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PROPERTIES OF RICE FLOUR AND EFFECTS OF SUCROSE AND
COCONUT MILK ON PROPERTIES OF RICE FLOUR

Ms. Duangrutai Thumrongchote

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Food Technology

Department of Food Technology

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RICE FLOUR

By Ms. Duangrutai Thumrongchote

Field of Study Food Technology

Thesis Advisor Associate Professor Saiwarun Chaiwanichsiri, Ph.D.

Thesis Co-Advisor Associate Professor Kalaya Laohasongkarm, Ph.D.,

Thesis Co-Advisor Professor Toru Suzuki, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial
Fulfillment of the Requirements for the Doctoral Degree

..... Dean of the Faculty of Science
(Professor Supot Hannongbua, Dr. rer. Nat.)

THESIS COMMITTEE

..... Chairman
(Professor Vanna Tulyathan, Ph.D.)

..... Thesis Advisor
(Associate Professor Saiwarun Chaiwanichsiri, Ph.D.)

..... Thesis Co-Advisor
(Associate Professor Kalaya Laohasongkarm, Ph.D.)

..... Thesis Co-Advisor
(Professor Toru Suzuki, Ph.D.)

..... Examiner
(Assistant Professor Pasawadee Pathepasen, Ph.D.)

..... External Examiner
(Professor On-anong Naivikul, Ph.D.)

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DUANGRUTAI THUMRONGCHOTE: PROPERTIES OF RICE FLOUR AND EFFECTS OF SUCROSE AND COCONUT MILK ON PROPERTIES OF RICE FLOUR.

THESIS ADVISOR: ASSOC. PROF. SAIWARUN CHAIWANICHSIRI, Ph.D.,

THESIS CO-ADVISOR: ASSOC. PROF. KALAYA LAOHASONGKARM, Ph.D., and

PROF. TORU SUZUKI, Ph.D., 128 pp.

This research consists of three parts. The first part aimed to investigate the physicochemical (chemical composition, amylose content, % damaged starch, flour particle size, and % crystallinity), functional (water absorption index, water solubility index, and swelling power), thermal, pasting and gel textural properties of flours from 6 varieties of non-waxy Thai rice, i.e. Pathum Thani 1 (P1), Hawm Suphan Buri (HS), Suphan Buri 1 (S1), Suphan Buri 2 (S2), Suphan Buri 3 (S3), and Suphan Buri 60 (S60). Second part aimed to study the effects of sugar and coconut milk on thermal, pasting, gel textural properties, and retrogradation of P1 flour. Third part involved the study of the effect of sucrose (0-20%, w/w) on the gelatinization of rice flour treated under the high hydrostatic pressure (HHP). The results showed that flours from 6 varieties had different properties, i.e. amylose content 18.64-34.19%, damaged starch 2.52-6.38%, onset temperature (T_{onset}) 70.48-77.72°C, % crystallinity 23.14-31.30, and hardness 24.87-61.15 gf. Based on these properties the flour samples could be divided into 2 groups: flours from P1, HS, S2, and S60 having amylose content of 18-22% had T_{onset} 70°C, % retrogradation 32-40, and low final viscosity and setback; second group was flours from S1 and S3 having amylose content of 28-33% had T_{onset} 77°C, % retrogradation 55-59, and high final viscosity and setback. In the second part it was found that adding sucrose increased thermal properties (T_{onset} , T_{peak} , T_{end}) of flour suspensions in both water and coconut milk systems. From pasting measurement it showed that sucrose and coconut milk increased setback from 893 cP (flour in water system) to 1796 cP and 1359 cP (flours with 50% sucrose in coconut milk and in water). After storage the gels at 4°C for 30 days, it was found that coconut milk retarded retrogradation while sucrose accelerated the process. The rate constants (k) of rice gel in coconut milk and gel in water with 50% sucrose were 0.308 and 0.419 day⁻¹ which were different from k of flour in water (0.355 day⁻¹). The SEM micrographs of the gels stored at 4°C for 15 days showed that there was noticeable difference between the surfaces of the gels in water and coconut milk. Lastly, HHP at 600 MPa and 40°C for 30 minutes could gelatinize 20% (w/w) flour suspensions completely. Addition of sucrose would retard the gelatinization process. From the polarized light micrographs it showed that HHP gelatinized the flour without destroying starch structure.

Department : Food Technology.....

Student's Signature

Field of Study : Food Technology.....

Advisor's Signature

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Co-Advisor's Signature

Co-Advisor's Signature

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CHAPTER I

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important crop of Thailand. In 2009, rice flour was exported and earned 3,867.70 million baht (MOC, 2010). Rice flour is used to produce various food products such as traditional Asian foods, baked products, noodles, extruded products or as additive of other ingredients. The qualities of these products depended on the physicochemical properties of rice flour (Bhattacharya, Zee, and Corke, 1999; Riva, Fessas, and Schiraldi, 2000; Park and Baik, 2002; Jangchud, Boonthrapong, and Prinyawiwatkul, 2004). Previous studies revealed that rice flour from different varieties were different in the amylose, amylopectin, starch, protein, lipid and ash contents (Fan and Marks, 1998; Tan and Corke, 2002; Zhou *et al.*, 2002; Varavinit *et al.*, 2003; Zhong *et al.*, 2009; Wang *et al.*, 2010; Yu *et al.*, 2010; Zhu *et al.*, 2010). The difference in chemical composition of rice flour affected the functional, thermal and pasting properties (Biliaderis and Tonogai, 1991; Dipti *et al.*, 2002; Varavinit *et al.*, 2003; Derycke *et al.*, 2005; Zhu *et al.*, 2010). Starch, as main composition of rice flour, is composed of two glucose polymers: amylose and amylopectin (Champagne, 1996). Both amylose and amylopectin affected the functional, pasting, gelatinization and retrogradation properties of rice flour (Lii, Tsai and Tseng, 1996; Singh *et al.*, 2000; Nakamura *et al.*, 2002; Varavinit *et al.*, 2003). Amylose acts as an inhibitor of swelling and is responsible for the structure changes of flour gel in short-term (less than 1 day), while amylopectin is responsible for the longer term structural changes (Bhattacharya, *et al.*, 1999). Besides amylose and

amylopectin, protein and lipids, which are minor components of rice flour, also affect the properties of rice and rice flour such as restricting the expansion of starch in flour during gelatinization or retarding amylopectin retrogradation (Tester and Morrison, 1990; Champagne, 1996; Toshio and Ichiro, 2000; Tan and Corke, 2002; Tulyathan and Leeharatanaluk, 2007; Likitwattanasade and Hongsprabhas, 2010; Yu *et al.*, 2010).

Retrogradation is one of the most important quality parameters of Thai dessert from rice flour. Many factors such as rice variety, amylose content, lipid, sugar and cooking process influence the retrogradation in Thai dessert from rice flour during storage (Riva *et al.*, 2000; Varavinit *et al.*, 2003; Lii, Lai and Shen, 2004; Zhou *et al.*, 2007; Yu, Ma and Sun, 2009). In addition, lipid and sucrose can also affect the retrogradation properties of flour. Previous studies revealed that lipids could form lipid-amylose complex which retarded retrogradation of rice flour (Chang and Liu, 1991; Godet, Bizolt and Buléon, 1995; Zhou *et al.*, 2007) and sucrose increased rate of retrogradation in rice, wheat, and oat starches (Chang and Liu, 1991; Jang *et al.*, 2001). Therefore, the chemical composition of Thai rice flour need to be investigated.

Coconut milk and sugar are important ingredients in many Thai desserts. Addition of both ingredients can affect the interaction between starch and other minor components in flour. Adding lipid, monoglycerides or fatty acid into rice flour or starch brought to reduce the gelatinization enthalpy, increase storage modulus, retard retrogradation and increase cooking time of rice flour or starch (Germani, Clacco, and Rodriguez-Amaya, 1983; Billiaderis and Tonogi, 1991; Kaur and Singh, 2000). However, coconut milk is a colloidal system composed of

lipids, proteins and some trace of carbohydrate and ash (Seow and Gwee, 1997; Tansakul and Chaisawang, 2006) and the changes of properties of rice flour or starch due to coconut milk has not been reported. So the second part of this study aimed to investigate the effects of coconut milk and sucrose on the gelatinization, pasting and retrogradation properties of rice flour which will be useful for development of the quality of rice flour products.

Gelatinization of starch in heating process principally involves swelling and disruption of starch granules (Wetzel and Charalambous, 1998). Many researchers attempted to use high hydrostatic pressure (HHP) in starch and found that HHP process could gelatinize starch (Stolt, Oinonen, and Autio, 2001; Katapo, Song, and Jane, 2002; Kawai, Fukami, and Yamamoto, 2007; Oh *et al.*, 2008). The degree of gelatinization increased with the treatment pressure and time. Most starches gelatinized completely at pressure up to about 600 MPa at temperature lower than 50 °C (Kato *et al.*, 2000; Ahmed *et al.*, 2007; Liu, Selomuyo, and Zhou, 2008). The SEM micrographs showed that there was almost no apparent change in starch granular growth and size for starches gelatinized by HHP (Kato *et al.*, 2000; Katapo *et al.*, 2002; Liu *et al.*, 2008; Yamamoto *et al.*, 2009). The different phenomena of gelatinization will give products with different properties. However, there is no data on the effect of sugar on HHP starch gelatinization. So the last part of this study aimed to investigate the influence of sucrose on the rice flour treated under HHP which will be useful for application in food products.

1.1 Objectives

The main objectives of this research were to investigate

1. the physicochemical, functional, thermal, pasting and gel texture properties of rice flour from six non-waxy rice varieties and the relationships among these properties,
2. the effects of adding coconut milk and sucrose on the gelatinization, pasting and gel hardness properties of rice flour, and
3. the effects of pressure and holding time of HHP process and adding sucrose on the gelatinization properties of rice flour suspension.

CHAPTER II

LITERATURE REVIEW

2.1 Rice and Rice Flour

2.1.1 Rice

Rice is the food crop that grows in both lowland and upland. It is primarily grown on the vast areas, low-lying river basins and delta areas. There are two species of rice: *Oryza sativa* L., which is found throughout the world, and *O. glaberrima* Steud, which its economical importance is limited to West and Central Africa, from Senegal to Sudan. Rice (*Oryza sativa* L.) can be divided into three sub-species according to evolution and geographical origin: temperate race (i.e. Japonica), tropical race (i.e. Indica) and the third sub-species of rice called Javanica, which does not fit to either Indica or Japonica (Marshall and Wadsworth, 1992).

Rice is the main source of food energy of Thai people and is the most important crop in Thailand. In 2009, Thailand produced rice about 23.25 million tons and has income from rice exports 183,422 million baht (MOC, 2010). The shape of Thai rice grain is long, slender, and translucent. Rice in Thailand has many varieties. Different varieties have different chemical composition and quality of cooked rice. The main factor influencing the property is amylose content. The low-amylose type (less than 20% amylose) always has a soft texture and is sticky when cooked. The intermediate-amylose type (21–25%) produces

rather soft cooked rice, whereas the high-amylose type (> 25%) has a hard texture. To obtain an optimum cooked-rice quality, high amylose milled rice requires more cooking water than that having lower amylose content (Cheaupun, Wongpiyachon, and Kongseree, 2004).

2.1.2 Rice flour

In Thailand, most rice flour is produced from broken rice by dry-, semi-dry, or wet- milling (Naivikul, 2007). The broken rice is then ground to form rice flour.

2.1.2.1 Manufacture of rice flour

In industry, rice flour can be prepared from three methods (wet-, semi-dry, and dry-milling) (Fig. 2.1). Soaking, milling, drying and regrinding are involved in wet milling. Usually rice is soaked in water for more than 4 h, drained, and ground by a stone mill with water. After wet milling, excess water is removed by drying, and the flour is gently reground to obtain the wet-milled flour. In semi-dry milling, rice is soaked, drained, and ground by using disc mill or pin mill without adding any water. For dry milling, rice is cleaned and directly ground to different sizes by various mills (including roller, pin mill, hammer mill and burr) (Yeh, 2004; Naivikul, 2007).

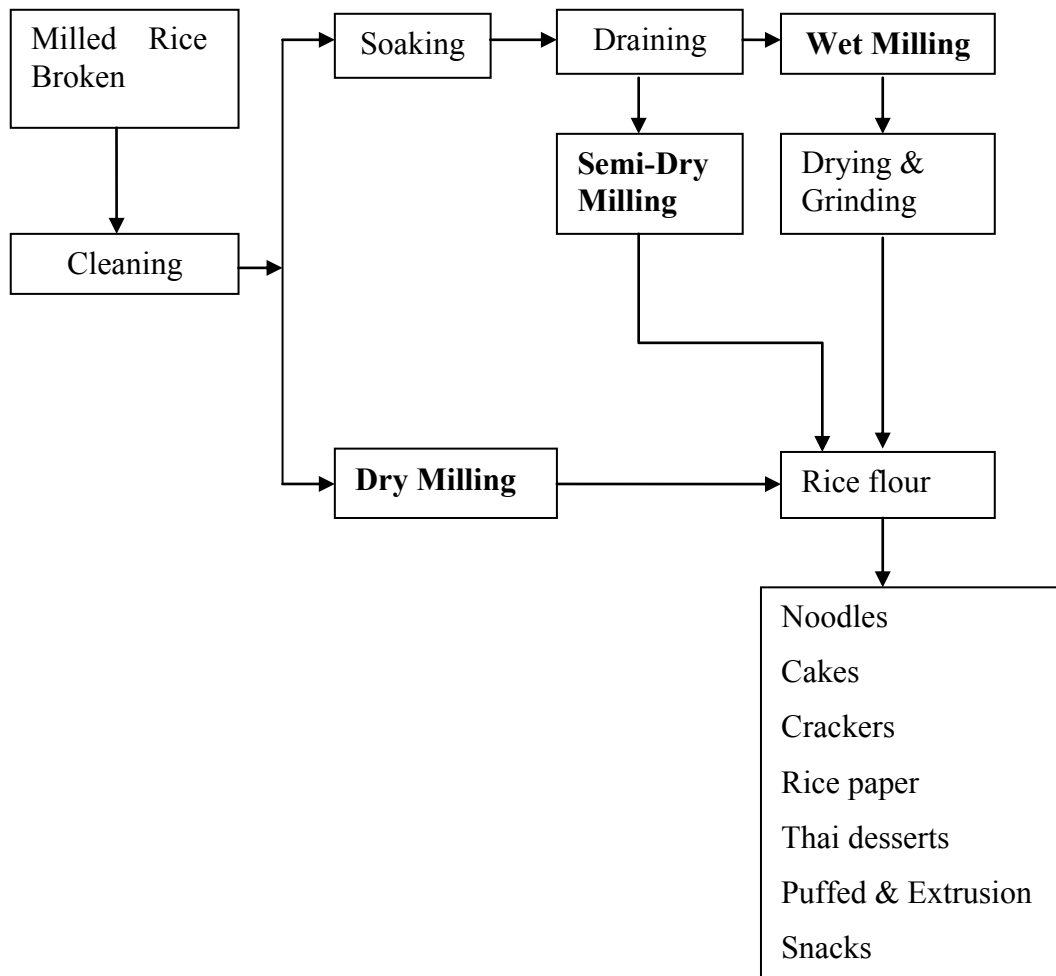


Figure 2.1 Manufacture of rice flour and their application.

Source: Adapted from Yeh (2004) and Naivikul (2007)

2.1.2.2 Utilization of rice flour

In Thailand, there are two kinds of rice flour: rice flour and waxy rice flour. Rice flour is used for the preparation of rice noodles, extrusion-cooked baby foods, unleavened breads, Japanese rice products such as rice cake (*rakugan*), rice crackers (*senbei* and *arare*), rice pudding (*uiro*), and Thai rice products such as rice paper, rice layer cake, (*khanom chan*), rice sponge cake

(*khanom thuai fu*), rice filled custard (*khanom sod sai*) (Juliano and Sakurai, 1972; Naivikul, 2007).

The amylose content in rice flour affects the quality of rice product. For example, rice noodle which is a major rice product in Asia is made from high amylose rice flour (>25%). Sookdang (1998) reported that only high-amylose rice could give a good quality of instant flat noodle. Flour from intermediate-amylose rice yielded a softer product with higher cooking loss, while flour from low-amylose rice had no potential to make usable rice noodles, even when blended into a composite with flour from high-amylose rice. Bhattacharya *et al.* (1999) found that the amylose content of rice flour was closely correlated to the texture of the rice noodle as well as other physicochemical properties of the flour, including swelling volume, Rapid Visco Analyser (RVA) pasting properties and gel texture. Rice paper, the most popular rice products in the northeastern part of Thailand, is produced from high amylose rice flour (> 27%) (Phothiset and Charoenrein, 2007), while an intermediate amylose rice flour (20- 25%) is used for making extruded (dried) pasta and low amylose rice flour is used for making baby foods, beer and breakfast cereal in the United States (Juliano, 1985). For Thai rice cake made from mixture of Jasmine rice flour (20.92 % amylose content) and Yellow-11 rice flour (33.34 % amylose content), it was found that the amylose content in mixed rice flour affected the springiness and puffiness of the rice cake. The springiness of rice cake decreased with increasing ratio of Jasmine rice flour-to-Yellow-11 (Jangchud *et al.*, 2004).

2.2 Properties of rice flour

Flour from rice (*Oryza sativa* L.) has specific nutritional properties. It is hypoallergenic, colorless and has bland taste. The quality of rice products is greatly dependent on the properties of rice flour. Amylose and amylopectin were found as important factors that affected physicochemical and functional properties of rice flour. Wang *et al.* (2010) reported that amylose content was positively correlated to rice gel hardness, while the branch-chain amylopectin played different roles in rheological behavior of starch dispersions. The short chain (DP 6-11) of amylopectin was positively correlated to G' and G'' of starch dispersions, while the long chains (DP 18-23) of amylopectin was negatively correlated (Wang *et al.*, 2010). Rice flour gel hardness was positively correlated to amylose content, while protein and lipid in rice flour significantly influenced on the mechanical properties of flour gel (Yu *et al.*, 2010). The properties of rice flour, which affect qualities of rice-based products, were reviewed.

2.2.1 Physicochemical properties

2.2.1.1 Chemical composition

The chemical composition of rice flour from different varieties was difference in the amount of amylose, protein, lipids and ash (Table 2.1).

2.2.1.1.1 Rice starch

Rice starch has the smallest granule of starch (~ 30 nm in diameter) (Ohtani *et al.*, 2000). The starch granules are accumulations of numerous starch molecules that can be fractionated into linear chain amylose and

highly branched amylopectin. The two molecules are organized into semi-crystalline structure in starch granule (Zhou *et al.*, 2002). A model of the starch granule is schematically shown in Fig. 2.2. The more or less concentric layers (Fig. 2.2 a) show alternating high and low refractive indices, densities, crystallinity and resistance to acid and enzymatic hydrolysis and presumably represent growth rings. These growth rings arise from a periodicity in the biosynthesis. The dense layer in a growth ring consists of about 16 repeats of alternating crystalline (5-6 nm) and amorphous (2-5 nm) lamellae (semi-crystalline layer, Fig. 2.2 b). It has a thickness between 120 and 400 nm. The less dense layer is largely amorphous, contains more water and at least as thick as the dense layer. Starch granules are thus partially crystalline with a degree of crystallinity of 20-40 %. The crystalline lamellae are made up by amylopectin double helices, which are packed in a parallel fashion, whereas the amylopectin branch points are in amorphous zones (Fig. 2.2c). The crystallinity of the granule is thus mainly ascribed to double helices formed by amylopectin branches rather than to amylose (Jacobs and Delcour, 1998).

Table 2.1 Chemical composition of rice flour (% on the dry weight)

| Samples | Moisture (%) | Amylose (%) | Protein (%) | Lipids (%) | Ash (%) | References |
|---|---------------------|--------------------|--------------------|-------------------|----------------|--|
| Rice flour from market | | | | | | |
| - Riviana Foods, Inc., USA | 10.7 | 30.0 | 8.7 | 0.8 | 0.7 | Liang and King (2003) |
| - Maha Sarakham, Thailand | 11.77 | 35.36 | 6.83 | 0.21 | 0.22 | Wanyo, Chomnawang, and Siriamornpun (2009) |
| Rice flour from <i>Indica</i> rice | | | | | | |
| a. Thai rice varieties | | | | | | |
| - Kor Khor 6 | 12.53 | 5.64 | 6.15 | 0.17 | 0.14 | Udomrati <i>et al.</i> (2003) |
| - Khao Dok Mali 105 | 10.63 | 17.46 | 6.95 | 0.17 | 0.17 | |
| - Luang 11 | 10.78 | 34.23 | 8.06 | 0.14 | 0.17 | |
| - Chainart 1 | 9.29 | 32.03 | 9.29 | 0.09 | 0.19 | Phothiset and Charoenrein (2007) |
| b. China varieties | | | | | | |
| - Simiao | ca 10% | 23.59 | 8.33 | 0.89 | - | Yu <i>et al.</i> (2010) |
| Rice flour from <i>Japonica</i> rice | | | | | | |
| - Daohuaxiang , China | ca 10% | 13.78 | 6.93 | 1.44 | - | Yu <i>et al.</i> (2010) |

- **Amylose**

Rice amylose is a mixture of branched and linear molecules with a degree of polymerization (DP) of 1,100 - 1,700 and 700 - 900, respectively. The branched fraction in rice constituted 25-50% by number and 30-60% by mass of amylose (Zhou *et al.*, 2002). The amylose content of rice is specified as waxy, 0-2%; low, 12-20%; intermediate, 20-25%; and high, 25-33% (Juliano, 2004). The structure characteristics of amylose from rice are shown in Table 2.2.

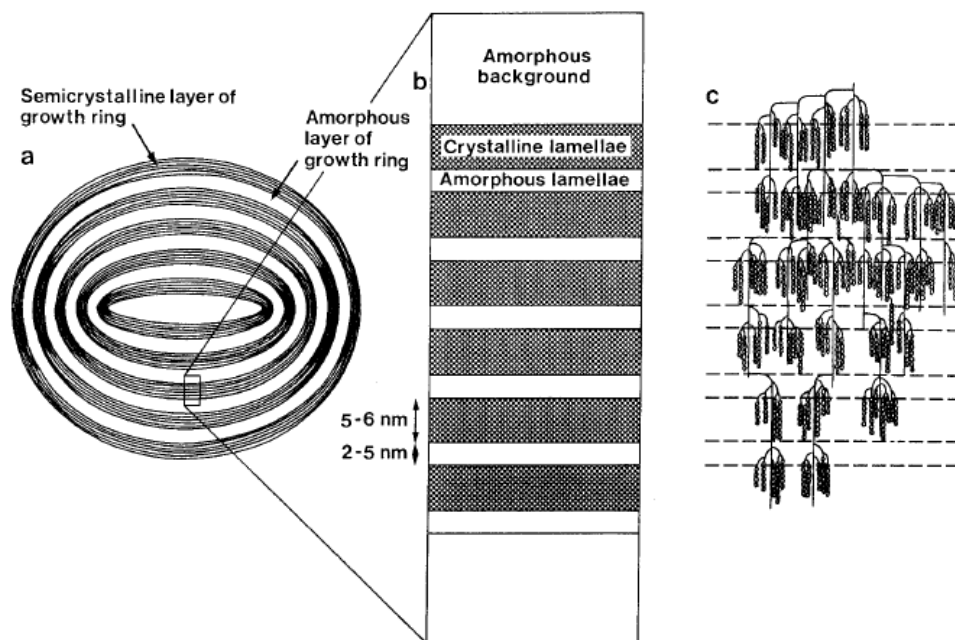


Figure 2.2 Schematic representation of starch granule structure:

- single granule with alternating amorphous and semicrystalline layers, representing growth ring;
- expanded view of the semicrystalline layer of a growth ring, consisting of alternating crystalline and amorphous lamellae;
- the cluster structure of amylopectin within the semicrystalline layer of the growth ring.

Source: Jacobs and Delcour (1998)

Table 2.2 The structure characteristics of rice amylose

| Characteristics | Indica | | Japonica |
|--------------------------------------|--------------------|-----------------|------------|
| | Kaohsiung Sen 7 | Taichung Sen 10 | Tainung 67 |
| Number-average DP (DP _n) | 1075 | 1289 | 1004 |
| Average chain length (CL) | 430 | 537 | 287 |
| Average chain number (NC) | 2.5 | 2.4 | 3.5 |
| β-Amylolysis limit (%) | 83.5 | 84.2 | 79.8 |

Source: Shao *et al.* (2007).

The molecular weight of rice amylose is now elucidated by mean of size exclusion chromatography with multi-angle laser light scattering and refractive index. Ong and Blanshard (1995a) reported that the molecular weight of amylose from eleven cultivars of Indica, which had amylose content between 8.0 - 30.5% by using size exclusion chromatography- high performance liquid chromatography method, was in the range of $(3.42-7.22) \times 10^5$ g/mol.

Amylose is one of the two components of rice starch which greatly affects the quality and gelatinization properties of cooked rice. It is commonly used as an objective index for cooked rice texture. Low amylose levels are associated with cohesive, tender, and glossy cooked rice. Conversely, high levels of amylose cause rice to absorb more water and consequently expand more during cooking (Dipti *et al.*, 2002). Wang *et al.* (2010) reported that rice amylose content was negatively correlated to swelling power and positively correlated to gel hardness. Bao *et al.* (2006) reported that the amylose content served as a good indicator for gel hardness of rice flour paste.

The influence of amylose on thermal properties, contradictory results were found in different reports. Varavinit *et al.* (2003) reported that the gelatinization temperature (determined by DSC) of Thai rice flour was highly positively correlated with amylose level. On the contrary, the gelatinization temperatures of corn starches were not correlated with amylose content (Sandhu and Singh, 2007). Thus, the differences in thermal properties of starches could not be explained by only amylose content.

- **Amylopectin**

Rice amylopectin is highly branched biopolymer consisting of linear chains of α (1, 4) linked glucose residues joined together by α (1,6) linkages. Chain lengths of 20-28 glucose units are similar for both non-waxy and waxy amylopectin, with a degree of polymerization (DP) of 5,000 - 15,000 glucose units and 220 – 1,050 chains per molecule (higher for waxy than non-waxy rice). Recent studies revealed that shorter amylopectin chains (DP < 12) had a negative influence, while longer amylopectin chains (12 < DP < 24) had a positive influence on the gelatinization temperatures of rice starches (Nakamura *et al.*, 2002; Noda *et al.*, 2005; Vandeputte *et al.*, 2003a; Srichuwong *et al.*, 2005a). Longer chains would make long helices and strengthen hydrogen bonds between chains, spanning the complete crystalline region. On the other hand, the existence of shorter chains, forming short or weak double helices, would produce inferior crystalline structures (Jane *et al.*, 1999). Wang *et al.* (2006) found that average exterior chain length and average interior chain length of amylopectin were significantly related to pasting properties (peak, setback and final viscosity)

of yam starches. The rheological properties of starch gel were influenced by the amylose to amylopectin ratio, amylopectin weight and amylopectin chain length (Chang and Lina, 2007; Chung *et al.*, 2008; Xie *et al.*, 2009). In waxy rice starch, the molecular weight and the average chain length of amylopectin correlated positively with rheological properties. Srichuwong *et al.* (2005b) reported that amylopectin chain lengths also affected the enzyme digestibility of starch granules.

2.2.1.1.2 Rice protein

Rice protein is the second most abundant constituent of rice flour (Table 2.1). It is more nutritious because of its higher lysine content than other cereal proteins. The content of protein in rice is affected by environmental conditions such as soil and applicability of nitrogen fertilizer. Protein is found in different parts of rice grain (including the endosperm, the polish and the bran, most being within the endosperm cells) and located in protein bodies between the starch granules (Cagampang *et al.*, 1966). Rice protein affected swelling power of rice flour (Derycke *et al.*, 2005), the pasting properties (Hamaker and Griffin, 1993; Tan and Corke, 2002; Liang and King, 2003; Ibanez *et al.*, 2007; Xie *et al.*, 2008; Zhu *et al.*, 2010), retrogradation properties (Wu *et al.*, 2010) and textural properties of cooked rice (Xie *et al.*, 2009). Derycke *et al.* (2005) reported that the reduction of disulfide bond in rice flour by dithiotreitol (DTT) increased swelling power, leaching of carbohydrate during cooking and stickiness, while decreased water absorption and hardness of cooked rice. Hamaker and Griffin (1993) reported that protein restricted starch granule swelling during gelatinization and made the swollen granules less susceptible to

disruption by shear so that the value of Brookfield viscosity of removed protein rice flour was higher than that of the un-removed protein rice flour. The protein content in rice flour was reported to negatively correlate to peak viscosity and hot paste viscosity. The lower protein rice flour had higher peak viscosity (Tan and Corke, 2002; Ibanez *et al.*, 2007; Xie *et al.*, 2008). Liang and King (2003) studied the effects of protein removal on pasting properties of rice flour and X-ray diffraction (XRD) pattern of rice flour gel which stored in the refrigerator for 3 days and found that protein removal reduced peak viscosity, trough and final viscosity. Zhu *et al.* (2010) reported that protein was the main cause of the different pasting properties among the rice starch and rice flour. Likitwattanasade and Hongsprabhas (2010) found that cysteine play a significant role in pasting properties of rice flour. It decreased holding strength, final viscosity and setback of rice flour. Wu *et al.* (2010) studied retrogradation properties of high amylose rice flour (protein content 8.1 g/ 100 g. flour) and rice starch (protein content 0.5 g/ 100 g. starch) kept at 4⁰C for 7 days by using DSC, and found that the retrogradation enthalpy of rice flour (5.93 J/g dry flour) was higher than that of rice starch (5.13 J/g dry starch). For textural properties, Xie *et al.* (2009) reported that cooked rice texture of high protein rice tends to be tougher and chewier than low protein rice.

2.2.1.1.3 Rice lipid

Lipid content in rice flour was ranged from 0.09 to 1.44% (Table 2.1). It may be associated with native starch granules in three ways. On the exterior of the granule are non-starch lipids that bind to protein adhering to

the granular surface; these are removed by suitable clean-up procedures. In the interior of the granule are monoacyl non-starch and true starch lipids. Monoacyl non-starch lipids enter the granule generally during steeping and wet-milling for starch isolation. Major fatty acids in rice lipids are linoleic, palmitic, and oleic or myristic acids (Champagne, 1996). Rice lipids, although as minor constituents of rice flour, were shown to have significant effects on the properties of rice flour. Biliaderis and Tonogai (1991) reported that lipids in rice retarded retrogradation and the effect was more pronounced for low-amylose starches. Liang and King (2003) studied the effects of lipid removal on pasting properties of rice flour and X-ray diffraction (XRD) pattern of rice flour gel which stored in the refrigerator for 3 days and found that lipid removal reduced final viscosity and setback. For XRD pattern, they also found that the removal of lipid caused loss of the V-pattern when compared with rice flour control.

2.2.1.2 Crystalline of rice flour

The starch is main composition in rice flour, and consists of 2 main structural components, the amylose and amylopectins. Amylose is linear or slightly branched, has a degree of polymerization (DP) (ca 3000) and an average molecular weight (Mw) $((4 - 7) \times 10^5 \text{ g/mol})$ lower than that of amylopectin (DP around 6000 to 8000) and Mw around $((1 - 1.4) \times 10^6 \text{ g/mol})$ (Chen and Bergman, 2007). The chains of amylopectin can easily form double helices. These double helical structures form many small crystalline regions in the dense layers of starch granules. The crystalline structure of rice flour or rice starch was A type X-ray diffraction pattern (Fig. 2.3) (Srichuwong *et al.*, 2005a; Shu *et al.*, 2006;

Singh *et al.*, 2007; Yu *et al.*, 2010). The double helices of the A type crystal were formed in a monoclinic lattice (unit cell parameter $a = 2.14$ nm, $b = 1.172$ nm, $c = 1.069$ nm, and $\alpha = \beta = 90^\circ$, $\gamma = 123.5^\circ$) having the maltotriose as a repeating unit and four water molecules (3.6%) per unit cell (Imberty *et al.*, 1988). The schematic conformation of A-type structure contained ordered arrays of double helices shown in Fig. 2.4. There is 1.1 nm between the hole of the two double helices. Calculated densities for the crystalline region of the A type crystal is 1.14 (Gidley and Bociek, 1985).

The crystallinity was determined using wide-angle X-ray diffraction. Rice flours were tightly packed into sample holder. The diffraction data were recorded over an angular ranged from at least 4 to 30 (2θ). The % crystallinity was calculated as the ratio of area of the crystalline sharp peak over the total area (Fig. 2.5) (Cheetham and Tao, 1998).

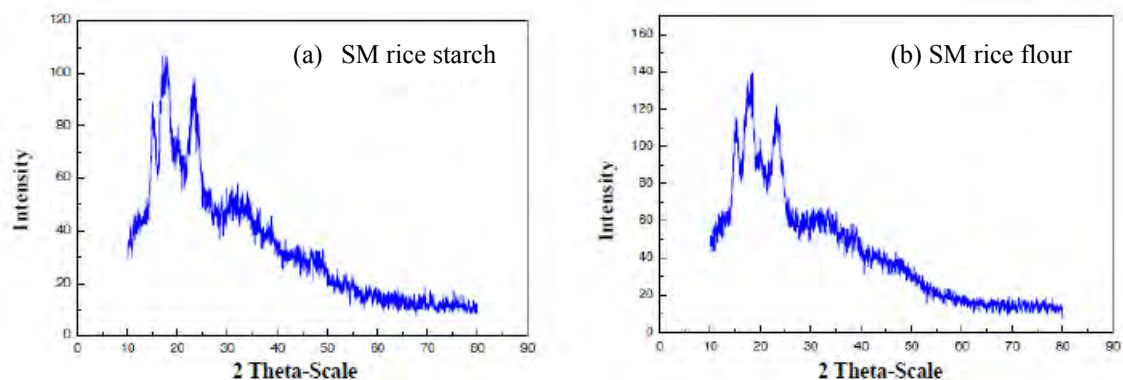


Figure 2.3 X-ray diffraction pattern of *Indica* rice Simiao variety (SM):
(a) Starch and (b) Flour.

Source: Yu *et al.* (2010)

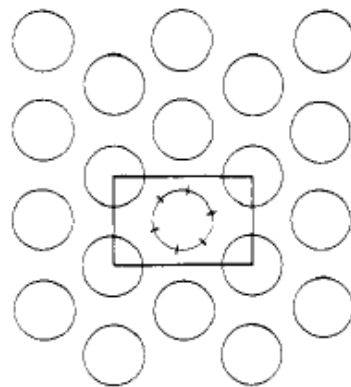


Figure 2.4 Representation of helix packing and unit cells in A type (1 → 4)- α -D-glucan. Each circle represents a view down the centre of a double helix. Positions of C-1 sites are shown by spokes for helix within unit cells.

Source: Gidley and Bociek (1985)

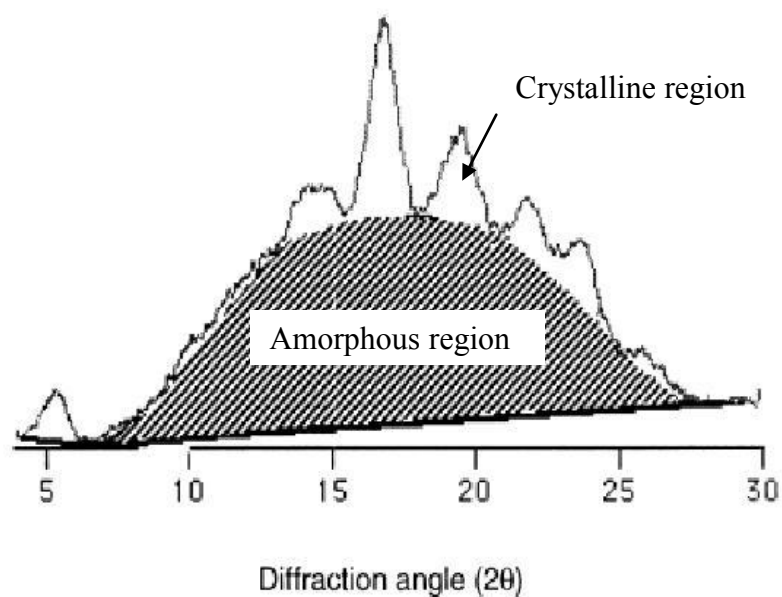


Figure 2.5 A type crystal; showing crystalline region (white zone) and amorphous region (line zone).

Source: Cheetham and Tao (1998)

2.2.1.3 Damaged starch

Damaged starch is the destruction of starch granule structure to extent that it affects water absorption. It is resulted from various processes such as milling of grains, hydrolysis or reaction of enzymes (Dubat, 2004).

Extensive damaged starch causes disruptions in the molecular structure of starch. Craig and Stark (1984) found that extraction of damaged granules of wheat flour with cold water preferentially leached out low molecular weight amylopectin. Aqueous extracts of a commercially milled wheat-flour contained pentosan and α -d-glucan, the latter consisting of 99% of amylopectin and <1% of linear glucan. Chen, Lu and Lii (1999) found that the grinding-milled waxy rice flour gave the higher solubility (20-30%) during heating at 65, 75, 85, and 95⁰C compared with other milling methods (turbo, cyclone, and hammer mill) due to the higher level of damaged starch. Damaged starch has a strong influence on the properties of flour-based products such as bread, cracker, noodles, etc. Damaged starch notably increases the hydration potential of the flour. Biscuits produced from wheat flours with low damaged starch content had better quality because water loss from this biscuits was less than that of biscuit from high damaged starch wheat flour (Dubat, 2004). For wheat noodle, Park and Baik (2002) reported that damaged starch increased water absorption of wheat noodle dough resulting in thicker and softer noodle than that from low water absorption wheat flour.

2.2.1.4 Particle size

Particle size of flour is a very important factor affecting the quality of product. The particle size of wheat flour was assigned by Codex standard that 98% or more of flour shall pass through a 212 micron (No. 70 sieve) (CODEX, 1985). There are many researchers studied effect of flour particle size on the properties of flour and quality of baking products. Chen *et al.* (1999) found that particle size of rice flour influenced the onset temperature of gelatinization (T_{onset}). The finest flour (10-30 μm) had the lowest initial T_{onset} , while the coarsest flour (100-300 μm) had the highest. Wang and Flores (2000) reported that particle size of the wheat flour affected the tortilla texture. They also found that tortillas made from the medium fractions (38–53 μm and 53–75 μm), especially the 53–75 μm fractions, had longer rupture distance and better fold ability. The finest fraction (< 38 μm) yielded tortillas with shorter rupture distance and worse fold ability. Park, Kim, and Kim (2001) studied effect of flour particle size on the properties of bread and found that medium particle size fraction flour (53-75 μm) showed the highest loaf volume (LV) and small particle size fraction flour (< 53 μm) showed the lowest LV. Crumb grain scores of bread determined by baking expert and elongation ratio determined by Crumb Scan V 3.0 (AIB) were higher for smaller particle size fractions.

2.2.2 Thermal properties

Thermal analysis of rice flour by DSC has been carried out by several researchers to study gelatinization and retrogradation phenomena of rice flour in water. For this part, it has been reviewed under the topic of gelatinization and retrogradation measured by using DSC.

2.2.2.1 Gelatinization

Gelatinization describes the irreversible collapse of molecular order within a starch granule when heated in excess water. The gelatinization of rice flour is important in processing rice flour-based food products. They are useful in determining the amount of heat and time required for cooking into finished products. The gelatinization temperature of various rice flours (Lemont, Mars, Calmochi and S-201) (Fig. 2.6) indicated that variety affected the gelatinization properties of rice flour (Normand and Marshall, 1989). Singh *et al.* (2000) studied thermal and physicochemical properties of three types of rice flour [Indica (27.7% amylose content), Japonica (19.9% amylose content), and Japonica waxy (1.5% amylose content)] and found that glass transition temperature (T_g) of Indica rice flour was the highest (221.8°C), while the T_g of Japonica non-waxy rice flour and Japonica waxy rice flour were almost the same (210.7 °C and 209.3 °C). After defatted, the T_g value decreased for Japonica rice flour but increased for Indica rice flour. Saif, Lan and Sweat (2003) studied gelatinization properties of rice flour from different rice varieties (3.49, 17.3, and 23 % of amylose content) and found that amylose content significantly affected the gelatinization temperatures (T_{onset} , T_{peak} , and T_{end}) and enthalpy of gelatinization (ΔH). Both T_{onset} and T_{peak} increased, and T_{end} decreased with the increased amylose content. ΔH had a high significant negative correlation with the levels of amylose content of the rice flour.

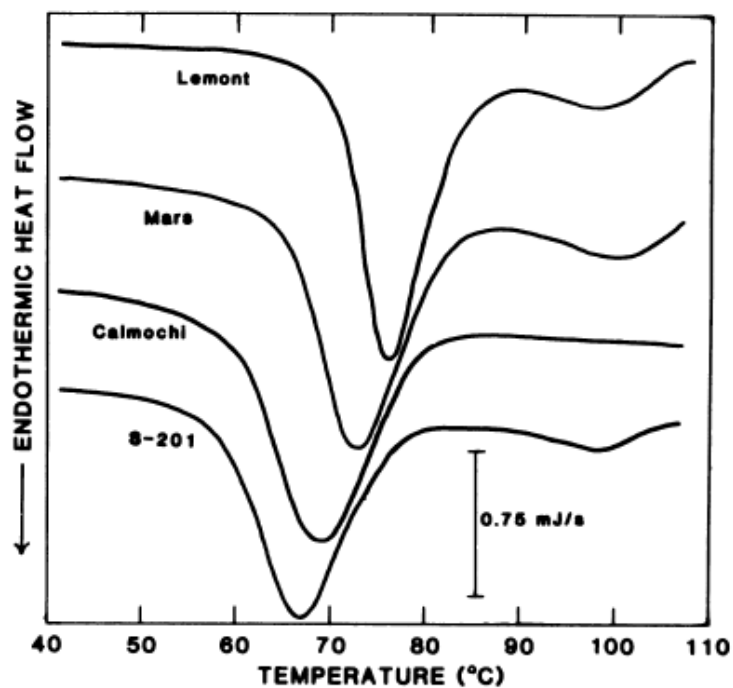


Figure 2.6 DSC thermal curves of different milled rice flour varieties at 70% water content (w/w). The heating rate was 1.0°C/ min.

Source: Normand and Marshall (1989)

2.2.2.2 Retrogradation

Starch retrogradation is the phenomenon occurred on cooling and storage of gelatinized starch. This phenomenon is interesting to food scientists and food technologists because it affects quality, acceptability and shelf-life of starch-containing foods. There are many researchers studied the retrogradation process of rice starch using DSC and found that high amylose rice starch retrograded more rapidly than low amylose rice starch (Iturriaga, Lopez, and Anon, 2010; Yu *et al.*, 2010) and amylose has a synergic effect on the retrogradation rate (Iturriaga *et al.*, 2010). Besides of amylose, water content had

influence on the retrogradation of starch. Zeleznak and Hosney (1986) studied the role of water in the retrogradation of wheat starch gels and bread crumb using DSC and found that retrogradation in wheat starch gels was controlled by the amount of water present during aging.

Yu *et al.* (2010) studied retrogradation properties of starch and flour from different rice cultivars and found that amylose content increased retrogradation of rice starch. Besides, they also found that retrogradation enthalpy and % retrogradation of rice flours were higher than starches.

2.2.2.3 *Retrogradation kinetics*

Retrogradation of gelatinization starch molecules involves formation and subsequent aggregation of double helices of amylose and amylopectins chains. During retrogradation, crystal can grow from different nucleation centers and, therefore, the amount of crystallized material present at a given time is a combined function of crystal growth rate and the density of nucleation. The increase in enthalpy change (ΔH) during starch retrogradation can be described by the Avrami equation [Eq. 2.1], which gives the fraction of uncrystallized starch at time (t): (Avrami, 1939; Fan and Marks, 1998; Lii *et al.*, 2004) and expresses as Eq. [2.2].

$$\text{Avrami equation: } U(t) = \exp(-kt^n) \quad [2.1]$$

$$\log(-\ln U(t)) = n \log t + \log k \quad [2.2]$$

where $U(t)$ is $(\Delta H_{\infty} - \Delta H_t) / (\Delta H_{\infty} - \Delta H_0)$ the fraction of crystallization

resting to take place which is equal to

ΔH_0 is the ΔH (J/ g starch) at storage time of 0 day

ΔH_t is the ΔH (J/ g starch) at storage times of any t days

ΔH_{∞} is limiting of ΔH (J/ g starch) at storage for infinite times

$(t \rightarrow \infty)$

k is the crystal growth rate constant (day^{-1})

n is the Avrami exponent which reflects the growth morphology

Replace $U(t)$ in Equation (2.2) leads to

$$\log[-\ln (\Delta H_{\infty}-\Delta H_t) / (\Delta H_{\infty}-\Delta H_0)] = n \log t + \log k \quad [2.3]$$

The values of k and n can be determined from the slope and intercept of the plot of $\log [-\ln (\Delta H_{\infty}-\Delta H_t) / (\Delta H_{\infty}-\Delta H_0)]$ vs. $\log t$. The value of n was a constant whose value lied between 1 and 4, depending on the nucleation mechanism. The n value was an indicator for growth of instantaneous nucleation such as $n \sim 1$ indicating linear growth, $n \sim 2$ indicating disk-like growth and $n \sim 3$ indicating spherulithic growth (Harnish and Muschik, 1983).

2.2.3 Functional properties

2.2.3.1 Water solubility and absorption

Water solubility of flour indicates the level of degradation of flour during processing. The processing of flour results in changes in the

molecular structure of starch. Juliano (1985) reported that during cooking of 47 non-waxy milled rice varieties for 40 min, solids lost in the cooking water ranged from 2.9 to 11.8%. The solids represented 1.6-10.7% of total amylopectins and 6.3-28% of total amylose of the milled rice. Horndok and Noomhorm (2007) reported that the solubility value of rice starch was 6.65%, which was higher than that of rice flour (3.2%), and the solubility value related with gel hardness. The gel hardness value of rice starch was 49.82 g_f, which was higher than that of rice flour (27.48 g_f). Kadan, Bryant, and Miller (2008) studied the water solubility index (WSI) of rice flour by soaking rice flour in excess distilled water (30⁰C) for 30 min, and then the suspension was centrifuged at 3000 x g. WSI of rice flour was found to relate with flour particle size. The fine particle size flour (148.3 μm) had high WSI (1.52), while the coarse particle size flour (165.1 μm) had low WSI (1.31).

The water absorption value of flour is another important quality factor in rice products. It is defined as the amount of water absorbed by flour. Water absorption is usually determined by the increase in weight of rice during treatment or by the decrease in volume of cooking water. There are many researcher studied water absorption index (WAI) by using temperature around 30⁰C (Chiang and Yeh, 2002; Kadan *et al.*, 2008). Chiang and Yeh (2002) found that the WAI of rice starch was in the range of 2.1-2.3 when the damaged starch was lower than 5%, but for damaged starch > 5%, the WAI increased with increasing damaged starch. Kadan *et al.* (2008) reported that WAI value of rice flour was around 1.0. For rice noodle, high absorption values of flour are desirable in making rice noodle because the added moisture improves texture of

noodle. Tungtrakul *et al.* (2007) reported that particle size of high amylose rice flour from Thai rice variety affected the WAI and WSI of flour and the smaller particle size gave the higher value of these two properties. The noodle from high WAI rice flour had the highest tensile strength as well as the highest hardness and stickiness.

2.2.3.2 Swelling power

The most important property of starch in a commercial application is its ability to swell and produce a viscous paste when heated with water. The swelling behavior of native rice starch is primarily a property of the amylopectin content, amylose acts as an inhibitor of swelling, especially in the presence of lipids that can form amylose-lipid complex. It is suggested that the rigidity of starch granular structure might be proportional to its amylose content and inversely proportional to the degree of granular swelling (Lii *et al.*, 1996). Singh *et al.* (2000) reported that the swelling power increased slowly in rice flour from 50 °C to 70 °C and remained almost constant up to 90 °C. But in rice starch this property increased gradually from 50 to 80 °C and had a sudden jump from 80 to 90 °C. Swelling power was high in rice starch compared to its flour at 90 °C.

2.2.3.3 Pasting properties

When the flour is heated in excess water up to gelatinization temperature, heat transfer and moisture transfer phenomena occur. The granule swells to several times of its initial size as a result of crystalline order loss and absorption water inside the granular structure. The pasting viscosity

during swelling and gelatinization can be recorded using Brabender Visco Amylograph, Rapid Visco Analyzer (RVA), or other viscometers. The viscosity is continuously recorded as the temperature is increased, held constant for a time and then decreased. At the initial step, the viscosity increased 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, causing the further decrease in viscosity. On cooling, some starch molecules partially reassociated to form a precipitate or gel in which amylose molecules aggregate into a network (Booth, 2007).

The pasting properties have been reported to correlate with amylose content in cereal starch and flour. Pérez *et al.* (1993) showed that setback values of rice flour increased with increasing amylose content (15.4-36.2%), while breakdown values decreased with increasing amylose content. Varavinit *et al.* (2003) reported that the data obtained from RVA viscogram (peak viscosity (PV), trough, breakdown (BD), final viscosity (FV), setback viscosity (SB), and pasting temperature) can be used for identification the kind of Thai rice flour. They separated rice flour into 3 groups according to the amylose content; high amylose rice flour (21.95 – 26.42%), medium amylose rice flour (14.63 - 15.45%), and low amylose rice flour (4.47 – 5.28%). They found that low amylose rice flour had the highest PV and BD, and the lowest SB and pasting temperature.

2.3 Factors affecting properties of rice flour

Many factors have been reported to affect the properties of rice flour. The literature pertinent to this topic has been reviewed under the following heading of milling method and ingredients.

2.3.1 Milling method

It was found that milling method affected the particle size of flour and % damaged starch in flour (Yeh, 2004), which consequently affected the properties of rice flour. The type of mill or grinder used profoundly affects the particle size of rice flour. Chen *et al.* (1999) studied the effect of three milling methods (dry-milling, semi-dry hammer milling, and wet-milling) on the physicochemical properties of two waxy rice varieties in Taiwan (TCW 70 and TCSW 1) and found that rice flour particles ranged from coarse particles (100-300 μm), produced from hammer and semi-dry hammer milling, to very fine (10-30 μm), produced from wet-milling. The very fine particles had the lowest T_{onset} and T_{peak} measured by DSC.

The damaged starch caused by milling affected the structure, swelling properties and solubility of flour. Suksomboon and Naivikul (2006) studied effect of milling method on the properties of Thai rice starch and found that rice starch from dry-milling process contained more damaged starch than that of wet-milling (Table 2.3). The amount of damaged starch in rice starch affected the enthalpy of gelatinization and pasting properties of rice starch. The enthalpy gelatinization of rice starch increased with increasing amount of damaged starch. The peak viscosity and final viscosity of dry-milled rice starch measured by RVA were significantly ($p \leq 0.05$) lower than those of wet-milled rice starches. For rice

flour, León *et al.* (2006) found that the amount of damaged starch affected retrogradation of rice bread. The breadcrumb firmness increased when the damaged starch content in rice flour increased.

Table 2.3 The amount of damaged starch from dry-milling and wet-milling

| Rice varieties | Damaged starch (%) | |
|----------------|--------------------|-------------|
| | Dry-milling | Wet-milling |
| Pathum Thani 1 | 5.20 | 4.34 |
| RD 7 | 4.42 | 3.74 |
| Leuang 11 | 4.23 | 3.53 |

Source: Suksomboon and Naivikul (2006)

2.3.2 *Ingredients*

There are many researchers studied effect of adding ingredients such as sugar, protein, or lipid on the properties of rice flour (Chungcharoen and Lund, 1987; Billiaderis and Tonogi, 1991; Kohyama and Nishinari, 1991; Aee, Hie and Nishinari, 1998; Ahmad and Williams, 1999; Chiotelli, Rolée, and Meste, 2000; Kaur and Singh, 2000). For this topic, it has been reviewed only the effect of sugar and lipid on the properties of rice flour.

2.3.2.1 **Sugar**

Sugar strongly influences the quality of dessert products from flour such as cake, bread, Thai traditional dessert. Effect of sugars on the gelatinization and retrogradation of wheat starch have been studied by many

techniques such as DSC (Chungcharoen and Lund, 1987; Kohyama and Nishinari, 1991; Aee *et al.*, 1998; Ahmad and Williams, 1999; Chiotelli *et al.*, 2000), rheological property changes (Gunaratne, Ranaweera and Corke, 2007), loss of birefringence (Rumpold, Bauer, and Knorr, 2005) or nuclear magnetic resonance (Johnson, Davis and Gordon, 1990). Sugar is known to function as antistaling ingredients. In excess water, as sucrose was added to system, the gelatinization temperature was found to increase in rice flour and rice starch (Chungcharoen and Lund, 1987), sweet potato starch (Kohyama and Nishinari, 1991), wheat starch (Chiotelli *et al.*, 2000), waxy corn starch (Chiotelli *et al.*, 2000), acorn starch (Aee *et al.*, 1998) and sago starch (Ahmad and Williams, 1999). For the enthalpy of gelatinization (ΔH_{gel}), there was conflicting report. When sucrose was added, it was found that ΔH_{gel} decreased for rice flour and rice starch (Chungcharoen and Lund, 1987) but increased for sweet potato starch (Kohyama and Nishinari, 1991), wheat starch (Chiotelli *et al.*, 2000), waxy corn starch (Chiotelli *et al.*, 2000), acorn starch (Aee *et al.*, 1998) and sago starch (Ahmad and Williams, 1999). The increases in gelatinization temperature and ΔH_{gel} can be attributed to reduced ability of the sugar to decrease the mobility of the amorphous regions within starch granule and is a consequence of their increasing hydrodynamic size and also due to specific starch-sugar interactions. The starch granule swelling and amylose leaching were reduced in the presence of sugar.

2.3.2.2 Lipids

Lipids have long been known to affect gelatinization and texture of starch-thickened foods. Fatty acid can form complex with amylose.

It was reported that the addition of monoglycerides into starch systems showed different effect depended on kind of starch. Biliaderis and Tonogai (1991) added monoglycerides [anionic lipid, sodium dodecyl sulfate (SDS); emulsifier lipid, glycerol monostearate; cationic lipid, cetyltrimethylammonium bromide (CTAB); zwitterionic lipid, L- α -lysophosphatidylcholine (LPC)] into rice starch and other starch systems (wheat, pea, and garbanzo bean starch) and found that there were amylose-lipid complex in the systems. The addition of monoglycerides reduced the ΔH_{gel} of starches as measured by DSC, and increased storage modulus of rice and wheat starch gels, while the storage modulus of pea and bean starch gels changed slightly. They also found that lipids retarded retrogradation of rice starch gels during storage (35% w/w, at 6 °C for 96 hr). Kaur and Singh (2000) studied the effect of addition of saturated fatty acids [myristic (C 14:0), palmitic (C 16:0), or stearic acid (C 18:0)] in rice flour system and found that the amylose in rice flour formed complex with fatty acid. The amylose-lipid complex formation increased with increasing in levels of all fatty acid (1.5, 3.0, and 4.5%) in rice flour paste which was cooked in visco-amylograph at 95°C for 30-90 min. Complexing of amylose with all fatty acids increased with increasing cooking time. Zhou *et al.* (2007) studied the effect of addition of fatty acids (stearic and linoleic acid) on the properties of rice starch, and found that addition of all fatty acids increased pasting time, setback, and final viscosity of rice starch. For triglyceride, Chiotelli and Meste (2003) reported that the addition of triglycerides had no effect on gelatinization and rheological behavior of potato starch during heating.

2.3.2.3 Coconut milk

Coconut milk is an oil-in-water emulsion. It is obtained from extraction of coconut flesh and is one of the popular ingredients in Thailand, especially for Thai dessert. The chemical composition of coconut milk had very wide variations because of differences in variety, geographical location, maturity, method of extraction and the degree of dilution with added water (Cancel, 1979). Coconut milk contains water, fat, carbohydrate, protein and ash with the major components being water and fat. Phosphorus, calcium and potassium are major minerals. According to Thai standard (TIS 582-2528, 1985), coconut milk product must contain $\geq 18\%$ fat, $\geq 22\%$ total solid and $\leq 0.3\%$ lauric acid.

2.4 Effect of high hydrostatic pressure on gelatinization

High-pressure technology has been known since the beginning of the 20th century. Douzals *et al.* (1996) reported that wheat starch gelatinization started below 300 MPa and was completely achieved at 600 MPa. For rice starch, Oh *et al.* (2008) reported that the effect of high hydrostatic pressure (HHP) on rice and waxy rice starch gelatinization depended on type of starch, the pressure level, temperature, and the duration of treatment. At 500 MPa and 40⁰C for 30 min, both starches lost all birefringence. This topic was reviewed under the principle of HHP, HHP induced starch gelatinization and factors related with HHP induced starch gelatinization.

2.4.1 Principle of HHP

High hydrostatic pressure (HHP) is a non-thermal processing. The principle of HHP treatment is the immediately and uniform pressure distribution among the food regardless of size, shape and the composition. Food is contained in a heat sealed plastic bag and placed in a pressure vessel filled with water. High pressure is then generated in the vessel by a pressure pump (Fig. 2.7) and transferred throughout the product in the plastic bag (Buzrul *et al.*, 2008; Yaldagard, Mortazavi, and Tabatabari, 2008). During the pressure is applied to food in pressure vessel, there is an increase of temperature due to the compressive work against intermolecular forces. The increasing of temperature depended on the compression rate, initial temperature and composition of food. Buzrul *et al.* (2008) studied compression heating of selected pressure transmitting fluids and liquid foods during high hydrostatic pressure treatment and found that water and liquid foods were not affected by compression rate within 100 – 300 MPa/min, while these compression rate affected the compression heating values of ethanol and ethylene glycol.

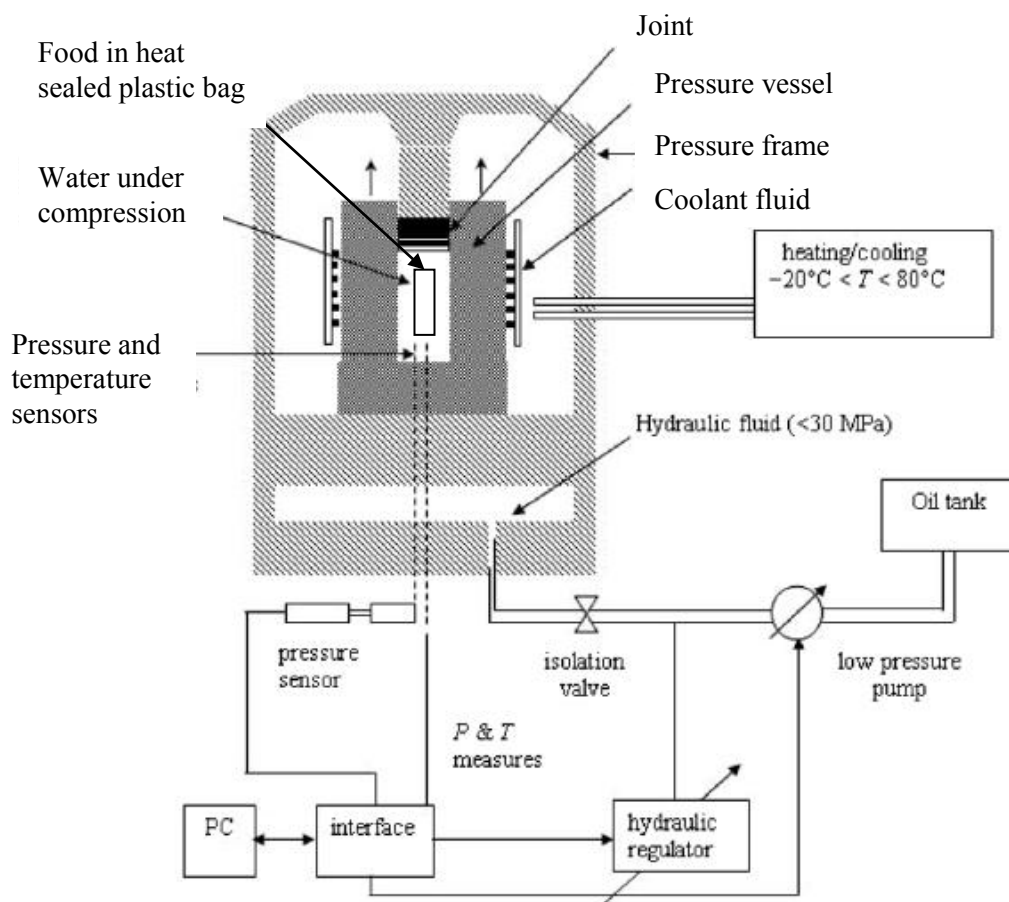


Figure 2.7 Schematic diagram of high hydrostatic pressure equipment

Source: Adpted from Buzrul *et al.* (2008)

2.4.2 HHP induced starch gelatinization

HHP has been shown to affect starch polymers causing gelatinization starch (Douzals *et al.*, 1996; Douzals *et al.*, 1998). Yamamoto *et al.* (2009) reported that the mechanism of HHP-induced starch gelatinization differed from heat-induced one. In heat-induced gelatinization, starch granules absorbed water and swelled; the water more than 14 molecules per one glucose molecule were required for a complete gelatinization. The starch granule was destroyed

during heating process and lost crystalline. When gelatinized starch recrystallized during storage, it affected the texture of food products. This phenomenon is known as retrogradation. For HHP-induced gelatinization, starch granules can swell and loss crystalline, while maintaining the granular shape (Fig. 2.8 and Fig. 2.9) (Oh *et al.*, 2008). During HHP-gelatinized starch, it was found that amylose was hardly released (Oh *et al.*, 2008) so that retrogradation of HHP-induced gelatinized starch was much slower than that of heat-induced gelatinized starch.

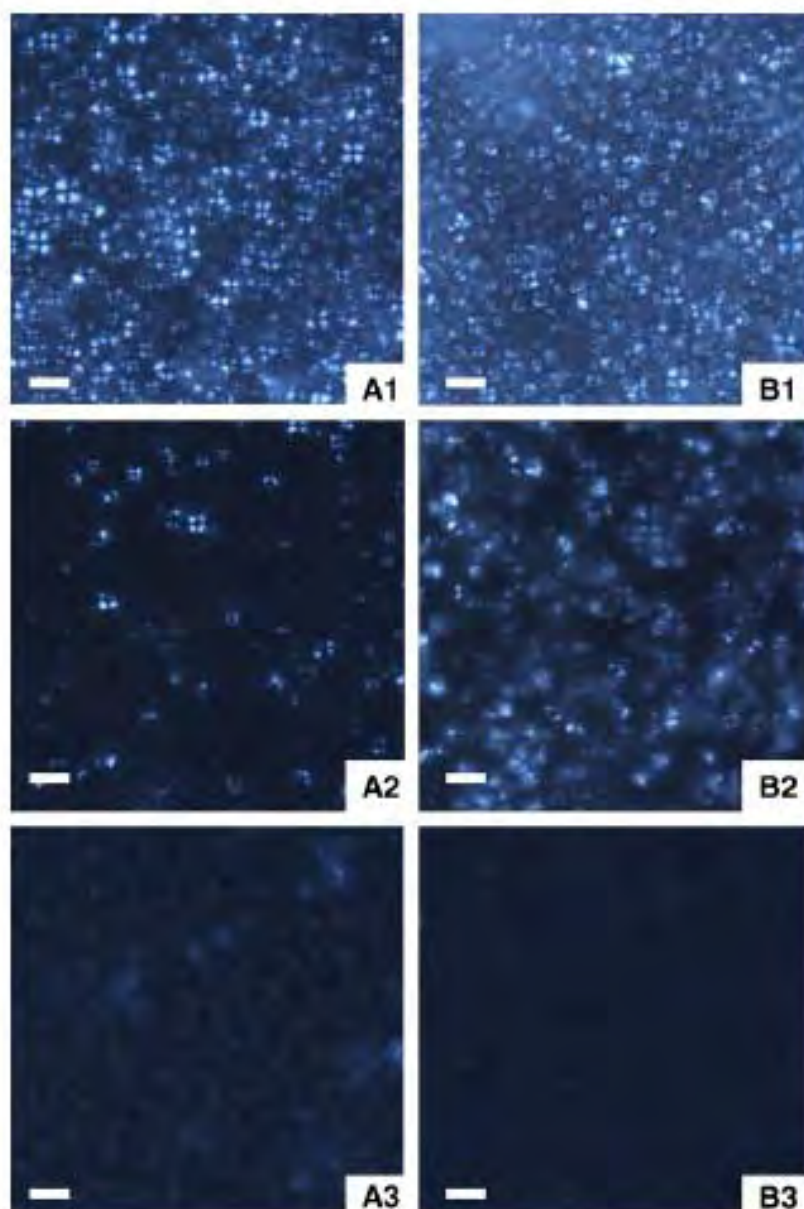


Figure 2.8 Polarized light micrographs. (A) Normal rice starch suspension after [A1] no pressure treatment, [A2] pressure treatment at 400 MPa, and [A3] pressure treatment at 500 MPa. (B) Waxy rice starch suspension after [B1] no pressure treatment, [B2] pressure treatment at 350 MPa, and [B3] pressure treatment at 500 MPa. The bar is 20 μm . The temperature at treatment was 40 $^{\circ}\text{C}$ and the treatment duration was 30 min.

Source: Oh *et al.* (2008)

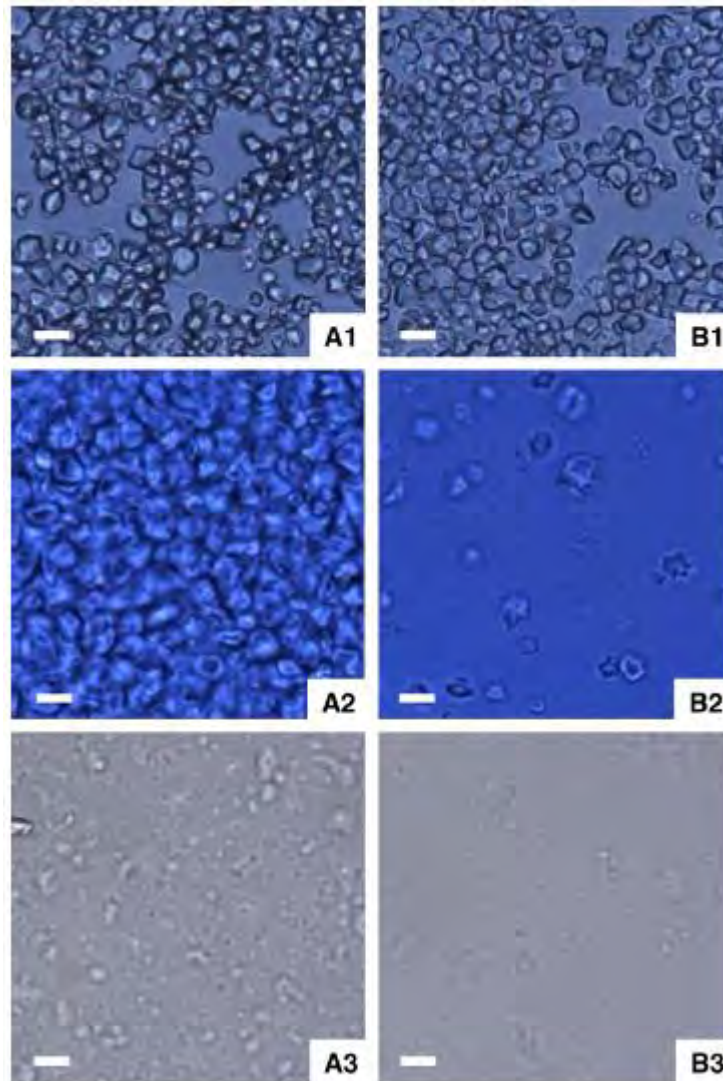


Figure. 2.9 Light micrographs without polarizing filter. (A) Normal rice starch suspension; (B) Waxy rice starch suspension. After no pressure treatment [A1 and B1], after pressure treatment at 500 MPa [A2 and B2], and after pressure treatment at 500 MPa and subsequent pasting [A3 and B3]. The bar is 20 μm . The temperature at treatment was 40 $^{\circ}\text{C}$ and the treatment duration was 30 min.

Source: Oh *et al.* (2008)

2.4.3 Factors related with HHP induced starch gelatinization

In thermal processing the impact of heat treatment is quantified by the application of time and temperature integrators. For HHP, the effect high hydrostatic pressure induced starch gelatinization depends on pressure, time, and temperature. Thus, this part will review about the effect of pressure, time, and temperature on HHP-induced starch gelatinization.

Bauer and Knorr (2005) studied the effect of different treatment pressures, temperatures at pressurization, treatment duration, and kind of starch on the degree of starch gelatinization and found that degree of starch gelatinization increased with increasing treatment pressure at constant time of wheat starch. They also found that at constant temperature and pressure, the degree of starch gelatinization increased with increasing treatment time, and at constant pressurized time, the degree of starch gelatinization increased with increasing temperatures at pressurization and treatment pressure.

Oh *et al.* (2008) studied the effects of different treatment pressures, temperatures at pressurization, and treatment duration on rice starch gelatinization. They found that the degree of gelatinization depended on the type of starch, the pressure, temperature, and duration of treatment. For 10 % (w/w) of starch-water suspension, they also found that at pressure higher than 300 MPa, the degree of swelling of rice starch was lower than that of waxy rice starch, while the amount of leached amylose in rice starch increased with increasing treatment pressure up to 400 MPa. The amount of leached amylose did not change significantly from 400 to 700 MPa.

CHAPTER III

MATERIALS AND METHODS

The experiment was separated into 3 parts according to the objectives as follows.

Part 1. Properties of Thai rice flour: Physicochemical, functional, pasting and gel textural properties

This part was aimed to investigate the physicochemical, functional, thermal, pasting and gel textural properties of rice flour from six non-waxy rice varieties and the relationship among these properties were analyzed.

3.1.1 Materials

Six varieties of Thai rice (*Oryza sativa* L.) namely Pathum Thani 1 (P1), Hawm Suphan Buri (HS), Suphan Buri 1 (S1), Suphan Buri 2 (S2), Suphan Buri 3 (S3) and Suphan Buri 60 (S60), were chosen to represent different ranges of amylose of non waxy rice. They were harvested during year 2006- 2007 from Pathum Thani Rice Research Institute and Suphan Buri Rice Research Institute, Thailand.

Flour was produced by wet milling at Cho Heng Rice Vermicelli Factory Co. Ltd., Thailand. Rice was steeped in water at the ratio of water: rice of 2:1 (w/w) for 4 h and grounded by double-disk stone mill. The slurry was

centrifuged by basket centrifuge for 10 min before drying in a hot-air oven at 40 °C for 12 h to reduce the moisture content to approximately 10%. The dried sample was grounded using hammer mill with a 0.5-mm sieve and kept in Al-laminated bag (12µm PET/ 6.5µm Al/50µm LDPE) at 4°C until used. The rice flour sample was sieved through a 100 mesh before using.

3.1.2 Methods

3.1.2.1 Physicochemical properties

Chemical composition, granule morphology, particle size distribution and crystallinity of the rice flour samples were analyzed using the following methods:

3.1.2.1.1 Chemical composition

The samples were analyzed for moisture (AACC method 44-15A, 1995), crude protein (AACC method 46-13A, 1995), crude fat (AOAC method 920.39, 2000), ash (AACC method 08-01, 1995), starch (Glucoamylase method, AACC, 1995), appearance amylose (Juliano, 1971), and damaged starch (AACC method 76-31, 1995).

3.1.2.1.2 Granule morphology

The granule morphology of the samples was examined under Scanning Electron Microscope (SEM JEOL model JSM-6480 LV, Tokyo, Japan) with a magnification of 5,000 X at accelerating voltage of 20kV.

3.1.2.1.3 Particle size distribution

Two hundred gram of rice flour was passed through a series of 4 sieves with mesh size ranging from 63 to 200 μm . The sieve shaker (Retsch, Germany) was operated at speed # 50 (150 rpm) for 30 min. The particle collected on each sieve was weighed and percentage of each particle size range was calculated.

3.1.2.1.4 Crystallinity

The crystallinity of rice flour granules was determined using the X-ray diffractometer (XRD) (Bruker AXS, model D8 Advance, Germany). The X-ray source was Cu radiation (wavelength = 1.5406 nm) and operating conditions were 40 kV and 30 mA. About 0.1 mg rice flour was put into the XRD sample slide, and placed on the sample holder. Data was collected over the 2θ range from 5° to 40° at a scanning speed of 0.1 deg / min, and a step size of 0.02° (Qi *et al.*, 2004). The % crystallinity was calculated as the ratio of area of the crystalline shape peak, as double helices of polymer in starch, over the total area (Cheetham and Tao, 1998).

3.1.2.1.5 Amylose leaching

The apparent amylose leaching in supernatant was determined by the blue-value method. Accurately 1.000 g rice flour sample in centrifuge tube was suspended in 15 ml of deionized water and shaken in water-bath at 80°C for 30 min. The content was centrifuged at 2,500 rpm (Universal 32R, Hettich Zentrifugen, Germany) for 10 min. The supernatant was carefully poured into an aluminum dish (of known weight) and accurately weighed. After

that, the supernatant 1 ml was pipetted into 100 ml volumetric flask. Then 1 ml of 1 M acetic acid and 2 ml of 2% (w/v) iodine solution were added to the supernatant and adjusted to 100 ml by using deionized water. The solution was placed into dark room for 30 min. The blue color was measured colorimetrically at 620 nm. Potato amylose (Fluka, Switzerland) was used as standard. Apparent amylose contents in supernatant were determined in triplicate. The amylose leaching was calculated from amylose standard curve in Appendix A.

3.1.2.2 Functional properties

Water absorption index (WAI), water solubility index (WSI), and swelling power (SP) of flour samples were analyzed using the following methods:

3.1.2.2.1 Water absorption index and water solubility index

The WAI and WSI of rice flour samples were determined following the method described by Kadan *et al.* (2008). One gram (1.0000 g) of dried flour sample was accurately weighed and suspended in 6 ml of distilled water and shaken in water bath at 80 °C for 30 min. The content was centrifuged at 2,500 rpm (Universal 32R, Hettich Zentrifugen, Germany) for 10 min. The supernatant was carefully poured into an aluminum dish (of known weight) before drying at 105 °C for 10 h and weighing. The sediment was weighed. The WAI and WSI were calculated from equations [3.1] and [3.2].

$$\text{WSI (\%)} = \frac{\text{weight of dried solids in supernatant} \times 100}{\text{dry weight of flour}} \quad [3.1]$$

$$\text{WAI} = \frac{\text{weight of wet sediment}}{\text{dry weight of flour}} \quad [3.2]$$

3.1.2.2.2 Swelling power (SP)

The SP of rice flour samples was determined by measuring water uptake of the samples (Schoch, 1964). The rice flour 500 mg was weighed into centrifuge tube and 15 ml of distilled water was added. The suspension was heated in water bath at 80 °C for 30 min and then centrifuged at 4,000 rpm for 20 min. The supernatant was carefully poured into aluminum dish (of known weight) before drying at 105 °C to constant weight and weighing. The sediment was collected and weighed. SP was calculated using equation [3.3].

$$\text{SP} = \frac{\text{weight of sediment}}{\text{weight of flour} - \text{weight of dried solids in supernatant}} \quad [3.3]$$

3.1.2.3 Thermal properties

A differential scanning calorimeter (Pyris Diamond DSC 7, Perkin Elmer Inc., MA, USA) was employed to measure the gelatinization and retrogradation of rice flour samples according to the method described by Thirathumthavorn and Trisuth (2008). The sample (about 6 mg) and distilled water (about 24 mg) were accurately weighed into a stainless steel DSC pan and hermetically sealed. The content was equilibrated at room temperature for

overnight. The sample was heated from 25 °C to 110 °C at 10⁰C/min. An empty pan and indium were used as reference and calibration standard, respectively. The onset (T_{onset}), peak (T_{peak}), and end (T_{end}) temperatures, and the gelatinization enthalpy (ΔH_{gel}) of samples were obtained from thermogram. The samples were kept at 4⁰C for 7 days to allow them to retrograde, and then rescanned from 25 °C to 110 °C at 10⁰C/min for retrogradation studies. The onset ($T_{\text{onset_retro}}$), peak ($T_{\text{peak_retro}}$), and end ($T_{\text{end_retro}}$) temperature and the enthalpy of regelatinization (ΔH_{retro}) were obtained from thermogram. All measurements were done in triplicate. The percentage of retrogradation was calculated from equation [3.4].

$$\% \text{ Retrogradation} = \frac{\Delta H_{\text{retro}}}{\Delta H_{\text{gel}}} \times 100 \quad [3.4]$$

3.1.2.4 Pasting properties

A Rapid Visco-Analyzer (Newport Scientific model RVA-4C, Sydney, Australia) was employed to evaluate the pasting characteristics of rice flour samples according to the AACC 76-21 method. About 3.50 g of rice flour was weighed and poured into 25 g distilled water in aluminum RVA canister. The content was quickly stirred using a plastic paddle for 10 times before insertion into Rapid Visco-Analyzer. The temperature profile consisted of equilibrating the flour suspension at 50 °C for 1 min, then heated to 95⁰C within 3 min 42 s at 12.2⁰C/min, and held at 95⁰C for 2 min 30 s. It was subsequently cooled to 50 °C within 3 min 48 s at 11.8⁰C/min, and held at 50 °C for 2 min. The rotation speed was maintained at 160 rpm. The pasting characteristics: peak viscosity (PV),

trough (T), breakdown (BD), final viscosity (FV), and setback from trough (SB) were determined from Newport Scientific's ThermoCline for Windows software. All measurements were done in triplicate.

3.1.2.5 Textural properties of rice flour gel

The textural properties of rice flour gel obtained from RVA were determined following the method described by Sandhu and Singh (2007). After RVA testing, the paddle was removed and the canister was sealed with parafilm[®]. Samples were kept at room temperature ($25 \pm 2^{\circ}\text{C}$) for 3 h before texture measurement by Instron Texture Analyzer (Instron 5565, USA). Load cell of 10 N and a cylindrical flat-ended probe of 3 mm diameter were used. Distance was set at 10 mm and a test speed was 1.0 mm/s. Hardness, cohesiveness, adhesiveness, and springiness were computed using the instrument software. All measurements were done in triplicate.

3.1.2.6 Data analysis

The data were statistically analyzed using SPSS software version 17. The Analysis of Variance (ANOVA) and Duncan's New Multiple Range Test (Steel and Lorrie, 1980) were used to determine in effect of main parameters and compare difference between means. Pearson correlation analysis from SPSS program (Version 17) was used to determine the correlation between physicochemical properties and functional properties. The significance level was established at the 95% confidence limit.

Part 2. Effects of sucrose and coconut milk on the gelatinization, pasting and retrogradation properties of Thai rice flour

Coconut milk and sugar are important ingredients in many Thai desserts. Even though, there were many researchers study effect of fatty acids on the properties of flours (Kaur and Singh, 2000; Chiotelli and Meste, 2003; Zhou *et al.*, 2007). However, there was no report of coconut milk, which is complex system of oil in protein solution, in flour. It is expected that oil in coconut milk will interact with starch in rice flour differently from only oil or fatty acids with starch. Thus the effect of coconut milk and sugar on the gelatinization, pasting and gel hardness properties of rice flour was investigated.

3.2.1 Materials

Rice flour from Pathumthani 1 variety (P1) was chosen because it had low amylose content and gelatinization temperatures. UHT coconut milk, (Chaokoh Brand) was purchased from local supermarket in Bangkok, Thailand. Sucrose used was an analytical grade from Ajax Finechem Pty Ltd., Australia.

3.2.2 Methods

3.2.2.1 Effects of sucrose and coconut milk on thermal properties of rice flour

20 % (w/w) rice flour suspension was prepared from mixing 20 g of flour in 80 g distilled water or coconut milk. Different amount of sucrose (0, 10, or 20 g) was dissolved in 100 g of flour suspension with intermittent

stirring. The sample (about 30 mg) were accurately weighed into a stainless steel DSC pan and hermetically sealed. The content was equilibrated at room temperature (about 28⁰C) overnight. The sample was heated from 25 ⁰C to 95 ⁰C at 10⁰C/min. An empty pan and indium were used as reference and calibration standard, respectively. The T_{onset} , T_{peak} , and T_{end} , and ΔH_{gel} of samples were obtained from thermogram.

3.2.2.2 Effects of sucrose and coconut milk on pasting properties of rice flour

Rice flour suspension was prepared from 3.5 g flour and 25 g distilled water or coconut milk. Sucrose (0, 1.75 or 3.5 g) was added to rice flour suspension. The pasting properties of rice paste were measured by RVA according to the same condition as in section 3.1.2.4.

3.2.2.3 Retrogradation kinetics

The sample obtained from the RVA (about 30 mg) was accurately weighed into a stainless steel DSC pan, hermetically sealed, and kept at 4 ⁰C for 1, 3, 7, 15, and 30 days. The sample was heated from 25 to 95 ⁰C, at a rate of 10⁰C/min. The regelatinization enthalpy (ΔH_{retro}) and temperatures ($T_{\text{onset_retro}}$, $T_{\text{peak_retro}}$, and $T_{\text{end_retro}}$) were obtained from thermogram. All measurements were done in triplicate.

The Avrami model was employed to describe the kinetics of flour retrogradation (McIver *et al.*, 1968):

$$\theta = (\Delta H_1 - \Delta H_t) / (\Delta H_1 - \Delta H_0) = \exp(-kt^n) \quad [3.5]$$

Where θ was the no crystallization part at time (t). ΔH_0 was the enthalpy of gelatinization flour after removing sample from RVA and assumed to be 0 J/g. ΔH_t and ΔH_t were the enthalpies of gelatinization of gelatinized samples after kept at 4 °C for 30 days and time t , respectively. The k was the rate constant (day^{-1}), and n was the Avrami exponent which were calculated from equation [3.6].

$$\log \{ \ln [(\Delta H_t - \Delta H_0) / (\Delta H_t - \Delta H_t)] \} = n \log t + \log k \quad [3.6]$$

3.2.2.4 Effects of sucrose and coconut milk on hardness and structure of rice flour

The samples obtained from the RVA were kept at room temperature ($25 \pm 2^\circ\text{C}$) for 3 h and at 4 °C for 7 days before hardness measurement using Instron Texture Analyzer (Instron 5565, USA) as in section 3.1.2.5 and structure of rice flour gel using complexing index method, SEM, and X-ray diffraction.

3.2.2.4.1 Complexing Index

The complexing index (CI) of the sample obtained from the RVA was measured (Kaur and Singh, 2000). Five gram of sample was taken from the RVA canister immediately after completing the heating-cooling cycle. It was then mixed with 25 ml distilled water in 50 ml-capped tube for 2 min

using vortex mixer (Vortex-Genie 2 model G-560E, Bohemia, USA) and centrifuged for 15 min at 3000 rpm (Hettich Zentrifugen model Universal 32R, Germany). The supernatant (500 μ l) was drawn from the tube and mixed with 15 ml of distilled water, followed by 2 ml of iodine solution (2.0% KI and 1.3% of I₂ in distilled water). The absorbance of the dispersion (Abs) was measured at 690 nm using the flour paste in water as reference (Abs_{Reference}). The complexing index (CI) was calculated as follows:

$$CI = \frac{[Abs_{Reference} - Abs] \times 100}{Abs_{Reference}} \quad [3.7]$$

3.2.2.4.2 Surface morphology by using Scanning Electron Microscope (SEM)

The sample obtained from the RVA was kept at 4 °C for 15 day before freeze drying and grinding in Waring blender and sieving through 50 mesh sieve. The sample was mounted on scanning electron microscope (SEM) stub using double sided adhesive tape and was coated with gold. Scanning electron micrographs were taken from SEM (JEOL model JSM-6480 LV microscope, Tokyo, Japan) at the accelerating voltage of 15 kV and magnification of 1000X.

3.2.2.4.3 X-ray diffraction analysis

The powder sample from freeze dried sample in no. 3.2.2.5.2 was mounted on glass sample holder. The X-ray diffraction pattern was obtained from X-ray diffractometer (Bruker AXS, model D8 Advance, Germany).

The X-ray source was Cu radiation (wavelength = 1.5406 nm) and operating conditions were 40 kV and 30 mA. Data was collected over the 2θ range from 5° to 40° at a scanning speed of 0.01 deg/min, and a step size of 0.02° . Peaks in traces were analyzed using Eva Program interfaced with the equipment.

3.2.2.5 Data analysis

All analyses were carried out in triplicate. Linear regressions of the data were analyzed by using Microsoft Excel software package (Microsoft Corporation, USA) and the ANOVA was analyzed by using SPSS software version 17.

Part 3. Effect of sucrose on the thermal and rheological properties of Thai rice flour treated with high hydrostatic pressure

High hydrostatic pressure (HHP) process is a nonthermal process which has been used. The HHP product has higher quality. It has been reported that HHP can gelatinize Basmati rice flour (Ahmed *et al.*, 2007), rice starch (Oh *et al.*, 2008), wheat and tapioca starch (Bauer and Knorr, 2005), and potato starch (Bauer and Knorr, 2005; Kawai *et al.*, 2007). However there is no report on the effect of sugar on the HHP product. Therefore, the aim of this part was to investigate the effects of pressure and holding time of HHP process and adding sucrose on the gelatinization properties of rice flour suspension.

3.3.1 Materials

Rice flour from Pathumthani 1 variety (P1) was used. Sucrose was analytical grade from Sigma-Aldrich, Germany.

3.3.2 Methods

3.3.2.1 Sample preparation

The 20% (w/w) of rice flour suspension was prepared by mixing 20 g of rice flour in 80 g of 2 % (w/w) waxy corn starch solution in distilled water. Sucrose was added into suspension at 0, 10, and 20 wt%. The sample was filled into a polymer pouch (10 x 3 cm) before heat-sealing and kept at 25 ± 2 °C overnight. The waxy corn starch was used to prevent the sedimentation of rice flour during HHP treatment and was proven not to interfere with the measurement (Kawai *et al.*, 2007).

3.3.2.2 High pressure treatment

The sample was treated under high hydrostatic pressure (HHP) range of 0.1- 600 MPa and at 40 °C for 60 min with a HHP-generating system (Model Dr.Chef: Teramecs Co. Ltd., Japan). After the treatment, the pressure was released to ambient pressure at 0.1 GPa/min, and the thermal and rheological properties of the sample were measured by using a Differential Scanning Calorimeter (Pyris Diamond DSC 7, Perkin Elmer Inc., MA, USA) and a controlled stress rheometer (AR 2000 , TA instrument, New Castle, USA).

3.3.2.3 Thermal properties

The HHP-treated of rice flour with various levels of sucrose were measured for their thermal properties. The sample (about 30 mg) was accurately weighed into a stainless steel DSC pan and hermetically sealed. Temperature scanning from 20 to 120⁰C at a rate of 10⁰C/min, was used. An empty pan (air) and indium were used as a reference and calibration standard, respectively. Thermal curves resulting from scanning were determined for T_{onset}, T_{peak}, and T_{end}. Enthalpy of gelatinization was calculated from the area of the peak endotherm using the Thermal Analysis Pyris Software interfaced with the DSC. All measurements were done in triplicate.

The percentage of un-gelatinized flour at any time (A_t) was determined by comparing the enthalpy of gelatinization of the rice flour suspension treated with HHP ($\Delta H_{\text{treated}}$) with and without sucrose with that of un-treated HHP rice flour ($\Delta H_{\text{un-treated}}$). Calculations were made using the following equation (Riva *et al.*, 2000):

$$\begin{aligned} A_t &= \text{Un-gelatinized flour (\%)} \\ &= \frac{\Delta H_{\text{treated}}}{\Delta H_{\text{un-treated}}} \times 100 \end{aligned} \quad [3.8]$$

And the degree of flour gelatinization of the sample was calculated from equation [3.9]

$$\text{Degree of gelatinization (\%)} = 100 - A_t \quad [3.9]$$

3.3.2.4 Analysis of gelatinization kinetics

The extent of starch gelatinization was found to follow the first-order kinetics (Bakshi and Singh, 1980);

$$\frac{dA_t}{dt} = -kA \quad [3.10]$$

$$\ln (A_t/ A_i) = -kt \quad [3.11]$$

Where A_i is the initial percentage of un-gelatinized flour taken to be 100%, A_t is the percentage of un-gelatinized at time t , and k is the first-order rate constant (s^{-1}).

3.3.2.5. Rheological properties

The rheological properties of the samples were analyzed using controlled stress rheometer (AR 2000, TA instrument, New Castle, USA) employing parallel plate geometry of 20 mm diameter and 1000 μm gap. The sample temperature was controlled at 20 $^{\circ}\text{C}$ by using water circulating unit. For each test, the sample was placed between the rheometer plates for 2 minutes for stress relaxation and temperature equilibration before the actual measurement.

The measurement was performed in linear viscoelastic region after temperature equilibration over a frequency range between 0.1 and 100 Hz which enables the material to retain the structure. Rheological parameters [elastic modulus (G'), viscous modulus (G''), and viscosity (η)] were directly

obtained from the manufacturer supplied computer software (Rheology Advantage Data Analysis Program, TA Instruments, New Castle, DE). The deviations did not exceed 5% between duplicate runs, as the experiment was repeated. The average of the triplicate runs was reported.

3.3.2.6 Morphology of rice flour treated with HHP

Each sample was put on a glass slide and a cover slip was placed on top of the sample for microscopic examination. A polarizing light microscope (Nikon Corporation, Tokyo, Japan) with a 50x objective was used to observe birefringence of the starch granules. The microscope was also used with and without the polarizing filter to observe the appearance of the sample.

3.3.2.7 Data analysis

The analysis was carried out in triplicate. Linear regressions of the data were analyzed by using Microsoft Excel software package (Microsoft Corporation, USA) and the ANOVA was analyzed by using SPSS software version 17.

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Part 1. Properties of Thai rice flour: Physicochemical, functional, thermal, pasting and gel textural properties

4.1.1 Physicochemical properties of rice flour

The proximate compositions of non-waxy rice flour from six varieties were shown in Table 4.1. It was found that varieties significantly affected protein content ($p \leq 0.05$). Rice flour from Hawm Suphan Buri (HS) variety contained the highest protein content (6.90%), while Suphan Buri 3 (S3) variety contained the lowest protein content (5.28%). The moisture content, fat content and ash content were found to be 5.67 to 5.88 %, 0.27 to 0.30% and 0.32 to 0.37%, respectively.

Table 4.1 Chemical composition of rice flour from different rice varieties

| Rice varieties | Proximate content (% db) | | | |
|----------------|--------------------------|------------------------|-------------------|-------------------|
| | Moisture ^{ns} | Protein (N x 5.95) | Fat ^{ns} | Ash ^{ns} |
| P1 | 5.67±0.09 | 6.83±0.08 ^a | 0.28±0.04 | 0.32±0.02 |
| HS | 5.88±0.60 | 6.90±0.17 ^a | 0.28±0.05 | 0.37±0.01 |
| S2 | 5.82±0.72 | 5.98±0.08 ^b | 0.30±0.03 | 0.36±0.03 |
| S60 | 5.81±0.55 | 5.64±0.07 ^c | 0.27±0.08 | 0.32±0.03 |
| S1 | 5.86±0.23 | 5.85±0.09 ^b | 0.30±0.01 | 0.36±0.03 |
| S3 | 5.74±0.53 | 5.28±0.08 ^d | 0.29±0.03 | 0.33±0.04 |

Means with different letters in each column were significantly different ($p \leq 0.05$). ns means in the same column were not significantly different ($p > 0.05$).

From Table 4.2 the amylose content (AC), starch content (SC), damaged starch (DS) and % crystallinity were found to be 18.64 to 33.05% db, 84.44 to 86.14% db, 2.52 to 6.38% db, and 23.15 to 31.31%, respectively. These rice flours could be classified into 3 groups according to its amylose content as follows (Juliano, 2004)

- Low amylose rice flour having AC of less than 20%
- Intermediate amylose rice flour having AC of 20 – 25 %
- High amylose rice flour having AC of more than 25%

However, the result in this experiment showed that the rice flour samples could be divided into only 2 groups; P1, HS, S2, and S60 (AC 18.63 - 21.58 %db) and S1 and S3 (AC 28.22 - 33.05 %db).

The crystallinity patterns of rice flour samples (Fig. 4.1) showed a typical A-type diffraction pattern with strong peaks at 15.2° , 17.1° , 18.2° , and 23.2° (2θ) as observed by many researchers (Ong and Blanshard 1995a; Iturriaga *et al.* 2004; Shu *et al.*, 2006; Phothiset and Charoenrein, 2007; Wu *et al.*, 2010). Table 4.2 showed that % crystallinity of the samples were separated into 2 groups corresponding to their amylose contents, i.e. amylose content of 18.63 - 21.58% had % crystallinity of 23.15 – 25.75 and amylose content of 28.22 - 33.05% had % crystallinity of 30.56 – 31.31. This may be because amylose chain either linear or slightly branched can easily form double helices in amorphous zone resulting in higher % crystallinity (Srichuwong *et al.*, 2005a). Besides amylose, protein in flour may influence granule structure and crystallinity (Yu *et al.*, 2010). Therefore, flours from P1 and HS, which had higher protein and lower amylose content, had lower % crystallinity.

For damaged starch, it was found that damaged starch ranged from 2.5 – 6.4 % and did not depend on rice variety or amylose content. Usually damaged starch in flour or starch was affected by milling and grain hardness (Chen *et al.*, 1999; Suksomboon and Naivikul, 2006; Takata *et al.*, 2010). Dry milling causes more damage than wet milling. However, in this experiment only wet milling was used.

For amylose leaching, it was found that amylose leaching of rice flour samples ranged from 0.0513 – 1.222 mg/100 ml. Rice flour from S3 variety had high amylose leaching than the other. This difference may be because the amylose inside the granules leached out simultaneously when starch granules swelled (Tester and Morrison, 1990).

Table 4.2 Amylose content, starch content, damaged starch, crystallinity and amylose leaching of rice flour from different rice varieties

| Rice Varieties | Amylose content (% db) | Starch content ^{ns} (% db) | Damaged starch (% db) | Crystallinity (%) | Amylose leaching at 80°C (mg/100ml) |
|----------------|--------------------------|-------------------------------------|-------------------------|-------------------------|-------------------------------------|
| P1 | 18.64±0.57 ^c | 84.44± 2.87 | 5.44± 0.06 ^b | 24.04±0.15 ^c | 0.053±0.003 ^d |
| HS | 19.46±0.57 ^{dc} | 84.80± 2.89 | 6.38± 0.07 ^a | 23.15±0.22 ^t | 0.060±0.005 ^d |
| S2 | 21.58±1.23 ^c | 85.16± 1.93 | 4.18±0.03 ^d | 24.39±0.16 ^d | 0.058±0.003 ^d |
| S60 | 20.73±0.93 ^{cd} | 85.33± 2.75 | 4.62±0.05 ^c | 25.75±0.14 ^c | 0.418±0.006 ^b |
| S1 | 28.22±1.07 ^b | 85.08± 1.54 | 2.52±0.06 ^e | 30.56±0.34 ^b | 0.375±0.009 ^c |
| S3 | 33.05±1.00 ^a | 86.14± 1.38 | 4.52± 0.08 ^c | 31.31±0.24 ^a | 1.222±0.025 ^a |

Means with different letters in each column were significantly different ($p \leq 0.05$). ns means in the same column were not significantly different ($p > 0.05$).

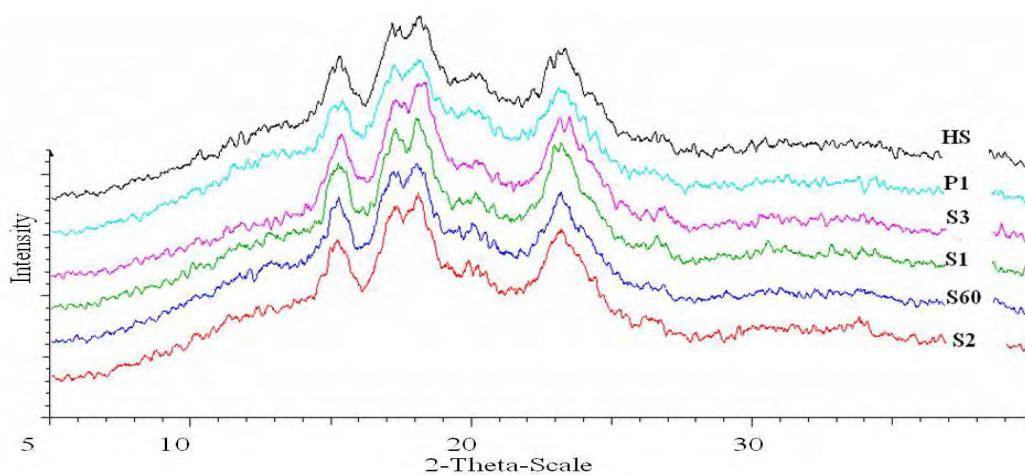


Fig. 4.1 The crystalline pattern of rice flour from different rice varieties

Table 4.3 shows that the distribution of flour particle size from different rice varieties, ranged from less than 63 to more than 200 μm . About 50% of rice flour particle size ranged from 91 to 125 μm . The granule morphology of rice flour samples showed angular polyhedral shape (Fig. 4.2).

Table 4.3 Particle size distribution of rice flour from different rice varieties by vibration sieve

| Rice varieties | Particle size distribution (weight %) | | | | |
|----------------|---------------------------------------|-----------------------------------|----------------------------------|---------------------------------|--------------------------------|
| | > 200 μm^{ns} | 126-200 μm^{ns} | 91-125 μm^{ns} | 63-90 μm^{ns} | < 63 μm^{ns} |
| P1 | 6.56 \pm 1.11 | 24.12 \pm 1.16 | 47.42 \pm 0.60 | 18.24 \pm 1.30 | 3.64 \pm 0.37 |
| HS | 6.47 \pm 1.13 | 21.86 \pm 0.51 | 49.48 \pm 0.08 | 18.48 \pm 1.81 | 3.71 \pm 0.26 |
| S2 | 7.02 \pm 0.71 | 24.56 \pm 1.80 | 48.41 \pm 0.09 | 16.57 \pm 1.00 | 3.44 \pm 0.17 |
| S60 | 6.58 \pm 1.11 | 23.79 \pm 0.75 | 46.65 \pm 0.78 | 19.38 \pm 0.93 | 3.59 \pm 0.19 |
| S1 | 6.26 \pm 1.21 | 23.63 \pm 0.21 | 47.99 \pm 0.56 | 18.38 \pm 1.02 | 3.72 \pm 0.16 |
| S3 | 6.45 \pm 0.86 | 23.09 \pm 0.85 | 47.59 \pm 1.14 | 18.54 \pm 0.27 | 4.31 \pm 0.84 |

ns means in the same column were not significantly different ($p > 0.05$).

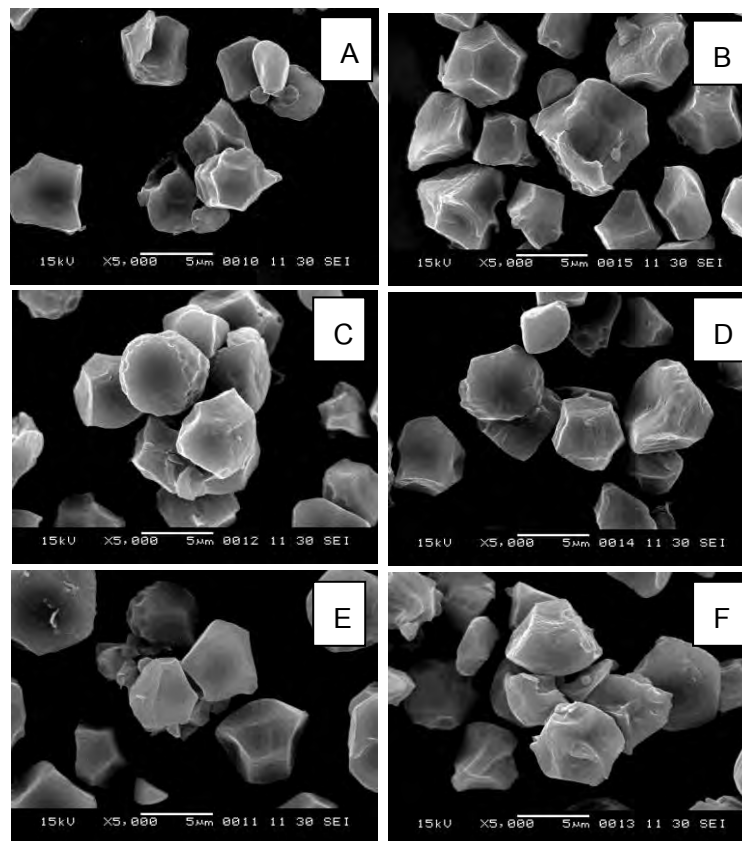


Fig. 4.2 SEM micrographs (x5000) of rice flour granules from different rice varieties; P1 (A), HS (B), S2 (C), S60 (D), S1 (E) and S3 (F)

4.1.2 Functional properties of rice flour

From Table 4.4, the WAI, WSI and swelling power of rice flour from different rice variety at 80 °C were significantly different ($p \leq 0.05$). It was found that rice flour from S3 variety had higher WAI, WSI and swelling power than the others. This difference may be because S3 flour had the highest amylose content.

Table 4.5 showed the correlation coefficients between amylose content, % crystallinity, protein content, damaged starch and amylose leaching with functional properties of rice flour. It was found that the correlation between amylose content, % crystallinity or amylose leaching was positively correlated

with WSI of flour ($r > 0.6$) and stronger than that of WAI and swelling power, while the correlation coefficient of protein and WSI was negatively weaker correlated ($r = -0.545$). It may be because disulfide bond in rice protein restricted starch granule from swelling (Derycke *et al.*, 2005).

Table 4.4 Water absorption index, water solubility index, and swelling power of flour from different rice varieties

| Varieties | WAI | WSI (%) | SP |
|------------|-----------------------------|-----------------------------|-----------------------------|
| P1 | 6.838 ± 0.365 ^b | 0.787 ± 0.063 ^b | 6.892 ± 0.368 ^b |
| HS | 6.909 ± 0.220 ^{ab} | 0.748 ± 0.108 ^b | 6.962 ± 0.216 ^b |
| S2 | 6.684 ± 0.694 ^b | 0.727 ± 0.104 ^b | 6.734 ± 1.008 ^b |
| S60 | 6.477 ± 0.451 ^b | 0.897 ± 0.181 ^{ab} | 6.535 ± 0.451 ^b |
| S1 | 7.100 ± 0.709 ^{ab} | 0.909 ± 0.211 ^{ab} | 7.165 ± 0.821 ^{ab} |
| S3 | 8.008 ± 0.317 ^a | 1.068 ± 0.098 ^a | 8.095 ± 0.315 ^a |

Means with different letters in each column are significantly different ($p \leq 0.05$).

Table 4.5 Correlation coefficients of physicochemical and functional properties of rice flour

| | Amylose content | % Crystallinity | Protein | Damaged starch | Amylose leaching |
|------------|---------------------|---------------------|---------------------|----------------|---------------------|
| WAI | 0.593 ^{**} | 0.516 [*] | ns | ns | 0.588 ^{**} |
| WSI | 0.666 ^{**} | 0.664 ^{**} | -0.545 [*] | ns | 0.694 ^{**} |
| SP | 0.600 ^{**} | 0.523 [*] | ns | ns | 0.595 ^{**} |

* Significant at $\alpha = 0.05$ level

** Significant at $\alpha = 0.01$ level

ns not significant at $\alpha = 0.05$ level

4.1.3 Thermal properties

Table 4.6 shows the effect of rice varieties on gelatinization and retrogradation properties of flour. The gelatinization parameters (ΔH_{gel} , T_{onset} , T_{peak} , and T_{end}) of flour from different rice varieties were significantly different ($p \leq 0.05$). Flours containing high amylose content and % crystallinity (S1 and S3 flours) had higher gelatinization parameters (T_{onset} , T_{peak} , T_{end} , and ΔH_{gel}). This may be due to the rigid amorphous regions of the starch granule by the interaction among amylose chains. The stability of amorphous region may be increased, resulting in higher energy for gelatinization and gelatinization temperature (Chungcharoen and Lund, 1987; Zhou *et al.*, 2009).

The retrogradation parameters in term of enthalpy, temperature and % retrogradation of rice flour from different rice varieties were significantly different ($p \leq 0.05$). ΔH_{retro} and % retrogradation of rice flour samples increased with increasing AC and % crystallinity (Table 4.2). The ΔH_{retro} reflected the melting of recrystallized amylopectin and amylose (Sasaki *et al.*, 2000; Lii *et al.*, 2004; Wang *et al.*, 2010). The S1 and S3 had higher % retrogradation than those of S2, S60, HS and P1. It may be because amylose could associate more easily resulting in more crystal nuclei and hence faster retrogradation (Biliaderis, 1992; Lii *et al.*, 2004).

Table 4.7 showed the correlation coefficients between amylose content, % crystallinity, protein content, damaged starch and amylose leaching with thermal properties of rice flour. It was found that the correlation coefficient of amylose content or % crystallinity was highly positive correlated with T_{onset} , ΔH_{retro} and % retrogradation ($r > 0.8$) and stronger than that of protein

content, damaged starch and amylose leaching. The correlation coefficients of protein content and damaged starch were negatively correlated with T_{onset} , ΔH_{retro} and %retrogradation. These results were similar to those reports of Dipti *et al.* (2002) and Yu *et al.* (2010) but different from that reported by Vandeputte *et al.* (2003a), who found that the amount of amylose content in normal rice starch (amylose content >17.0%) did not significantly influence gelatinization parameters. This may be due to different chemical composition in rice flour, storage time of rice flour, growth season and growth climate (Juliano *et al.*, 1969; Hamaker and Griffin, 1993; Singh *et al.*, 2000; Noda *et al.*, 2003; Derycke *et al.*, 2005; Noosuk *et al.*, 2005; Srichuwong *et al.*, 2005b).

Table 4.6 Gelatinization and retrogradation parameters of rice flour from different rice varieties

| Rice varieties | Thermal parameters of flour gelatinization | | | | Thermal parameters of retrogradation at 4 ⁰ C, 7 days | | | | Retrogradation (%) |
|----------------|--|--------------------------------------|-------------------------------------|------------------------------------|--|--|---|--|--------------------------|
| | ΔH (J/g) | T _{onset} (⁰ C) | T _{peak} (⁰ C) | T _{end} (⁰ C) | ΔH_{retro} (J/g) | T _{onset-retro} (⁰ C) | T _{peak-retro} (⁰ C) | T _{end-retro} (⁰ C) | |
| P1 | 9.50±0.12 ^c | 70.48±0.12 ^b | 75.46±0.26 ^c | 81.73±0.04 ^b | 3.08±0.13 ^b | 43.94±3.33 ^b | 54.20±0.43 ^a | 60.01±0.45 ^b | 32.42±0.32 ^e |
| HS | 9.37±0.26 ^d | 70.68±0.12 ^b | 75.33±0.24 ^c | 80.13±0.82 ^c | 3.37±0.27 ^b | 41.50±3.23 ^c | 54.04±1.32 ^a | 59.90±1.64 ^b | 35.97±2.88 ^d |
| S2 | 9.58±0.3 ^c | 70.50±0.27 ^b | 75.48±0.35 ^c | 80.45±0.30 ^c | 3.84±0.36 ^b | 39.59±1.82 ^d | 53.28±1.71 ^b | 59.21±1.53 ^b | 40.08±3.47 ^c |
| S60 | 9.61±0.13 ^c | 70.15±0.05 ^b | 75.69±0.32 ^c | 80.71±0.39 ^c | 3.47±0.20 ^b | 40.47±2.99 ^c | 53.28±1.17 ^b | 60.86±1.26 ^b | 36.34±2.80 ^{cd} |
| S1 | 10.47±0.12 ^a | 77.72±0.67 ^a | 82.48±0.20 ^a | 86.47±0.14 ^a | 5.78±0.36 ^a | 43.02±2.64 ^b | 54.93±1.70 ^a | 62.93±1.16 ^{ab} | 55.21±1.07 ^b |
| S3 | 9.94±0.21 ^b | 77.54±0.46 ^a | 81.39±0.43 ^b | 85.75±0.75 ^a | 5.74±0.23 ^a | 45.33±3.56 ^a | 54.88±1.82 ^a | 63.54±2.30 ^a | 59.73±1.84 ^a |

Means with different letters in each column were significantly different ($p \leq 0.05$).

ΔH_{retro} means enthalpy of regelatinization

Table 4.7 Correlation coefficients of physicochemical and thermal properties of rice flour

| | Amylose content | Crystallinity | Protein | Damaged starch | Amylose leaching |
|---------------------------|------------------------|----------------------|----------------|-----------------------|-------------------------|
| ΔH_{gel} | ns | ns | ns | -0.533* | ns |
| T_{onset} | 0.931** | 0.942** | -0.559* | -0.632** | 0.712** |
| ΔH_{retro} | 0.939** | 0.940** | -0.672** | -0.708** | 0.710** |
| $T_{\text{onset_retro}}$ | ns | ns | ns | ns | ns |
| % Retrogradation | 0.970** | 0.937** | -0.701** | -0.629** | ns |

* Significant at $\alpha = 0.05$ level

** Significant at $\alpha = 0.01$ level

ns not significant at $\alpha = 0.05$ level

4.1.4 Pasting properties

Pasting properties of rice flour from different rice varieties (Table 4.8) significantly differed ($p \leq 0.05$). It was found that high amylose rice flours (S1 and S3) had higher setback, final viscosity and pasting temperature than that of low amylose rice flours (S60, S2, HS and P1), while low amylose rice flours had higher breakdown value. These results were similar to the report of Varavinit *et al.* (2003) and Pérez *et al.* (1993), who found that amylose content had a positive correlation with setback value, final viscosity and pasting temperature. The low amylose rice flour had low final viscosity, setback and pasting temperature. Flour having low amylose would swell easier indicating a weaker binding force in that starch granule and upon heating its viscosity could increase at lower temperature (Hoover *et al.*, 1996). S1 and S3 flours had higher setback which corresponded to higher % retrogradation from DSC (Table 4.6). This may be because the granules of S1 and S3 flour

(high amylose rice flour) swelled harder when the granules were destroyed by gelatinization and had more amylose leached out to cause the higher final viscosity.

Table 4.9 showed the correlation coefficients between amylose content, % crystallinity, protein content, damaged starch and amylose leaching with pasting properties of rice flour. It was found that most of the pasting parameters were highly correlated with amylose content and amylose leaching of rice flour samples. Almost all correlation between amylose content and pasting parameters agreed with that reported by Varavinit *et al.* (2003) except peak viscosity. It was also found that the correlation coefficient values between % crystallinity and pasting parameters were nearly that of amylose content, while the correlation coefficient values of protein content were also significant but in opposite direction.

Table 4.8 Pasting properties of rice flour from different rice varieties.

| Varieties | PV (cP) | trough (cP) | BD (cP) | FV (cP) | SB (cP) | Peak time (min) | Pasting temperature (°C) |
|------------|------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|--------------------------|---------------------------|
| P1 | 3611.67 ± 26.95 ^d | 1713.67 ± 34.59 ^e | 1898.00 ± 50.74 ^c | 2607.33 ± 43.66 ^f | 892.667 ± 10.26 ^e | 5.96 ± 0.07 ^b | 74.47 ± 0.06 ^c |
| HS | 4356.00 ± 16.00 ^b | 1987.00 ± 12.50 ^b | 2371.67 ± 29.02 ^a | 3252.00 ± 35.79 ^c | 1265.00 ± 23.64 ^d | 6.03 ± 0.04 ^b | 74.58 ± 0.90 ^c |
| S2 | 3800.70 ± 18.03 ^c | 1650.30 ± 26.51 ^f | 2147.00 ± 23.07 ^b | 2994.00 ± 26.56 ^d | 1344.33 ± 20.08 ^c | 5.77 ± 0.06 ^d | 81.21 ± 0.98 ^b |
| S60 | 3376.33 ± 11.02 ^e | 1887.70 ± 14.50 ^c | 1489.00 ± 16.08 ^d | 2845.30 ± 37.50 ^e | 1841.33 ± 15.01 ^b | 6.05 ± 0.04 ^b | 80.08 ± 0.48 ^b |
| S1 | 3381.70 ± 9.07 ^e | 1839.00 ± 20.80 ^d | 1542.30 ± 36.65 ^d | 4404.70 ± 10.97 ^b | 2568.33 ± 17.16 ^a | 5.88 ± 0.04 ^c | 85.27 ± 0.73 ^a |
| S3 | 4487.00 ± 17.35 ^a | 3316.30 ± 12.68 ^a | 1158.33 ± 29.55 ^e | 5844.70 ± 38.80 ^a | 2528.33 ± 47.98 ^a | 6.39 ± 0.09 ^a | 86.76 ± 0.54 ^a |

PV means peak viscosity

BD means breakdown viscosity

FV means final viscosity

SB means setback viscosity from trough to final viscosity

Table 4.9 Correlation coefficients of physicochemical and pasting properties

| | Amylose content | Crystallinity | Protein | Damaged starch | Amylose leaching |
|---------------------|------------------------|----------------------|----------------|-----------------------|-------------------------|
| peak viscosity | ns | ns | ns | ns | ns |
| trough | 0.784** | 0.657** | -0.563* | ns | 0.935** |
| breakdown | -0.752** | -0.847** | 0.815** | ns | -0.858** |
| final viscosity | 0.964** | 0.891** | -0.640** | ns | 0.888** |
| setback | 0.892** | 0.945** | -0.808** | -0.704** | 0.775** |
| peak time | 0.576* | 0.520* | ns | ns | 0.856* |
| pasting temperature | 0.847** | 0.849** | -0.898** | -0.789** | 0.699** |

* Significant at $\alpha = 0.05$ level

** Significant at $\alpha = 0.01$ level

ns not significant at $\alpha = 0.05$ level

4.1.5 Textural properties of rice flour gels

The textural parameters of rice flour gels obtained from RVA from different rice varieties varied significantly ($p \leq 0.05$) (Table 4.10). When flour gel was prepared using RVA, a constant shear was applied and caused fragmentation of starch granules as the evidence from the breakdown viscosity value. Fragmentation enabled more starch molecules to solubilize in water, which then interacted with each other to form a gel network structure. Thereafter, the extent of starch fragmentation and the structures of starch molecules might determine the gel hardness and springiness. Flour gels (S1 and S3) were found to have higher hardness and springiness than lower amylose rice flour gels (P1, HS, S2, and S60). The gel samples were left at room temperature for about 3 h before the texture measurement so the hardness of gel in this case may be due to the rigid gel network of amylose in amorphous zone and higher amylose

leaching in suspension (Table 4.2) rather than the retrogradation of amylopectin. This result was similar to the reports of Vandeputte *et al.* (2003b) and Yu *et al.* (2009).

Table 4.11 showed the correlation coefficients between amylose content, % crystallinity, protein content, damaged starch and amylose leaching with hardness and springiness of rice flour. It was found that all texture parameters were highly correlated with amylose content, % crystallinity and amylose leaching ($r > 0.8$). These results were similar to the report of Iturriaga *et al.* (2010). The gel hardness was mainly caused by starch gel retrogradation, which was associated with crystallization of amylose in short time, lead to harder gels (Yu *et al.*, 2009). Amylose content, % crystallinity and the amount of amylose leaching of rice flour samples were positively correlated with hardness and springiness, whereas protein content and % DS were weakly negatively correlated with hardness and springiness. These results were similar to the reports of Vandeputte *et al.* (2003a), Iturriaga *et al.* (2010) and Yu *et al.* (2010). The gel hardness was mainly caused by starch gel retrogradation, which was associated with crystallization of amylose in short time, lead to harder gels (Yu *et al.*, 2009). The textures of rice starch gel were mainly depended on amylose content, the amount of amylose leaching and amylose retrogradation in the short time.

Table 4.10 Texture parameters of rice flour gels from different rice varieties

| Rice Varieties | Hardness (gf) | Springiness |
|-----------------------|---------------------------|--------------------------|
| P1 | 24.87 ± 1.28 ^c | 0.55 ± 0.01 ^d |
| HS | 25.92 ± 1.57 ^c | 0.59 ± 0.04 ^c |
| S2 | 31.64 ± 1.53 ^b | 0.62 ± 0.01 ^c |
| S60 | 30.73 ± 2.14 ^b | 0.59 ± 0.03 ^c |
| S1 | 57.10 ± 2.55 ^a | 0.69 ± 0.02 ^b |
| S3 | 61.15 ± 1.91 ^a | 0.74 ± 0.25 ^a |

Means with different letters in each column were significantly different ($p \leq 0.05$).

Table 4.11 Correlation coefficients of physicochemical and textural properties

| | Amylose content | Crystallinity | Protein | Damaged starch | Amylose leaching |
|--------------------|----------------------------|----------------------|----------------------|---------------------------|-----------------------------|
| Hardness | 0.968 ^{**} | 0.978 ^{**} | -0.725 ^{**} | -0.677 ^{**} | 0.968 ^{**} |
| Springiness | 0.935 ^{**} | 0.880 ^{**} | -0.733 ^{**} | -0.569 [*] | 0.935 ^{**} |

** Significant at $\alpha = 0.01$ level

4.2 Part 2. Effects of sucrose and coconut milk on gelatinization, pasting and retrogradation properties of Thai rice flour

The P1 flour, represent of rice flour having low amylose group, was selected to study in this part because it has low amylose content, amylose leaching, gelatinization temperature, % retrogradation, and higher swelling power, which these properties would help to preserve shape and improve texture of dessert from rice flour.

4.2.1 Effects of sucrose and coconut milk on thermal properties of rice flour

The effects of coconut milk and sucrose on the thermal properties of rice flour are shown in Fig. 4.3 and Table 4.12. It was found that thermal parameters (T_{onset} , T_{peak} , T_{end} and ΔH_{gel}) of rice flour suspensions in either water or coconut milk increased with increasing sucrose ($p \leq 0.05$). This agrees with previous studies on rice starch (Chungcharoen and Lund, 1987; Ahmed *et al.*, 2007), wheat starch (Chiotelli *et al.*, 2000; Richardson *et al.*, 2002), waxy corn (Chiotelli *et al.*, 2000; Jang *et al.*, 2001), waxy corn starch (Chiotelli *et al.*, 2000), acorn starch (Aee *et al.*, 1998), sweet potato starch (Kohyama and Nishinari, 1991), tapioca starch (Thirathumthavorn and Trisuth, 2008), and sago starch (Ahmad and Williams, 1999). This may be because sucrose reduced the water activity in the system by forming hydrogen bonds with water as a solvent at the ratio of 4.6 water molecules per 1.0 sucrose molecule resulting in increasing concentration of flour in system and stabilized amorphous regions of the starch

granule by the interaction with starch chains (Botlan and Desbois, 1995) so that more heat was required to melt the amorphous phase.

When compare between the flour suspension systems it was found that coconut milk increased the thermal parameters of flour suspensions. This may be also due to the decreasing amount of water to interact with rice flour in the system as coconut milk contained only 77.5% water (from nutrition label). In addition, lipid could also form complex with amylose and higher energy is required to disrupt the complex (Biliaderis and Tonogai, 1991).

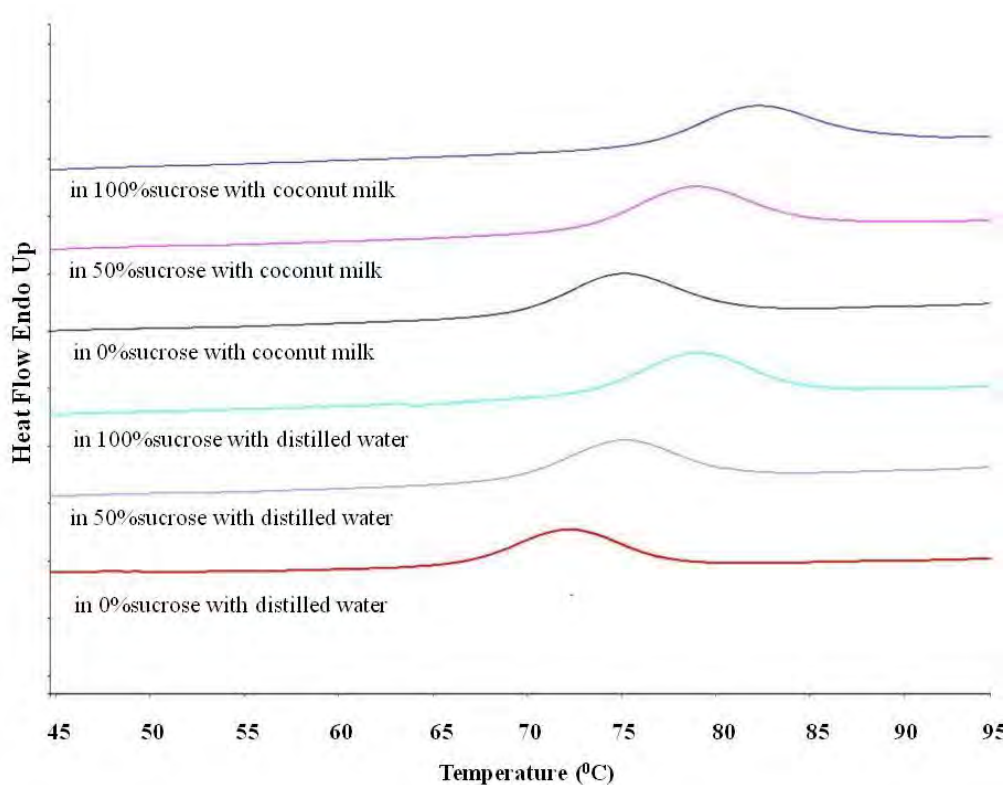


Fig. 4.3 DSC thermogram of 20 % (w/w) rice flour suspension with coconut milk and sucrose

Table 4.12 Effects of sucrose and coconut milk on thermal properties of 20 % (w/w) rice flour suspension

| Flour suspension with | ΔH (J/g) | T_{onset} ($^{\circ}\text{C}$) | T_{peak} ($^{\circ}\text{C}$) | T_{end} ($^{\circ}\text{C}$) |
|-----------------------|---------------------------|---|--|---|
| <u>Water system</u> | | | | |
| 0% sucrose | 10.62 ^b ±0.70 | 66.83 ^c ±0.18 | 72.49 ^d ±0.54 | 78.03 ^d ±0.83 |
| 50% sucrose | 11.74 ^{ab} ±0.57 | 69.56 ^c ±0.26 | 75.15 ^c ±0.11 | 80.74 ^c ±0.18 |
| 100% sucrose | 11.02 ^{ab} ±0.98 | 73.02 ^b ±0.23 | 78.81 ^b ±0.14 | 84.41 ^b ±0.13 |
| <u>Coconut system</u> | | | | |
| 0% sucrose | 11.64 ^{ab} ±0.07 | 71.07 ^b ±2.02 | 75.10 ^c ±0.06 | 80.91 ^c ±0.04 |
| 50% sucrose | 13.33 ^a ±0.58 | 72.53 ^b ±0.81 | 78.71 ^b ±0.18 | 84.47 ^b ±0.56 |
| 100% sucrose | 13.77 ^a ±1.47 | 77.01 ^a ±0.69 | 82.49 ^a ±0.59 | 88.64 ^a ±0.51 |

Means with different letters in each column were significantly different ($p \leq 0.05$). % sucrose based on flour weight.

4.2.2 Effects of sucrose and coconut milk on pasting properties of rice flour

Table 4.13 shows that addition of sucrose to rice flour in both systems of water and coconut milk would increase the peak viscosity, trough, final viscosity, setback, peak time, and pasting temperature while the breakdown decreased. The similar results were observed by various researchers (Ahmad and Williams, 1999; Pongsawatmanit *et al*, 2002; Gunaratne *et al.*, 2007; Chantaro and Pongsawatmanit, 2010).

Addition of coconut milk significantly increased the peak viscosity, trough, breakdown, final viscosity, setback and pasting temperature and decreased the peak time compared to the flour in water system (0% sucrose). These results were similar to the report of Kaur and Singh (2000). However, the results of

peak viscosity, setback and pasting time did not agree with the reports of Zhou *et al.* (2007) and Tang and Copeland (2007). The difference in pasting parameters may be due to two different processes occurring in RVA: (i) lipid in coconut milk, (an oil-in-water emulsion) may interfere with the aggregation of amylose and amylopectin chains (ii) there was amylose-lipid complex formation (Kaur and Singh, 2000) between amylose with fatty acids in coconut milk (Marina, Che Man and Amin, 2009). The micrograph of rice flour in coconut milk paste (0% sucrose) obtained from RVA by using a standard microscope (Olympus Model CH2, Japan) showed that there were lipid granules from coconut milk dispersed in rice flour paste (Fig. 4.4).

Table 4.13 Effect of sucrose and coconut milk on the pasting properties of rice flour

| % Sucrose in system | PV (cP) | trough (cP) | BD (cP) | FV (cP) | SB (cP) | Peak time (min) | Pasting temperature(°C) |
|----------------------------|-------------------------------|------------------------------|-------------------------------|-------------------------------|-------------------------------|--------------------------|--------------------------------|
| <u>Water system</u> | | | | | | | |
| 0% sucrose | 3614.50 ± 26.95 ^d | 1838.67 ± 10.59 ^c | 1776.00 ± 48.74 ^c | 2582.33 ± 10.66 ^f | 744.67 ± 10.26 ^c | 5.96 ± 0.05 ^b | 74.81 ± 0.06 ^c |
| 50% sucrose | 3884.33 ± 50.33 ^d | 2319.33 ± 18.90 ^d | 1565.00 ± 21.07 ^c | 3678.67 ± 10.07 ^d | 1359.33 ± 9.24 ^c | 6.35 ± 0.04 ^b | 76.52 ± 0.49 ^c |
| 100% sucrose | 4080.00 ± 84.86 ^d | 3613.33 ± 65.59 ^b | 466.67 ± 27.47 ^f | 5061.67 ± 92.50 ^b | 1448.33 ± 28.54 ^d | 7.00 ± 0.10 ^a | 79.15 ± 0.10 ^b |
| <u>Coconut system</u> | | | | | | | |
| 0% sucrose | 8378.67 ± 44.75 ^b | 1960.33 ± 17.04 ^c | 6418.33 ± 39.80 ^a | 3756.33 ± 73.91 ^d | 1796.00 ± 88.07 ^c | 4.51 ± 0.03 ^f | 76.27 ± 0.46 ^c |
| 50% sucrose | 8539.00 ± 146.18 ^a | 2580.67 ± 55.43 ^c | 5958.33 ± 199.16 ^b | 4625.67 ± 196.19 ^c | 2045.00 ± 143.10 ^b | 4.81 ± 0.17 ^c | 80.27 ± 0.88 ^b |
| 100% sucrose | 7896.33 ± 146.74 ^c | 3903.33 ± 26.58 ^a | 3993.00 ± 124.74 ^c | 6311.67 ± 199.25 ^a | 2408.33 ± 176.80 ^a | 5.38 ± 0.87 ^d | 84.53 ± 0.42 ^a |

Means with different letters in each column were significantly different ($p \leq 0.05$)

PV means peak viscosity

BD means breakdown viscosity

FV means final viscosity

SB means setback viscosity from trough to final viscosity

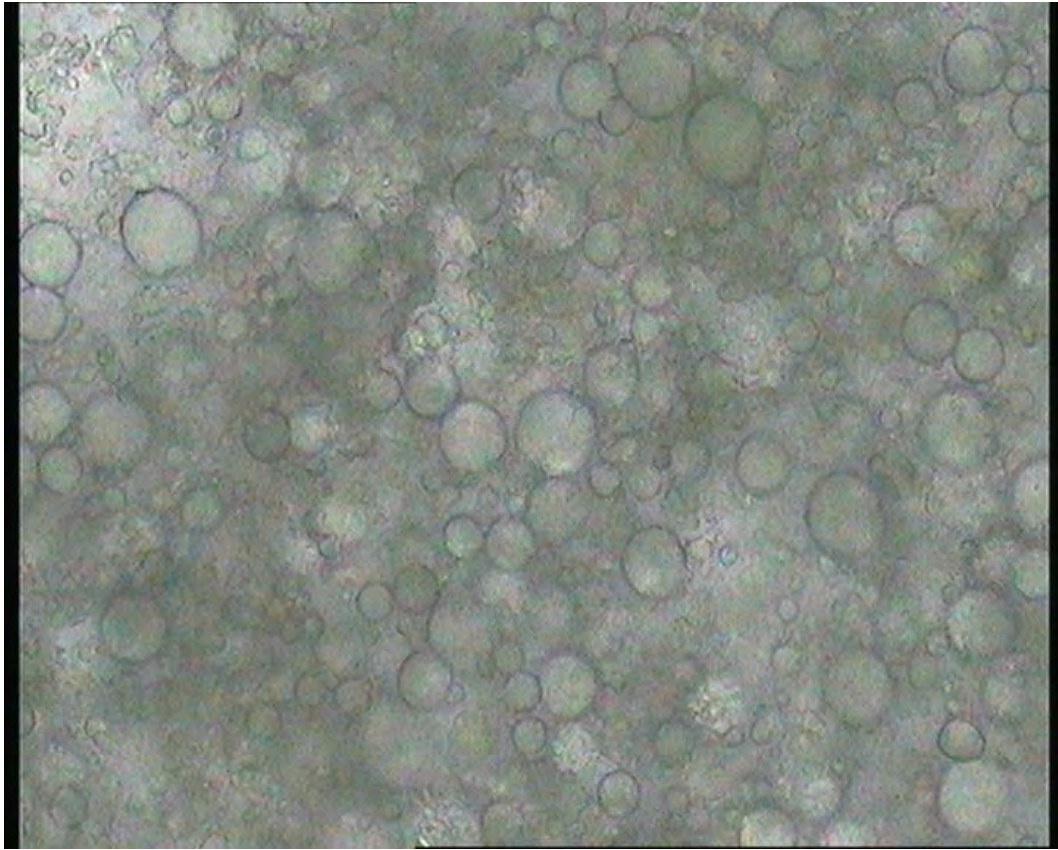


Fig. 4.4 Micrograph (1000 magnifications) of rice flour from RVA presented in coconut milk.

4.2.3 Retrogradation kinetics

Table 4.14 showed the enthalpy of retrogradation (ΔH_{retro}) and the Avrami parameters (n and k) of retrograded rice flour pastes with and without coconut milk and sucrose kept at 4°C for 1, 3, 7, 15 and 30 days. The Avrami exponents (n) and rate constant (k) of recrystallization of rice flour were 0.833 and 0.355 day^{-1} , which was consistent with the works of Fan and Marks (1998) and Yao *et al.* (2002). As n indicated the crystallite growth (Harnish and Muschik, 1983), the result showed that n value ranged from 0.748 to 0.846 and addition of sucrose did not affect the n value after keeping samples at 4°C for 30 days. The n values of the samples in this study came from heating and stirring were less than 1 (which mean that the crystallite growth mode was linear). It may be because the chain length of amylose and amylopectin was shorten during stirring and heating in RVA. Coconut milk was found to affect the Avrami parameters of rice flour retrogradation as the values of n and k decreased in pastes in coconut milk system. These results were consistent with the report of Eliason and Ljunger (1988). The k of rice flour with coconut milk was 0.308 day^{-1} , which was less than that in distilled water (0.355 day^{-1}). The k value of rice flour increased with increasing sucrose which agreed with the reports of Farhat *et al.* (2000) and Jang *et al.* (2001). Thus, the effect of adding coconut milk and sucrose on the retrogradation kinetic of rice flour gelatinization from heating and stirring process indicated that coconut milk retarded the retrogradation properties of rice flour, while sucrose increased it.

Table 4.14 Effects of sucrose and coconut milk on gelatinization enthalpy and Avrami parameters of retrograded rice flour

| % Sucrose in system | ΔH_{retro} (J/g) | | | | | Avrami parameter | | |
|-------------------------------------|---------------------------------|------------|------------|-------------|------------|------------------|----------------------------------|-----------------------|
| | 1 day | 3 days | 7 days | 15 days | 30 days | <i>n</i> | <i>k</i> (day ⁻¹) | <i>r</i> ² |
| <u>Water system</u> 0% sucrose | 0.188±.006 | 0.359±.029 | 0.558±.020 | 0.600±.005 | 0.629±.048 | 0.833 | 0.355 | 0.983 |
| 50% sucrose | 0.212±.006 | 0.382±.023 | 0.594±.019 | 0.606±.055 | 0.633±.029 | 0.817 | 0.419 | 0.950 |
| 100% sucrose | 0.229±.012 | 0.414±.035 | 0.603±.029 | 0.613±.003 | 0.628±.006 | 0.846 | 0.429 | 0.932 |
| <u>Coconut system</u> 0% sucrose | 0.179±.028 | 0.275±.006 | 0.535±.010 | 0.572±.026 | 0.636±.012 | 0.778 | 0.308 | 0.943 |
| 50% sucrose | 0.202±.007 | 0.291±.015 | 0.539±.036 | 0.589±.029 | 0.642±.035 | 0.748 | 0.344 | 0.948 |
| 100% sucrose | 0.205±.018 | 0.289±.025 | 0.565±.046 | 0.595 ±.023 | 0.645±.039 | 0.768 | 0.348 | 0.923 |

4.2.4 Effects of sucrose and coconut milk on hardness and structure of rice flour

The hardness of rice flour pastes was measured by using Instron texture analyzer to determine the effect of coconut milk and sucrose on flour retrogradation during aging at 4 °C for 7 days. When the gelatinized flour was cooled, the amylose retrograded immediately, the amylopectin remained in an amorphous state. During storage, hardness increased. These changes had been correlated with the amylopectin retrogradation (Perez *et al.*, 1993; Narkrugsakul and Saeleaw, 2009). The hardness of rice flour at 25 °C for 3 h was the initial hardness. The change of hardness of rice flour paste was shown in Fig. 4.5 and Table 4.15. It was found that the hardness of rice flour paste increased with adding coconut milk or sucrose in the system and aging increased hardness of all rice flour samples. The change of hardness (Δ Hardness) of rice flour gel in coconut milk system (77.86%) was less than that in water system (153.45%) and adding sucrose increased Δ Hardness of rice flour gel both in coconut milk and water system.

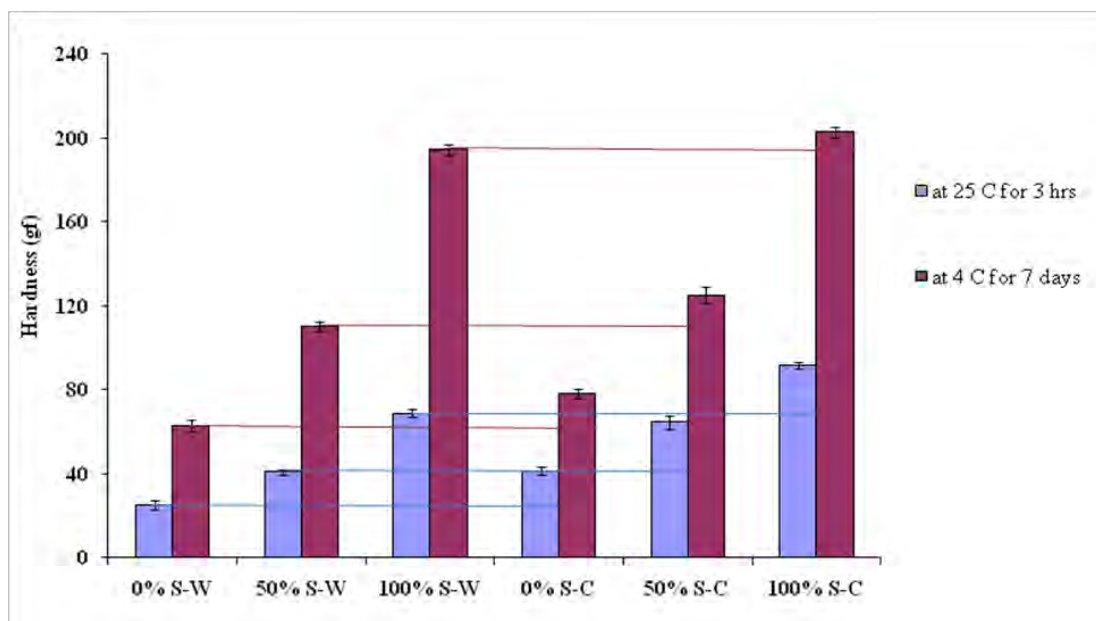


Fig. 4.5 Effects of sucrose and coconut milk on the hardness of rice flour from RVA during storage at 4⁰C for 7 days compared with the initial rice flour (at 25⁰C for 3 h)

Table 4.15 The effects of addition of sucrose and coconut milk on hardness of rice flour from RVA

| % Sucrose in system | Hardness (gf) | | Δ Hardness (%) |
|-----------------------|-------------------------|--------------------------|----------------|
| | 3 h, 25 ⁰ C | 7 days, 4 ⁰ C | |
| <u>Water system</u> | | | |
| 0% sucrose | 24.88±1.29 ^e | 63.05±1.79 ^f | 153.45 |
| 50% sucrose | 41.04±2.42 ^d | 110.13±1.26 ^d | 168.38 |
| 100% sucrose | 68.04±1.07 ^b | 194.57±2.65 ^b | 182.28 |
| <u>Coconut system</u> | | | |
| 0% sucrose | 41.33±1.75 ^d | 78.18±2.17 ^e | 77.86 |
| 50% sucrose | 64.71±1.85 ^c | 125.15±1.70 ^c | 90.47 |
| 100% sucrose | 91.83±1.61 ^a | 201.78±1.76 ^a | 120.83 |

Means with different letters in each column were significantly different ($p \leq 0.05$)

The CI showed the loss of iodine-binding capacity of starch paste which was consistent with the formation of starch-lipid complexes. Amylose helices occupied by lipid had lower capacity to bind iodine and would give a lower absorbance than flour alone when mixed with iodine (Tang and Copeland, 2007). The result showed that amylose formed complex with lipid in coconut milk around 45% and sucrose had no effect on complex formation (Table 4.16). SEM microgram of rice flour (Fig. 4.6) showed the effects of sucrose and coconut milk on SEM morphology of rice flour gel from RVA kept at 4 °C for 15 days. It can be seen that the surface of rice flour crystal in coconut milk system differed from that in water system. It may be because oil droplets from coconut milk were stabilized by the thin films of flour at the oil-water interface (Fanta *et al.*, 1999).

Table 4.16 Complexing index (CI) of rice flour from RVA

| % Sucrose in system | OD at 690 nm | CI (%) |
|----------------------------|---------------------|---------------|
| <u>Water system</u> | | |
| 0% sucrose | 0.165±0.001 | Reference |
| 50% sucrose | 0.165±0.003 | 0 |
| 100% sucrose | 0.165±0.001 | 0 |
| <u>Coconut system</u> | | |
| 0% sucrose | 0.091±0.001 | 45.05 |
| 50% sucrose | 0.092±0.001 | 44.44 |
| 100% sucrose | 0.092±0.001 | 44.44 |

The crystallites of rice flour in water and coconut milk systems after being kept at 4 °C for 15 days (Fig. 4.7) showed that sucrose and coconut milk affected the crystal of rice flour retrogradation. It was found that addition of sucrose changed the XRD patterns of freeze-dried rice flour gel at 16.8 °(2θ), while addition of coconut milk showed weak peak at 8.8 , 12.6 and 19.9 °(2θ), which match the V-pattern observed for amylose-lipid complex (Godet, Bizot, and Buleon, 1995; Tang and Copeland, 2007). This result also found that there were weak peak of 4 Å at around 22 °(2θ) in water system and at around 23 °(2θ) in coconut system and addition of sucrose increase size of crystal at 16.8 °(2θ) in coconut milk system.

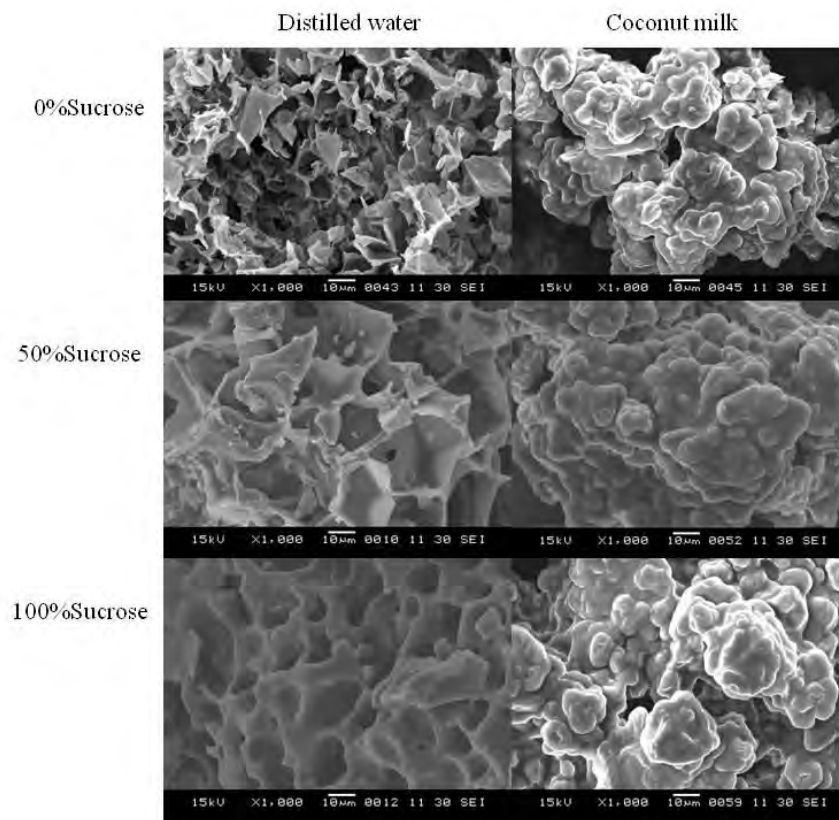


Fig. 4.6 SEM microgram of rice flour from RVA presented in distilled water

and in coconut milk with different sucrose content

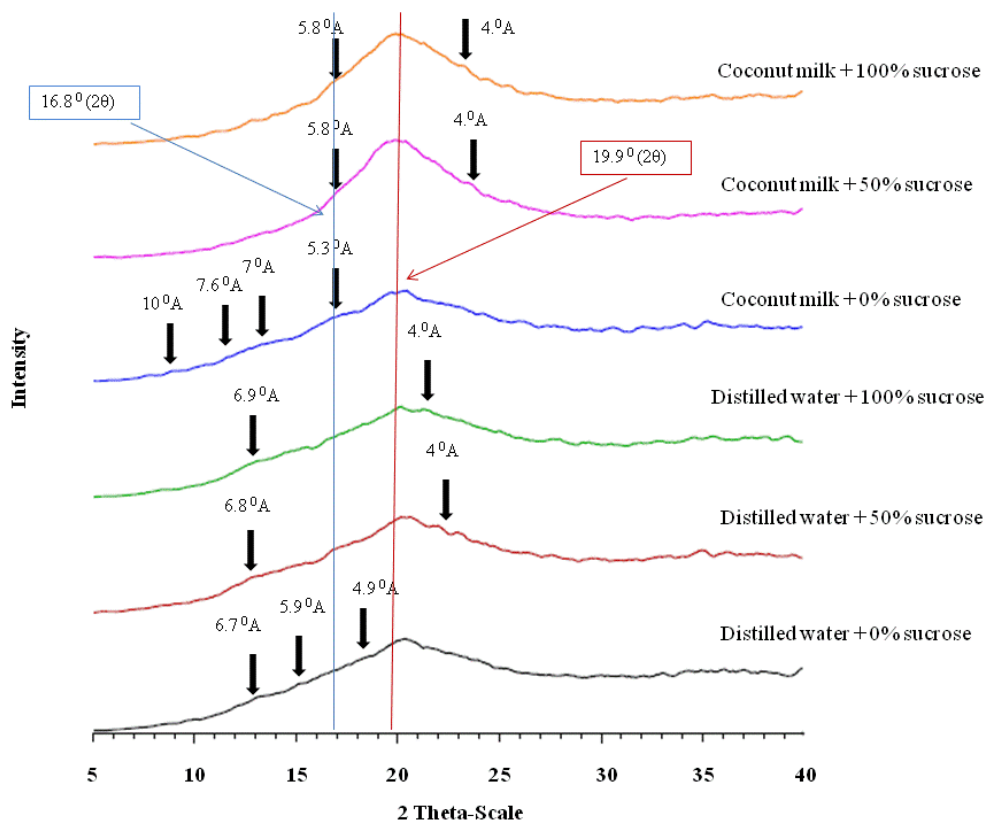


Fig. 4.7 XRD patterns of rice flour presented in distilled water and in coconut milk with adding sucrose [0, 50, 100% (w/w)], after kept at $4^\circ C$ for 15 days

4.3 Part 3. Effect of sucrose on the thermal and rheological properties of Thai rice flour treated with high hydrostatic pressure

4.3.1 Effect of High Hydrostatic Pressure (HHP) condition on the gelatinization of rice flour

Fig.4.8 showed the ΔH_{gel} of 20 % (w/w) rice flour suspension treated under HHP at various pressures and holding time. It was found that ΔH_{gel} of HHP-treated rice flour suspensions decreased with increasing holding time and treatment pressure. This result was consistent with those of rice starch (Oh *et al.*, 2008), Basmati rice flour and starch (Ahmed *et al.*, 2007), wheat and tapioca starch (Bauer and Knorr, 2005), and potato starch (Bauer and Knorr, 2005; Kawai *et al.*, 2007). At pressure 200 MPa, the result showed that as holding time increased the ΔH_{gel} of rice flour suspensions decreased slightly, but above 200 MPa, ΔH_{gel} of samples decreased significantly. The condition for complete gelatinization of rice flour suspension (ΔH_{gel} value ~ 0 J/g) was found to be 600 MPa at 40 °C for 30 min which different from the condition for japonica rice starch (500 MPa at 40°C for 15 min) (Tan *et al.*, 2009), Basmati rice starch (550 MPa at 28.5 °C for 15 min) and Basmati rice flour (650 MPa at 28.5 °C for 15 min) (Ahmed *et al.*, 2007). The difference may be because protein in rice flour retarded starch gelatinization during HHP treatment (Ahmed *et al.*, 2007).

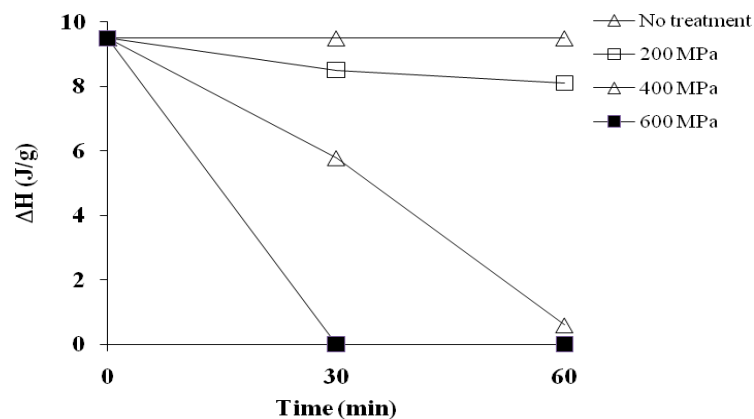


Fig. 4.8 Effects of pressure and holding time on enthalpy of gelatinization of 20% (w/w) rice flour suspension treated with HHP at 40⁰C.

4.3.2 Effect of sucrose on the gelatinization of rice flour treated with HHP

Fig. 4.9 and Fig. 4.10 show that the T_{onset} and ΔH_{gel} of 20 % (w/w) rice flour suspensions increased with increasing sucrose. The rice flour suspensions with 10 % (w/v) sucrose and without sucrose showed complete gelatinization ($\Delta H_{\text{gel}} = 0$ J/g and no T_{onset}), while sample with 20 %(w/v) sucrose showed partial gelatinization ($\Delta H_{\text{gel}} = 1.284$ J/g and $T_{\text{onset}} = 75.4$ ⁰C) after HHP treatment at 600 MPa at 40 ⁰C for 60 min. This may be because sucrose stabilized the amorphous regions of the starch (Spies and Hosney, 1982) and also immobilized water (Koyama and Nishinari, 1991), so that the loss of helix structure in amorphous lamellae due to the water during HHP process has been reduced.

Fig. 4.11 shows that the degree of rice flour gelatinization increased with holding time and pressure. This result was consistent with those of wheat starch, potato starch and tapioca starch (Bauer and Knorr, 2005).

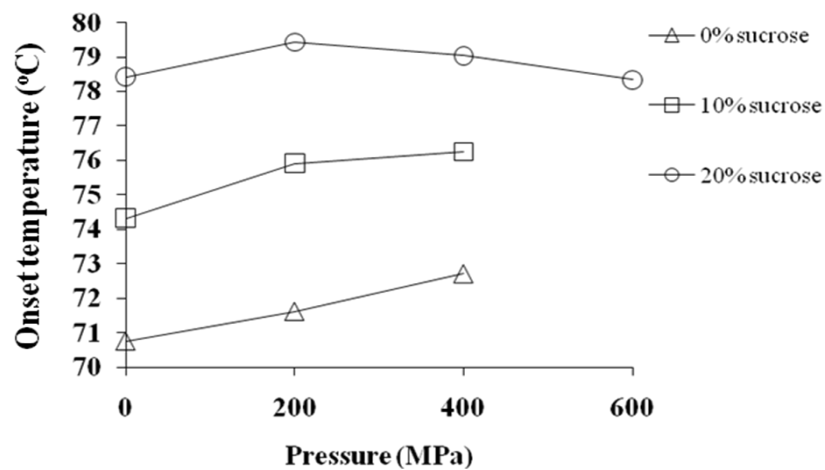


Fig. 4.9 Effect of sucrose on the onset temperature of 20 % (w/w) rice flour suspension treated with vary pressure at 0.1, 200, 400 and 600 MPa at 40 °C for 60 min.

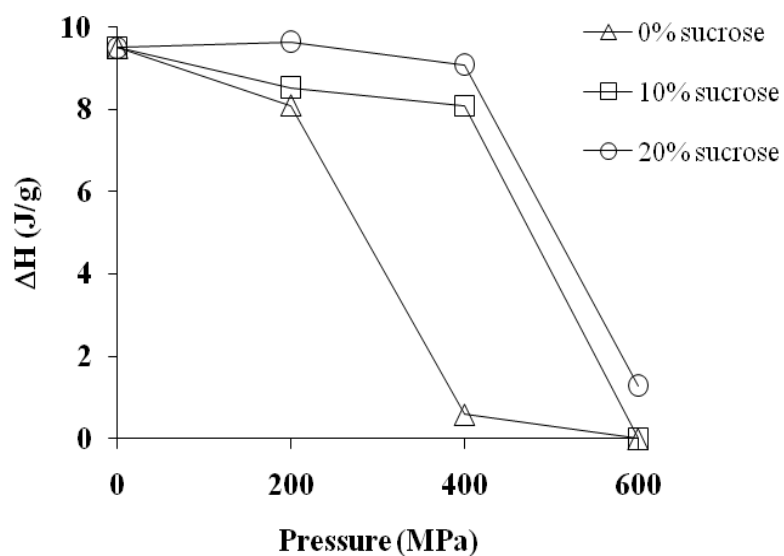


Fig. 4.10 Effect of sucrose on gelatinization enthalpy (ΔH) of 20 % (w/w) rice flour suspension treated with various pressure (0.1, 200, 400 and 600 MPa) at 40 °C for 60 min.

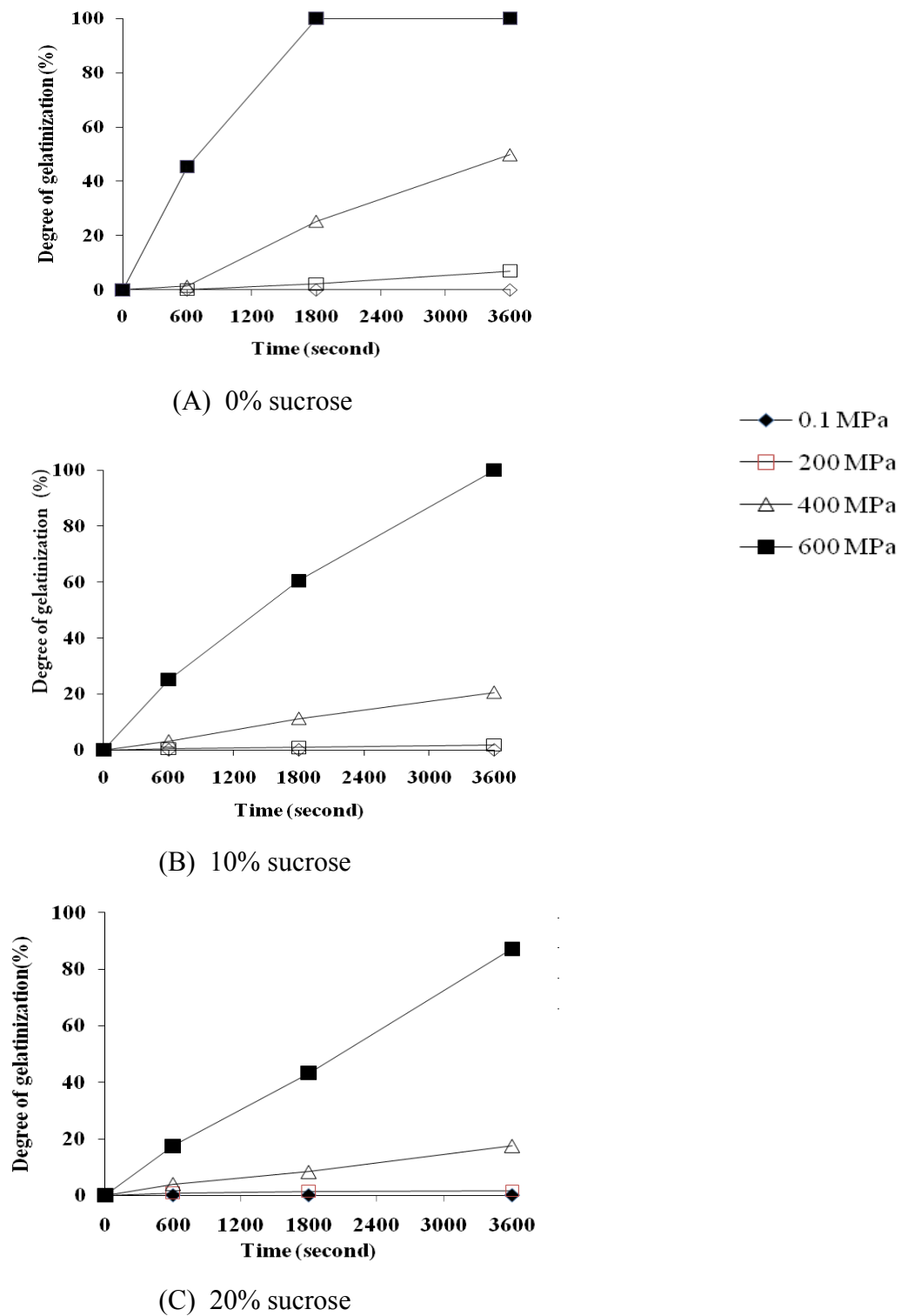


Fig. 4.11 Effects of holding time and pressure on the degree of gelatinization of 20 % (w/w) rice flour suspension with various sucrose levels at 40°C

4.3.3 A kinetic model for pressure induced gelatinization of rice flour at different sucrose concentration

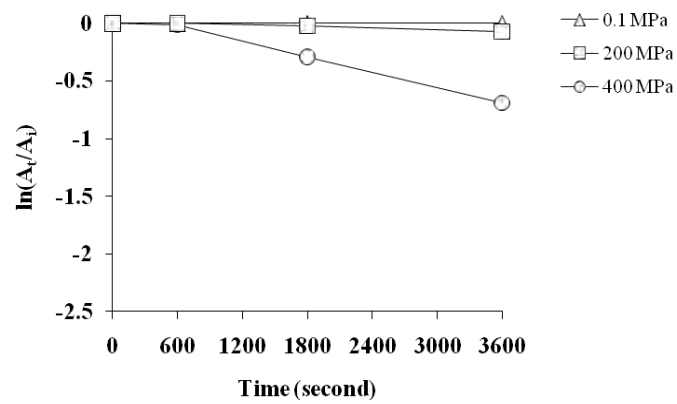
Fig. 4.12 shows that the extent of rice flour gelatinization can be described by a first-order kinetic model. The rate constant (k) is determined from slope of the plot of $\ln (A_t/ A_i)$ versus the treatment time (t) covered the pressure range of 200 - 600 MPa (Table 4.17). It was found that rice flour treated pressure 0.1 MPa, 200 MPa and 400 MPa at 40⁰C for 10 min had no gelatinization. The k value increased with increasing treatment pressure and decreased with increasing sucrose concentration. This indicated that sucrose had effect on the pressure-induced rice flour gelatinization, when the holding temperature was held constant at 40 ⁰C. The rice flour suspension was appeared to resist gelatinization by treatment pressure, when sucrose presented in the system and the effect was stronger at higher sucrose concentration. At 20% (w/v) sucrose concentration, the gelatinization was incomplete at 600 MPa for 60 min.

Table 4.17 First-order rate constants (k) of 20 % (w/w) rice flour suspension treated with HHP at various sucrose concentrations and treatment pressures at 40°C for 3600 second.

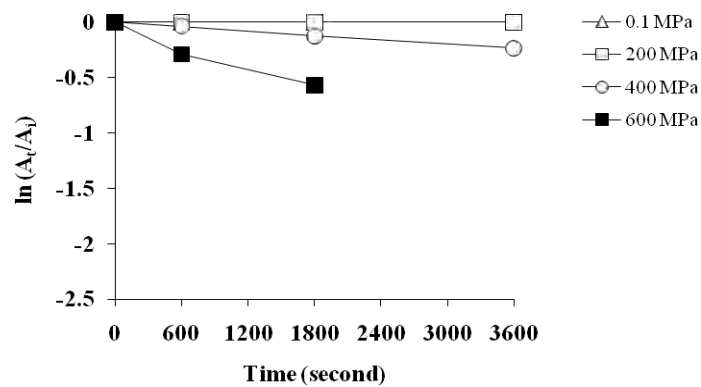
| Pressure (MPa) | k (s ⁻¹) at various sucrose concentration (%) | | |
|----------------|---|--|--|
| | 0% | 10% | 20% |
| 0.1 | n/a | n/a | n/a |
| 200 | 2x10 ⁻⁵ (R ² = 0.958) | n/a | n/a |
| 400 | 2x10 ⁻⁴ (R ² = 0.964) | 7x10 ⁻⁵ (R ² = 0.998) | 5x10 ⁻⁵ (R ² = 0.992) |
| 600 | CG | CG | 5x10 ⁻⁴ (R ² = 0.930) |

n/a = no gelatinization was observed under the experimental conditions.

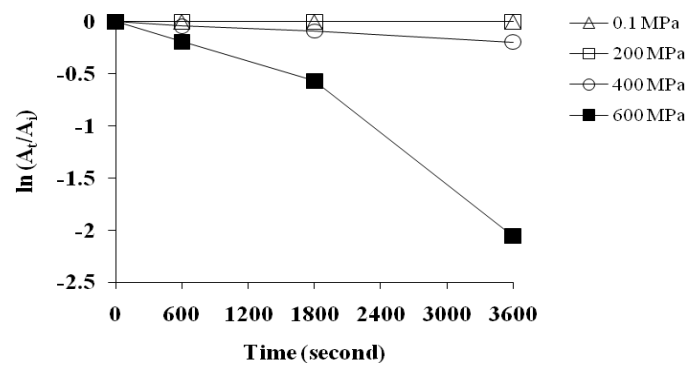
CG = complete gelatinization



(A) 0% sucrose



(B) 10% sucrose



(C) 20% sucrose

Fig. 4.12 Effects of holding time and pressure on the ratio of percentage of ungelatinized flour of 20 % (w/w) rice flour suspension treated with HHP with various sucrose levels at various holding time (A_t) to that present initially (A_i), at 40⁰C

4.3.4 Effect of sucrose on the rheological properties of rice flour gel treated with HHP

Rheological properties of 20 % (w/w) rice flour suspension treated with HHP (600 MPa, 40⁰C for 60 min) with and without sucrose showed various rheological characteristics (Fig. 4.13). It was found that the viscosities of the rice flour suspensions without sucrose and with 10 % (w/v) sucrose and treated with HHP at 600 MPa for 60 min were shear thinning while that for 20 % sucrose was very low.

Effect of sucrose concentration on dynamic viscoelastic of 20 % (w/w) rice flour suspension treated with HHP with and without sucrose was shown in Fig. 4.14. It was found that sucrose decreased the G' and G'' of rice flour suspensions treated with HHP. Addition of sucrose up to 10% (w/v) decreased these values slightly while significant lower values were observed at 20% (w/v) sucrose.

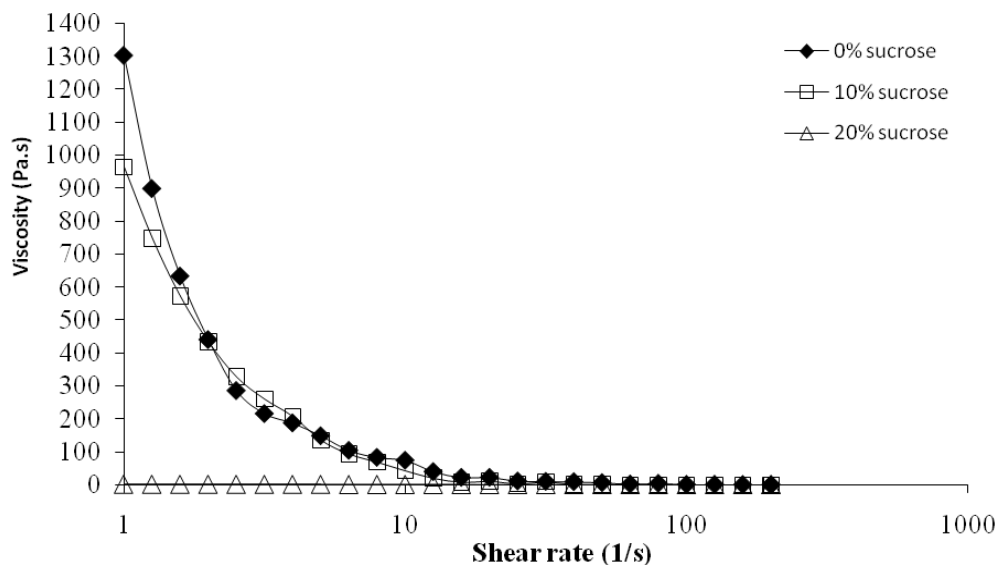


Fig. 4.13 Effect of sucrose on viscosity of 20 % (w/w) rice flour suspension treated with HHP at 600 MPa and 40⁰C for 60 min

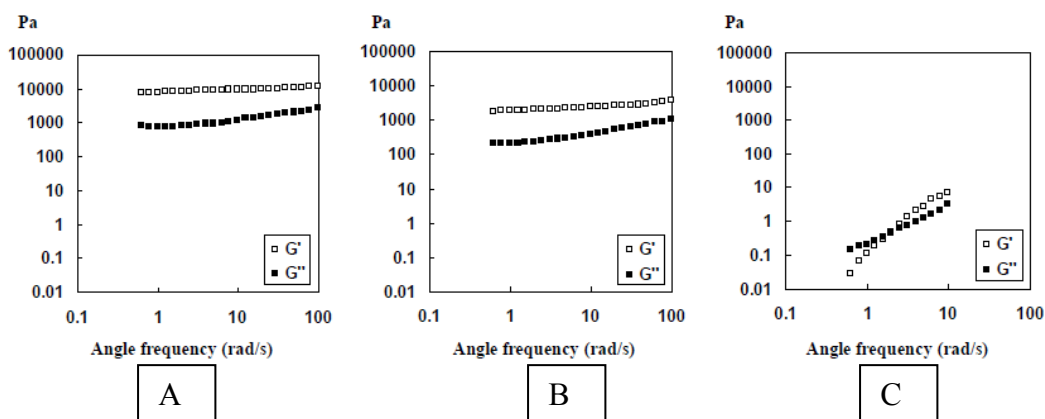


Fig. 4.14 Effect of sucrose concentration on gel strength of 20 % (w/w) rice flour suspension with and without sucrose: A, 0% sucrose; B, 10% sucrose; C, 20% (w/v) sucrose treated with HHP at 600 MPa, 40⁰C for 60 min.

4.3.5 Morphology of rice flour treated with HHP

The granule of rice flour from 20 % (w/w) rice flour suspension with 20% (w/v) sucrose treated with 600 MPa and 40 °C for 60 min. was observed by using polarized light microscope (Fig. 4.15). It was found that the granular shape of rice starch was conserved after HHP gelatinization and the polarized microscopic images showed no birefringence indicating a complete gelatinization of starch.

Fig. 4.16 shows that rice flour suspension in the presence of 20 %(w/v) sucrose treated at 100 °C for 60 min was gelatinized completely as determined by DSC ($\Delta H = 0$ J/g). The heat-treated sample showed no birefringence which indicated complete loss of crystallinity while the granules in the heat-treated sample were swollen and burst in the light microscopic images. The sample treated with HHP was not gelatinized completely ($\Delta H \sim 1$ J/g). The starch granule of sample treated with

HHP indicated retained crystallinity. After storage at 25 °C for 1 day, the starch granules in the HHP-treated sample were less swollen and disintegrated than the heat-treated ones so that these phenomena may not have amylose leaching to form network.

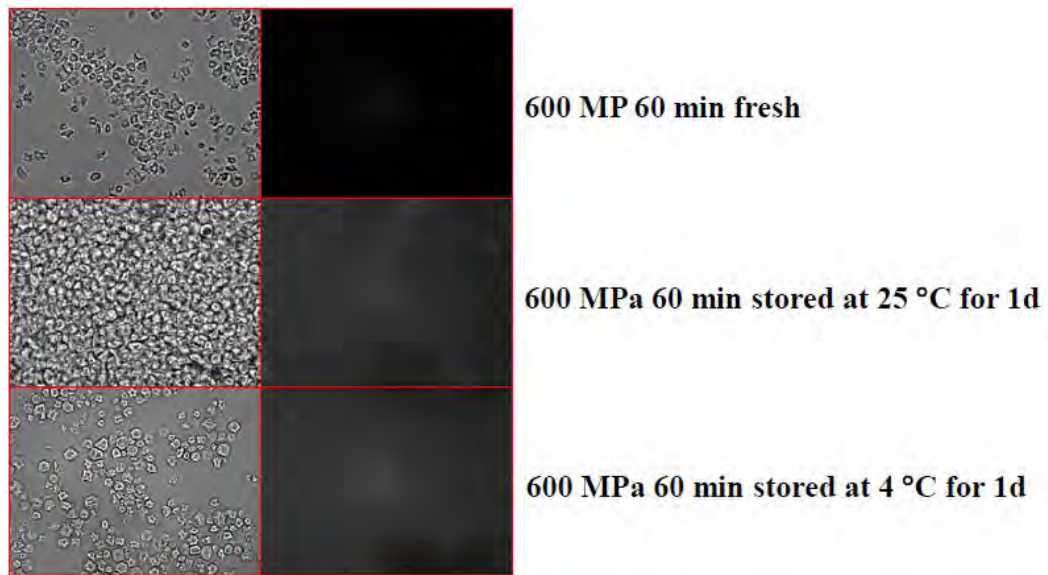


Fig. 4.15 Polarized light micrographs of stored at 4⁰C and 25⁰C for 1 day HHP treated -gelatinized 20 % (w/w) rice flour suspension

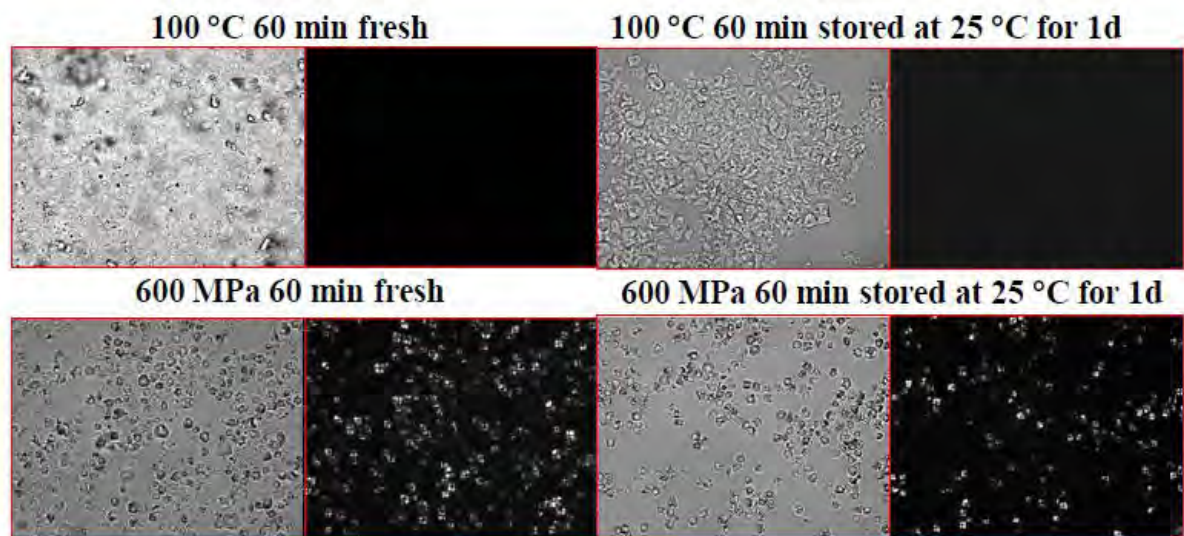


Fig. 4.16 Light micrographs without and with polarized filter of heat treated - gelatinized and HHP treated -gelatinized 20 %(w/w) rice flour suspension with 20 % (w/v) sucrose

CHAPTER V

CONCLUSIONS

From the results, it can be concluded that:

1. Variety of rice affected physicochemical properties, i.e. protein content, amylose content, % damaged starch and % crystallinity of flour; functional properties, i.e. water absorption index (WAI) and swelling power (SP); thermal properties, i.e. T_{onset} , T_{peak} , T_{end} , ΔH_{gel} and % retrogradation; pasting properties, i.e. final viscosity, setback, and pasting temperature; and textural properties, i.e. hardness, cohesiveness, adhesiveness, and springiness.

Six non-waxy rice flour used in this study could be divided into 2 groups by their amylose content, thermal and pasting properties as:

- Rice flour having intermediate amylose content (18 - 28%), i.e. P1, HS, S2 and S60, had T_{onset} around 70 °C, low final viscosity (< 3,300 cP), setback (< 2,000 cP), and % retrogradation (< 40%).
- Rice flour having high amylose content (28 - 33%), i.e. S1 and S 3, had T_{onset} around 77 °C, high final viscosity (> 4,000 cP), and setback (> 2,500 cP), and % retrogradation (> 40 %).

The physicochemical, functional, pasting, and gel texture properties of these flours were found to be as follows:

- Proximate composition was found to be moisture content ~ 6 %, protein content 4.64 - 6.9%, fat content ~ 0.3%, and ash ~ 0.3%.

- The crystalline structure of rice flour was A-type starch and % crystallinity of rice flour was 23.15 – 31.31 % depending on its amylose content. Flour having low amylose had lower % crystallinity.

- Amylose leaching of rice flour at 80 °C was 0.053 – 1.222 mg/100 ml with higher in flour having high amylose.

- Water absorption index (WAI) and water solubility index (WSI) at 30 °C of rice flour sample were found to be 6.477 - 8.008 and 0.727- 1.068 %. They were positively correlated with amylose content, %crystallinity and amylose leaching of rice flour.

- Swelling power at 80 °C (SP) was found to be 6.535 – 8.095 and increased with increasing amylose content.

- Gelatinization temperatures, pasting and gel textural properties of rice flour depended on the amylose content in rice flour. Flour having high amylose tended to have higher gelatinization temperatures, viscosity, setback and % retrogradation. The % retrogradation of rice flour was positively correlated with amylose content.

2. Adding sucrose into flour suspensions would increase thermal and pasting properties except breakdown in both water and coconut milk systems. Replacing water with coconut milk drastically increased peak viscosity, breakdown, and setback while trough and final viscosity increased slightly. The Avrami parameters indicated

that coconut milk retarded the rate of retrogradation of flour, while sucrose accelerated the rate of retrogradation of flour both in coconut milk and distilled water.

3. The 20 %(w/w) rice flour suspension treated with HHP was found to completely gelatinized at 600 MPa and 40 °C for 60 min. and the rice flour granule was not destroyed by pressure. The addition of sucrose in rice flour suspension retarded swelling of flour during HHP process and reducing rate of gelatinization. Adding sucrose at 20 % (w/v), 20 %(w/w) rice flour suspension treated with HHP was not gelatinized completely ($\Delta H \sim 1 \text{ J/g}$).

Suggestions on the implementation of research results are:

1. For adding sucrose, sucrose inhibited swelling of rice flour so that sucrose should be added in the last step before cooking.
2. For adding coconut milk, it should gradually add enough coconut milk into rice flour to form soft dough and then dissolved the dough in the remaining coconut milk before cooking in order to prevent effect of amylose-lipid complex formation. Care must be taken not to add too much coconut milk in the first step.

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APPENDIXES

APPENDIX A

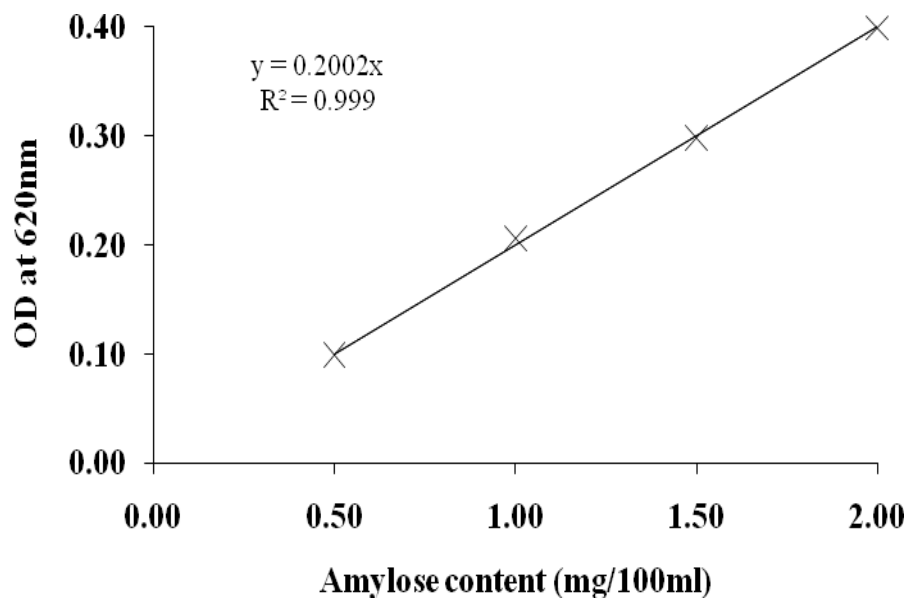
Amylose standard curve for amylose leaching determination at 620 nm

Iodine solution 2% (w/v) preparation

Accurately weigh 0.2 g iodine and 2.0 g potassium iodine were suspended in deionized water to obtain total volume of 100 ml into 100 ml volumetric flask.

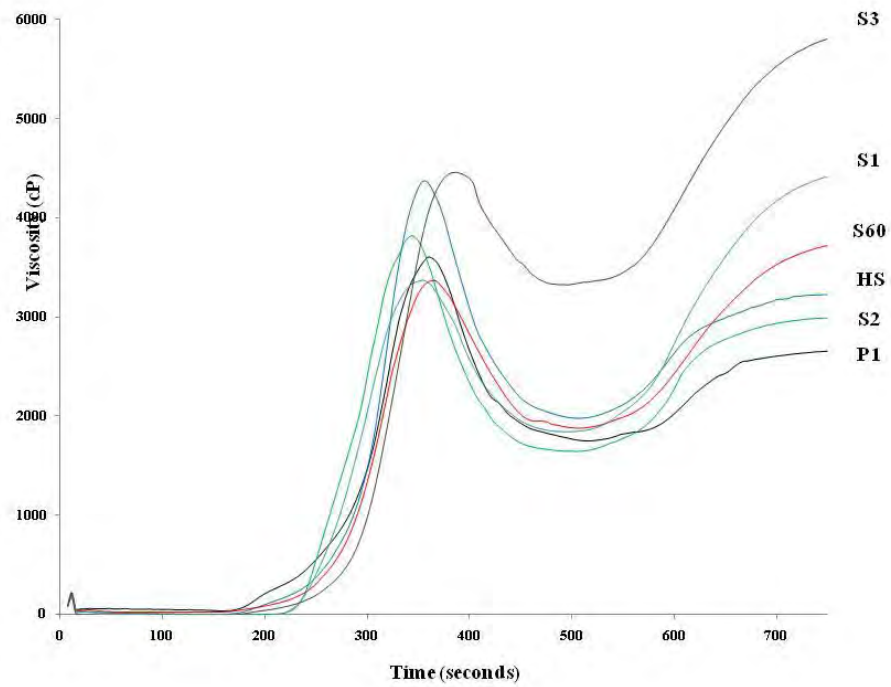
Standard amylose solution preparation

Accurately weigh 50.00 mg potato amylose standard from Fluka (Switzerland), 0.5 ml of 95 % ethanol and 4.5 ml of 1N NaOH were added into 50 ml volumetric flask. The solution was heated and shaken in shaker water bath at 95 °C for 10 min and then cools in ice bath. After that the solution was adjusted in 50 ml by using deionized water. Pipette solution into a 100 ml volumetric flask at 0.5, 1.0, 1.5 and 2.0, respectively and then added 1 ml of 1 M acetic and 2 ml of 2% (w/v) iodine in each flask. After that every volumetric flasks was adjusted in 100 ml by using deionized water and kept in dark room for 20 min. The solutions were measured OD with spectrophotometer (Spectronic 20 Genesys Spectrometer) at 620 nm.



APPENDIX B

RVA profile of rice flour from 6 varieties



APPENDIX C

Nutrition facts from labeled in the UHT coconut milk packing, Ampol Food
Processing LTD., Nakornpathom, Thailand.

| NUTRITION FACTS | |
|------------------------------------|---------------|
| Serving Size 1/3 cup (80g) | |
| Servings Per Container about 6 | |
| Amount Per Serving | |
| Calories 150 Calories from Fat 120 | |
| | % Daily Value |
| Total Fat 14 g | 22 |
| Saturated Fat 10 g | 50 |
| Cholesterol 0 mg | 0 |
| Sodium 15 mg | 0 |
| Total Carbohydrate 2 g | 0 |

APPENDIX D

Statistical results of Functional properties of rice flour in Table 4.4

ANOVA

| | | Sum of Squares | df | Mean Square | F | Sig. |
|-----|----------------|----------------|----|-------------|-------|------|
| WAI | Between Groups | 4.305 | 5 | .861 | 2.427 | .097 |
| | Within Groups | 4.256 | 12 | .355 | | |
| | Total | 8.561 | 17 | | | |
| WSI | Between Groups | .247 | 5 | .049 | 2.614 | .080 |
| | Within Groups | .227 | 12 | .019 | | |
| | Total | .474 | 17 | | | |
| SP | Between Groups | 4.505 | 5 | .901 | 2.485 | .091 |
| | Within Groups | 4.351 | 12 | .363 | | |
| | Total | 8.856 | 17 | | | |

WAI

Duncan

| FLOUR | N | Subset for alpha = .05 | |
|-------|---|------------------------|--------|
| | | 1 | 2 |
| 4.00 | 3 | 6.4770 | |
| 3.00 | 3 | 6.6837 | |
| 1.00 | 3 | 6.8377 | |
| 2.00 | 3 | 6.9093 | 6.9093 |
| 5.00 | 3 | 7.1000 | 7.1000 |
| 6.00 | 3 | | 8.0083 |
| Sig. | | .264 | .052 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

WSI

Duncan

| FLOUR | N | Subset for alpha = .05 | |
|-------|---|------------------------|--------|
| | | 1 | 2 |
| 3.00 | 3 | .7273 | |
| 2.00 | 3 | .7485 | |
| 1.00 | 3 | .7874 | |
| 4.00 | 3 | .8972 | .8972 |
| 5.00 | 3 | .9087 | .9087 |
| 6.00 | 3 | | 1.0684 |
| Sig. | | .166 | .172 |

Means for groups in homogeneous subsets are displayed.
 a Uses Harmonic Mean Sample Size = 3.000.

SP

Duncan

| FLOUR | N | Subset for alpha = .05 | |
|-------|---|------------------------|--------|
| | | 1 | 2 |
| 4.00 | 3 | 6.5353 | |
| 3.00 | 3 | 6.7340 | |
| 1.00 | 3 | 6.8920 | |
| 2.00 | 3 | 6.9617 | |
| 5.00 | 3 | 7.1657 | 7.1657 |
| 6.00 | 3 | | 8.0950 |
| Sig. | | .264 | .083 |

Means for groups in homogeneous subsets are displayed.
 a Uses Harmonic Mean Sample Size = 3.000.

BIOGRAPHY

Mrs. Duangrutai Thumrongchote was born on December 30, 1965 in Bangkok, Thailand. She obtained a B.Sc. degree in Food and Biotechnology from Chulalongkorn University in 1987. Since 1983, she has worked for the Rajamangala University of Technology. In 1990, she studied M.Sc. in Food technology from Chulalongkorn University, and obtained M.Sc. degree in 1993. In 2005, she received a scholarship from Rajamangala University of Technology to further her PhD at department of Food Technology, Chulalongkorn University. After being finished her PhD degree, she will join the Department of Food Technology and Nutrition, Faculty of Home-Economics at Rajamangala University of Technology Krungthep.