture content is higher, it is essential to dehydrate them either by lyophilization or critical point drying (Blond and Simatos, 1996). If it is preferred to avoid the inconvenience of dehydration, it is possible to use a cryotransfer stage. This special device allows the sample to be observed directly, after very fast freezing on a liquid nitrogen cooled stage (-170 °C). Since the sample is preliminary fractured and coated with metal under vacuum at -170 °C, the temperature is never interrupted. The results with this technique are outstanding (Blond and Simatos, 1996).

The remarkable advantages of SEM are apparently admitted. However, this technique also presents certain drawbacks, like the inability to study hydrated media without resorting to complex techniques, and multitude of problems related to the preparatory techniques, which do not occur without fairly significant disruption in the sample material or the production of foreign elements that must be eliminated. These factors must be taken into account when interpreting the photographic documentation (Blond and Simatos, 1996). As stated above that samples must be observed in dehydrated state, biological samples exhibit at three major drawbacks: 1) they are poor conductor, and thus poor emitters, causing strong discharges during observation, which disrupts the electromagnetic detection and consequently, the image; 2) the low energy electrons penetrate easily, so that the image is formed from both true secondary electrons and second generation electrons, which weakens image clarity, but accentuates its relief; 3) some substances, such as starch, are very sensitive to the action of high energy electrons, and break up when the intensity is too high (Blond and Simatos, 1996).