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# Effects of Lipids, Amino Acids, and Beta-Cyclodextrin on Gelatinization, Pasting, and Retrogradation Properties of Rice Starch.

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# EFFECTS OF LIPIDS, AMINO ACIDS, AND BETA-CYCLODEXTRIN ON GELATINIZATION, PASTING, AND RETROGRADATION PROPERTIES OF RICE STARCH

A Dissertation

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## ABSTRACT

The primary purpose of this study was to determine the addition of lipids, amino acids, and β-cyclodextrin effects on commercial rice starch gelatinization, pasting, and retrogradation properties by using differential scanning calorimetry (DSC), rapid visco analysis (RVA), and x-ray diffractometry (XRD).

Analyses from commercial rice starch showed that the presence of 0.6% monopalmitin (MP) increased the peak viscosity (PV), minimum viscosity (MV), and final viscosity (FV) by 38, 27, and 72 RVU respectively. The pasting temperature was also increased by 6 °C, whereas the total setback (TSB) was increased by 45 RVU. Incorporation of lysophosphatidylcholine (LC) and lysophosphatidylethanolamine (LE) also showed strong influence on pasting properties of commercial rice starch. Charged amino acids showed a more significant influence on starch pasting properties than the neutral amino acids. Addition of 6% aspartic acid decreased the MV, FV, and TSB by 39, 63, and 25 RVU respectively, whereas the breakdown (BKD) was increased by 33 RVU. The presence of 6% glutamic acid showed a similar pattern to the aspartic acid. However, the presence of 6% arginine and lysine reduced the pasting temperature up to 15 °C. Lipid removal from white rice flour reduced the FV and TSB by 48 and 53 RVU respectively. Protein removal by alkaline protease digestion further reduced the PV, MV, and FV by 62, 19, and 78 RVU respectively. Brown flour showed a different pattern from the white flour.

The presence of amino acids and β-cyclodextrin inhibited the amylose-lipid complex formation, resulting in the reduction of the 2$^{nd}$ endothermic transition enthalpy. Beta-cyclodextrin reduced both the 1$^{st}$ and 2$^{nd}$ endothermic transition enthalpies.

**Addition of lipids increased the relative crystallinity and enhanced the V-type crystalline structure. The presence of β-cyclodextrin enhanced the V-pattern of x-ray diffraction, whereas the addition of amino acids increased the 4.4 peak.**

# CHAPTER 1
# INTRODUCTION

Rice is one of the leading food crops in the world. The world rice production reached a record of 400 million metric tons (milled basis) in 1999/2000 (Bryant et al 2001), with the United States producing 6.6 million metric tons. Although the cost of rice is greater per pound compared to corn and wheat, its application in value-added products could give the industry new avenues of use, thus increasing its demand.

Due to lack of gluten, rice flour differs from wheat flour in baking properties, and cannot retain gases generated during baking. However, there is a steady demand for rice flours for use in baby foods, breakfast cereals, baked biscuits, dusting powders, and breading mixes. Some unique functional properties of rice, such as flavor carrying capability, hypoallergenicity, and bland flavor make it more attractive to food manufacturers for value-added products. Those applications include gluten-free rice bread (Deis 1997), tortillas, beverages, processed meats, and low-fat sauces, puddings, or salad dressings (McCue 1997). Modification of rice flour can change its functional properties, which might be used as a fat substitute in other value-added products.

Starch is the major food source of carbohydrates, making up 70 to 80% of the calories consumed by humans. It is an important ingredient in the food industry. The properties of starch can be improved by modification, resulting in a wider food application.

In the US market, the annual sales volumes for food starch are 1 to 1.5 billion pounds with a 60:40 ratio of modified versus native starch (Krause 2001). Modified starches can be used to thicken, stabilize, texturize, reduce fat, or manage moisture in food ingredients. Among the wide food applications, the top uses for food starch are

soups, sauces, gravies, bakery, processed meats, dairy and coatings. There are several trends in the modified starch market for value-added products. One trend is the instant starch, which develops viscosity under lower temperatures than traditional cook-up starches. Those instant starches can be used in convenience foods for microwave applications. Another trend is the modified starch gels and clear coatings to meet consumers' healthy and flavorful foods requirement. Modified starch gels provide low-fat alternatives for processed meat applications. Clear starches are used in meat coatings, which create invisible moisture barriers, keeping fried foods hot, improving crispiness, and extending heat lamp hold times (Krause 2001). According to preliminary estimates by Menlo-Park, California-based SRI Consulting, the US food starch market of the year 2000 was growing by 2 to 3 percent, especially for the value-added modified food starches.

The functional properties of starch are generally regarded as the primary factors that influence the texture and quality of starch-based food products. The physicochemical properties of starch can be expressed in the processes of temperature dependent starch-water interaction, which are known as gelatinization, pasting, and retrogradation (Dengate 1984, Atwell et al 1988). Cereal starch granules usually contain residual levels of lipids and proteins, which significantly influence the starch physicochemical properties. In starchy food manufacturing, emulsifiers, fats, and other protein containing ingredients (such as skimmed milk and egg white) are commonly used. Knowledge of the starch, lipid, and protein interactions allows us to predict the influences of those food ingredients on the quality of final products and to apply it in value-added product development through starch modifications.

The research was divided into three phases. The main objectives of phase I of the research were (1) to determine the lipid removal and protein removal effects on pasting properties of rice flour; (2) to determine the lipids, amino acids, and β-cyclodextrin effects on pasting properties of rice starch; (3) to compare those additive effects on commercial rice starch versus rice starch isolate. Those studies were carried out using a Rapid Visco Analyzer (RVA) to record the paste viscosity changes over time during heating, holding, and cooling periods. In addition, those lipids and amino acids that substantially influenced starch pasting properties were selected for the phase II study.

In phase II of the project, the main research objectives were (1) to determine the heating rates and moisture contents influences on thermal characteristics of rice starch using a Differential Scanning Calorimetry (DSC); (2) to examine the selected lipids and amino acids effects on thermal characteristics of rice starch; (3) to test β-cyclodextrin inclusion effect on thermal characteristics of rice starch.

In phase III of this study, the main objectives were (1) to study the XRD pattern of rice starch and rice flours; (2) to determine the lipid removal and protein removal effects on rice flour XRD patterns; (3) to examine the influence of lipids, amino acids, and β-cyclodextrin on starch granule XRD pattern.

The research is presented in three chapters. Chapter 3 describes the materials, amylose content measurement, lipids extraction and starch isolation methods, and RVA testing procedures. Chapter 4 describes the sample preparation and DSC thermal profiles. In chapter 5, the XRD pattern specifications are introduced, followed by the

testing results and comparison. In chapter 6, general conclusions and recommendations for future research are presented.

# CHAPTER 2
# LITERATURE REVIEW

This section presents a review of related literature pertinent to understanding the composition and structure of starch granules, the health value of starch-based food products, the functional attributes related to starch-water interactions, as well as the influences of starch granule associated lipids and proteins. In addition, common food additives influences on the physicochemical properties of starches during the manufacturing process were presented.

## 2.1. STARCH

### 2.1.1. General Background Information

Cereal grains store energy in the form of starch. The amount of starch contained in a cereal grain is generally between 60 and 75% of the weight of the grain (Hoseney 1986). Starch provides us an excellent source of energy in our diet, and often at low cost. In addition to its nutritive values, starch is important because of its unique chemical and physical characteristics, which significantly influence the physical properties of many food products.

Granular starch has been commonly used as dusting agents for candy, carrying agents for baking powder, and starch molds for gum drop manufacture (Jane 1997). Small granular or small particle starch and microcrystalline starch are proposed for fat substitutes or fat mimetics (Jane et al 1992, Chiou et al 1991). Starch pastes have a wide application in food products for thickening, fillings, oil mimetics, and providing body texture to beer and soft drinks (Jane et al 1997). In addition, starch is used as ingredients in the food industry to provide structure to bread, cakes, and pudding, and to give

desirable textures to final products, such as crispy coatings to fried chicken and fish (Hegenbart 1996, Jane 1997).

Compared with lipid or fat, starch has a relatively low caloric value and contains no cholesterol. Thus, starch meets consumers' expectations for health benefits, which may lead to the gradual dietary pattern change of consumers from fat and protein to starch and complex carbohydrates (Jane 1997). In recent decades, dietary carbohydrates have drawn the attention of many scientists and legislators for public health concern. Evidence showed that resistant starches and certain high-amylose cereal starches could significantly reduce the level of serum glucose and insulin, and thus may reduce the risks for some diseases such as colon cancer, coronary heart disease, and glycemia (Goddard et al 1984, Juliano and Goddard 1986, Bjorck and Asp 1994, Englyst and Hudson 1997, Unlu and Faller 1998).

### 2.1.2. Chemical Composition of Starch

Starch is composed of two fractions: a linear fraction, amylose, and a branched fraction, amylopectin. In both molecules, glucose is the basic building block. Commercial starches usually contain residual levels of lipids, proteins, phosphorus, and other minerals. The physical properties of starch are mainly determined by the ratio and structure of amylose and amylopectin. However, those non-starch constituents can also impact the characteristics and functionality of starch-based products. The molecular composition and physical properties of starch granules had been largely reviewed by Zobel (1988a).

**Amylose**

Amylose is an essentially linear polymer, which is built up by α-(1→4) linked -D-glucose units (Fig. 2.1). Very few branches (accounting for 0.3% of the total linkages) are reported to be randomly present in amylose (Takeda et al. 1992; Shibanuma et al. 1994). Amylose contains 1,500 to 6,000 glucose units. The extended form of amylose is composed of 6 glucosyl residues that repeat in 21 Angstroms (Fig.2.2a). This is the typical structure of one strand in the double helix model found in retrograded amylose crystallites (Zobel 1988a). When complexing with other molecules, amylose changes to the collapsed helix form, which also has 6 residues but repeats in about 8 Angstroms (Fig. 2.2b). Only hydrogen atoms exist in the interior surface of the helix, while the hydroxyl groups are on the exterior of the helix. Therefore, the interior surface of the helix is hydrophobic while the exterior surface is hydrophilic. Guest molecules can occupy the cavity when their structures are matched. Kuge and Takeo (1968) reported that the average amylose complexing temperature was 63 °C after investigating 38 agents. Under high temperature extrusion and heat/moisture treatments conditions, starches may form these collapsed helices with granule-associated lipids (Zobel 1988a, Charbonniere et al 1973).

The amylose content of milled rice or rice starch can be determined by the iodine colorimetry method proposed by Juliano et al (1981). Based on the amylose content, milled rice can be classified as waxy (1-2%), low (7-20%), intermediate (20-25%), and high (>25%) rice (Juliano, 1979). Thus, waxy rice starch usually contains 0.8 to 1.3% amylose, whereas the nonwaxy rice starch contains 7 to 33% amylose (Juliano 1985). Cooking and eating qualities of rice have long been associated with amylose

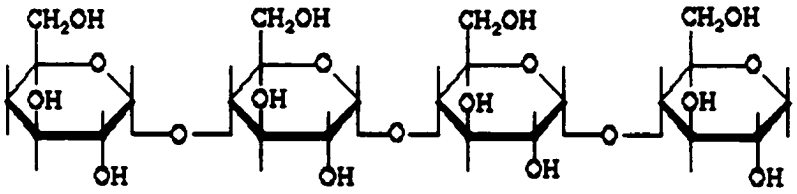


Fig. 2.1. Schematic diagram of amylose.

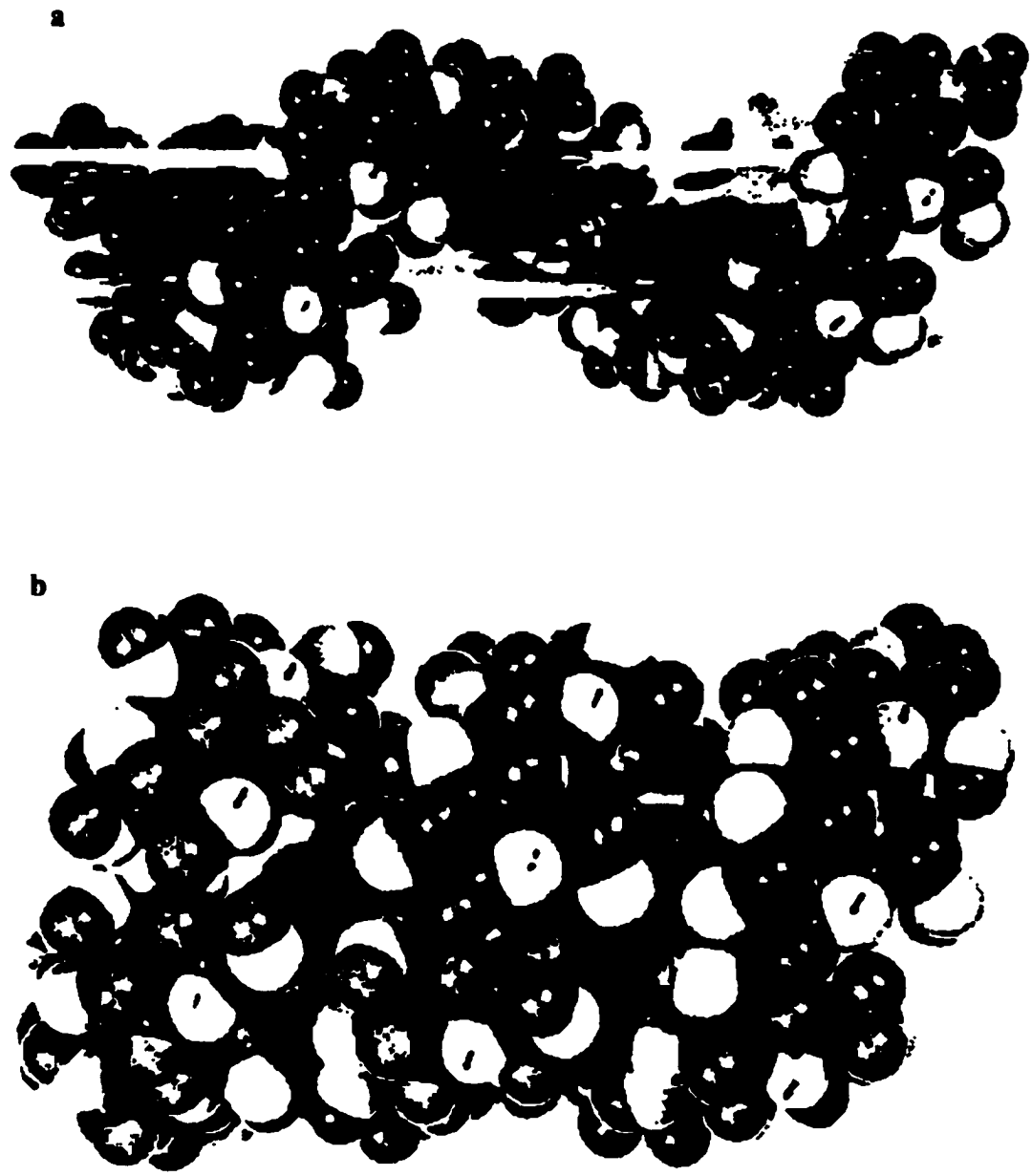


Fig. 2.2. Space-filling model of amylose (Zobel 1988a): (a) in an extended helical configuration; (b) in a collapsed helical configuration.

content. Low-amylose rices are generally known to be sticky and moist, whereas those with high amylose are non-sticky, flaky, and dry (Juliano et al 1965). Exceptions also exist as some rices with the same amylose content may differ substantially in hardness, stickiness, and gel consistency (Perez and Juliano 1982; Juliano et al 1987), which indicates that the structure of amylopectin molecules and other components (protein and lipids) of rice play a role in governing the physicochemical properties of rice. Rheological studies of aqueous amylose gels (Clark et al 1989) indicated that increasing amylose concentration or decreasing amylose chain length could result in more rapid gelation.

**Amylopectin**

Amylopectin is a very large, highly branched molecule. It consists of short, linear α-(1→4) glucan chains which are connected at the α-(1→6) branch points (Fig. 2.3). The branch points constitute 4-5% of the total linkages. Amylopectin can produce a double helix through intertwining a branch chain with another (Fig. 2.4). Zobel (1988a) described the shape of amylopectin molecules as the "cluster" model, which inhibits the formation of intermolecular interactions (hydrogen bonds) and thus results in a softer gel compared with the amylose gel. Temperatures required to reverse association and solubilize amylopectin gels usually vary from room temperature to 95°C. On the other hand, amylose gels generally require autoclave temperatures (110-160°C) for reverse. Amylopectin can also form a complex with limited agents such as iodine. However, those complexes are usually unstable due to the shorter length and disposition of the linear segments of amylopectin.

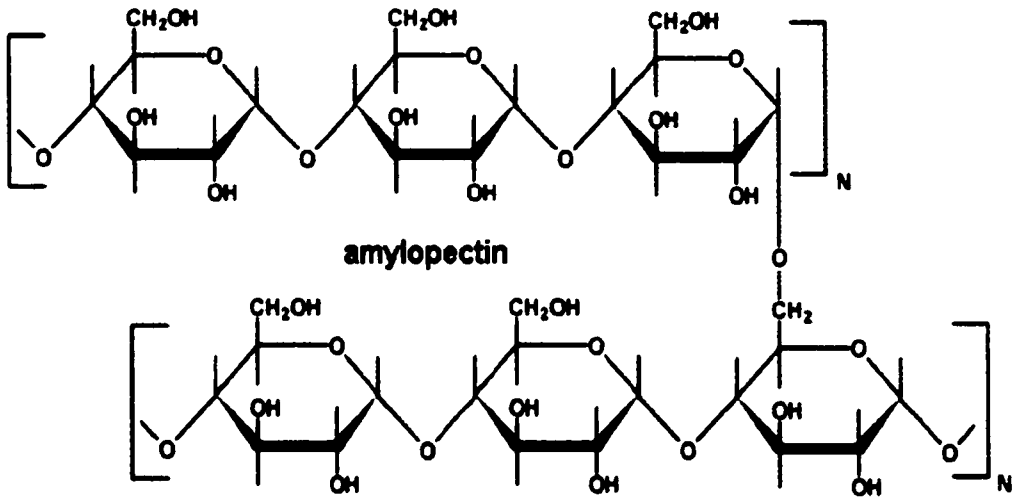


Fig. 2.3. Schematic diagram of amylopectin

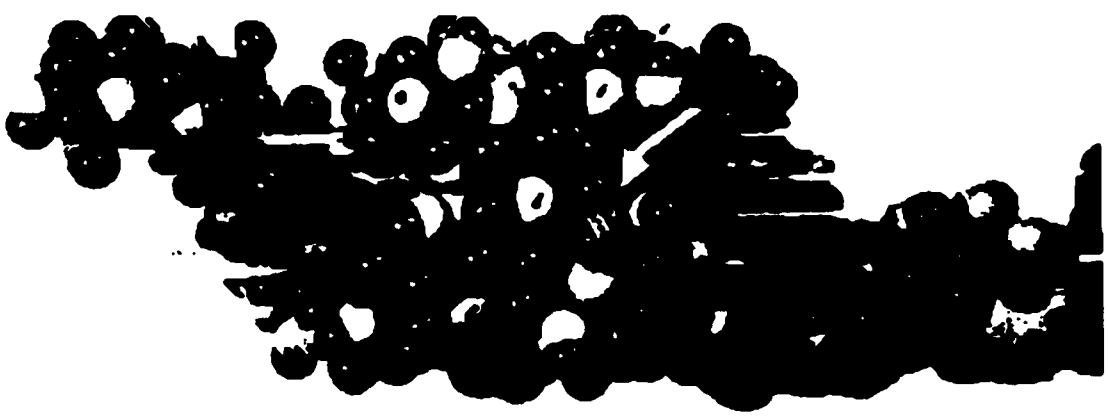

Fig. 2.4. Space-filling model of amylopectin with molecular segments packed in a double helix arrangement (Zobel 1988a). Large (white) arrow shows location of an α-1,6 bond.

More long-chain linear portions in amylopectin molecules were observed to produce a harder gel and increase the iodine binding capacity of the rice starch (Juliano et al 1987). Like the amylose content, amylopectin structure also changes with the varieties of rice (Takeda et al 1987, Juliano et al 1987). The swelling behavior of cereal starch is generally considered as the property of its amylopectin content, and amylose acts as both a diluent and an inhibitor of swelling, especially in the presence of lipid (Tester and Morrison 1990).

### 2.1.3. Starch Granule Structure

A model of the starch granule structure (Fig. 2.5) was proposed by Jenkins et al (1994) and reviewed by Jacobs and Delcour (1998). The granules are composed of concentric layers (dense layers and less dense layers), the growth rings, which exhibit the alternating high and low refractive indices, densities, crystallinities, and resistance to acid and enzymic hydrolysis (French 1984). The dense layer consists of 16 alternative crystalline and amorphous lamellae, while the less dense layer is largely amorphous (French 1984, Jenkins et al 1994). Thus, starch granules are partially crystalline with a degree of crystallinity of 20-40% (Hizukuri 1996, Jacobs and Delcour 1998). The crystalline lamellae are made up of amylopectin double helices, which are packed in a parallel fashion. Thus, the crystallinity of the granule is mainly attributed to double helices formed by amylopectin branches rather than to amylose (Jenkins et al 1993).

The structures and transformations of the starch crystal forms (A, B, C, and V) were reviewed by Zobel (1988b). P. Scherrer was the first person that proved the crystalline structure of the starch granules (Zobel 1988b). Since the 1920's, several

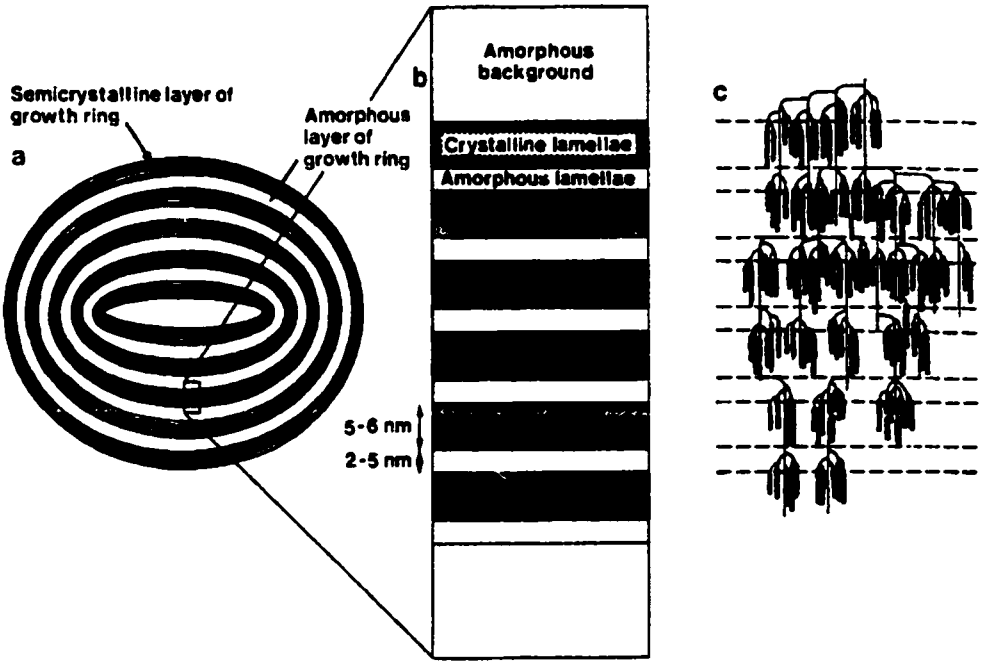


Fig. 2.5. Schematic representation of starch granule structure (Jenkins et al. 1994): (a) a single granule with alternating amorphous and semicrystalline layers, representing growth rings; (b) expanded view of the semicrystalline layer of a growth ring, consisting of alternating crystalline and amorphous lamellae; (c) the cluster structure of amylopectin within the semicrystalline layer of the growth ring.

investigators have been using X-ray diffraction (XRD) to explore the crystalline structures of starches from different species and their connections to the staling of baking products (Katz 1928, 1934a, 1934b; Katz and Mark 1924). In his findings, Katz (1928, 1934a, 1934b) designated his X-ray film patterns schematically as A, B, C, and V.

Under XRD, in general, cereal starches give A patterns, tubers show B patterns, and certain root and seed starches give C patterns (Zobel and Senti 1960; Zobel 1988a, 1988b). Levels of crystallinity in granular starches can be measured through the separation and integration of the areas under the X-ray diffraction peaks (Zobel and Senti 1959, Sterling 1960, Nara et al. 1978). Studies have shown that starches with B- and C- type XRD patterns (such as potato starch and banana starch) are more resistant to enzymatic and acidic hydrolysis compared with A-type starch (such as maize, wheat, and rice starch) (Takaya and Sugimoto 1980, Williamson et al 1992, Jane 1997). Sarko and Wu (1978) observed the packing of the double-helices in the crystalline structures and that B-starch has an orthogonal unit cell consisting of an open channel at the center, whereas the A-starch has a hexagonal unit cell structure. The reason for B-starch being more resistant to enzymatic digestion is still unknown (Jane 1997).

Amylose content had little effect on granule crystallinity as both maize and waxy maize have the same crystallinity despite their difference in amylose contents. Several investigators (Hizukuri 1985, Murphy et al 1975, Germino and Caracci 1971, Davies et al 1980) observed that the crystal type of amylopectin changes with the weight-average chain length, i.e., short, long, and intermediate chains gave A, B, and C patterns, respectively. These findings could be used to explain the B pattern results for

maize samples with amylose contents higher than 49% (amylomaize), instead of the A pattern of normal cereal starches (Zobel 1988b). The distinguishing features for different crystal types are shown in Table 2.1.

V pattern is generally considered to be a result of amylose complex formation with agents, such as alcohols, fatty acids and phospholipids, certain surfactants (emulsifiers), and iodine, under selected heat/moisture treatments (Zobel 1988b, Fukui and Nikuni 1969, Dudacek et al 1985). The selected treatment (Dudacek et al 1985, Zobel 1988b) might include the combinations of moisture (18-45%), temperature (90-130 °C), and holding time (1-16 h). Under these selected conditions, potato starch undergoes B to A and B to C transformations, while sweet potato starch has been converted from C to A pattern (Sair 1967, Osman 1967). The resulting starches generally have higher gelatinization temperatures, decreased granule swelling and increased gel rigidity (Sair 1967; Osman 1967; Kulp and Lorenz 1981a, 1981b; Lorenz and Kulp 1982a, 1982b, 1983). Steeneken (1984) suggested that, after heat treatment of potato starch, amylose might be released from either its prior association with amylopectin or entrapment in the B structure, thus resulting in the increased gel strength and chemical reactivity of amylose. Separation of amylose from amylopectin was also observed after the V structure formation in annealed maize starch (Zobel 1988b). Theoretically, wheat starch treated to show A+V structures should result in an improved shelf-life in bread making (Zobel 1988b).

The two major amylose polymorphs A and B are double helical assemblies (Wu and Sarko 1978, Imberty et al 1988), while the amylose-iodine complexes are in single helical V-form (Immel and Lichtenthaler 2000). Hydrophobic topographic studies

Table 2.1. X-Ray Diffraction Patterns From Different Starches (Zobel 1988a)

| Starches | X-Ray Pattern | Peaks (Angstroms) |
|---|---|---|
| Cereal | A | 5.8, 5.2, 3.8 |
| Tuber | B | 15.8,16.0, 5.9, 5.2 |
| Tuber and Seed | C | 16.0, 5.8, 5.2, 3.8 |
| Helical Amylose Complexes | V | 12, 6.8, 4.4 |

(Immel and Lichtenthaler 2000) of amylose and amylose-iodine complex revealed that the double-stranded A-form is a rather compact structure with an irregular distribution of hydrophilic and hydrophobic regions over the entire outer surface, with the interior of the double helix being inaccessible even for small molecules. On the other hand, the V-form has a pronouncedly hydrophilic outside surface and a hydrophobic channel accessible for complexing ligands. Findings from Mikus et al (1946) suggested that the amylose-lipid complexes posses a similar structure as that of amylose-iodine complexes.

### 2.1.4. Health Value

Starchy foods are the most abundant staples in the world. Food processing significantly influences the site and rate of starch digestion, which results in substantial applications for human health (Englyst and Hudson 1997). During food processing, starches are often finely milled and fully gelatinized, which leads to the rapid digestion and absorption of the starch in the human small intestine. There are strong indications that the large amounts of rapidly available glucose derived from meals, lead to periodic elevated levels of plasma glucose and insulin that have been associated with noninsulin-dependent diabetes (Kraft and Nosal 1975) and cardiovascular disease (Flodin 1986). Bjorck and Asp (1994) reported that the development of carbohydrate-based foods with a low glycemic index might be beneficial to hyperinsulinemic individuals and to those who are carbohydrate-sensitive, insulin-resistant, or diabetic. Several investigators observed that rice with high amylose levels (24-30%) could significantly lower serum glucose and insulin response (Goddard et al 1984, Juliano and Goddard 1986, Miller et al 1992). Similar results (Behall and Howe 1995) were obtained from high amylose

(70%) corn starch for the reduction of serum glucose and insulin levels compared to a standard corn starch diet (30% amylose). The mechanisms of lowering the glycemic response for the high-amylose starches are still unknown. Guraya et al (1997) suggested that it might be related to the amylose-lipid complex formation and the reduced digestibility of the resulting starch (Eliasson and Krog 1985, Holm et al 1983).

Based on the digestibility, nutritionally, starch can be classified into three basic groups: (1) rapidly digestible starch (RDS), (2) slowly digestible starch (SDS), and resistant starch (RS). Resistant starch (RS) is starch that escapes digestion and absorption in human small intestine. Thus, it has physiological effects in the human body that are similar to that of dietary fiber (Berry 1986). Evidence has shown that resistant starch can reduce risks for some diseases, including colon cancer, coronary heart disease, and glycemia (Unlu and Faller 1998). Normal food processing generates only small amounts of RS. Fermentation of RS in the human large intestine was observed to reduce fecal ammonia, which is potentially beneficial to health (Birkett et al 1996). Resistant starch is further classified into three categories: (1) physically inaccessible starch, (2) RS granules (RS2), and (3) retrograded starch (RS3) (Englyst et al 1992).

To ensure public health and avoid soaring national health budgets, it is of utmost importance to have food labeling and nutritional advice on dietary carbohydrates, including dietary fiber (Englyst and Hudson 1997). The new knowledge of the importance of dietary carbohydrate provides a tremendous opportunity for the food industry contribute to public health by the development and marketing of starchy foods that are beneficial to human health.

### 2.1.5. Starch Preparation

Starch has been produced from cereals for centuries and some earlier reports describe starch preparation by soaking and kneading ground cereal in a cloth bag with water to permit the release of the starch granules through the cloth, leaving behind the sticky gluten and fibrous material. French (1975) provided a review of these early reports. Different methods have been developed for starch preparation from various sources (Watson 1964, Wolf 1964, Willigen 1964). In the literature, two major techniques of starch extraction are often cited (Grant 1998). These two procedures are grain steeping and dough washing. Grain-steeping is the process of soaking whole grain in water with or without the use of chemicals to inhibit enzyme activity. Softening the grain in this manner avoids the starch granule damage normally acquired during dry milling of cereal to flour. Flour is the starting material for the dough-washing method of starch extraction. Hart and Blanshard (1982) made the comparison of various starch isolation methods and their influences on the gelatinization behavior of wheat starch. Whistler et al (1958, 1959) isolated starch from corn using a wet-milling procedure and studied the effects of three types of drying on the physical properties and chemical reactivity of the granules. The three methods examined were freeze-drying, air-oven drying at 45 °C, and alcohol dehydration. Singh (1996) studied the isolation of starches from various food grains and measured the characteristic changes in these starches after acid modification at the granular and molecular level.

Rice starch can be isolated from endosperm by way of alkali, detergent, and protease digestions (Kim et al 1978; Yang et al 1984;Yamamoto et al 1973; Juliano 1984; Maningat and Juliano 1979, 1980; Lumdubwong and Seib 2000). High purity rice

starch with low surface protein-lipid contamination is desired to minimize rancidity during storage and for industry applications. Compared with maize and wheat starches, isolation of rice starch is more difficult. Lumdubwong and Seib (2000) ascribed the difficulty to the hydrophobic and thus swelling resistant properties of granule associated proteins (prolamin and glutelin), and the tiny size of starch granules, which are hard to sediment in water. They compared the sodium hydroxide method and the alkaline protease digestion method and found that starch isolated by protease digestion had lighter appearance but contained more lipid.

### 2.1.6. Starch Fractionation

Starch fractionation is usually used for amylose and amylopectin property studies and for incorporation of those fractions into new and existing food products and ingredients (Brooks and Griffin 1987). Different starch fractionation methods have been developed. One common method suitable for rice starch fractionation is based on the differential ability of the fractions to precipitate with aqueous 1-butanol (Schoch 1942, Juliano 1985). Starch is first gelatinized with dimethylsulfoxide, then mixed with 1-butanol and pentanols, and cooled to room temperature. Amylose-alcohol complex is then collected and washed with 1-butanol-saturated water, before redissolved in boiling water and recrystallized to ensure the purity of amylose. Amylopectin is then recovered from the mother liquor through precipitation by addition of ethanol and NaCl.

### 2.1.7. Starch Modification

The physical properties of the native unmodified starches and their solutions limit their applications in many food products manufacturing. Those shortcomings (Wurzburg 1986, Whistler et al 1984) may include (1) the lack of free-flowing

properties; (2) insolubility or insufficient swelling and viscosity development in cold water; (3) excessive viscosity after cooking; (4) the cohesive, rubbery paste textures of some cooked waxy starches; (5) weak-bodied, sensitive to break down under heat, shear, and acid associated processing conditions; (6) lack of clarity when the pastes are cooled.

Food processors usually prefer modified starches with better behavioral characteristics compared to their native ones. Methods have been developed to improve the properties of starch pastes by physical, chemical, enzymatic, and genetic modifications. Native starches usually require cooking before becoming a paste, which is not suitable for some starch paste applications (such as instant foods), especially when high-amylose starch is involved. To solve this technical problem, instant starch products have been developed. One conventional approach is to precook the starch slurry, followed by drum drying and grinding (Powell 1967). Products produced by this method usually display low viscosities, as a result of degradation of the starch during the processing. Other methods are also proposed to produce the granule cold-water-soluble (GCWS) starch through injection followed by nozzle-spray drying (Pitchon et al. 1981). Pastes from those starches usually display large viscosity due to the gelatinization of starch during processing (Jane et al. 1986).

Chemical modification is used to treat starches with chemicals to replace some hydroxyl groups with either ester or ether groups, or to chemically link the two neighboring hydroxyl groups (cross-linking). Most commonly used chemical modification approaches for commercial starch products include conversion, crosslinking, and stabilization (Atwell et al. 1988).

Native starches usually can not be used at much more than 6% solids due to the high viscosity. However, some products (such as soft gum candies) in the confection industry require a low viscosity starch at high solids to obtain the desired gel structure and set. Conversion reduces the viscosity of raw starches so that starches can be used at higher percentages with improved solubility, gel strength, and stability. Methods of conversion include acid hydrolysis, oxidation, dextrinization, and enzyme conversion. Each method of conversion provides starch products with distinctive functionality. In crosslinking, native starch is treated with a bi-functional reagent so that a small number of the starch polymer chains are chemically linked by the cross linking reagent. Crosslinking inhibits granule swelling on gelatinization and prevents swollen granules from disintegration, resulting in dramatic improvement of starch stability to acid, heat treatment, and shear forces. Therefore, cross-linked starch has been widely used in food processing. Stabilization is another chemical modification method, in which the blocking groups are reacted with starch polymers to inhibit retrogradation. Stabilization is very important in frozen foods as retrogradation of starch polymers is accelerated at cold temperatures. In addition, the introduction of some unique functional groups into starch polymers is needed for certain food processing. One example is the addition of lipophilic groups to starch molecules. These modified starches are used in encapsulation and emulsion stabilization.

Maltodextrin and cyclodextrin are two good examples of enzymatic modifications of starch. Maltodextrins, with different molecular sizes, are used as important additives to produce body texture for beers and soft drinks, to decrease the glass transition temperature of starch, and to serve as carriers (Robyt 1984).

Cyclodextrins are prepared by the reactions of starch and enzyme (cycloamylose glucanotransferase) (Kainuma 1984). There are three types of cyclodextrins (CD), α-, β-, and γ- CD, with six, seven, and eight glucose units respectively. The most interesting part of the structure of CDs is the hydrophobic cavities of the molecules, which can form inclusion complexes with various chemicals having hydrophobic structures. Thus, CDs can be used in food manufacturing to stabilize volatile flavor compounds, to protect lipids and vitamins from oxidation, and to remove unpleasant flavors from food products (Inouchi et al. 1991; Wang et al. 1993; Shieh and Hedges 1996). In the drug industry, CDs can be used to prevent drug oxidation, and to achieve speedy delivery of drugs by fast absorption and increased solubility of the complex.

Due to the health concerns over ingesting chemicals in chemically modified starches and environmental concerns over waste-water treatment, research efforts have focused on genetic modification of starch properties by breeding and genetic engineering (Preiss and Sivak 1995), by which the structures and properties of starch can be improved. For example, shortening the A and B1 chains of starch may result in the decrease of gelatinization temperature and the slowing down of the retrogradation process (Jane et al 1992). On the other hand, addition of B4 and B5 chains may improve the resistance of starch paste to shear thinning and increase the viscosity, with similar properties to cross-linked starches. Other applications may include the increase of molecular size of amylose to slow down retrogradation and to improve the mechanical properties of starch pastes and gels; and the reduction of lipid content, especially phospholipid, to increase the clarity of the starch paste (Pfannemuller et al 1971; Gidley and Bulpin 1987).

### 2.1.8. Starch Properties

Starch is the primary component of cereal flours, and consequently, plays an important role as a determinant of food product quality (Zeng et al 1997). Most of the functional attributes of starch are related to the temperature-dependent interactions of starch with water in the processes known as gelatinization, pasting, and retrogradation (Dengate 1984, Atwell et al 1988).

**Gelatinization**

Undamaged starch granules are insoluble in cold water, but can imbibe water reversibly. When starch is heated in water over a critical temperature, the hydrogen bonds responsible for the integrity of the granules are weakened. Water thus penetrates the granules and leads to the hydration and swelling of the polymers. This process is called gelatinization. The granule structural changes during gelatinization include changes of shape and size of granules, absorption of water and swelling, crystallite melting, and leaching of amylose (amylopectin ) from the granules (Atwell et al 1988). Gelatinization temperatures are considered as ranges covering the temperatures at which loss of birefringence is first noticed and less than 10% remains. This temperature range is greatly influenced by the binding forces within the granule which vary with species.

Because gelatinization of starch is an endothermic process, differential scanning calorimetry (DSC), which measures both the gelatinization temperature (onset temperature of gelatinization), and the heat energy required for gelatinization (enthalpies), is widely used. For native starches, the molecular order in double helices is significantly greater than the crystalline order (Gidley 1985). During gelatinization, both levels of structure, i.e. double-helical and crystalline, are disrupted concurrently

during gelatinization, and it is generally believed that gelatinization enthalpies ($\Delta H_{gel}$) primarily reflects the loss of double-helical order (Cooke and Gidley 1992).

One characteristic of starch, especially its linear amylose fraction, is the ability to form inclusion complexes with a variety of inorganic and organic ligands. The ligands enter the helical cavities of the amylose molecules and form molecular inclusion complexes. In the presence of ligand molecules, amylose undergoes rapid conformational ordering from coil to helix, which promotes aggregation of helices into partially crystalline V structures (Eliasson and Krog 1985). The complexes are heat-stable and insoluble in aqueous media at pH 7. Among the starch complexes known are those with iodine (Banks et al 1971), flavor components in foods (Osman-Ismail and Solms 1973), alcohols (Kuge and Takeo 1968), free fatty acids (Raphaelides and Karkalas 1988), emulsifiers (Krog 1971), and many surfactants (Kim and Robinson 1979).

During the gelatinization, part of the free lipids present in the cereal starches will probably form a helical inclusion complex with the amylose molecules. This complex formation is an exothermic process and will result in a decrease in the observed endothermic gelatinization enthalpy (Eliasson 1986). Studies (Eliasson and Krog 1985) from the thermal stabilities of amylose-monoglyceride complexes suggested that the thermal stability increased with increasing chain length and decreased with increasing unsaturation. It was stated also that formation of complexes prevents leaching of amylose during gelatinization, inhibits the swelling of starch granules heated in water, and reduces the water-binding capacity of the starch (Eliasson 1985).

In starch, a biopolymer of partially crystalline nature, the softening of the amorphous regions is required before melting of crystallites can take place (Slade and Levine 1987). When the temperature exceeds the glass transition temperature (Tg) during heating, the amorphous regions in starch granules are transformed from a rigid glassy to a mobile rubbery state (Jacobs and Delcour 1998). The second order glass to rubber transition is accompanied by an incremental change in heat capacity ($C_p$), preceding the gelatinization endotherm during the DSC scan. For rice starch, Biliaderis et al (1986) estimated the $C_p$ for this glass transition to be approximately 0.11 J/gK. In practice, the $\Delta H_{gel}$ is usually calculated by estimation of the area under the endotherm with a fitted straight base line (Fredriksson et al 1998).

Annealing is a process in which the starch granules are incubated with excessive water, for a period of time, at a temperature above the glass transition temperature but below the gelatinization temperature. Several investigators have studied the annealing effect using DSC (Jacobs et al 1995, Tester and Morrison 1990, Larsson and Eliasson 1991). Their findings indicated that annealing increased the gelatinization temperature and enthalpy, and decreased the range of gelatinization temperature.

The influence of ionic and nonionic solutes on starch gelatinization has been studied by many investigators (Chungcharoen and Lund 1987). Some salts accelerate the disruption of hydrogen bonds to assist gelatinization (Leach 1965, Lindqvist 1979), while others inhibit gelatinization by acting as salting-out agents (Ganz 1965, Lindqvist 1979). Sugars are also known to retard gelatinization by inhibiting swelling of starch granules (Bean and Yamazaki 1978, Savage and Osman 1978, Chungcharoen and Lund 1987). Leach et al (1959) reported that the swelling pattern of starch granules was

greatly influenced by the species of starch. Other factors might include amylose content, heat-moisture treatment, presence of fatty adjuncts, and chemical modification of the starch. Based on the final gelatinization temperature, rice starch may be classified as: low (≤69.5°C), intermediate (70-74 °C), and high (>74 °C) (Juliano 1979).

**Pasting**

The changes that occur following starch gelatinization, during further heating in excess water, are termed pasting. These changes include further swelling of granules, further leaching of molecular components (primarily amylose), and eventually disruption of the granules (around 85 °C) (Tester and Morrison 1990a,b). A Brabender Visco/Amylo/Graph or a Rapid Visco Analyzer (RVA) is usually used to evaluate starch pasting characteristics, specifically, to record the viscosity change over time during heating, holding, and cooling periods. The leached amylose forms a three-dimensional network (Hennig et al 1976, Eliasson 1985, Tester and Morrison 1990), with the swollen granules embedded in a continuous matrix (Wong and Lelievre 1981, Ring 1985). The changes that occur during gelatinization and pasting greatly influence the rheological properties of the starch suspension (Jacobs and Delcour 1998). Studies (Lii et al 1996, Miles et al 1985) show that the rheological properties of the starch during heating are mainly influenced by the granular structure and components, followed by the amount of leached-out amylose in the process.

Swelling is mainly determined by the amylopectin fraction (Juliano and Perdon 1975, Juliano et al 1987), while amylose acts as a restraint. Lower amylose content in starches tends to produce higher peak and breakdown viscosity (Halick and Kelly 1959, Moss and Miskelly 1984, Medcalf and Giles 1965, King et al 1994, Zeng et al 1997),

and lower pasting temperature (Oda et al 1980, Endo et al 1989). The association of lower apparent amylose content with lower final viscosity, negative setback, and less total setback was also observed by Zeng et al (1997). Juliano (1985) reported that amylose content, a laboratory indicator of cooked rice texture, positively correlated to amylograph breakdown viscosity, final viscosity at 95°C, viscosity on cooling to 50°C, and setback.

Structural analyses of starches by using chemical and enzymatic methods (Hizukuri et al 1970) and $P^{13}$-NMR (Lim et al 1994) have revealed that potato starch consists of substantial amounts of phosphate derivatives, whereas, normal cereal starches consist of high concentrations of phospholipids (Lim et al 1994). The phosphate monoester groups of potato and other starches carry negative charges which repel one another and result in greater viscosity and clarity. In normal cereal starches, the phospholipids complex with amylose and long-branch chains of amylopectin (Batres and White 1986), which create junction zones and result in turbidity (Craig et al 1989) and higher pasting temperature. Tapioca and most other root and tuber starches consist of a low concentration phosphate derivatives and no phospholipids (Lim et al 1994); thus, the starches have relatively high clarity and low pasting temperatures. Waxy starch contains little or no amylose and phospholipids and, thus, displays great clarity and a low pasting temperature (Jane 1997).

**Retrogradation**

When starch pastes cool, molecules reassociate in an ordered structure. The term retrogradation is used to describe the changes that occur upon cooling and storage of gelatinized starch (Atwell et al 1988). The initial stages in gelation of starch are

dominated by the gelation of the solubilized amylose (Miles et al 1985), which indicates that the solubilized amylose plays a key role in the gelation of starch (Lii et al 1996).

Retrogradation of cooked starch consists of two separable processes: (1) the short-term change and (2) the long-term change. The short-term changes that occur during cooling of starch gels have been attributed to the gelation and crystallization of the amylose fraction (Miles et al. 1985, Sievert and Wursch 1993, Fredriksson et al 1998). Retrograded amylose or resistant starch type III (Englyst et al 1992) is a physiologically important indigestible starch fraction. This fraction is heat stable and melts above 120 °C (Sievert and Pomeranz 1989).

The long-term changes that occur during storage of starch gels have been attributed to the recrystallization of amylopectin fraction (Eliasson 1985). The retrogradation behavior of amylopectin has been related to the starch source (Orford et al 1987, Kalichevsky et al 1990, Shi and Seib 1992) and concentration (Orford et al 1987, Slade and Levine 1987), storage temperature (Slade and Levine 1987, Eliasson and Ljunger 1988), and amylopectin structure (Fredriksson et al 1998). Lipids (Eliasson and Gudmundsson 1996) and short amylopectin unit chains (6-9 DP) (Shi and Seib 1992) have been shown to inhibit retrogradation. In high-amylose starches, the amylose fraction has been suggested to have synergetic effects on the amylopectin retrogradation process (Russell 1987). The change of crystal type from A (in native cereal starches) to B (in retrograded amylopectin) was also observed by Ward et al (1994). Many quality defects in food products (such as bread staling) are due to starch long term retrogradation.

## 2.2. STARCH ASSOCIATED LIPIDS

Starch associated lipids extracted from starch granules may be non-starch lipids or integral lipids (integral component of granules) (Morrison 1978, 1981; Juliano 1985). The non-starch lipids usually originate in the membranes and spherosomes of the cereal endosperm (Morrison 1978). These lipids, which are loosely associated with the granules, consist of triacylglycerides, diacylglycolipids, and phospholipids. Those lipids can be extracted from dry grains using non-polar solvents, such as diethyl ether, petroleum ether, and chloroform / methanol (2:1, v/v) (Morrison 1978, Choudhury and Juliano 1980a). Integral lipids (starch lipids) may be extracted with water-saturated butanol (WSB) after extraction of nonstarch lipids (Juliano 1985). Starch lipids extracted with cold WSB from brown rice amounted to 0.6-0.7% for nonwaxy rice and 0.2% for waxy rice (Juliano 1985). Corresponding values for milled rice were 0.5% for nonwaxy and 0.1% for waxy rice. Compared with the starch from normal rice genotypes, starches from waxy rice usually have much lower lipid contents, while starches from high amylose rice have higher lipid contents (Morrison 1995; Yasui et al 1996). To completely extract starch lipids, the refluxing WSB method (67% butanol and 33% water at 92 °C) is required. However, the starch granules will be gelatinized during the extraction process. The major starch lipids in rice are free fatty acids, lysophophatidylcholine (lysolecithin), and lysophosphatidylethanolamine (lysocephalin) (Maningat and Juliano 1980; Choudhury and Juliano 1980b). In rice starch, free fatty acids make up 30-60% of the granule integral lipids (Morrison 1993). The major fatty acids are: palmitic ($C_{16:0}$), linoleic ($C_{18:2}$), and oleic ($C_{18:1}$) (Juliano 1985, Maningat and Juliano 1980).

## 2.3. STARCH PROTEINS

Traditionally, proteins have been classified into four types according to their solubility: (1) albumins are proteins soluble in water; (2) globulins are proteins insoluble in pure water but soluble in dilute salt solutions and insoluble at high salt concentrations, and therefore exhibit salting in and salting out properties; (3) prolamins are proteins soluble in 70% ethyl alcohol; (4) glutelins are proteins soluble in dilute acids or bases.

Proteins are believed to be associated with starch granules and the amounts vary between and within species (Juliano 1985, Ellis et al 1998). Starch proteins can be classified as (1) surface protein or (2) integral protein. Surface proteins are those that can be extracted at temperatures below the gelatinization temperature, while the integral proteins are those that can be extracted at temperatures near or above the gelatinization temperature (Ellis et al 1998).

All cereal starch granules contain detectable amounts of protein. Due to the possible Maillard-type reactions taking place with polysaccharides, the amount and type of protein presented might limit cereal starch for food or non-food industrial applications (Ellis et al 1998). Starch proteins may also influence the isolation process and the physicochemical characteristics of extracted starch as well. Investigators reported that the rice starch granule is associated with two hydrophobic protein bodies: PB I (Prolamin) and PB II (Glutelin) (Adoracion et al 1993), which caused the difficulty for rice starch extraction (Lumdubwong and Seib 2000). Hamaker and Griffin (1993) found that proteins with disulfide bonds in rice flour restrict starch granule swelling during gelatinization and make the swollen granules easier to breakdown under stirring.

Proteins are formed from amino acids through amide linkages. There are about 20 amino acids in a protein hydrolysate. One of the major factors that affect protein physicochemical properties, such as structure, solubility, and fat-binding properties, is the hydrophobicity of the constituent amino acid residues (Damodaran 1996). Hydrophobicity is defined as the excess free energy of a solute dissolved in water compared to that in an organic solvent under similar conditions.

Protein content can be calculated from Kjeldahl nitrogen multiplied by the factor constant. Rice protein content is usually calculated based on the nitrogen content (16.8%) of the major rice protein, glutelin, using the factor constant 5.95 (Juliano 1985). Due to the highest glutelin and lowest prolamin content, rice protein, together with oat protein, has a higher lysine content than other cereals (Juliano 1985). The most abundant amino acids in rice protein are glutamic acid, aspartic acid, and arginine (Juliano 1985).

## 2.4. FOOD ADDITIVES USED IN STARCH-BASED FOOD MANUFACTURING

Additives are commonly used in food manufacturing to improve volume, texture, crumb, shelf-life and slicing characteristics of starch-based products (Aust and Doerry 1992, Tenny 1978, Maninder and Bains 1976, Farhat et al 2000). Lipids and emulsifiers are commonly used in food formulation to improve the stability of dispersed systems. Salts and sugars are used to improve the taste, flavor, and texture of the final products. Lipids and emulsifiers form complexes with amylose, whereas salts and sugars compete with amylose for water in the system. Thus, those additives influence the functional properties of starch granules.

### 2.4.1. Lipids and Emulsifiers

A majority of foods can be categorized as emulsions and foams. These are two-phase systems in which one of the phases (oil or air) is dispersed in the other phase. The stability of these dispersed systems depends on the presence of a surfactant (emulsifier), which can significantly reduce the interfacial tension between the phases. Two types of surfactants are usually used in foods: (1) low molecular weight surfactants, such as phospholipids, mono- and diglycerides, and sorbitan monostearate, and (2) polymeric surfactants, such as proteins and certain gums.

Emulsifiers are compounds that have both hydrophilic and hydrophobic ends on the same molecule. The roles of emulsifiers in foods have been discussed by Stutz et al.(1973). The lipophilic portions of emulsifiers can form a complex with the amylose fraction of starch during cooking, which leads to the increase of the gelatinization temperature. Starch granule swelling and solubility decline are reported due to the increase of complex formation (Eliasson 1985, Ryu and Walker 1993).

Emulsifiers are added to cake and bread formulations to increase air incorporation, decrease specific gravity, produce a finer fat dispersion, and as a result increase the final product volume (Baker and Gordon 1990). For example, saturated monoglycerides and sodium stearoyl lactylate are used in the bread-making industry to retard firming and staling of baked products (Krog et al 1989, Krog and Jensen 1970). Monoglycerides and related surface-active monoacyl lipids are used in the manufacture of instant mashed potato granules to prevent stickiness (Hoover and Hadziyev 1981), and in extruded starch-containing products to control texture (Launay and Lisch 1983). In cake manufacturing, emulsifiers added to cake formulation was reported to increase

air incorporation, decrease specific gravity, produce a finer fat dispersion, and as a result increase the final cake volume (Handelman et al 1961, Wootton et al 1967, Guy and Vettel 1973, Del Vecchio 1975). Common emulsifiers used in the baking industry include distilled monoglycerides (MG), diglycerides (DG), diacetyl tartaric acid ester of monoglycerides (DATEM), and lecithin.

Although low molecular weight emulsifiers (lipids) are more efficient in reducing the interfacial tension, foams and emulsions created by them are not as stable as those formed by proteins. This is because proteins, in addition to lowering interfacial tension, can form a membrane-like viscoelastic film around oil droplets or air bubbles via noncovalent interactions and disulfide cross-linking, which is not possible in the case of low molecular weight emulsifiers. For example, skimmed milk and egg white are used in rice cake formulation to enhance volume expansion and tenderness of chemically leavened rice cakes (Mohamed and Hamid 1998).

Fat is also used to create more tender products and shorter doughs. Fat lubricates the structure by being dispersed in the dough or batter during mixing and helps prevent the starch and protein from forming a continuous network. The flavor, texture, and appearance of baked products are affected by types and amounts of fat used (Sanchez et al. 1995).

**2.4.2. Sugars and Salts**

Sugars are very important in cake baking to delay starch gelatinization and result in a noncollapsing, porous cake structure (Bean and Yamazaki 1973, Yamazaki and Kissell 1978, Glover et al 1986). Usually, disaccharides retard swelling more than monosaccharides (Bean and Osman 1959, Glover et al 1986), except for maltose, which

acts like a monosaccharide (Kim and Walker 1992a, b). It was suggested (Kim and Walker 1992b) that the delay of starch gelatinization in a sugar solution is mainly due to the ability of sugar to (1) limit water availability to the starch granule, (2) lower the water activity, (3) form sugar bridges between starch chains, and (4) exert an antiplasticizing effect, relative to water.

Electrolytes (anions and cations) are known to affect the gelatinization temperature (GT) of starch, which are related to the effect on the water structure of the aqueous phase (Zobel 1984). Strongly hydrated ions (e.g. sulfate and lithium ions) tend to increase starch GT through increasing the water structure order but decreasing the concentration of "free" water molecules. It is also suggested (Chinachoti et al 1991a) that the influence of solute on GT might relate to various physical properties, such as water activity, solute-solvent average molecular weight, and the $D_2O$ mobility ($T_2$) of $^{17}O$ nuclear magnetic resonance. Investigators observed that NaCl and NaBr decreased the sharpness of the X-ray diffraction of potato starch (Takahashi et al 1981); addition of NaCl caused a small increase in the GT, and then a significant decrease when the solute concentration was raised to 5M (Lii and Lee 1993). Oosten (1982, 1990) proposed that starch could be viewed as a weak ion-exchanger; and the cation stabilized the starch structure while the anion promoted the rupture of the hydrogen bond. Interactions between salt and water, and the salt and starch are involved (Lii and Lee 1993, Chinachoti et al 1991b).

# CHAPTER 3
# EFFECTS OF LIPIDS, AMINO ACIDS, AND BETA-CYCLODEXTRIN ON PASTING PROPERTIES OF RICE STARCH USING RAPID VISCO ANALYSIS

## 3.1. INTRODUCTION

The most important practical property of starch is its ability to swell and produce a viscous paste when heated in water. After gelatinization, during further heating, starch granules swell and rupture and, as a result, more amylose (and / or amylopectin) leaches out into the solution to form a paste. Cooling a hot paste results in amylose molecule association and the formation of gels. There are many methods for determining the viscosity of a starch paste, and the selection of the proper method will depend on the purpose of the measurement. In recent years, the Rapid Visco Analyzer (RVA) has been used by a number of investigators to determine the pasting properties of starch, i.e., to record the viscosity change over time during heating, holding, and cooling periods. Compared with the Brabender Visco/Amylo/Graph, RVA has several advantages, such as smaller sample size requirement (3 g) and shorter period of testing time (usually less than 40 min, depending on the profile used) (Ravi et al. 1999). Besides that, evidence suggested that the starch pasting properties measured in the RVA simulate the actual food production process (Deffenbaugh et al. 1989).

Amylose is responsible for starch gelation, while amylopectin is considered as the major factor responsible for the granule swelling and viscosity change during pasting (Svegmark and Hermansson 1990, 1991; Svegmark et al. 1993). Results from Lii et al. (1996) indicated that the granular structure was more important than the leached-out amylose in contribution to the pasting properties. Tester and Morrison (1990) also proposed that the swelling behavior of cereal starch is mainly determined by

the amylopectin properties, whereas amylose acts as both a diluent and an inhibitor of swelling, especially in the presence of lipids.

Lipids are known to influence viscoelastic properties of cereal starches by forming the inclusion complexes with the helical structure of amylose (Maningat and Juliano 1980; Ghiasi et al.1982a; Juliano et al. 1987; Zobel 1988a). Those complexes might also affect the solubility of the granules, the amount of amlyose leaching out, and the pasting characteristics, such as peak time, peak viscosity, cold paste viscosity, breakdown, and setback value (Gray and Schocii 1962; Ryu and Walker 1993; Roach and Hoseney 1995; Ravi et al 1999).

Defatting reduced both gelatinization temperature and the pasting viscosity of the rice starch or flour (Manignat and Juliano 1980; Champagne et al. 1990; Hamaker and Griffin 1990). Addition of surfactants or emulsifiers may restrict the granule swelling and influence the rheological properties of starch paste. Those influences may vary with the type of surfactant used, the concentration of the surfactant, and other conditions, such as the presence of other additives (sugars, salts, and enzymes), the moisture content, and the heating temperatures (Kim and Walker 1992; Ryu and Walker 1993; Roach and Hoseney 1995; Ravi et al. 1999). However, further study is needed to compare and analyze individual starch lipid effects on the pasting characteristics of rice starch.

Cyclodextrin molecules contain hydrophobic cavities, which can form inclusion complexes with various hydrophobic compounds including lipids. Kim and Hill (1984) observed that β-cyclodextrin (β-CD) could increase the swelling power and solubility of

wheat starch granules during gelatinization, which was linked to the disruption of amylose-lipid complexes.

The protein and starch interaction has been reported during baking product manufacture and storage (Dreese et al 1988; Martin et al 1991a,b; Holm et al 1985; Bjorck et al 1986;Guerrieri et al 1997). Those interactions may influence the staling of the final products, the availability of starch to digesting enzymes, and the quality of the final products. Greenwell et al (1985) suggested that surface proteins in the starch granule may represent an obstacle to the access of amylolytic enzymes or may interact with them, modifying their surface distribution.

High-protein content rices were generally less tender than low-protein content rices after cooking (Onate et al 1964, Juliano et al 1965). Due to the hydrophobic properties of the proteins (prolamin and glutelin) in starch granule, starch isolation becomes more difficult compared with maize and wheat (Lumdubwong and Seib 2000).

Protein removal by solvent extraction may decrease the gelatinization temperature, increase the peak viscosity, and reduce the peak temperature of pasting (Cheng 1987; Marshall et al. 1990; Yang and Chang 1999). Studies from Juliano et al. (1964a) suggested that the protein content could influence the peak viscosity and set back values of milled rice, although those influences may vary from one variety to another.

After mixing reducing agent (dithiothreitol) or various proteinases with rice flour, Hamaker and Griffin (1990) observed that the pasting viscosity was reduced over the whole pasting temperature range, whereas no viscosity influence was observed when mixed with isolated rice starch. Based on those findings, they suggested that the

hydrolyzed product of rice proteins might interact with rice starch and result in the viscosity reduction of rice flour.

It is well known that cereal proteins vary in amino acid composition (Khan and Bushuk 1979). Studies suggested that protein and its subunit properties (such as solubility) are related to their difference in amino acid composition (Bushuk 1985). Further study is needed to exam the influence of amino acid structure and type on the pasting properties of rice.

Brown rice flours are obtained from raw, intact rice kernels. Those flours were reported to add a different flavor and chewy texture to baked products (Juliano 1985). However, due to the lipase activity, such flours usually contain large amount of free fatty acids, the enzymatic hydrolysates of lipid components. The study of pasting characteristics on brown rice flours and their isolated starch could provide some valuable information for expanding the use of brown flour and brown starch.

The objectives of this study are: (1) to determine the lipid and protein removal effects on pasting properties of rice flours; (2) to determine the lipids, amino acids, and β-cyclodextrin effects on pasting properties of rice starch; (3) to compare those additive effects on commercial rice starch versus rice starch isolate.

## 3.2. MATERIALS AND METHODS

### 3.2.1. Materials

Two types of commercially available rice flour (white, brown), obtained from Riviana Foods, Inc. (Abbeville, LA), were used in this study. Commercial rice starch (S-7260), purchased from Sigma Chemical Co (St. Louis, MO), was used as a control. Free fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid)

were chosen to represent different degrees of saturation. Those free fatty acids, along with phospholipids (lysophosphatidylcholine (LC), lysophosphatidylethanolamine (LE)) and glycerides (monopalmitin (MP), tripalmitin) were used to test the lipids effect. Amino acids used in this study included positive charged (arginine and lysine), negative charged (aspartic acid and glutamic acid), and neutral ones (leucine and alanine). Those lipids and amino acids, along with β-cyclodextrin (β-CD) (C-4767), amylose (A0512), amylopectin (A8515), and protease (P5147) were purchased from Sigma Chemical Co.(St. Louis, MO). Flour samples were kept in sealed containers to prevent moisture loss during the study period.

### 3.2.2. Lipids Extraction from Rice Flour

Defatting of rice flour was performed by the modified soxhlet extraction method (Yang and Chang 1999). A 30 g flour sample was weighed and transferred into a 80mm- high extraction thimble. The thimble was then covered with cotton and put into a Soxhlet extraction tube. A 100 ml of petroleum ether was added to a 500 ml flask, and then an Allihn condenser, the Soxhlet extraction tube, and the flask were connected and placed on a hot plate with temperature setting to 45 °C. A cooling system was then connected to the condenser with the coolant temperature setting at –5 °C.

The petroleum ether extraction was performed for 12 hrs and then the methanol extraction was followed with 100 ml methanol at 65°C for another 12-hr extraction. Finally, the defatted flour sample was air-dried under a vacuum hood.

### 3.2.3. Protein Removal of Rice Flour

The modified alkaline protease digestion method (Lumdubwong and Seib, 2000) was applied to remove protein from defatted rice flour. A 40 g defatted flour

sample was weighed and transferred into a 500 ml flask. Then a 150 ml of 0.001 M NaOH solution was transferred to the flask and 0.2 g protease powder was weighed and added to the mixture. The mixture was then stirred and adjusted to pH 10 by adding 1 M sodium hydroxide solution. Then the flask was covered with parafilm and placed in a shaking water bath for 18 hours at $55^0$C.

The slurry was then centrifuged at 3,000 g for 20 min. Finally, the supernatant was discarded while the sediment was washed twice with a 150 ml distilled water and centrifuged at 3,000g for 15 min. The residue was then suspended in 150 ml distilled water and adjusted to pH 7 by adding 1 M hydrochloric acid. After that, the pH-adjusted slurry was centrifuged at 10,000 g for 20 min. The supernatant was discarded, and the dark tailings layer atop the starch was carefully scraped away and discarded. The starch was finally washed three times with 100 ml distilled water until the tailing fraction became negligible after centrifuging. The isolated starch was dried in a convection oven at $40^0$C for 48 hours.

**3.2.4. Chemical Composition Analysis**

Two types of rice flours (white and brown), their defatted flours and starch isolates, and commercial starch were analyzed for moisture (method 985.14, AOAC 1995), lipid (method 945.16, AOAC 1995), protein (N x 5.95) (method 992.15, AOAC 1995), and ash content (method 920.153, AOAC 1995). The amylose content of the samples above was determined using the standard iodine colorimetry method proposed by Juliano et al (1981) with slight modification. Duplicate samples were used for above analysis.

### 3.2.5. Amylose Content Measurement

**Step One: Starch Dispersion Preparation**

The following samples were weighed in duplicate and transferred into 100-ml volumetric flasks: 100-mg samples of amylose (from potato), amylopectin (from potato), and rice flours (defatted or undefatted), and 90-mg samples of rice starch (commercial or isolated).

Then 1 ml of 95% ethanol was added to wash down any sample adhering to the sides of the flask. Next, 9.2 ml of 1-N NaOH was added to the amylose sample while 9.0 ml was added to all other samples. All of the samples were kept at room temperature for 15-24 h without shaking to disperse the starch. The solutions were then made to 100 ml volume with distilled water and mixed well.

**Step Two: Standard Solutions Preparation**

The standard solutions were prepared by mixing the amylose and amylopectin solutions with 2 ml 0.09-N NaOH as shown in Table 3.1.

**Step Three: Iodine Color Development**

Fifty ml of distilled water was added into 100-ml volumetric flasks. Then, five ml starch dispersion and 1.0 ml of 1-N acetic acid were added and mixed. Two ml of iodine solution (0.2% $I_2$ in 2.0% KI) was then added and the volume was made to 100 ml volume with distilled water and mixed. The mixture was kept for 20 min before the absorbance measurement at 620 nm. To prepare a blank, 5 ml of 0.09 N NaOH was added into a 100 ml volumetric flask containing 1 ml of acetic acid and 2-ml of iodine solution, which was then made to 100 ml volume using distilled water.

Table 3.1. Amylose and Amylopectin Content in Standard Solutions for Amylose Content Measurement

| Amylose (%) | Amylose Solution (ml) | Amylopectin Solution (ml) | 0.09N NaOH (ml) |
|---|---|---|---|
| 0 | 0 | 18 | 2 |
| 10 | 2 | 16 | 2 |
| 20 | 4 | 14 | 2 |
| 25 | 5 | 13 | 2 |
| 30 | 6 | 12 | 2 |

A standard curve was plotted using 5-ml aliquots of the standard solutions prepared previously. The amylose content of rice starch samples were determined from the curve. For rice flours (defatted or undefatted), amylose content was measured similarly.

### 3.2.6. Standard Rapid Visco Analyzer (RVA) Profile

Lipids and β-CD were used as additives to test their effects at two levels: 0.2% and 0.6% (starch dry base) for lipids, 2% and 6% (starch dry base) for β-CD. Commercial rice starch and the RSI from white flour were mixed with those additives, while the defatted flours and the RSI from brown flour were used for pasting test without the additives.

A rapid visco analyzer (Newport Scientific, Warriewood, Australia) was used to measure the apparent viscosity of samples as a function of temperature, time and stirring. The modified procedure of the RVA Rice Method (1997) was followed. Certain levels of additives were carefully weighed into a RVA canister. Distilled water (25 ml) was measured and transferred into the canister. Then a 2.65 g (dry basis) sample was weighed and transferred to the canister, and distilled water was added to reach a total weight of 28 g. A plastic paddle was placed into the canister and vigorously jogged through the sample up and down for 10 times. The canister with the paddle was then inserted into the instrument. The measurement cycle was initiated by lowering the motor tower of the instrument into position.

The starch suspension was stirred rapidly at 960 rpm for 10 sec before the shear input was decreased and held constant at 160 rpm for the heating and cooling cycles. The suspension was heated from $50^0$C to $95^0$C in 3 min and 48 sec, then held at

95$^0$C for 2 min and 30 sec before cooling to 50$^0$C over 3 min 48 sec. All pasting curves were performed in duplicate. The viscosity was expressed in RVU. Peak viscosity (PV), minimum viscosity (MV), final viscosity (FV), pasting temperature (PT), and time to peak (TP) were reported. The set back (SBK) value, total set back (TSB), and break down (BKD) viscosity were derived from the following formulas: SBK = FV – PV, TSB = FV – MV, BKD = PV – MV. Starch gels were collected and stored at refrigerator temperature for three days before the X-ray Diffraction test (see chapter 5).

### 3.2.7. Statistical Analysis

SAS (Statistical Analysis System) software (Version 8.0) was used for data analysis. Analysis of Variance (ANOVA), with Tukey's studentized range (HSD) test, was performed to examine the additive (lipids, amino acids, and β-cyclodextrin) effects on the pasting characteristics (PV, MV, FV, PT, TP, SBK, TSB, and BKD) of commercial starch and starch isolate. Similarly, the lipid and protein removal effects on the pasting characteristics of flours were examined. Duplicate samples were used and a significance level of $p \leq 0.05$ was applied.

## 3.3. RESULTS AND DISCUSSION

The RVA viscosity curves reflect the pasting characteristics of each tested starch. The pasting temperature (PT) is the temperature at which the viscosity starts to rise. Usually pasting temperature is higher than the gelatinization temperature. In other words, the granules are completely gelatinized, as judged by loss of birefringence, before the viscosity begins to rise and be detected by RVA. Lower pasting temperature usually means swelling faster. Peak viscosity (PV) usually reflects the extent of

granule swelling. Most of the time, we must cook through this stage in order to obtain a usable starch paste (Mazurs et al. 1957). Time to peak (TP) indicates the time required for cooking. The drop in viscosity from a maximum value (peak viscosity, PV) to a minimum value (minimum viscosity, MV) is termed as the breakdown value (BKD). BKD usually reflects the stability of the paste to breakdown during cooking, whereas the final viscosity (FV) at 50°C indicates the stability of the cooked paste in actual use. Total setback (TSB) is used to describe the extent of viscosity increase on cooling to 50 °C, which reflects the retrogradation tendency of the starch product.

### 3.3.1. Effects of Lipids and Protein Removal on Pasting Properties of Rice Flours

Brown flour contains more lipids (3.3%) and protein (10%) than the white flour (0.8% lipids and 8.7% protein) does (Table 3.2). Petroleum ether and methanol extraction completely removed the lipids from both flours whereas the alkaline protease digestion resulted in a protein residue of 1.4% in white starch isolate and 4.1% in brown starch isolate.

For white rice flour, lipid removal significantly reduced the final viscosity by 48 RVU and decreased the total setback by 53 RVU, resulting in a low cold paste viscosity and retrogradation tendency (Table 3.3, Fig. 3.1). Besides that, the pasting temperature of white flour was also reduced by 2 $^0$C after defatting. Therefore, the starch granules in white flour were easier to cook than in defatted white flour.

Compared to the defatted white flour, protein removal greatly reduced the peak viscosity, minimum viscosity, and final viscosity by 62, 19, and 78 RVU respectively. In addition, the total setback and the breakdown were also reduced by 60 and 45 RVU respectively. The proteins in the rice flour were believed to restrict starch granule

Table 3.2. Chemical Composition of Starches, Flours, and Defatted Flours[1]

| Sample | Moisture[2] | Protein[2] (N x 5.95) | Lipids[2] | Ash[2] | Amylose[2] |
|---|---|---|---|---|---|
| Commercial Starch | 11.1 | 0.6 | 0.0 | 0.3 | 25.3 |
| White Flour | 10.7 | 8.7 | 0.8 | 0.7 | 30.0 |
| Defatted White Flour | 11.6 | 8.0 | 0.0 | 0.6 | 29.4 |
| White Starch Isolate | 7.3 | 1.4 | 0.1 | 0.5 | 29.2 |
| Brown Flour | 9.1 | 10.0 | 3.3 | 1.8 | 28.5 |
| Defatted Brown Flour | 10.7 | 10.5 | 0.1 | 2.1 | 27.7 |
| Brown Starch Isolate | 6.7 | 4.1 | 0.1 | 1.7 | 28.7 |

[1]All values are calculated based on the dry weight of samples except moisture content.
[2]Unit: %.

Table 3.3. Effect of Lipid and Protein Removal on Pasting Properties of White Rice Flour [1,2,3]

| Sample | PV | MV | FV | PT | TP | SBK | TSB | BKD |
|---|---|---|---|---|---|---|---|---|
| Flour | 249.96[a] | 132.21[a] | 309.58[a] | 80.43[a] | 5.47[a] | 59.63[a] | 177.38[a] | 117.75[a] |
| Defatted Flour | 260.58[a] | 137.96[a] | 262.21[b] | 78.35[b] | 5.54[a] | 1.63[b] | 124.25[b] | 122.6[a] |
| Starch Isolate | 197.75[b] | 119.46[b] | 183.63[c] | 77.25[b] | 4.87[b] | -14.13[b] | 64.17[c] | 78.29[b] |

[1]PV=Peak Viscosity, MV=Minimum Viscosity, FV=Final Viscosity, PT=Pasting Temperature, TP=Time to Peak, SBK=Set Back. TSB=Total Set Back, BKD=Breakdown;
[2]Units: Viscosity (RVU), Temperature (°C), Time (min).
[3]Different letters within each column indicate values are significantly different at the level of $p \leq 0.05$.

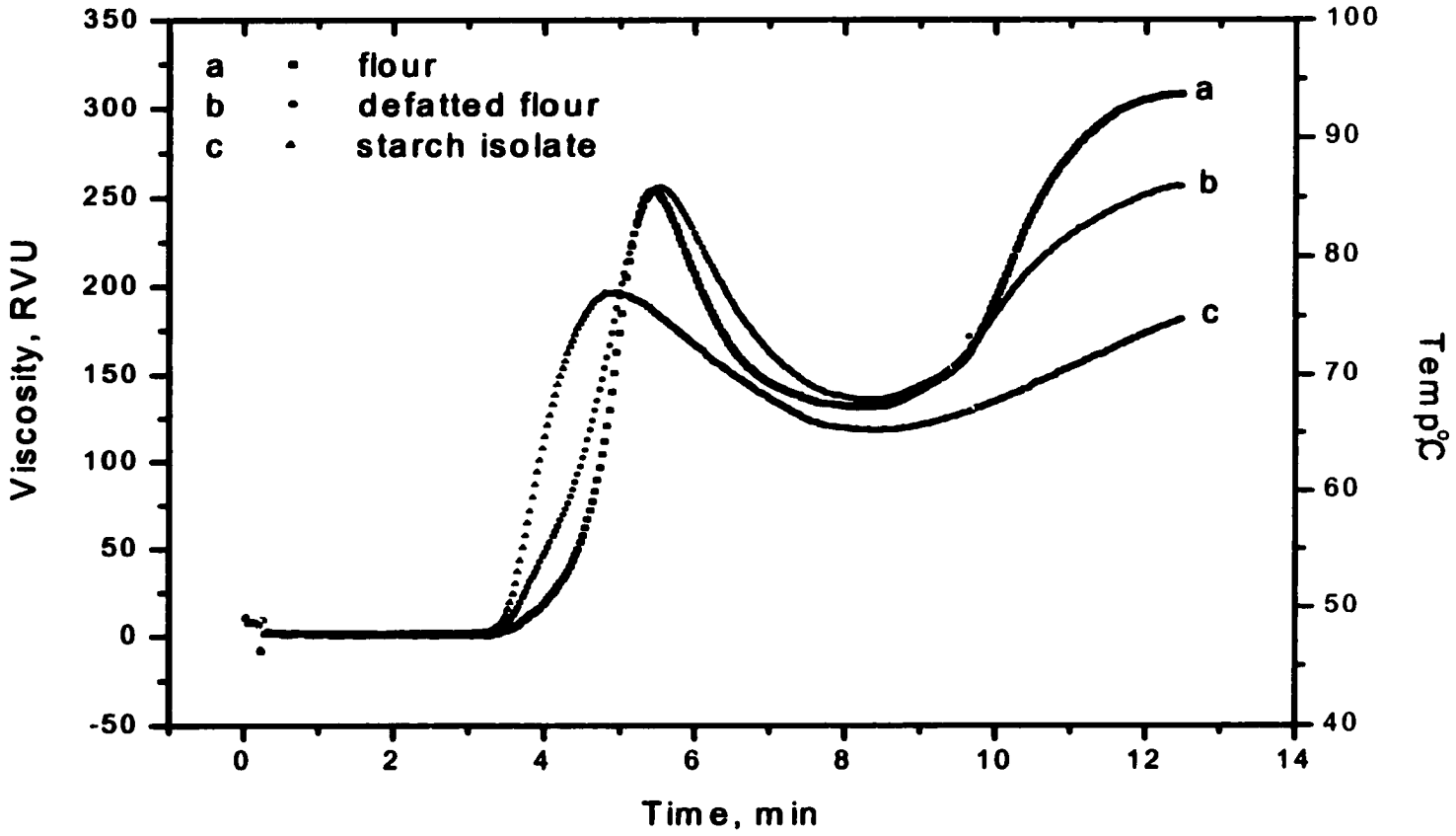


Fig. 3.1. Effects of lipids and protein removal on pasting properties of white rice flour

swelling and reduce the amylogram viscosity (Hamaker and Griffin 1993; Yang and Chang 1999). Protein removal by solvent extraction was reported to increase the paste viscosity (Yang and Chang 1999). In this study, alkaline protease digestion was used to remove proteins from the defatted white flour. Starch isolation by alkaline protease digestion was reported to generate amino acid salts as the co-products (Lumdubwong and Seib 2000). Therefore, the decrease of the paste viscosity (PV, MV, FV), in this study, might be due to the presence of amino acids in the isolated starch.

Brown flour (Table 3.4, Fig. 3.2) had a lower PV (54 RVU) and a greater PT ($92^0$C) than white flour (PV: 250 RVU, PT: $80^0$C), which might be related to the higher protein and lipids contents in brown flour. Brown flour showed no viscosity peak and breakdown, i.e., the viscosity continued to increase after passing the initial $95^0$C viscosity. This behavior reflects the greater stability of the swollen starch granule against mechanical disintergration. Defatting increased the PV, MV, FV by 14, 9, and 14 RVU respectively. The pasting temperature was reduced by $2^0$C while the TSB was increased by 4 RVU. Removing protein from defatted brown flour caused a further increase of PV by 14 RVU, whereas the MV was reduced by 22 RVU and the pasting temperature was further reduced by $9^0$C. The TSB was increase by 24 RVU.

### 3.3.2. Effects of Lipids, Amino Acids, and β-Cyclodextrin on Pasting Properties of Commercial Starch

**Lipids Effect**

Compared to the control (commercial starch), the presence of 0.2% monopalmitin (MP) greatly increased the PV, MV, and FV by 35, 33, and 41 RVU

Table 3.4. Effect of Lipid and Protein Removal on Pasting Properties of Brown Rice Flour [1,2,3]

| Sample | PV | MV | FV | PT | TP | SBK | TSB | BKD |
|---|---|---|---|---|---|---|---|---|
| Flour | 54[c] | 56.88[b] | 92.13[b] | 92.33[a] | 6.88[a] | 38.13[a] | 35.25[c] | -2.88[c] |
| Defatted Flour | 68.03[b] | 66.42[a] | 105.96[a] | 90.15[b] | 6.6[a] | 37.88[a] | 39.54[b] | 1.67[b] |
| Starch Isolate | 81.83[a] | 44.63[c] | 107.42[a] | 81.7[c] | 5.85[b] | 25.58[b] | 62.79[a] | 37.21[a] |

[1]PV=Peak Viscosity, MV=Minimum Viscosity, FV=Final Viscosity, PT=Pasting Temperature, TP=Time to Peak, SBK=Set Back. TSB=Total Set Back, BKD=Breakdown;
[2]Units: Viscosity (RVU), Temperature (°C), Time (min).
[3]Different letters within each column indicate values are significantly different at the level of $p \leq 0.05$.

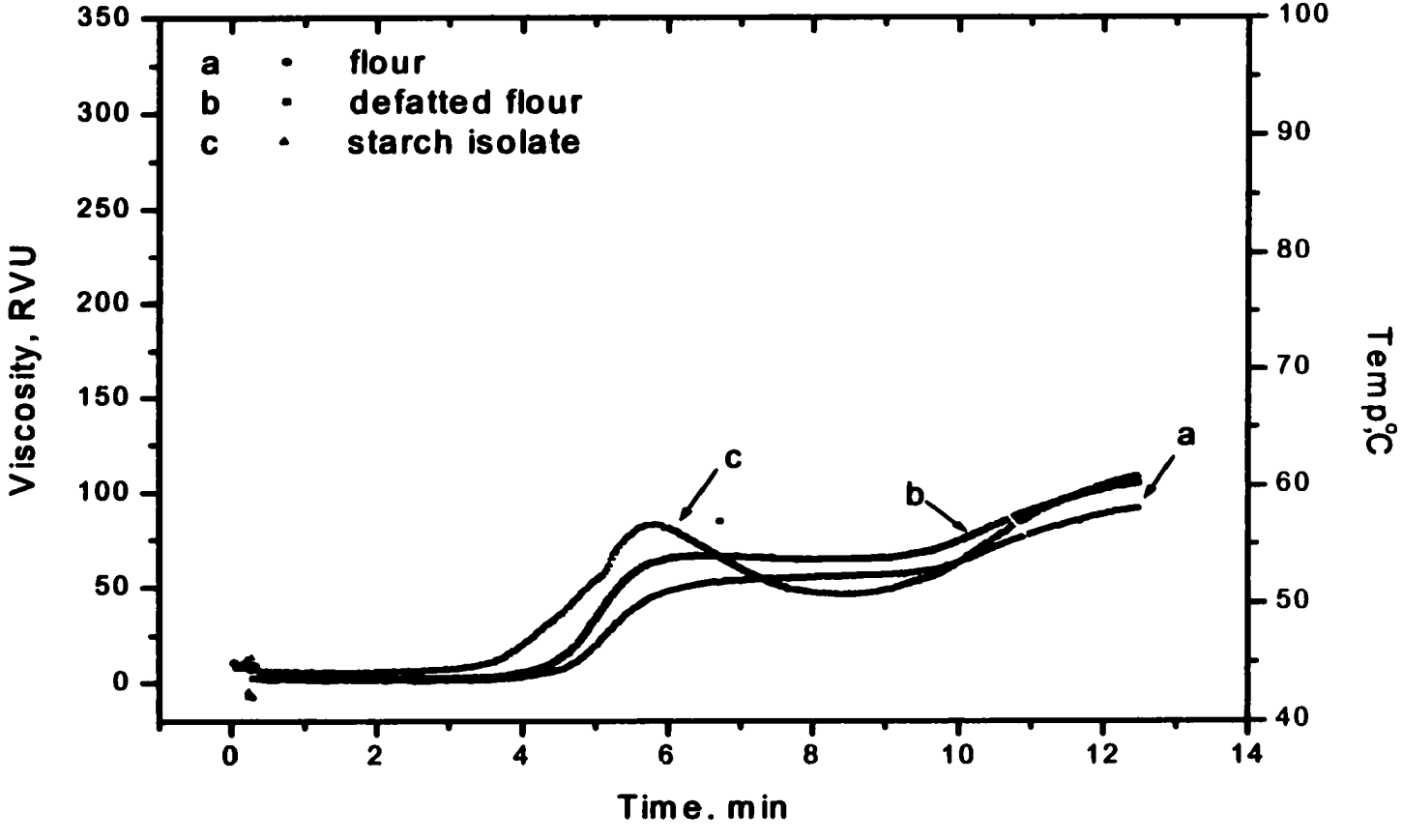


Fig. 3.2. Effects of lipids and protein removal on pasting characteristics of brown rice flour

Table 3.5. Effects of Lipids on Pasting Properties of Commercial Starch [1,2,3]

| Sample | Additive[4] (%) | PV | MV | FV | PT | TP | SBK | TSB | BKD |
|---|---|---|---|---|---|---|---|---|---|
| Control | ----- | 162.17$^{d}$ | 141.17$^{ef}$ | 217.34$^{ij}$ | 89.38$^{d}$ | 6.7$^{cde}$ | 55.17$^{gh}$ | 76.17$^{def}$ | 21.01$^{cdef}$ |
| Palmitic | 0.2 | 167.55$^{cd}$ | 152.13$^{c}$ | 244.25$^{defg}$ | 90.13$^{bcd}$ | 6.86$^{abc}$ | 76.71$^{cde}$ | 92.13$^{bc}$ | 15.42$^{fg}$ |
| | 0.6 | 162$^{d}$ | 142.21$^{def}$ | 260.5$^{bc}$ | 89.53$^{d}$ | 6.8$^{abcde}$ | 98.5$^{a}$ | 118.29$^{a}$ | 19.79$^{cdef}$ |
| Stearic | 0.2 | 168.84$^{bcd}$ | 150.75$^{cd}$ | 220.13$^{ij}$ | 90.13$^{bcd}$ | 6.8$^{abcde}$ | 51.29$^{h}$ | 69.38$^{f}$ | 18.09$^{defg}$ |
| | 0.6 | 166.09$^{d}$ | 144.84$^{cdef}$ | 227.38$^{hij}$ | 89.25$^{d}$ | 6.85$^{abc}$ | 61.29$^{fgh}$ | 82.54$^{cde}$ | 21.25$^{cde}$ |
| Oleic | 0.2 | 164.42$^{d}$ | 147.88$^{cde}$ | 235.71$^{fgh}$ | 91.2$^{abcd}$ | 6.83$^{abcd}$ | 71.3$^{ef}$ | 87.84$^{bcd}$ | 16.54$^{efg}$ |
| | 0.6 | 167.42$^{cd}$ | 149.29$^{cde}$ | 247.63$^{de}$ | 89.5$^{d}$ | 6.89$^{ab}$ | 80.21$^{bcde}$ | 98.34$^{b}$ | 18.13$^{defg}$ |
| Linoleic | 0.2 | 167.79$^{cd}$ | 145.83$^{cde}$ | 236.75$^{fgh}$ | 90.05$^{cd}$ | 6.73$^{bcde}$ | 68.96$^{efg}$ | 90.92$^{bc}$ | 21.96$^{cde}$ |
| | 0.6 | 166.05$^{d}$ | 136.21$^{f}$ | 252.13$^{cde}$ | 90.95$^{abcd}$ | 6.67$^{de}$ | 86.08$^{abcd}$ | 115.92$^{a}$ | 29.84$^{ab}$ |
| Linolenic | 0.2 | 167.83$^{cd}$ | 143.38$^{cdef}$ | 234.67$^{gh}$ | 89.78$^{cd}$ | 6.8$^{abcde}$ | 66.84$^{efg}$ | 91.3$^{bc}$ | 24.46$^{bc}$ |
| | 0.6 | 167.96$^{cd}$ | 136.13$^{f}$ | 253.66$^{cd}$ | 89.7$^{cd}$ | 6.64$^{e}$ | 85.71$^{abcd}$ | 117.54$^{a}$ | 31.84$^{a}$ |
| MP | 0.2 | 197.63$^{a}$ | 174.29$^{a}$ | 258.75$^{c}$ | 95.25$^{a}$ | 6.72$^{bcde}$ | 61.13$^{fgh}$ | 84.46$^{cd}$ | 23.34$^{cd}$ |
| | 0.6 | 199.88$^{a}$ | 167.84$^{ab}$ | 289.25$^{a}$ | 95.25$^{a}$ | 6.72$^{bcde}$ | 89.38$^{abc}$ | 121.42$^{a}$ | 32.04$^{a}$ |
| Tripalmitin | 0.2 | 165.79$^{d}$ | 147.67$^{cde}$ | 217.42$^{ij}$ | 89.88$^{cd}$ | 6.8$^{abcde}$ | 51.63$^{h}$ | 69.75$^{ef}$ | 18.13$^{defg}$ |
| | 0.6 | 167.88$^{cd}$ | 147.17$^{cde}$ | 216.63$^{j}$ | 90.5$^{bcd}$ | 6.8$^{abcde}$ | 48.75$^{h}$ | 69.46$^{ef}$ | 20.71$^{cdef}$ |
| LC | 0.2 | 170.25$^{bcd}$ | 152.42$^{c}$ | 242.29$^{efg}$ | 92.83$^{abcd}$ | 6.93$^{a}$ | 72.04$^{def}$ | 89.87$^{bc}$ | 17.83$^{defg}$ |
| | 0.6 | 176.88$^{bc}$ | 170.38$^{ab}$ | 271.04$^{b}$ | 93.93$^{abc}$ | 6.97$^{a}$ | 94.17$^{ab}$ | 100.67$^{b}$ | 6.50$^{h}$ |
| LE | 0.2 | 166.92$^{cd}$ | 147.08$^{cde}$ | 246.04$^{def}$ | 94.45$^{ab}$ | 6.93$^{a}$ | 79.12$^{cde}$ | 98.96$^{b}$ | 19.84$^{cdef}$ |
| | 0.6 | 177.80$^{b}$ | 164.59$^{b}$ | 228$^{hi}$ | 95.23$^{a}$ | 6.97$^{a}$ | 50.21$^{h}$ | 63.42$^{f}$ | 13.21$^{g}$ |

[1]MP=Monopalmitin, LC=Lysophosphatidylcholine, LE=Lysophosphatidylethanolamine, PV=Peak Viscosity, MV=Minimum Viscosity, FV=Final Viscosity, PT=Pasting Temperature, TP=Time to Peak, SBK=Set Back. TSB=Total Set Back, BKD=Breakdown;

[2]Units: Viscosity (RVU), Temperature ($^{o}$C), Time (min).

[3]Different letters within each column indicate values are significantly different at the level of $p \leq 0.05$.

[4]Additive percentage was based on the starch dry weight.

respectively (Table 3.5, Fig. 3.3). The pasting temperatures were increased by 6 $^{0}$C when 0.2% or 0.6% MP were added. However, addition of 0.6% MP greatly increased the FV and TSB by 72 and 45 RVU respectively. On the other hand, the presence of tripalmitin showed no influence on pasting properties of commercial starch (Table 3.5, Fig. 3.4).

At 0.6%, lysophosphatidylcholine (LC) increased the PV, MV, and FV by 14, 29, and 54 RVU, respectively, in comparison to the control (Table 3.5, Fig. 3.5). The pasting temperature was increased by 4$^{0}$C while the time to peak was increased by 0.27 min. However, the BKD of the commercial starch was reduced by 14 RVU. Compared to the control, addition of 0.6% lysophosphatidylethanolamine (LE) increased the PV, MV, and FV by 15, 23, and 11 RVU, respectively (Table 3.5, Fig. 3.6). The pasting temperature was increased by 6$^{0}$C. Compared to the control, the TSB was increased by 22 RVU when 0.2% LE was used. However, the TSB was reduced by 13 RVU when 0.6% LE was present.

Palmitic acid, oleic acid, linoleic acid, and linolenic acid increased the PV of the commercial starch at both levels (0.2, 0.6%) while the stearic acid showed no influence on PV (Table 3.5, Fig. 3.7~3.10). The presence of 0.6% palmitic acid, linoleic acid, and linolenic acid also increased the TSB by 42, 39, and 41 RVU respectively.

Among these lipids, monopalmitin caused the greatest increase of PV, MV, and FV. The highest TSB was observed when 0.6% MP was applied. Besides that, both MP and LE had the biggest increase on pasting temperature. Both LC and LE had greatest time to peak.

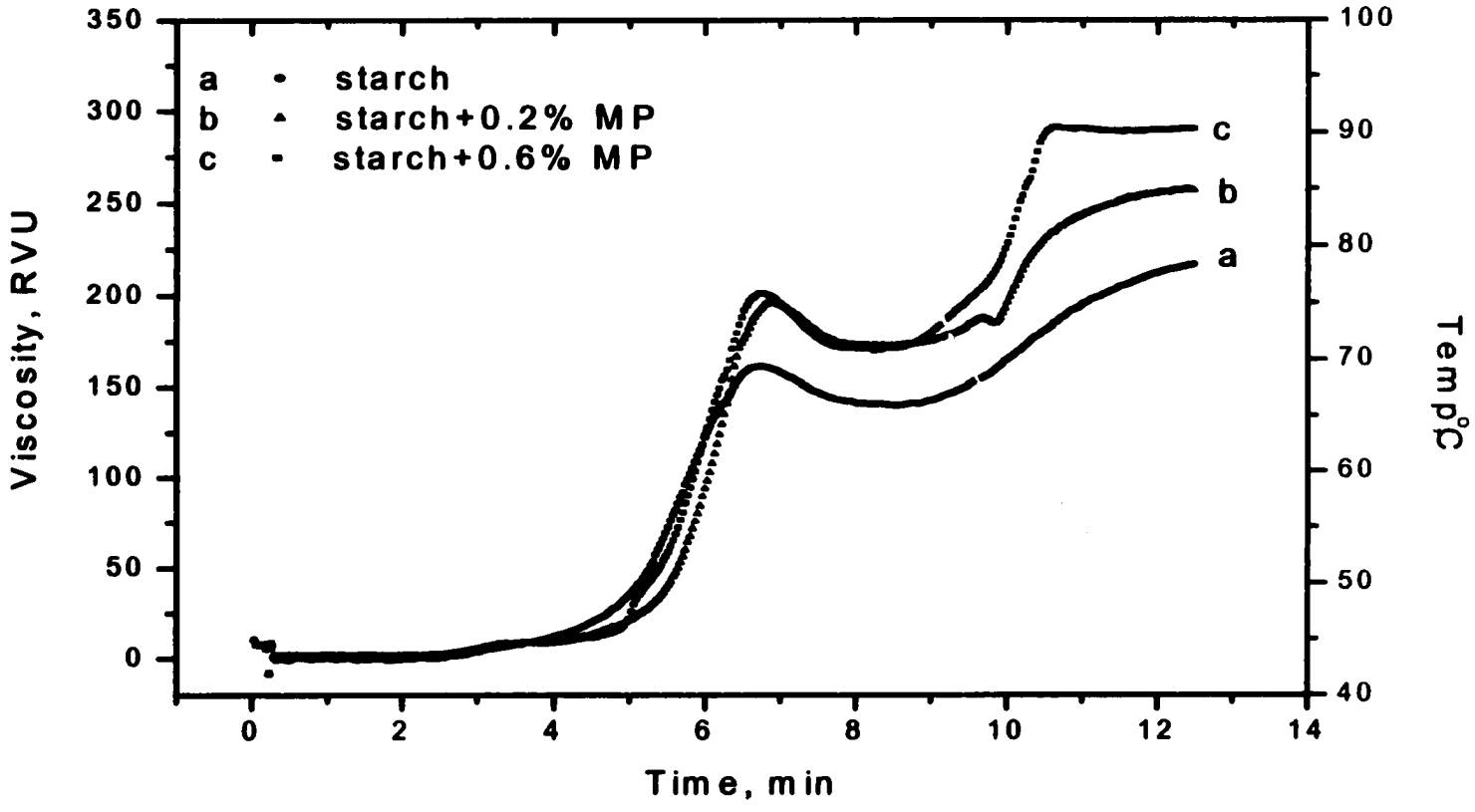


Fig. 3.3. Effects of monopalmitin on pasting properties of commercial starch

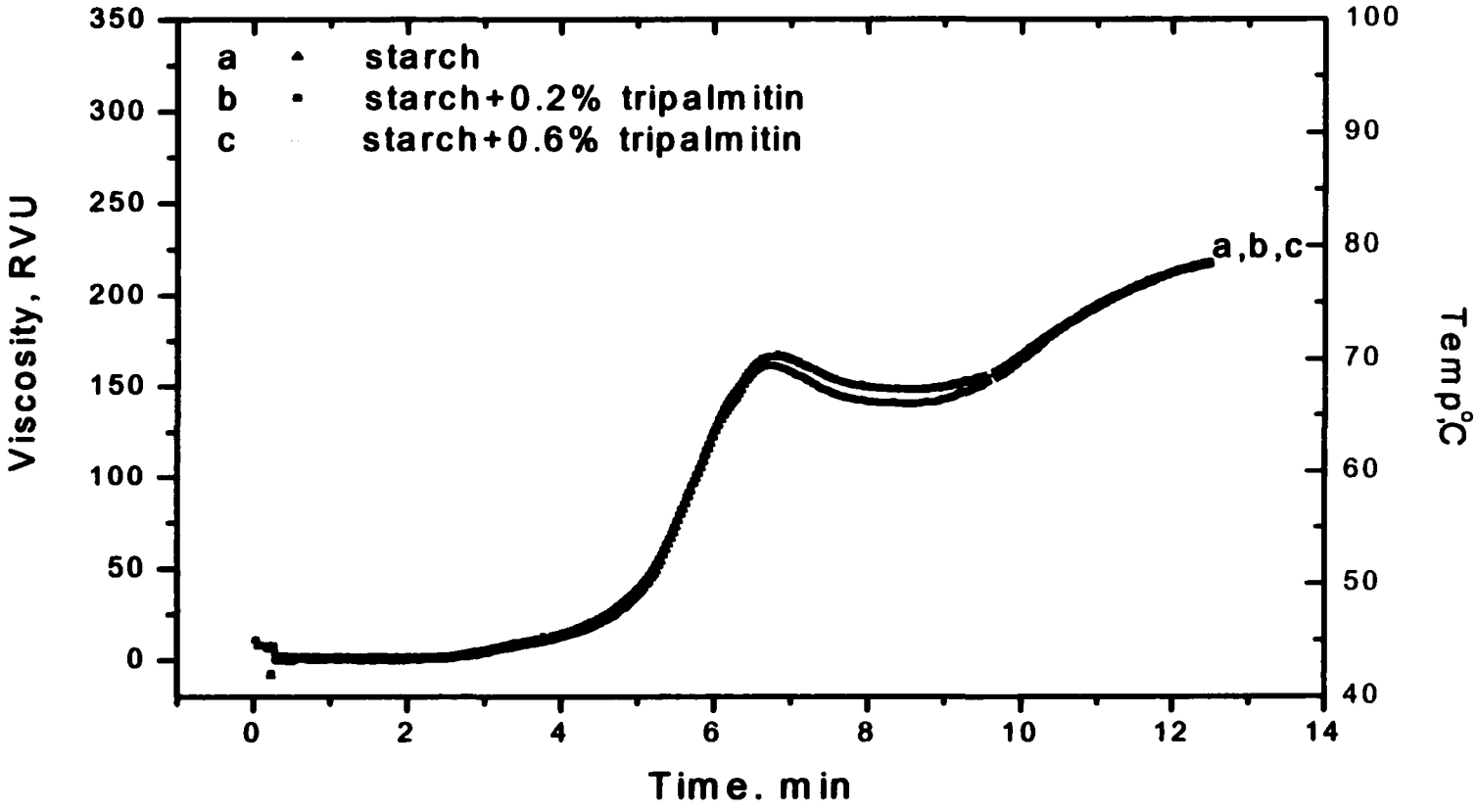


Fig. 3.4. Effects of tripalmitin on pasting properties of commercial starch

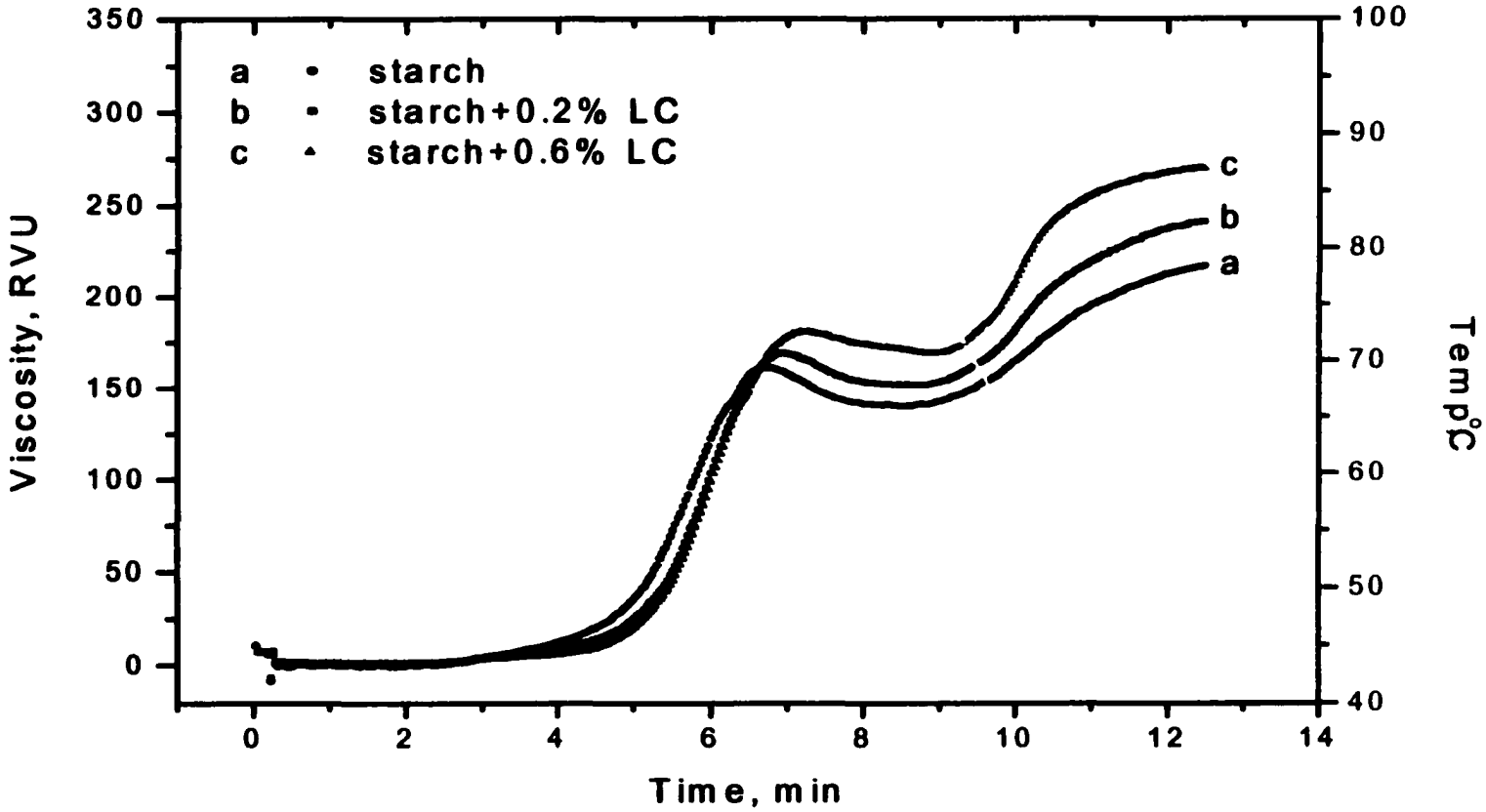


**Fig. 3.5. Effects of lysophosphatidylcholine (LC) on pasting properties of commercial starch**

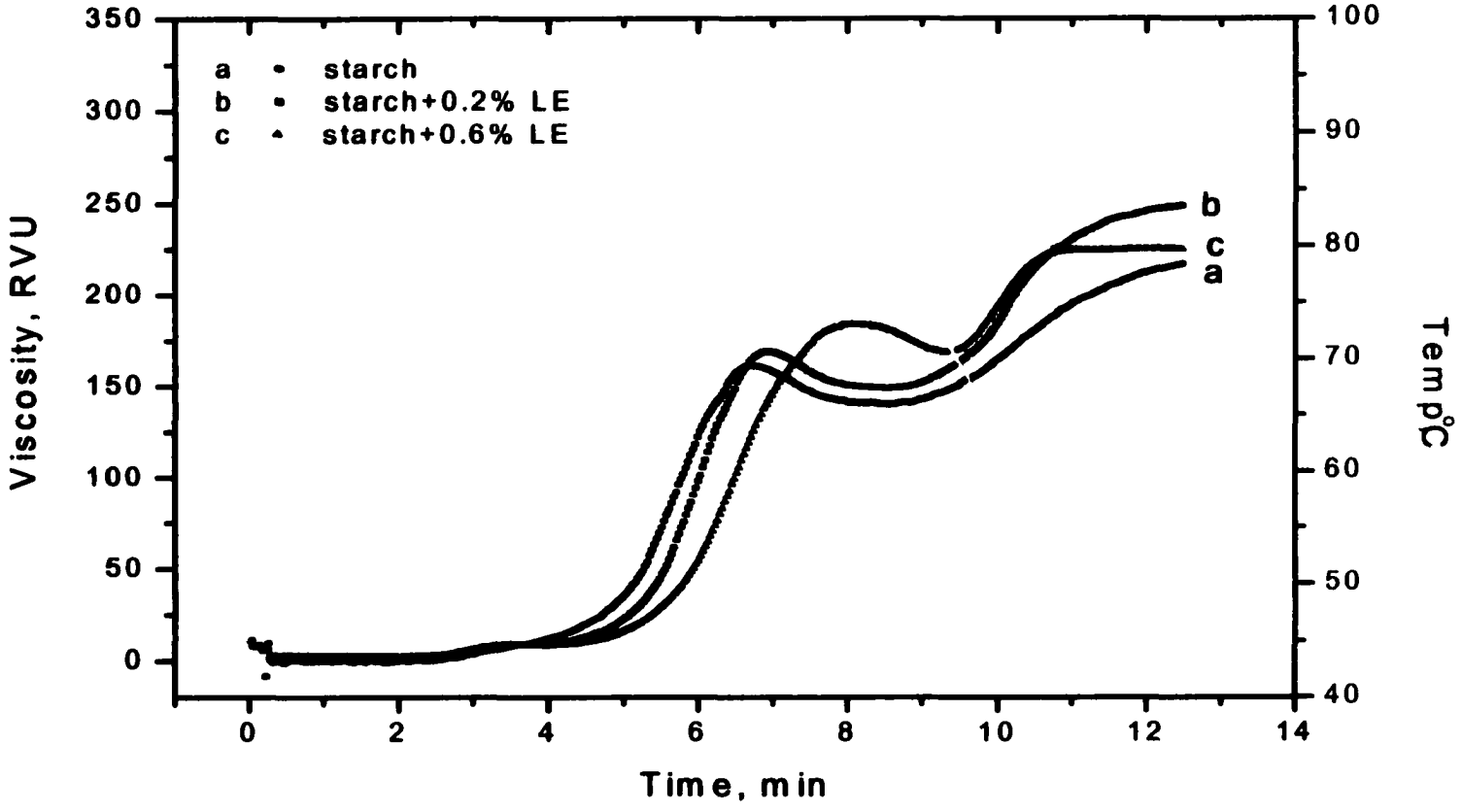


**Fig. 3.6. Effects of lysophosphatidylethanolamine (LE) on pasting properties of commercial starch**

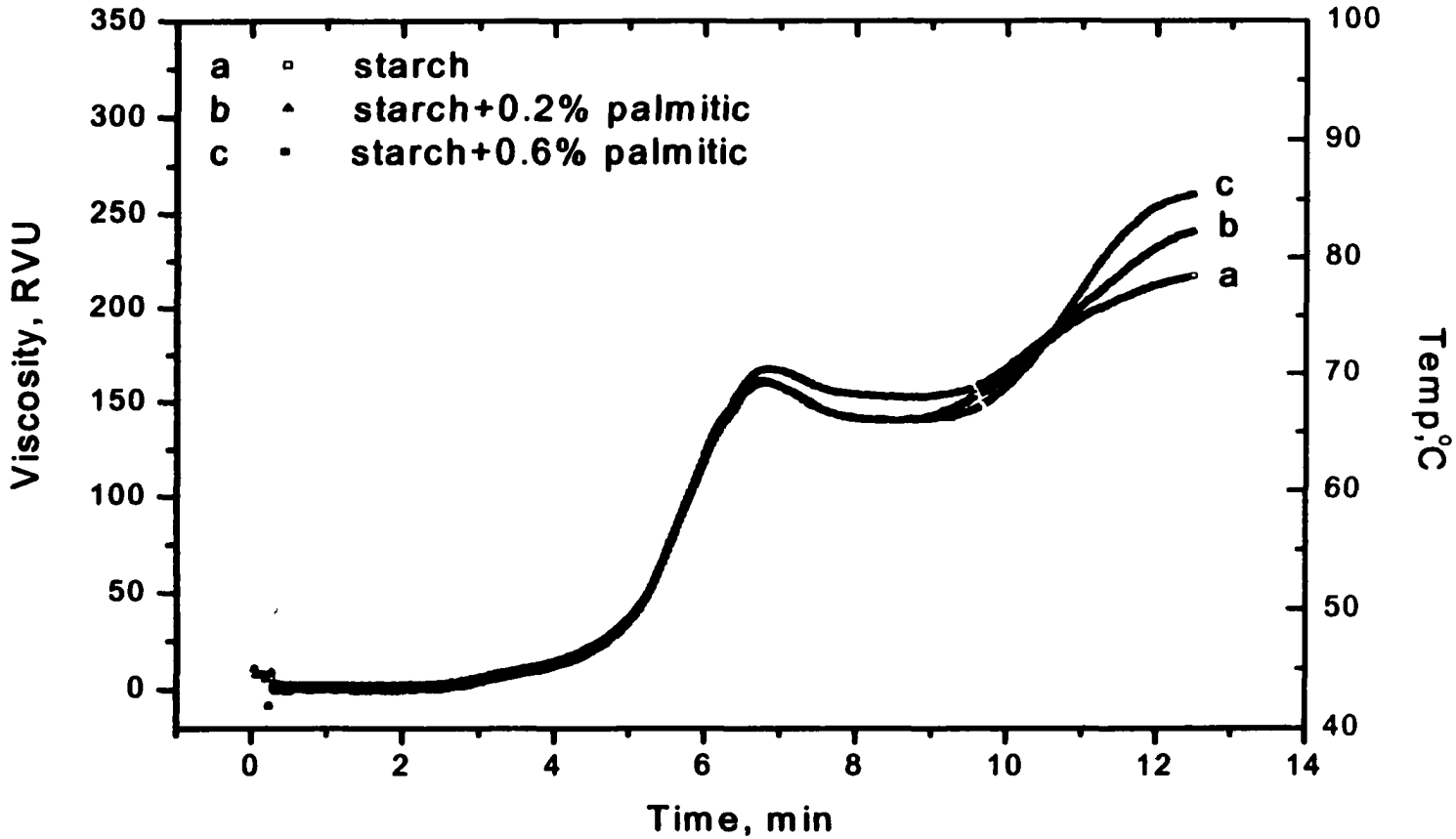


**Fig. 3.7. Effects of palmitic acid on pasting properties of commercial starch**

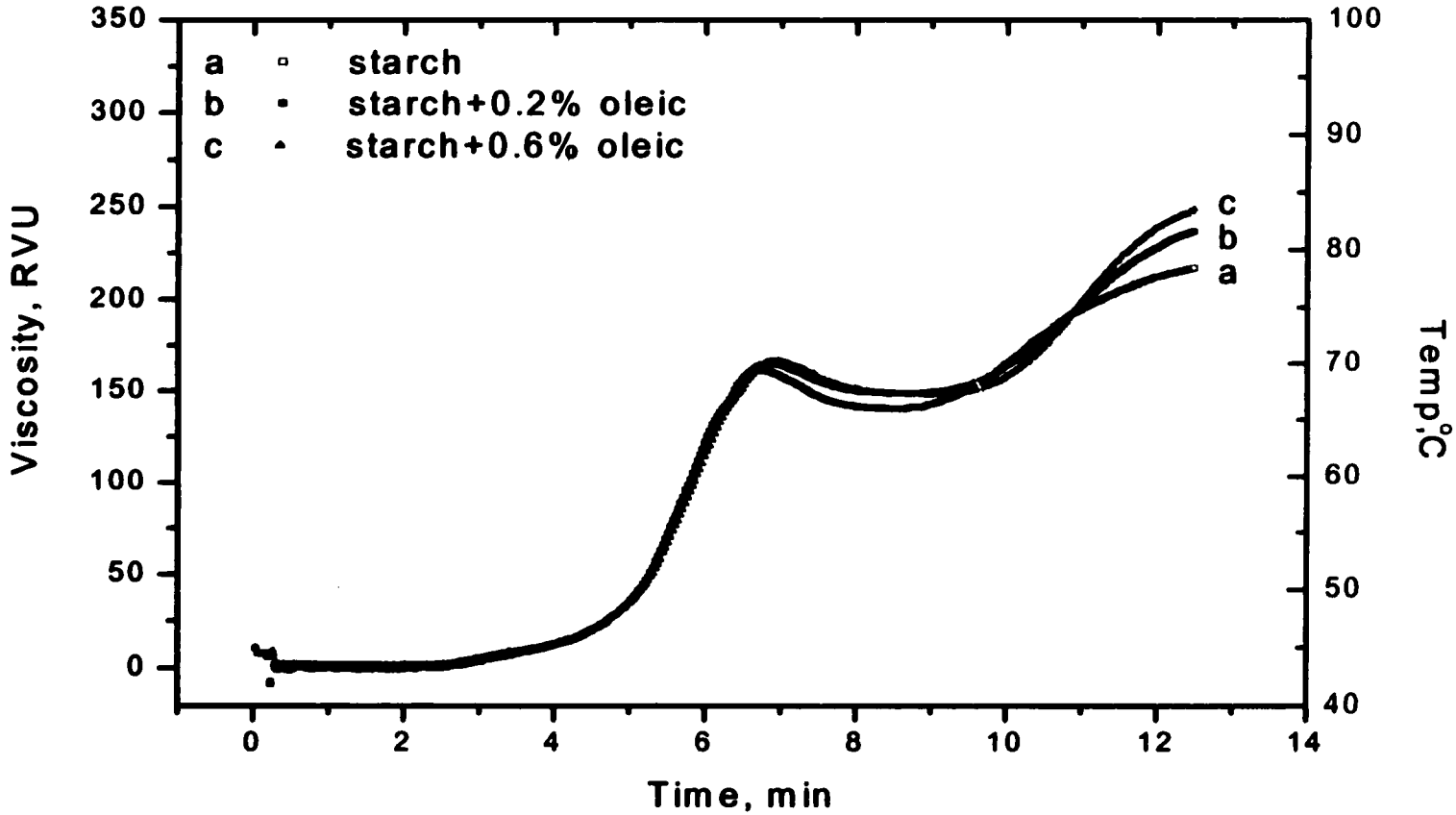


**Fig. 3.8. Effects of oleic acid on pasting properties of commercial starch**

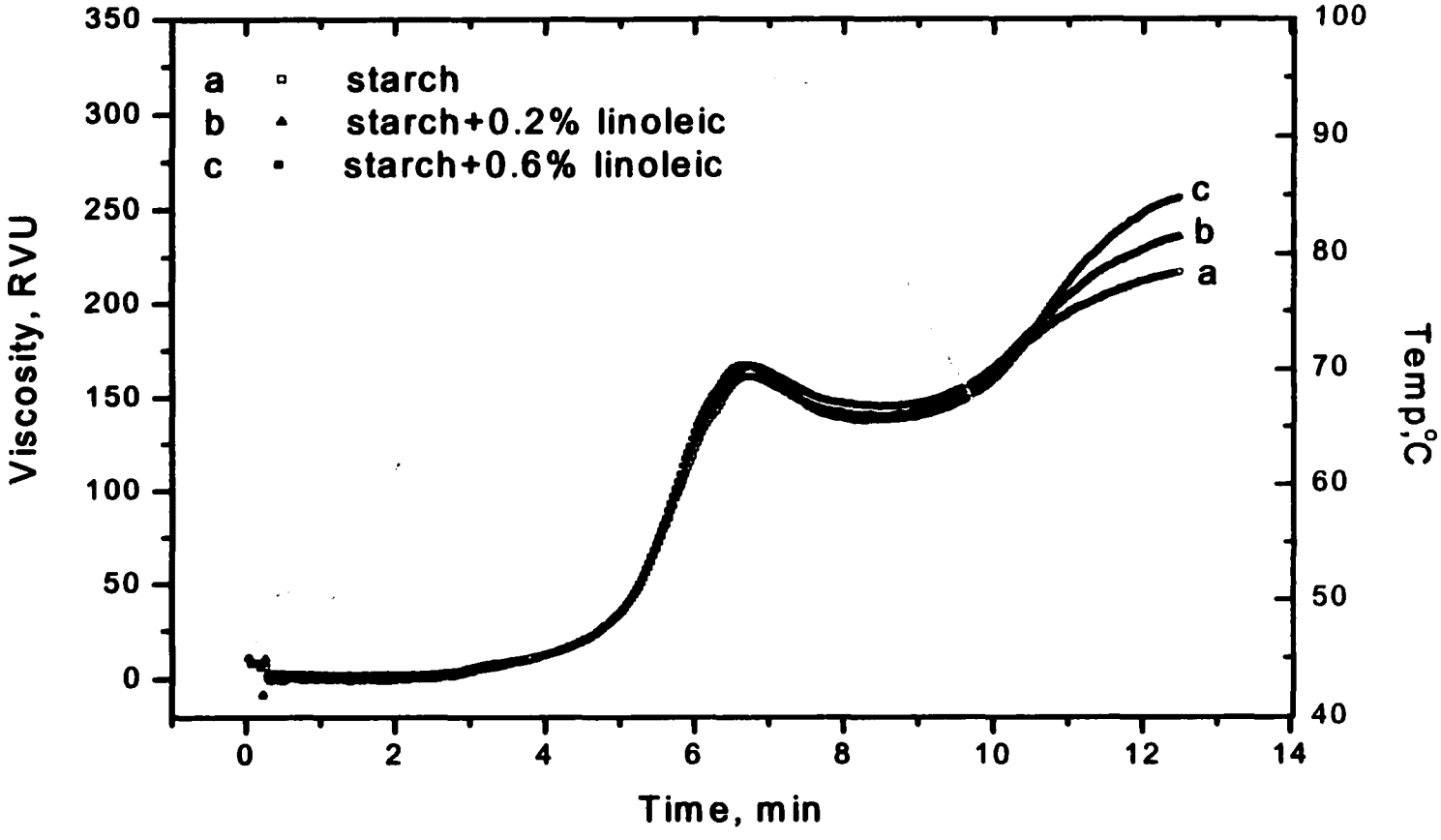


**Fig. 3.9. Effects of linoleic acid on pasting properties of commercial starch**

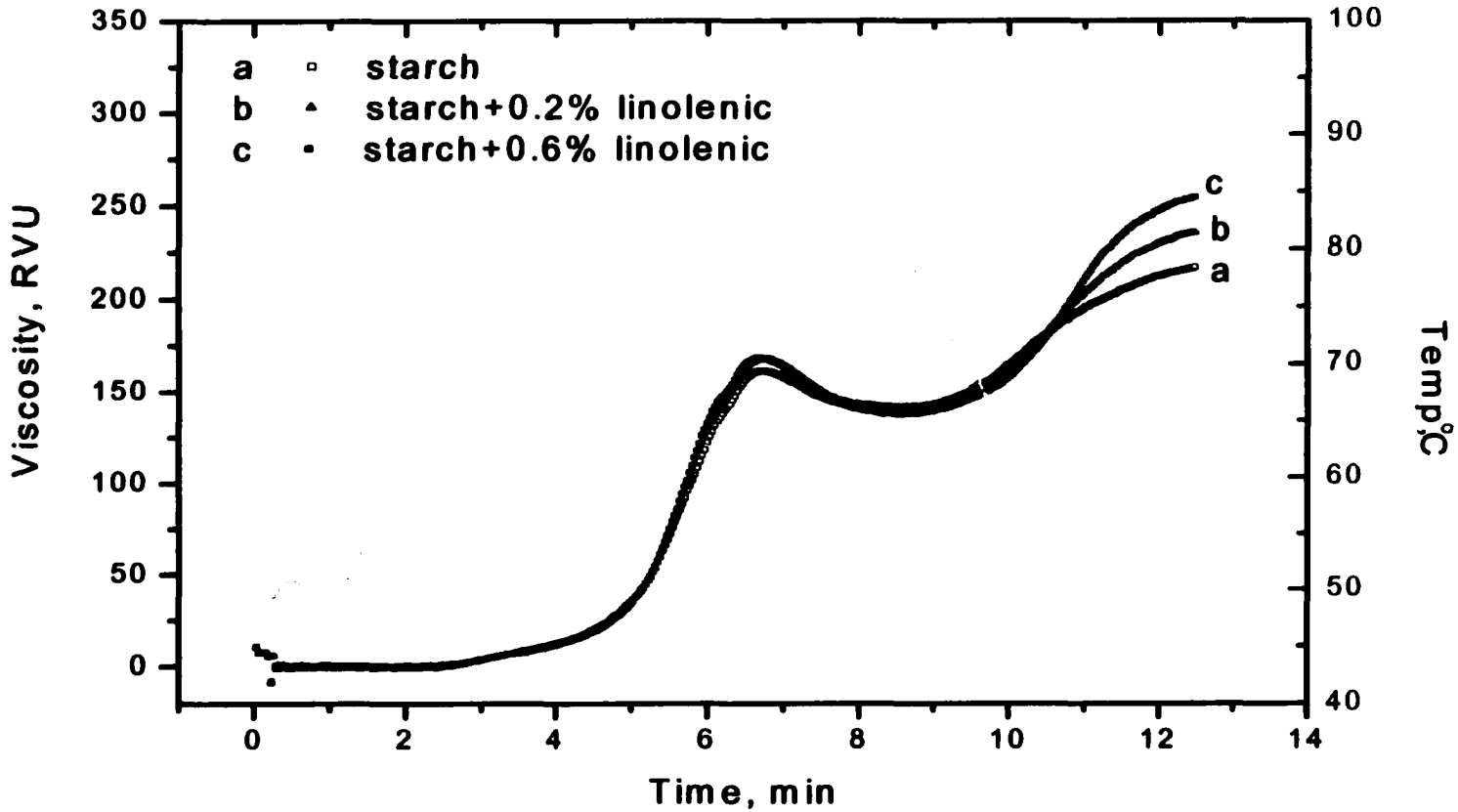


Fig. 3.10. Effects of linolenic acid on pasting properties of commercial starch

Table 3.6. Effects of Amino Acids on Pasting Properties of Commercial Starch [1,2,3]

| Sample | Additives[4] (%) | PV | MV | FV | PT | TP | SBK | TSB | BKD |
|---|---|---|---|---|---|---|---|---|---|
| Control | ----- | 162.17$^{bcd}$ | 141.17$^{c}$ | 217.34$^{a}$ | 89.38$^{a}$ | 6.7$^{bc}$ | 55.17$^{a}$ | 76.17$^{bc}$ | 21.01$^{gh}$ |
| Aspartic | 2 | 162.8$^{bcd}$ | 117.75$^{ef}$ | 169.42$^{d}$ | 91.28$^{a}$ | 6.49$^{d}$ | 6.63$^{g}$ | 51.67$^{g}$ | 45.05$^{de}$ |
| | 6 | 157.08$^{def}$ | 102.63$^{g}$ | 154$^{e}$ | 89.68$^{a}$ | 6.49$^{d}$ | -3.08$^{h}$ | 51.38$^{g}$ | 54.56$^{c}$ |
| Glutamic | 2 | 167.04$^{abc}$ | 127.17$^{d}$ | 182.46$^{c}$ | 89.83$^{a}$ | 6.63$^{cd}$ | 15.42$^{f}$ | 55.29$^{g}$ | 39.88$^{f}$ |
| | 6 | 150.25$^{f}$ | 110.67$^{f}$ | 165.42$^{d}$ | 91.55$^{a}$ | 6.62$^{cd}$ | 15.17$^{f}$ | 54.76$^{g}$ | 39.59$^{f}$ |
| Lysine | 2 | 158.08$^{de}$ | 112.5$^{f}$ | 191.12$^{b}$ | 74.58$^{b}$ | 5.88$^{f}$ | 33.05$^{d}$ | 78.63$^{b}$ | 45.58$^{d}$ |
| | 6 | 160.88$^{cde}$ | 119.75$^{e}$ | 191.71$^{b}$ | 75.15$^{b}$ | 6.05$^{e}$ | 30.84$^{de}$ | 71.96$^{cd}$ | 41.13$^{ef}$ |
| Arginine | 2 | 154.75$^{ef}$ | 93.54$^{h}$ | 181.84$^{c}$ | 74$^{b}$ | 5.41$^{g}$ | 27.09$^{e}$ | 88.3$^{a}$ | 61.21$^{b}$ |
| | 6 | 157.17$^{def}$ | 84.79$^{i}$ | 170.96$^{d}$ | 74.03$^{b}$ | 5.33$^{g}$ | 13.8$^{f}$ | 86.17$^{a}$ | 72.38$^{a}$ |
| Leucine | 2 | 167.96$^{abc}$ | 148.88$^{ab}$ | 220.34$^{a}$ | 89.5$^{a}$ | 6.81$^{ab}$ | 52.38$^{ab}$ | 71.46$^{d}$ | 19.09$^{hi}$ |
| | 6 | 168.71$^{ab}$ | 144.55$^{bc}$ | 219.54$^{a}$ | 90.03$^{a}$ | 6.7$^{bc}$ | 50.83$^{ab}$ | 75$^{bcd}$ | 24.17$^{g}$ |
| Alanine | 2 | 167.13$^{abc}$ | 149.88$^{ab}$ | 216.29$^{a}$ | 89.25$^{a}$ | 6.83$^{ab}$ | 49.17$^{bc}$ | 66.42$^{e}$ | 17.25$^{hi}$ |
| | 6 | 170.92$^{a}$ | 155.04$^{a}$ | 215.75$^{a}$ | 90.38$^{a}$ | 6.9$^{a}$ | 44.83$^{c}$ | 60.71$^{f}$ | 15.88$^{i}$ |

[1]PV=Peak Viscosity, MV=Minimum Viscosity, FV=Final Viscosity, PT=Pasting Temperature, TP=Time to Peak, SBK=Set Back. TSB=Total Set Back, BKD=Breakdown;
[2]Units: Viscosity (RVU), Temperature ($^{o}$C), Time (min).
[3]Different letters within each column indicate values are significantly different at the level of $p \leq 0.05$.
[4]Additive percentage was based on the starch dry weight.

**Amino Acids Effect**

The presence of 6% aspartic acid significantly decreased the MV, FV, and TSB by 39, 63, and 25 RVU, but increased the BKD by 33 RVU (Table 3.6, Fig. 3.11). Addition of 6% glutamic acid decreased the MV, FV, and TSB by 31, 52, and 22 RVU, but increased the BKD by 18 RVU (Table 3.6, Fig. 3.12). Both aspartic and glutamic acids had no influence on pasting temperature. Compared to the control, 6% lysine reduced the MV, FV, and TSB by 22, 26, and 14 RVU (Table 3.6, Fig. 3.13). It also reduced the pasting temperature by $14^0$C and increased the BKD by 20 RVU. Addition of 6% arginine reduced the MV and FV by 57 and 47 RVU, respectively, but increased the TSB by 10 RVU (Table 3.6, Fig. 3.14). It also reduced the pasting temperature by $15^0$C and increased the BKD by 51 RVU. Alanine (6%) increased the MV by 14 RVU but decreased the TSB by 16 RVU (Table 3.6, Fig. 3.15). Leucine showed no influence on pasting properties of commercial starch at both levels (Table 3.6, Fig. 3.16).

Among those amino acids, arginine caused the lowest MV and pasting temperature, the shortest time to peak, and the greatest break down. On the other hand, addition of aspartic acid resulted in the lowest FV, SBK, and TSB. The presence of lysine also caused the great reduction of pasting temperature, which was similar to arginine. Our results indicated that the negative charged amino acids (aspartic and glutamic acids) had a similar effect as that of the positive charged amino acids (lysine and arginine). Those charged amino acids significantly depressed the pasting viscosities (MV and FV), reduced the PT, TP, and SBK, but increased the BKD. Those neutral amino acids (alanine and leucine), on the other hand, were less effective

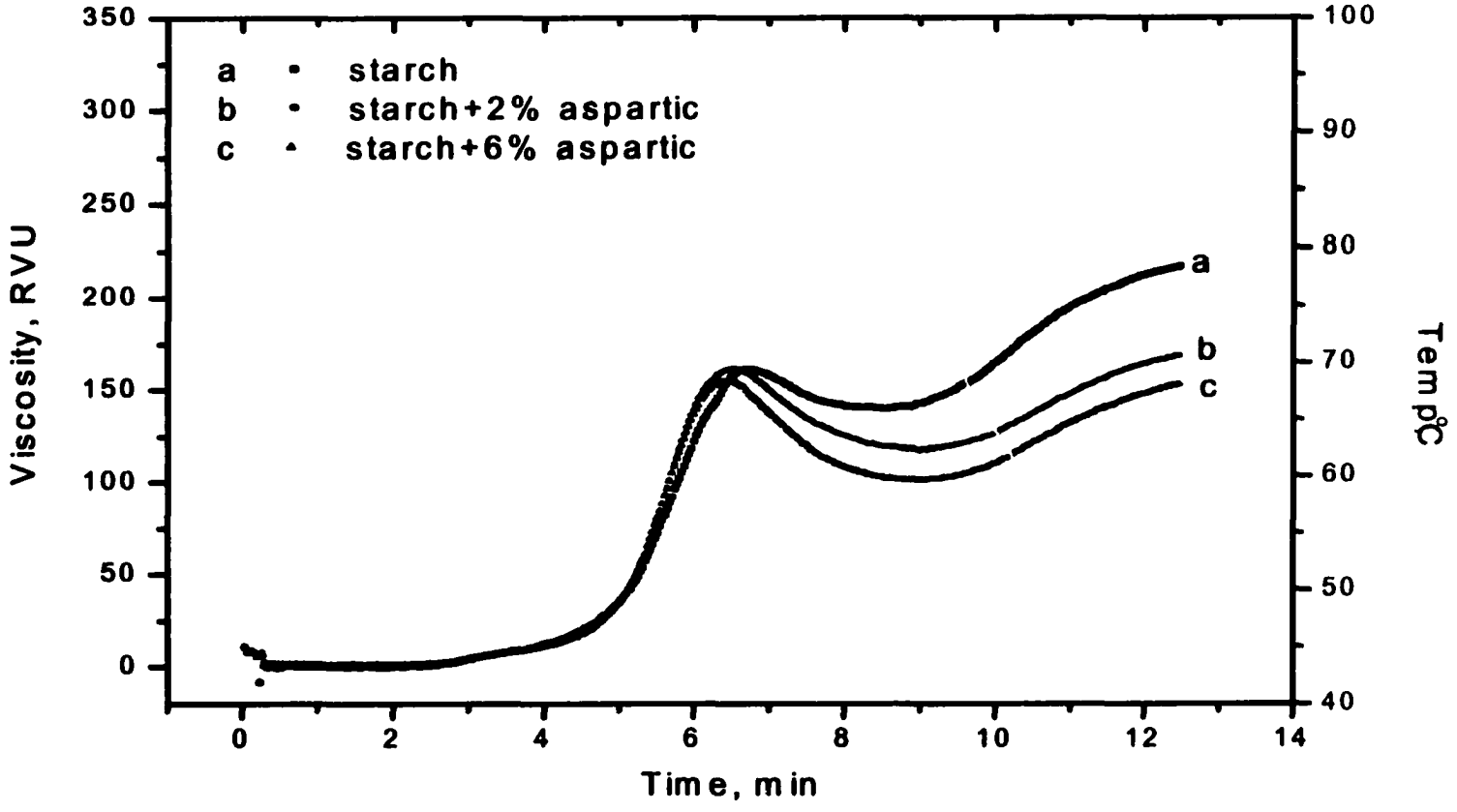


Fig. 3.11. Effects of aspartic acid on pasting properties of commercial starch

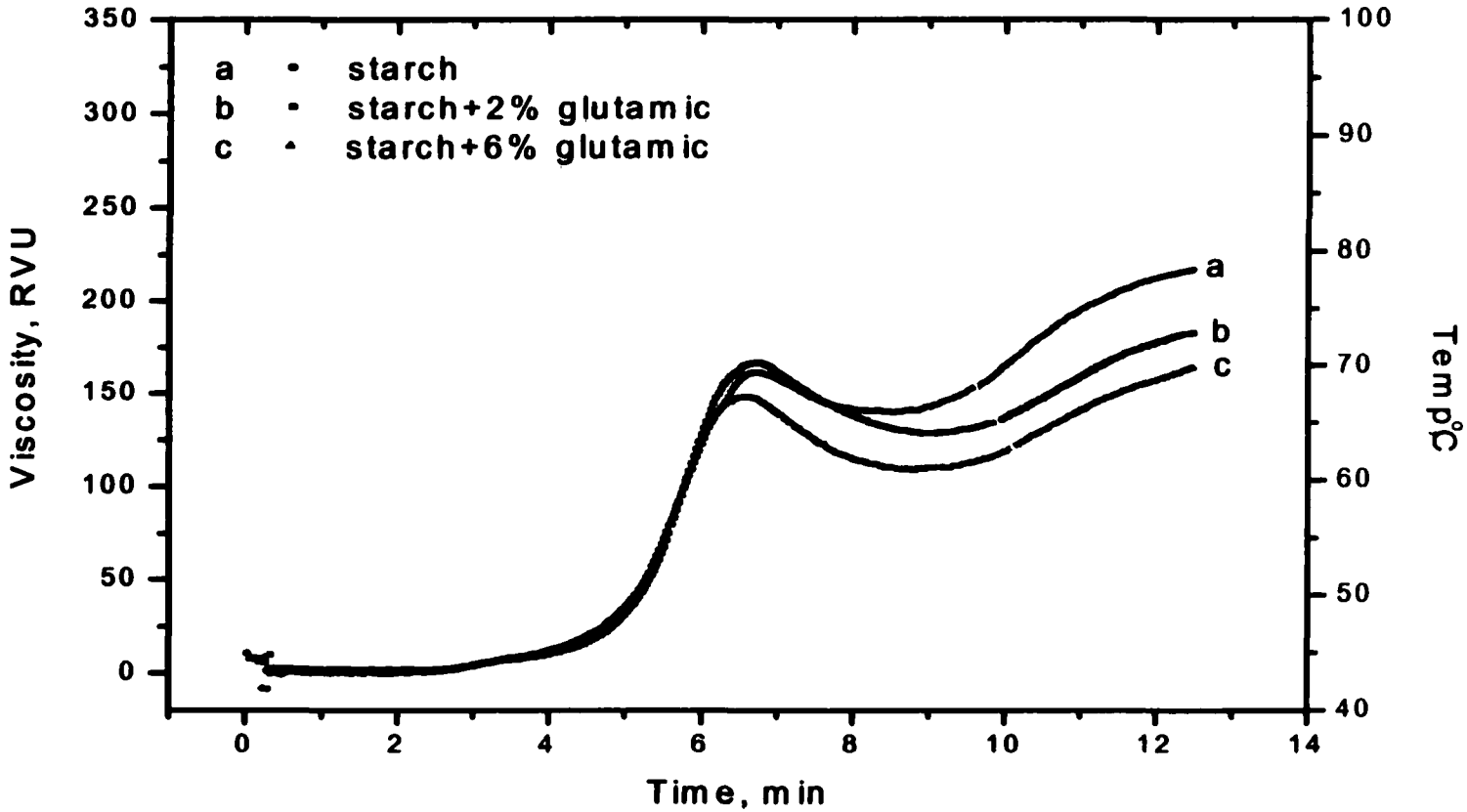


Fig. 3.12. Effect of glutamic acid on pasting properties of commercial starch

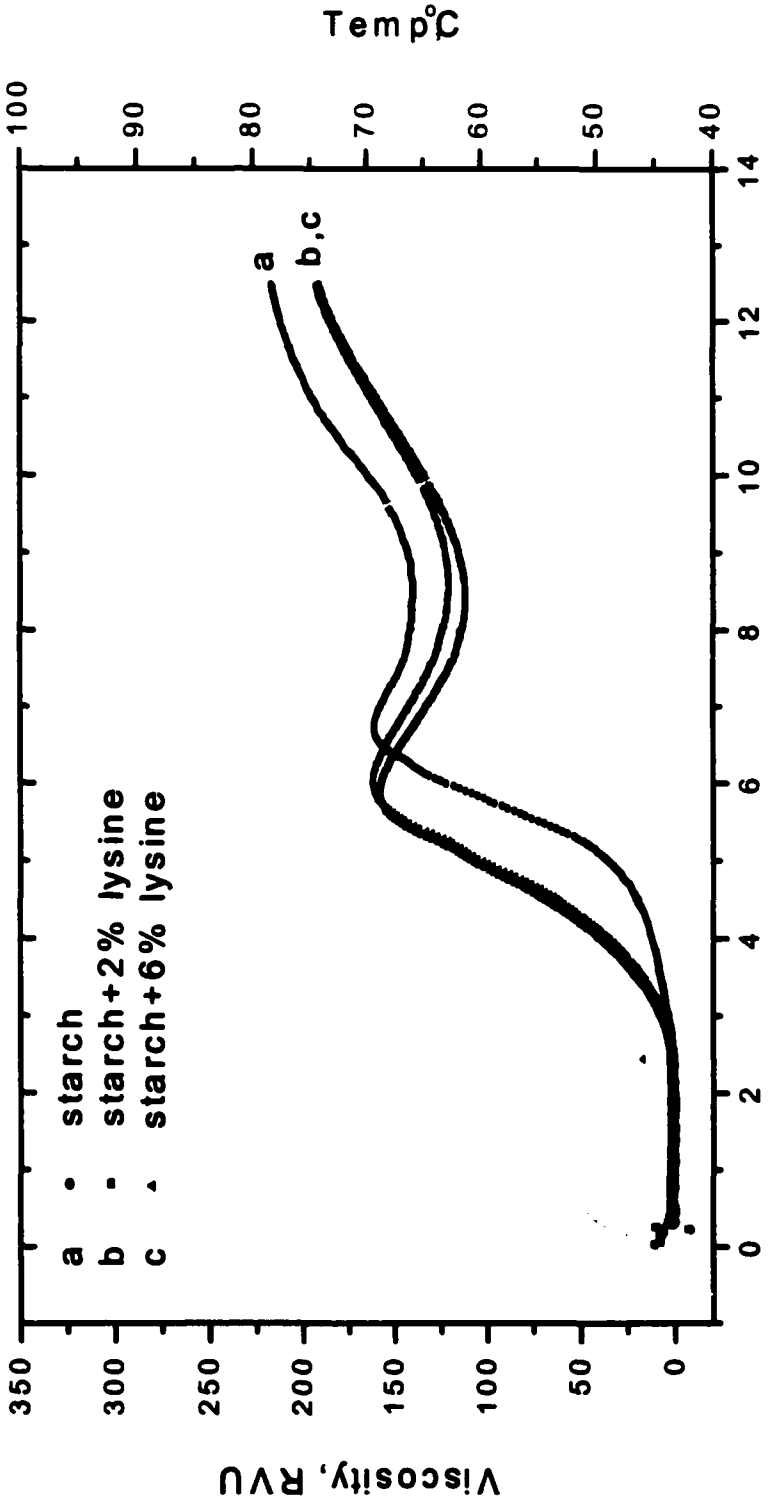


Fig. 3.13. Effect of lysine on pasting properties of commercial starch

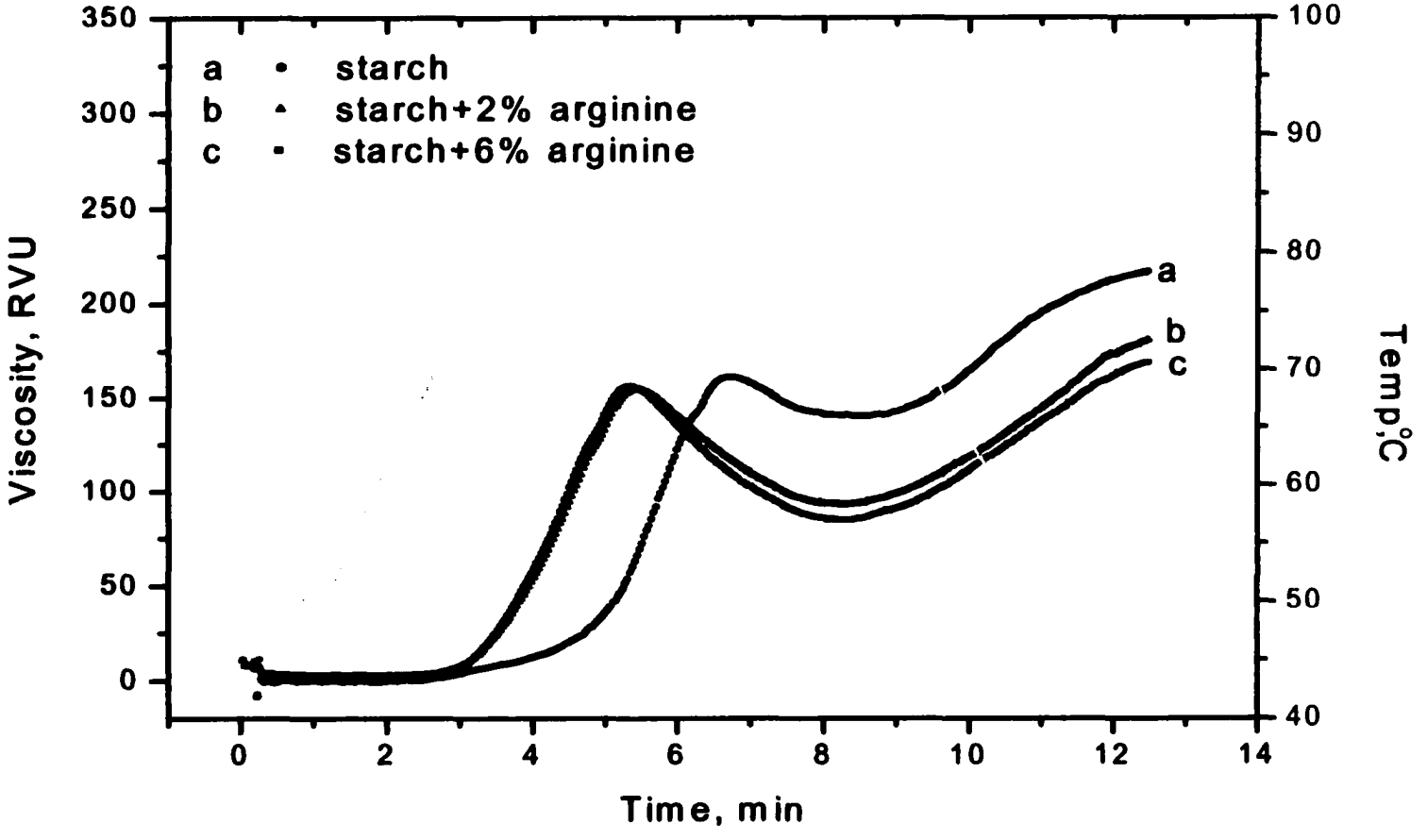


Fig. 3.14. Effect of arginine on pasting properties of commercial starch

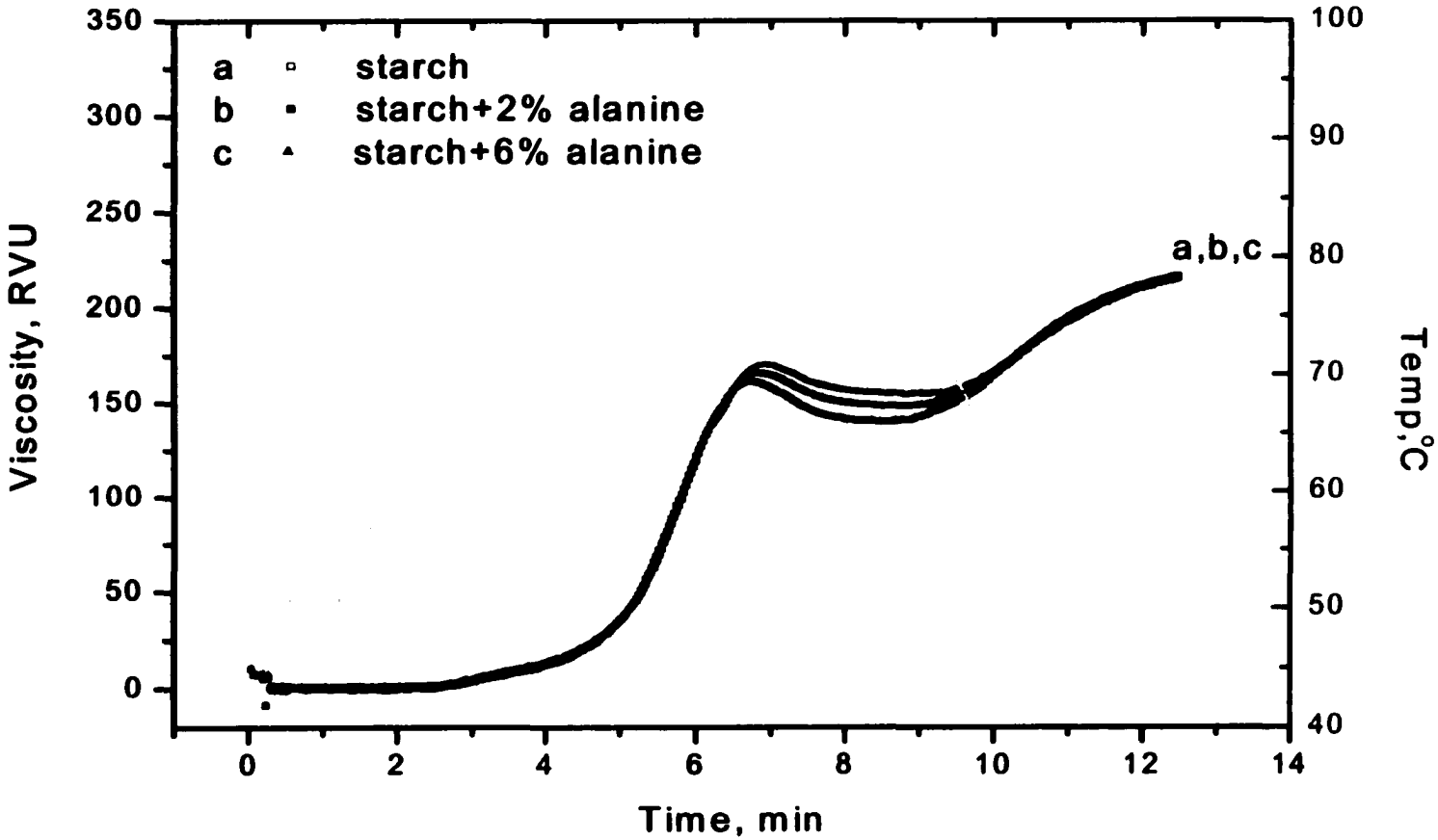


Fig. 3.15. Effect of alanine on pasting properties of commercial starch

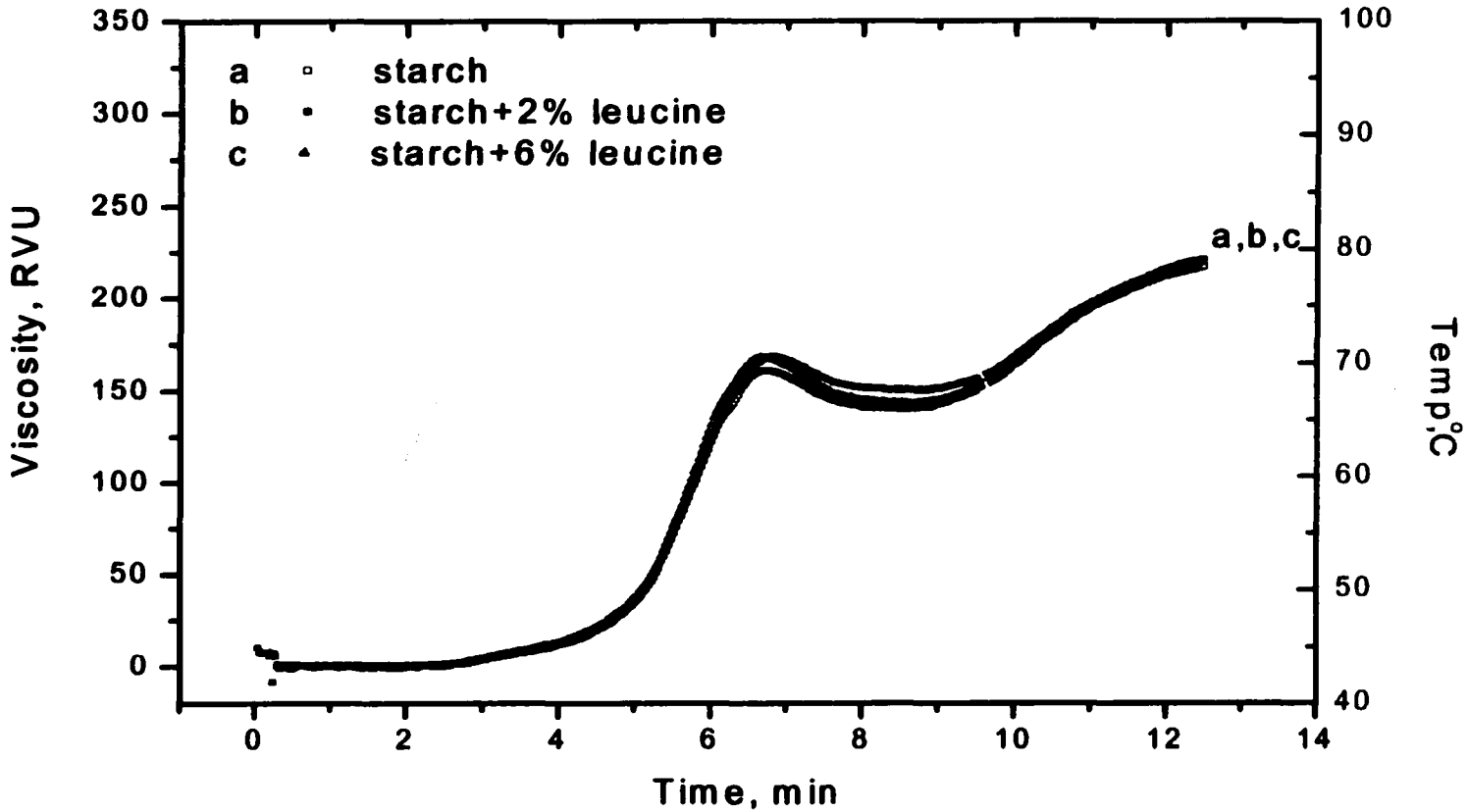


Fig. 3.16. Effect of leucine on pasting properties of commercial starch

Table 3.7. Effect of β-Cyclodextrin on Pasting Properties of Commercial Starch[1,2,3]

| Sample | Additive[4] (%) | PV | MV | FV | PT | TP | SBK | TSB | BKD |
|---|---|---|---|---|---|---|---|---|---|
| Control | ----- | 162.17[a] | 141.17[b] | 217.34[a] | 89.38[a] | 6.7[a] | 55.17[a] | 76.17[a] | 21.01[ab] |
| β-CD | 2 | 166.63[a] | 147.13[a] | 217.55[a] | 87.10[a] | 6.72[a] | 50.92[b] | 70.42[b] | 19.50[b] |
| | 6 | 162.67[a] | 140.92[b] | 208.79[b] | 85.93[a] | 6.59[a] | 46.13[c] | 67.87[b] | 21.75[a] |

[1]PV=Peak Viscosity, MV=Minimum Viscosity, FV=Final Viscosity, PT=Pasting Temperature, TP=Time to Peak, SBK=Set Back. TSB=Total Set Back, BKD=Breakdown;
[2]Units: Viscosity (RVU), Temperature (°C), Time (min).
[3]Different letters within each column indicate values are significantly different at the level of $p \leq 0.05$.
[4]Additive percentage was based on the starch dry weight.

than the charged ones. The influence of amino acids on starch paste viscosity has not been documented. However, several investigators found that addition of proteinase to rice flour could reduce the paste viscosity over the temperature range, and linked this phenomenon to the influence of protein hydrolyzed product on starch pasting (Hamaker and Griffin 1990). The result from our study supported the findings of Hamaker and Griffin (1990), i.e., amino acids could reduce the paste viscosity of starch.

**β-Cylodextrin Effect**

Addition of 2% β-CD increased the MV by 6 RVU when compared to the control (Table 3.7, Fig. 3.17). However, further increase of the concentration to 6% caused the MV to drop to a level similar to the control. In addition, the presence of 6% β-CD reduced the final viscosity by 9 RVU. The presence of 6% β-CD reduced the TSB by 9 RVU. Cyclodextrins were reported to increase the swelling power and solubility of wheat starch granules through amylose-lipid complex disruption and cyclodextrin-lipid inclusion (Kim and Hill 1984). Our result showed that β-CD had only a slight influence on pasting properties of rice starch.

### 3.3.3. Effects of Lipids, Amino Acids, and β-Cyclodextrin on Pasting Properties of White Starch Isolate

**Commercial Starch Versus White Starch Isolate**

White starch isolate contains a greater level of amylose and protein residues than the commercial starch did (Table 3.2). It was reported that lower amylose content starches tend to produce greater PV and BKD with lower FV and PT (Endo et al 1989; King et al. 1994; Zeng et al. 1997). Results from this study support that

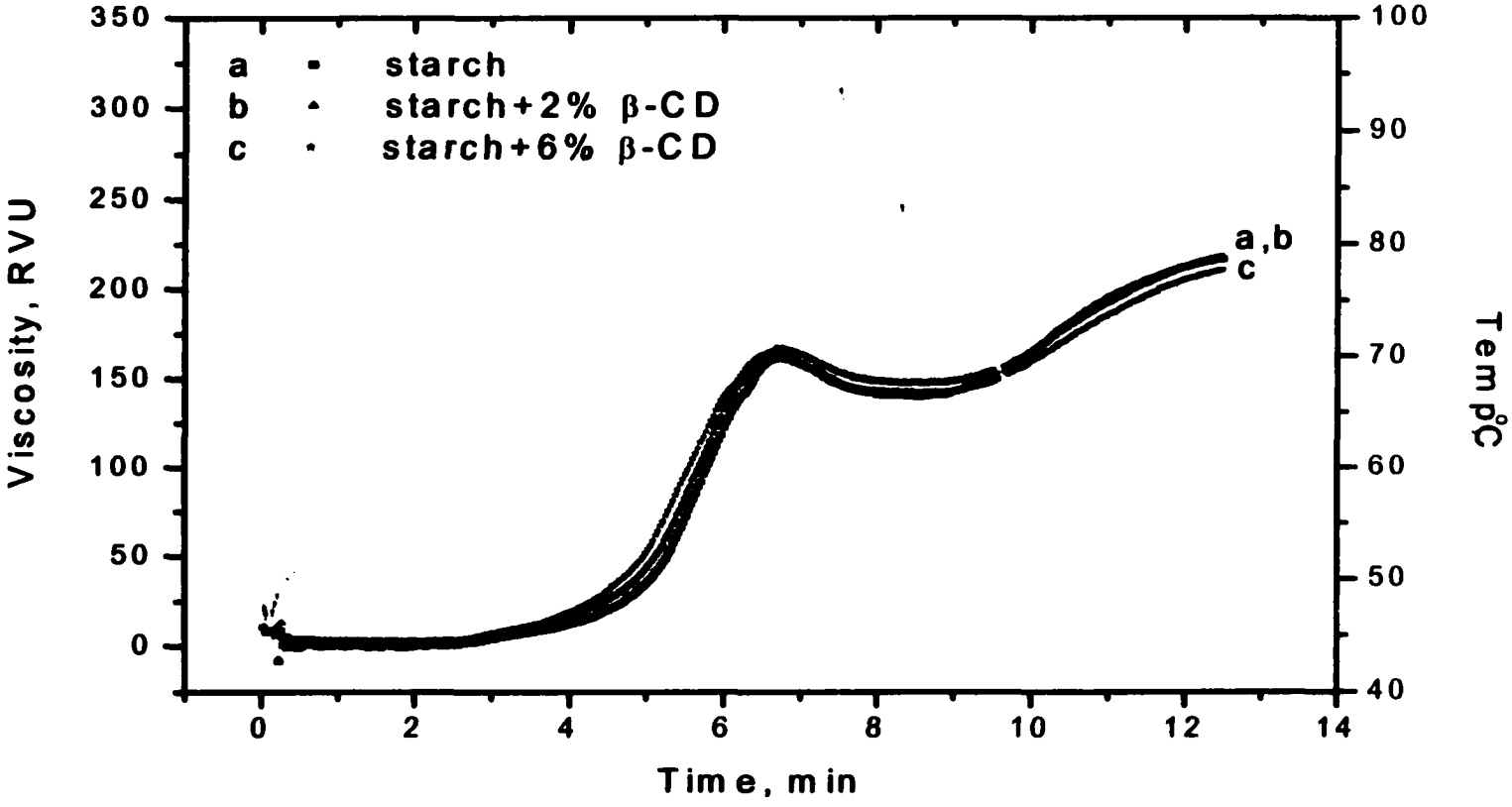


Fig. 3.17. Effect of β-cyclodextrin on pasting properties of commercial starch

Table 3.8. Effect of Lipids on Pasting Properties of White Starch Isolate [1,2,3]

| Sample | Additive[4] (%) | PV | MV | FV | PT | TP | SBK | TSB | BKD |
|---|---|---|---|---|---|---|---|---|---|
| Control | ----- | 197.75$^{bcde}$ | 119.46$^{bc}$ | 183.63$^{fghij}$ | 77.25$^{bc}$ | 4.87$^{fg}$ | -14.13$^{de}$ | 64.17$^{e}$ | 78.29$^{f}$ |
| Palmitic | 0.2 | 207.54$^{ab}$ | 107.50$^{cde}$ | 191.46$^{cfg}$ | 76.90$^{c}$ | 4.84$^{fg}$ | -16.08$^{e}$ | 83.96$^{bc}$ | 100.04$^{bcd}$ |
| | 0.6 | 201.33$^{abcd}$ | 99.92$^{def}$ | 186.75$^{fghij}$ | 76.93$^{c}$ | 4.86$^{fg}$ | -14.58$^{de}$ | 86.83$^{b}$ | 101.42$^{bc}$ |
| Stearic | 0.2 | 206.54$^{ab}$ | 121.33$^{bc}$ | 200.71$^{cdef}$ | 77.03$^{bc}$ | 4.86$^{fg}$ | -5.83$^{d}$ | 79.38$^{bcd}$ | 85.21$^{ef}$ |
| | 0.6 | 211.25$^{a}$ | 113.04$^{bcd}$ | 195.04$^{defg}$ | 77.35$^{bc}$ | 4.87$^{fg}$ | -16.21$^{e}$ | 82.00$^{bc}$ | 98.21$^{bcd}$ |
| Oleic | 0.2 | 198.13$^{bcde}$ | 108.17$^{bcde}$ | 180.83$^{ghij}$ | 77.03$^{bc}$ | 4.99$^{f}$ | -17.29$^{ef}$ | 72.67$^{cde}$ | 89.96$^{de}$ |
| | 0.6 | 1195.79$^{bcde}$ | 94.96$^{ef}$ | 169.79$^{ijk}$ | 77.85$^{bc}$ | 4.97$^{fg}$ | -26.00$^{fgh}$ | 74.83$^{bcde}$ | 100.83$^{bc}$ |
| Linoleic | 0.2 | 195.33$^{bcde}$ | 100.25$^{def}$ | 172.83$^{hijk}$ | 77.58$^{bc}$ | 4.95$^{fg}$ | -22.50$^{efgh}$ | 72.58$^{cde}$ | 95.08$^{cde}$ |
| | 0.6 | 193.13$^{cdef}$ | 86.29$^{f}$ | 161.33$^{k}$ | 77.28$^{bc}$ | 4.92$^{fg}$ | -31.79$^{hi}$ | 75.04$^{bcde}$ | 106.83$^{ab}$ |
| Linolenic | 0.2 | 211.83$^{a}$ | 108.71$^{bcde}$ | 183.38$^{fghij}$ | 77.33$^{bc}$ | 4.89$^{fg}$ | -28.46$^{gh}$ | 74.67$^{bcde}$ | 103.13$^{bc}$ |
| | 0.6 | 211.67$^{a}$ | 94.54$^{ef}$ | 171.67$^{ijk}$ | 77.55$^{bc}$ | 4.85$^{fg}$ | -40.00$^{i}$ | 77.13$^{bcd}$ | 117.13$^{a}$ |
| MP | 0.2 | 180.29$^{f}$ | 119.67$^{bc}$ | 206.13$^{cde}$ | 78.20$^{b}$ | 6.00$^{d}$ | 25.83$^{b}$ | 86.46$^{b}$ | 60.63$^{g}$ |
| | 0.6 | 185.21$^{ef}$ | 120.63$^{bc}$ | 247.63$^{a}$ | 77.75$^{bc}$ | 6.44$^{b}$ | 62.42$^{a}$ | 127.00$^{a}$ | 64.58$^{g}$ |
| Tripalmitin | 0.2 | 211.54$^{a}$ | 121.54$^{bc}$ | 189.38$^{efghi}$ | 77.15$^{bc}$ | 4.81$^{g}$ | -22.17$^{efg}$ | 67.83$^{de}$ | 90.00$^{de}$ |
| | 0.6 | 212.63$^{a}$ | 122.83$^{b}$ | 190.33$^{efgh}$ | 76.90$^{c}$ | 4.86$^{fg}$ | -22.29$^{efg}$ | 67.50$^{de}$ | 89.79$^{de}$ |
| LC | 0.2 | 212.75$^{a}$ | 185.21$^{a}$ | 235.29$^{ab}$ | 78.23$^{b}$ | 5.74$^{c}$ | 22.54$^{bc}$ | 50.08$^{f}$ | 27.54$^{h}$ |
| | 0.6 | 202.67$^{abc}$ | 198.17$^{a}$ | 217.63$^{bc}$ | 80.00$^{a}$ | 6.97$^{a}$ | 14.96$^{c}$ | 19.46$^{h}$ | 4.5$^{i}$ |
| LE | 0.2 | 205.46$^{abc}$ | 199.50$^{a}$ | 231.50$^{ab}$ | 77.63$^{bc}$ | 6.24$^{c}$ | 26.04$^{b}$ | 32.00$^{g}$ | 5.96$^{i}$ |
| | 0.6 | 189.04$^{def}$ | 191.79$^{a}$ | 211.83$^{cd}$ | 77.95$^{bc}$ | 6.97$^{a}$ | 22.79$^{bc}$ | 20.04$^{gh}$ | -2.75$^{i}$ |

[1]MP=Monopalmitin, LC=Lysophosphatidylcholine, LE=Lysophosphatidylethanolamine, PV=Peak Viscosity, MV=Minimum Viscosity, FV=Final Viscosity, PT=Pasting Temperature, TP=Time to Peak, SBK=Set Back. TSB=Total Set Back, BKD=Breakdown;

[2]Units: Viscosity (RVU), Temperature (°C), Time (min).

[3]Different letters within each column indicate values are significantly different at the level of $p \leq 0.05$.

[4]Additive percentage was based on the starch dry weight.

finding. Our result indicated that not only the amylose content influences the pasting characteristics, but other factors such as variety, starch preparation method, and minor component content (residual protein), might also play an important role in governing the pasting properties of rice starch.

**Lipids Effect**

Compared to the control (white starch isolate, WSI), 0.6% MP decreased the PV and BKD by 12 and 14 RVU respectively, but increased the FV and TSB by 64 and 63 RVU respectively (Table 3.8, Fig. 3.18). It also delayed the time to peak for 1.57 min. Tripalmitin slightly increased the PV by 15 RVU at both levels (Table 3.8, Fig. 3.19). It had no other influence on the pasting properties of the WSI.

The presence of 0.6% LC greatly increased the MV and FV by 79 and 26 RVU, resulting in a substantial drop in BKD (74 RVU) and TSB (45 RVU) (Table 3.8, Fig. 3.20). The pasting temperature was increased by $3^0$C, whereas the time to peak was increased by 2.1 min. With the presence of 0.6% LE, starch viscosity continued to increase after passing the initial $95^0$C, resulting no breakdown (Table 3.8, Fig. 3.21). The time to peak was increased by 2.1 min while the TSB was reduced by 44 RVU. Addition of 0.6% stearic acid and linolenic acid increased the PV about 14 RVU (Table 3.8, Fig. 3.22, 3.23). The presence of 0.6% palmitic, oleic, linoleic and linolenic acid decreased the MV by 20 to 33 RVU, and increased the BKD by 22 to 39 RVU (Table 3.8, Fig. 3.24~3.26).

**Amino Acids Effect**

The amino acids effects on pasting properties of white starch isolate were studied. Compared to the control, the presence of 6% arginine increased the PV by 31

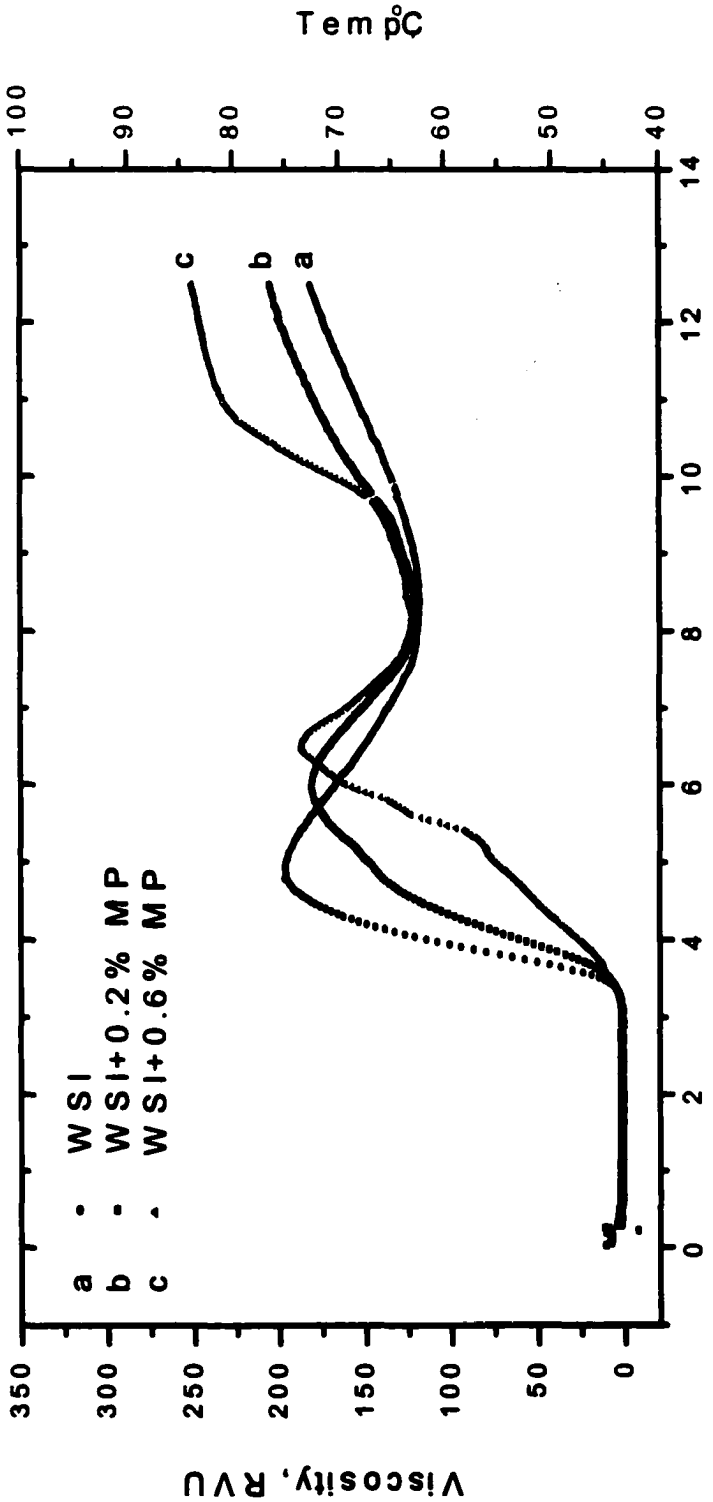


Fig. 3.18. Effect of monopalmitin (MP) on pasting properties of white starch isolate (WSI).

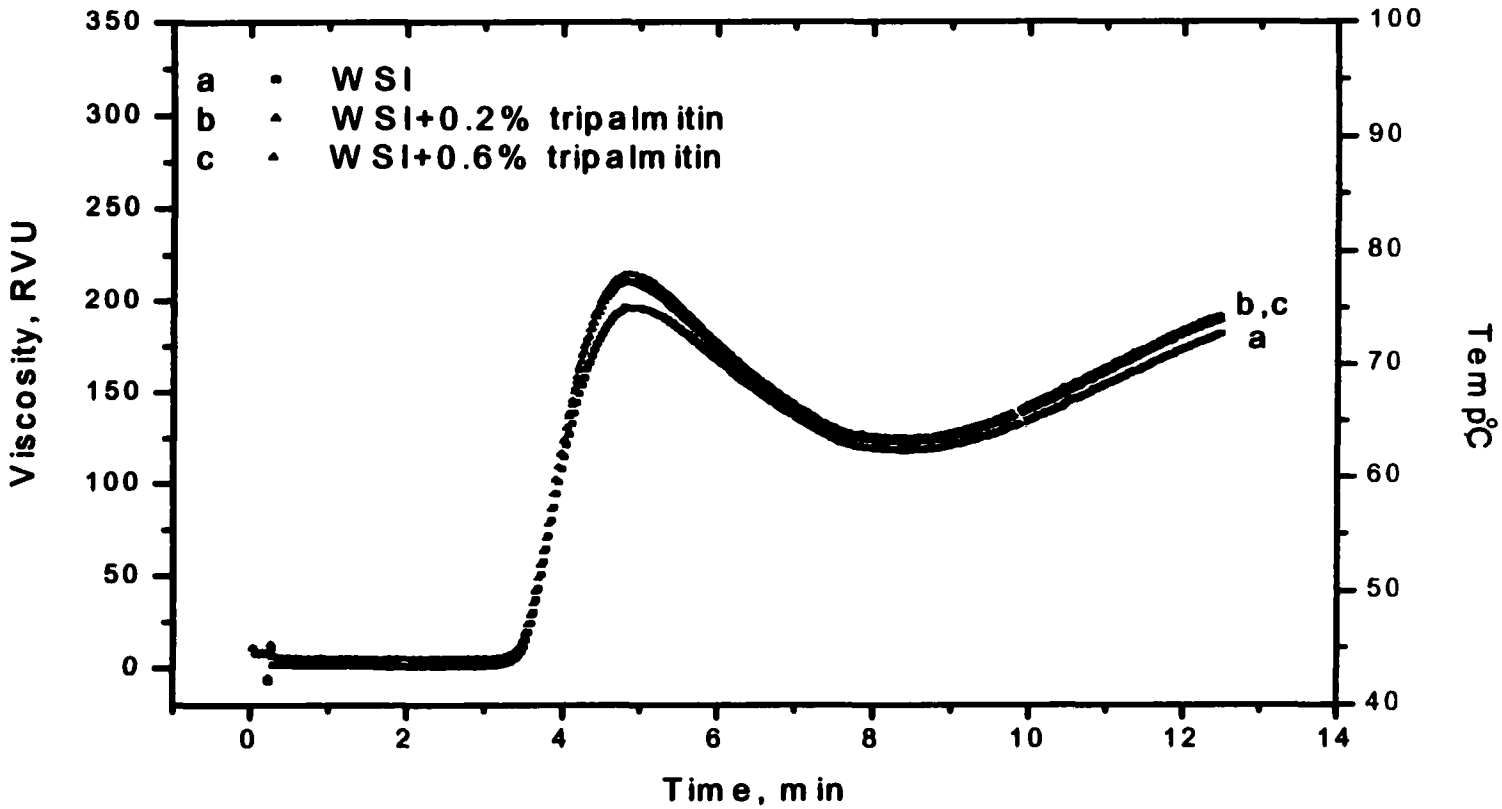


Fig. 3.19. Effect of tripalmitin on pasting properties of white starch isolate (WSI).

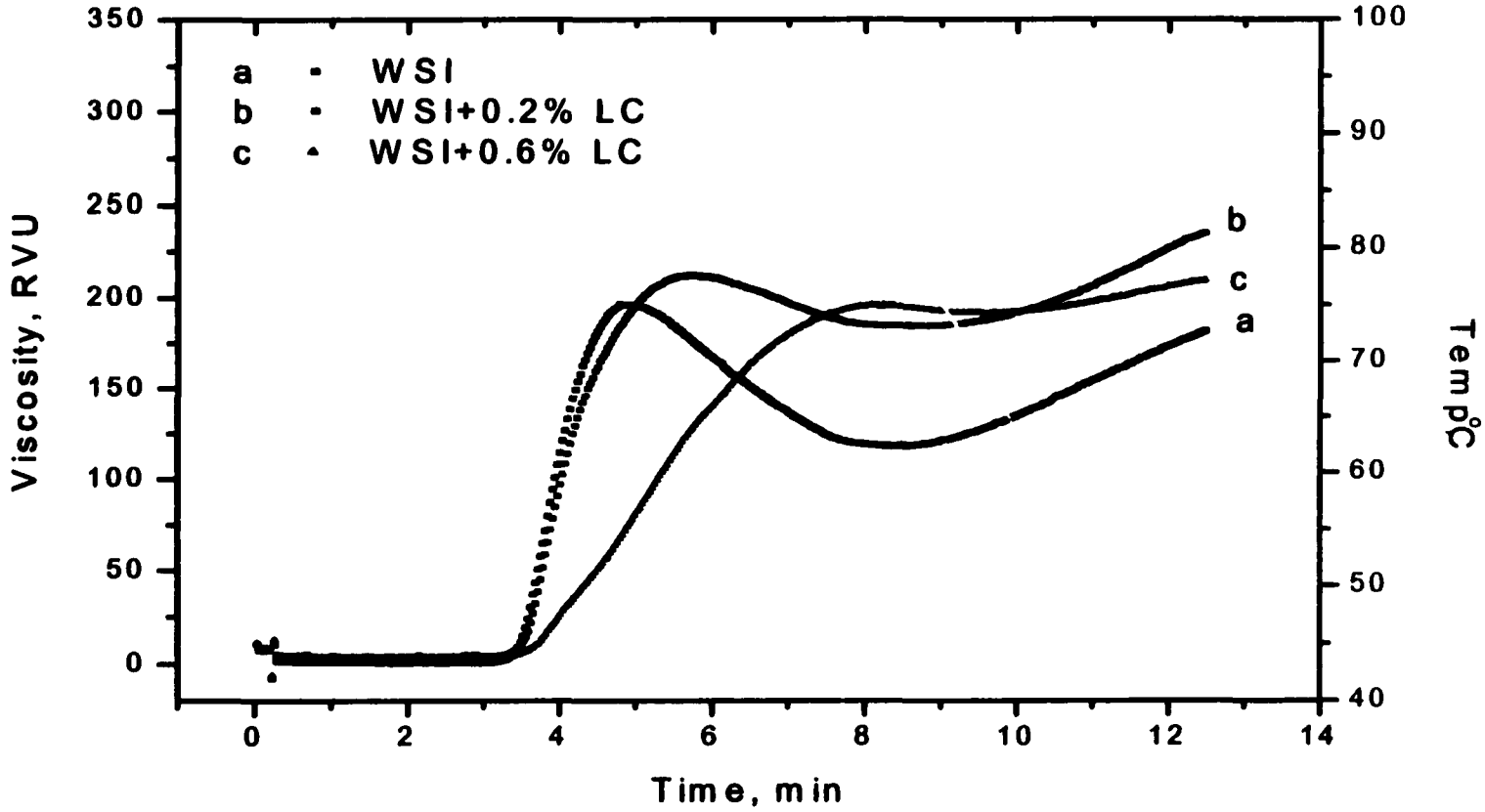


Fig. 3.20. Effect of lysophosphatidylcholine (LC) on pasting properties of white starch isolate (WSI).

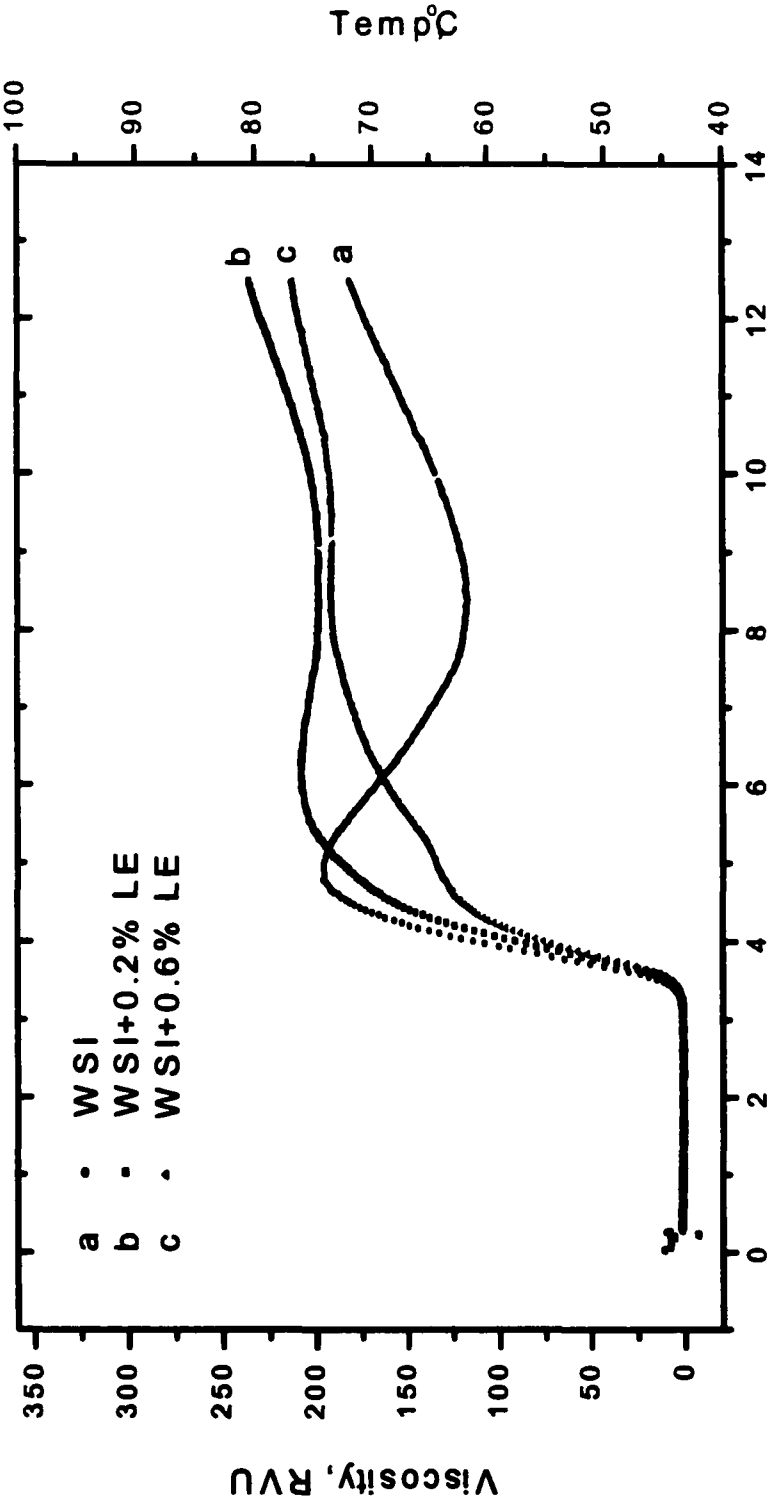


Fig. 3.21. Effect of lysophosphatidylethanolamine (LE) on pasting properties of white starch isolate (WSI).

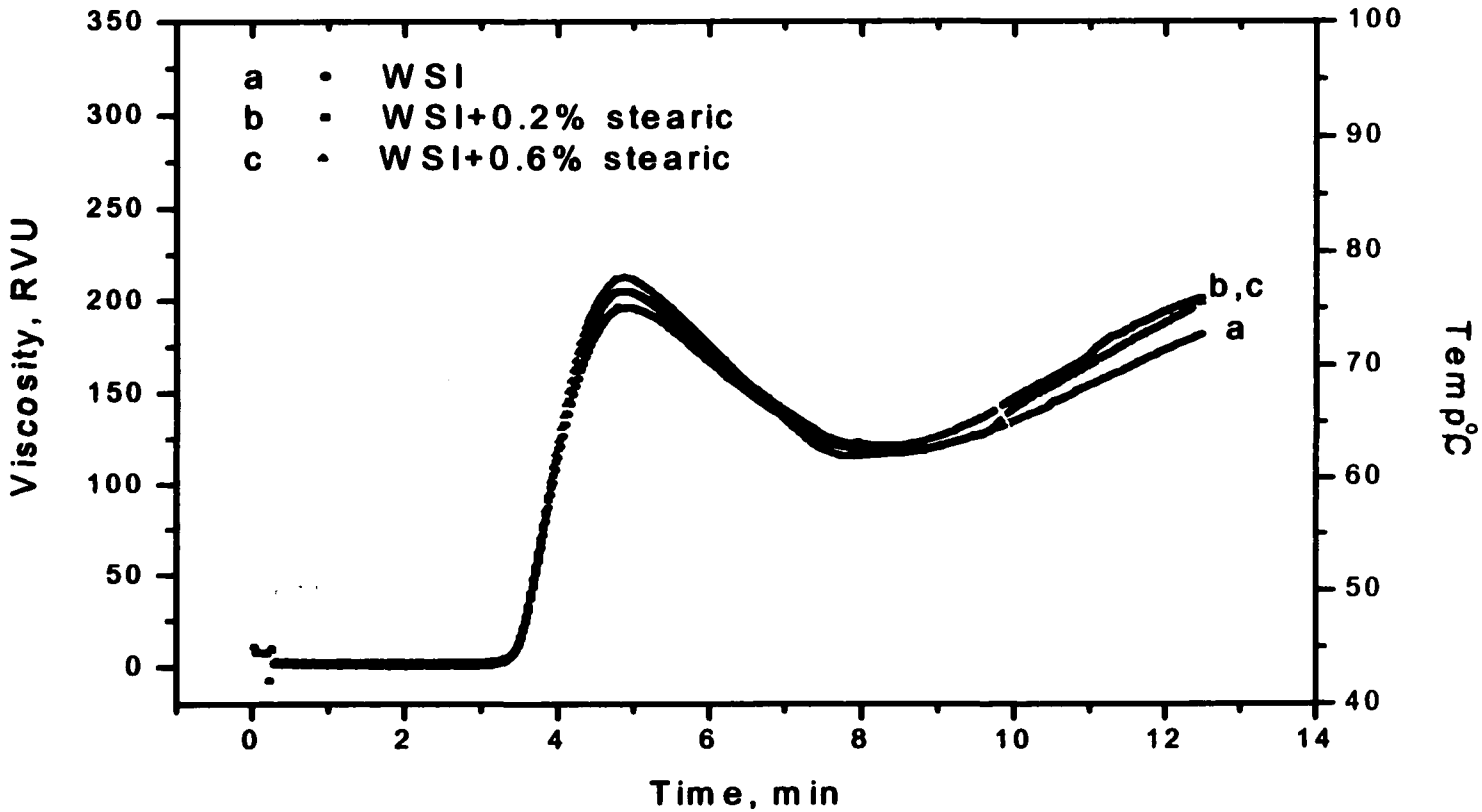


Fig. 3.22. Effect of stearic acid on pasting properties of white starch isolate (WSI).

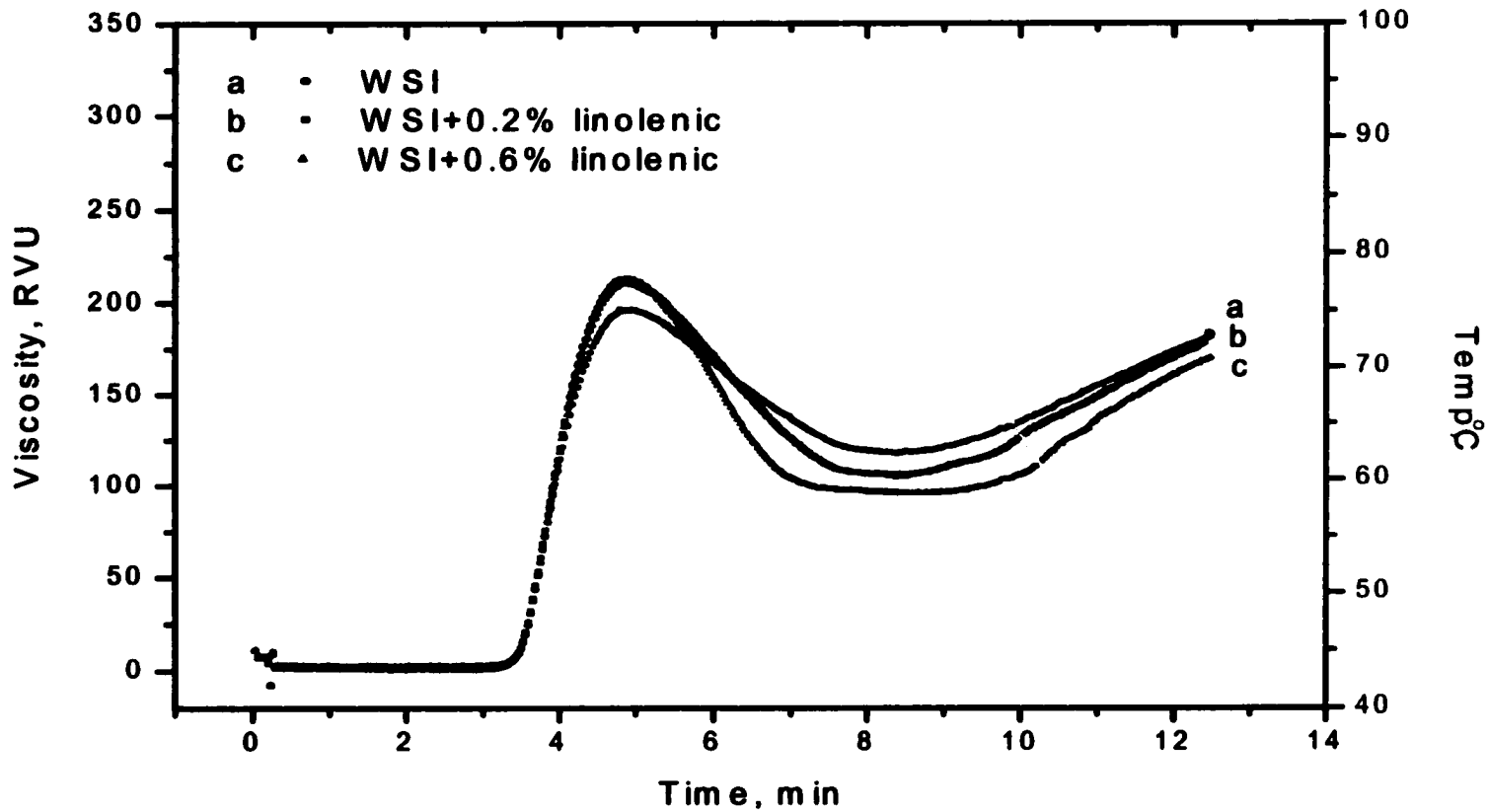


Fig. 3.23. Effect of linolenic acid on pasting properties of white starch isolate (WSI).

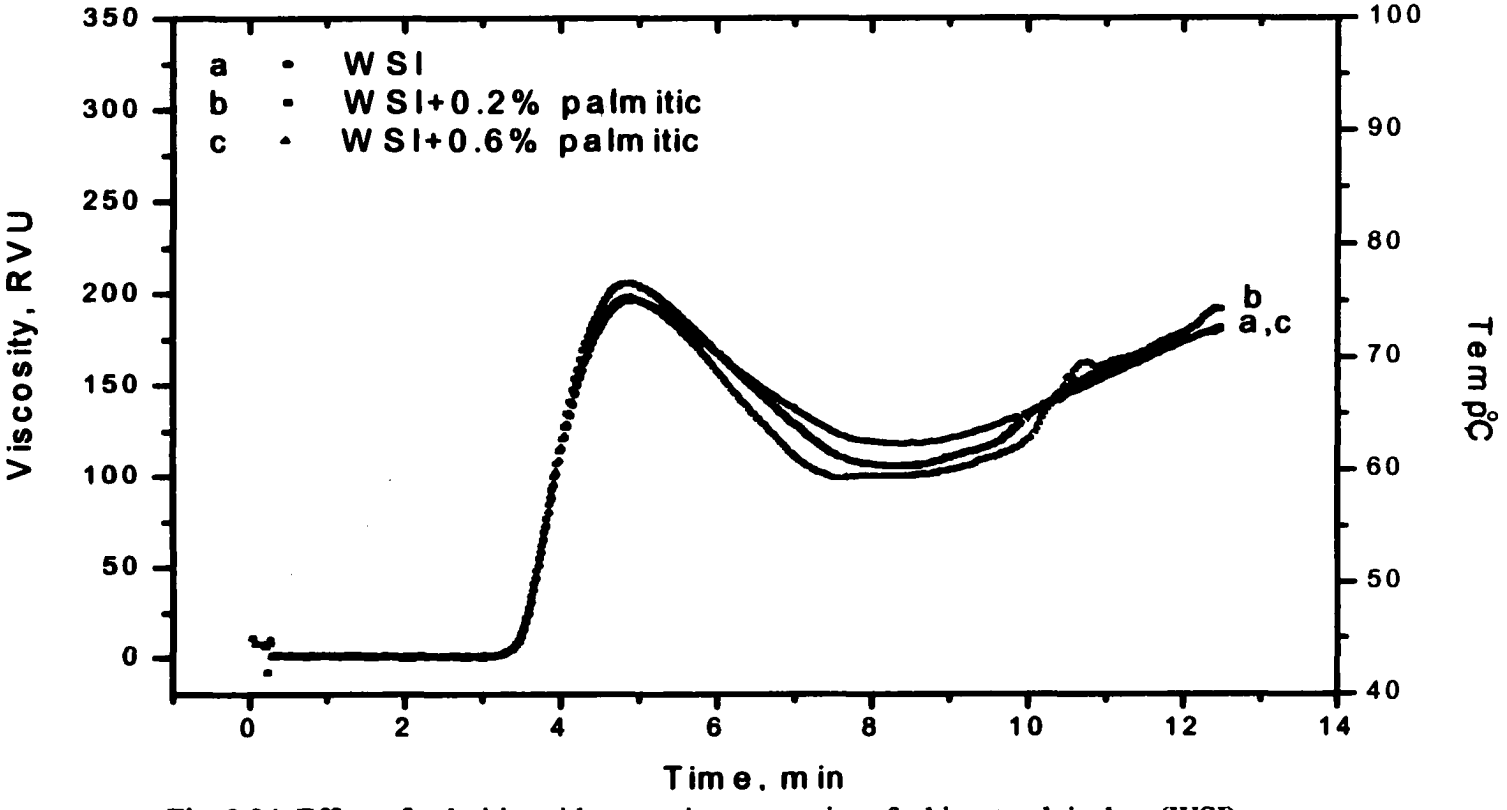


Fig. 3.24. Effect of palmitic acid on pasting properties of white starch isolate (WSI).

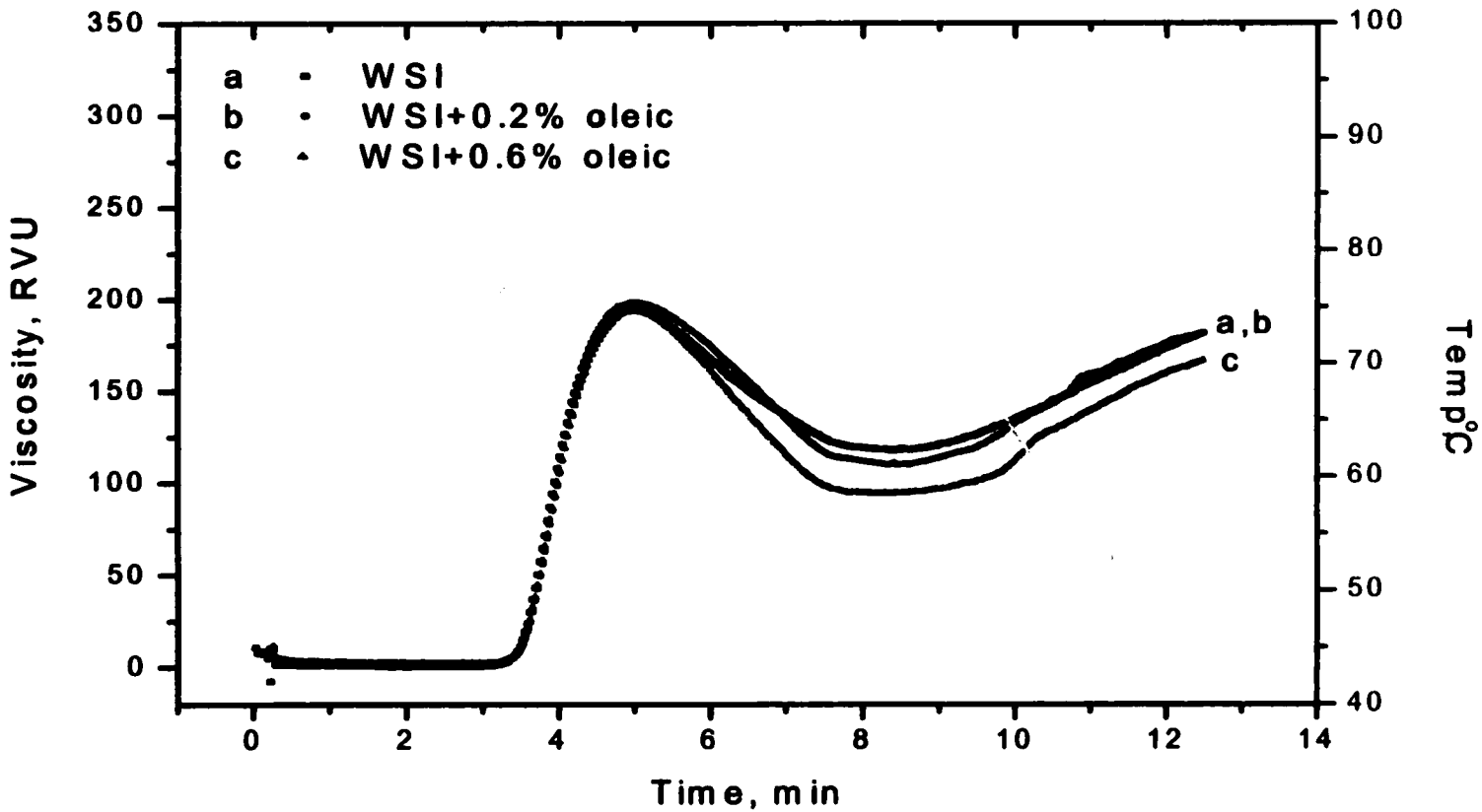


Fig. 3.25. Effect of oleic acid on pasting properties of white starch isolate (WSI).

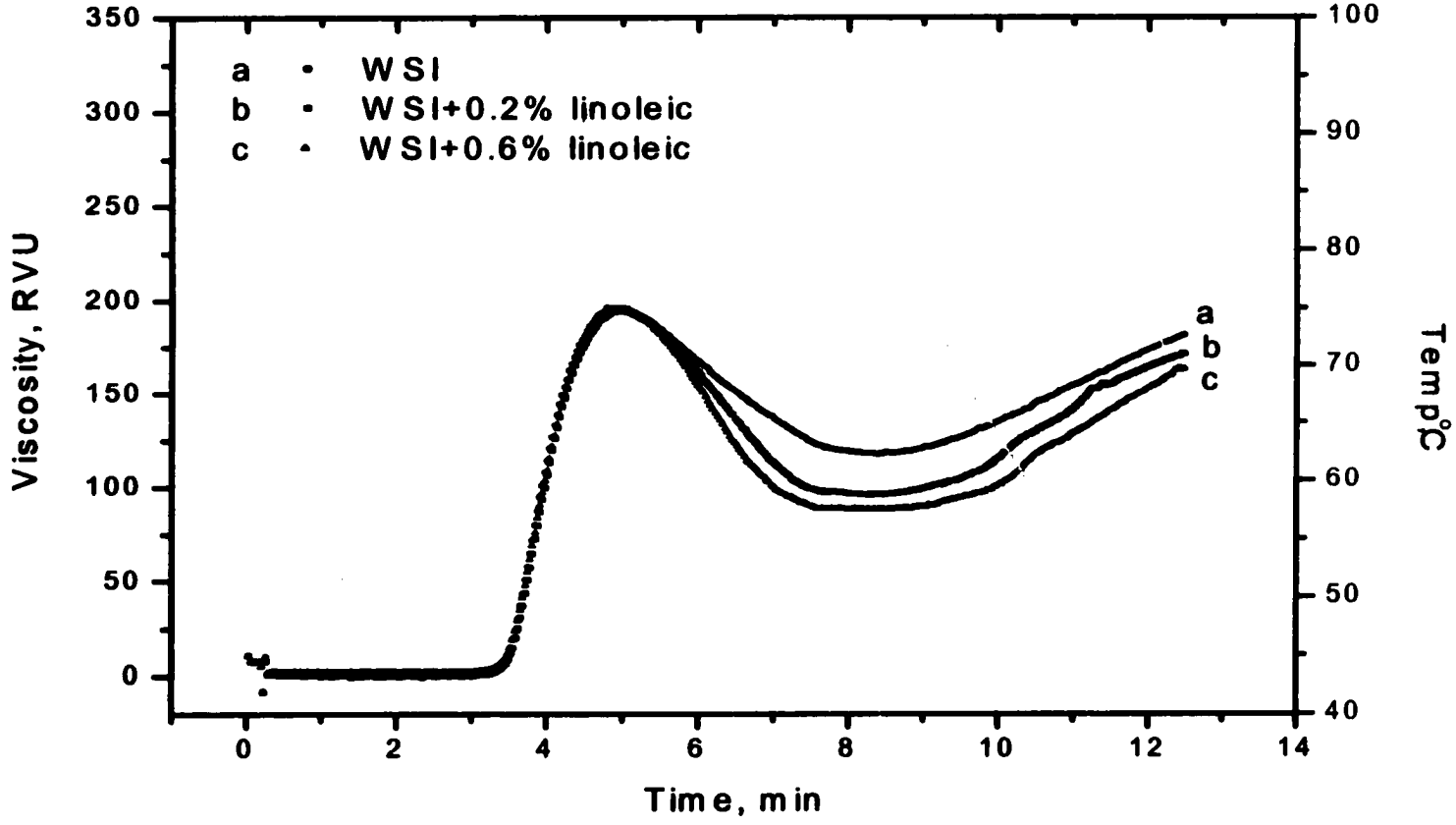


Fig. 3.26. Effect of linoleic acid on pasting properties of white starch isolate (WSI).

RVU while the other amino acids showed no influence on PV (Table 3.9, Fig. 3.27~3.32). Among those amino acids, 6% aspartic acid showed the greatest reduction in MV and FV by 26 and 45 RVU. At 6%, glutamic acid and arginine also reduced the MV and FV. The presence of 6% leucine also reduced the MV by 9 RVU. Alanine had no influence on pasting properties.

**β-Cyclodextrin Effect**

The presence of 6% β-CD decreased the MV and FV by 26 RVU, whereas the BKD was increased by 28 RVU (Table 3.10, Fig. 3.33). The pasting temperature and time to peak were not changed.

## 3.4. CONCLUSION

This study showed that, in general, the complex formation between amylose and lipids delayed commercial starch granule swelling, but increased the swelling extent. Addition of lipids increased gel viscosity and enhanced the retrogradation tendency, but did not affect the cooking stability. No specific influence pattern was observed regarding the degree of lipid saturation. Several investigators reported the anti-firming property of monoglycerides in bread making, and attributed this property to the formation of monoglycerides and amylose complexes (Krog and Jensen 1970, Eliasson and Krog 1985). However, results from this study indicated that amylose-lipid complex formation enhanced the retrogradation tendency of commercial starch. Removal of lipids from starch decreased the retrogradation tendency. Evidence indicated that amylopectin could interact with monoglycerides in model systems (Batres and White 1986, Huang and White 1993). Therefore, the anti-firming effect of lipids is mainly due to the amylopectin-lipid complex formation.

Table 3.9. Effect of Amino Acids on Pasting Properties of White Starch Isolate [1,2,3]

| Sample | Additives[4] (%) | PV | MV | FV | PT | TP | SBK | TSB | BKD |
|---|---|---|---|---|---|---|---|---|---|
| Control | ----- | 197.75$^{c}$ | 119.46$^{abc}$ | 183.63$^{ab}$ | 77.25$^{bc}$ | 4.87$^{ab}$ | -14.13$^{a}$ | 64.17$^{bc}$ | 78.29$^{b}$ |
| Aspartic | 2 | 209.13$^{bc}$ | 103.04$^{fg}$ | 153.13$^{d}$ | 78.05$^{abc}$ | 4.97$^{a}$ | -56.00$^{f}$ | 50.08$^{ef}$ | 106.08$^{cd}$ |
| | 6 | 211.25$^{bc}$ | 93.13$^{h}$ | 138.00$^{e}$ | 78.23$^{abc}$ | 4.86$^{ab}$ | -73.25$^{g}$ | 44.88$^{f}$ | 118.13$^{ab}$ |
| Glutamic | 2 | 203.54$^{c}$ | 113.00$^{cde}$ | 168.42$^{c}$ | 78.20$^{abc}$ | 4.99$^{a}$ | -35.13$^{cd}$ | 55.42$^{d}$ | 90.54$^{efg}$ |
| | 6 | 203.42$^{c}$ | 106.38$^{efg}$ | 156.88$^{d}$ | 78.18$^{abc}$ | 4.97$^{a}$ | -46.54$^{e}$ | 50.50$^{de}$ | 97.04$^{de}$ |
| Lysine | 2 | 206.67$^{bc}$ | 105.92$^{efg}$ | 174.00$^{bc}$ | 78.45$^{ab}$ | 4.81$^{ab}$ | -32.67$^{bc}$ | 68.08$^{abc}$ | 100.75$^{de}$ |
| | 6 | 207.33$^{bc}$ | 113.75$^{cde}$ | 177.04$^{bc}$ | 78.88$^{a}$ | 4.85$^{ab}$ | -30.29$^{bc}$ | 63.29$^{c}$ | 93.58$^{ef}$ |
| Arginine | 2 | 218.54$^{ab}$ | 106.04$^{efg}$ | 176.79$^{bc}$ | 78.60$^{a}$ | 4.76$^{b}$ | -41.75$^{de}$ | 70.75$^{a}$ | 112.50$^{bc}$ |
| | 6 | 228.08$^{a}$ | 99.88$^{gh}$ | 169.13$^{c}$ | 79.00$^{a}$ | 4.74$^{b}$ | -58.96$^{f}$ | 69.25$^{ab}$ | 128.21$^{a}$ |
| Leucine | 2 | 208.54$^{bc}$ | 115.71$^{bcd}$ | 183.63$^{ab}$ | 77.33$^{bc}$ | 4.81$^{ab}$ | -24.92$^{b}$ | 67.92$^{abc}$ | 92.83$^{ef}$ |
| | 6 | 204.33$^{c}$ | 110.75$^{def}$ | 176.71$^{bc}$ | 77.03$^{c}$ | 4.81$^{ab}$ | -27.63$^{bc}$ | 65.96$^{abc}$ | 93.58$^{ef}$ |
| Alanine | 2 | 206.80$^{bc}$ | 122.97$^{ab}$ | 190.00$^{a}$ | 77.19$^{c}$ | 4.92$^{ab}$ | -16.80$^{a}$ | 67.03$^{abc}$ | 83.83$^{fgh}$ |
| | 6 | 204.58$^{bc}$ | 124.25$^{a}$ | 189.88$^{a}$ | 77.09$^{c}$ | 4.92$^{ab}$ | -14.70$^{a}$ | 65.63$^{abc}$ | 80.33$^{gh}$ |

[1]PV=Peak Viscosity, MV=Minimum Viscosity, FV=Final Viscosity, PT=Pasting Temperature, TP=Time to Peak, SBK=Set Back. TSB=Total Set Back, BKD=Breakdown;

[2]Units: Viscosity (RVU), Temperature ($^{o}$C), Time (min).

[3]Different letters within each column indicate values are significantly different at the level of $p \leq 0.05$.

[4]Additive percentage was based on the starch dry weight.

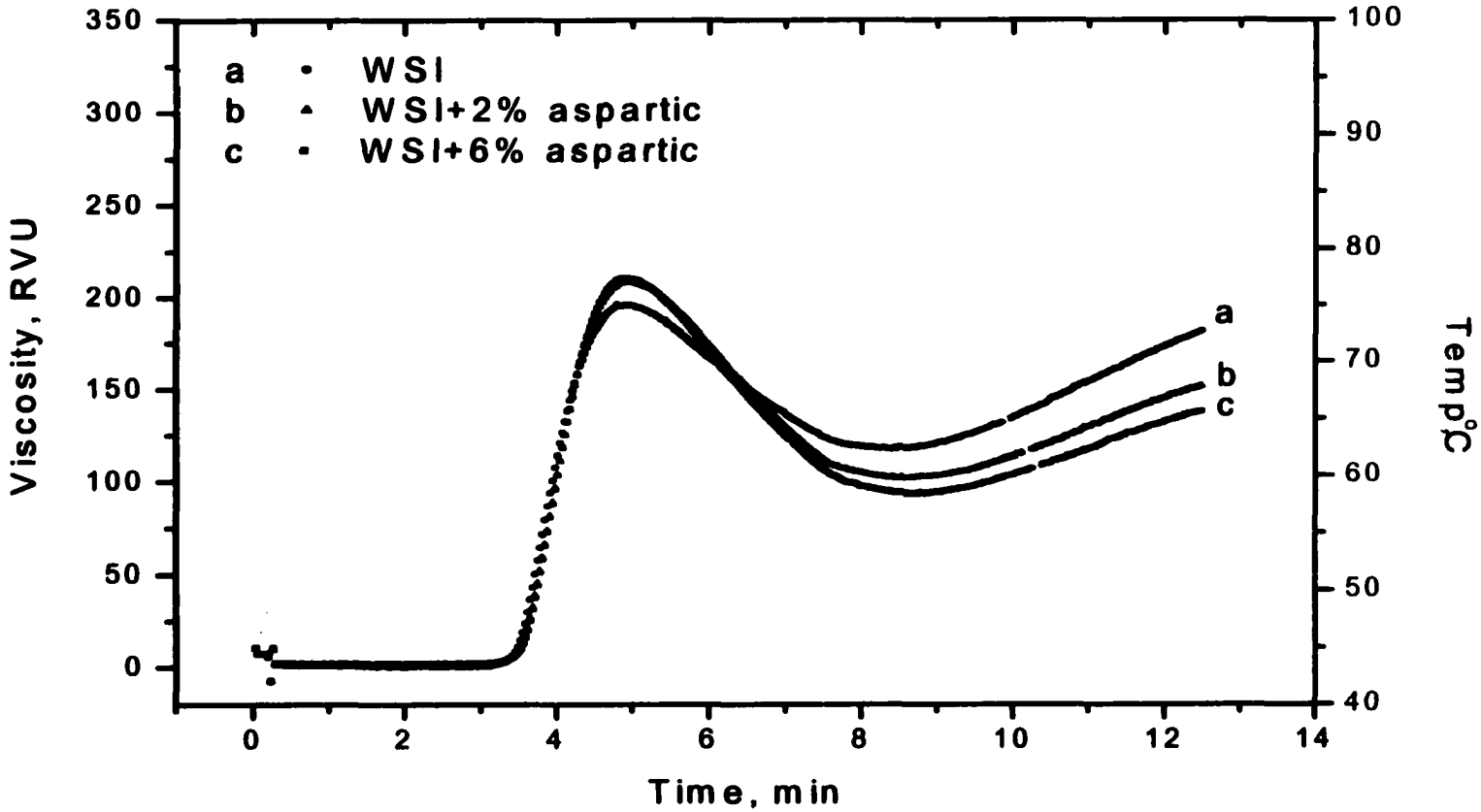


Fig. 3.27. Effect of aspartic acid on pasting properties of white starch isolate (WSI).

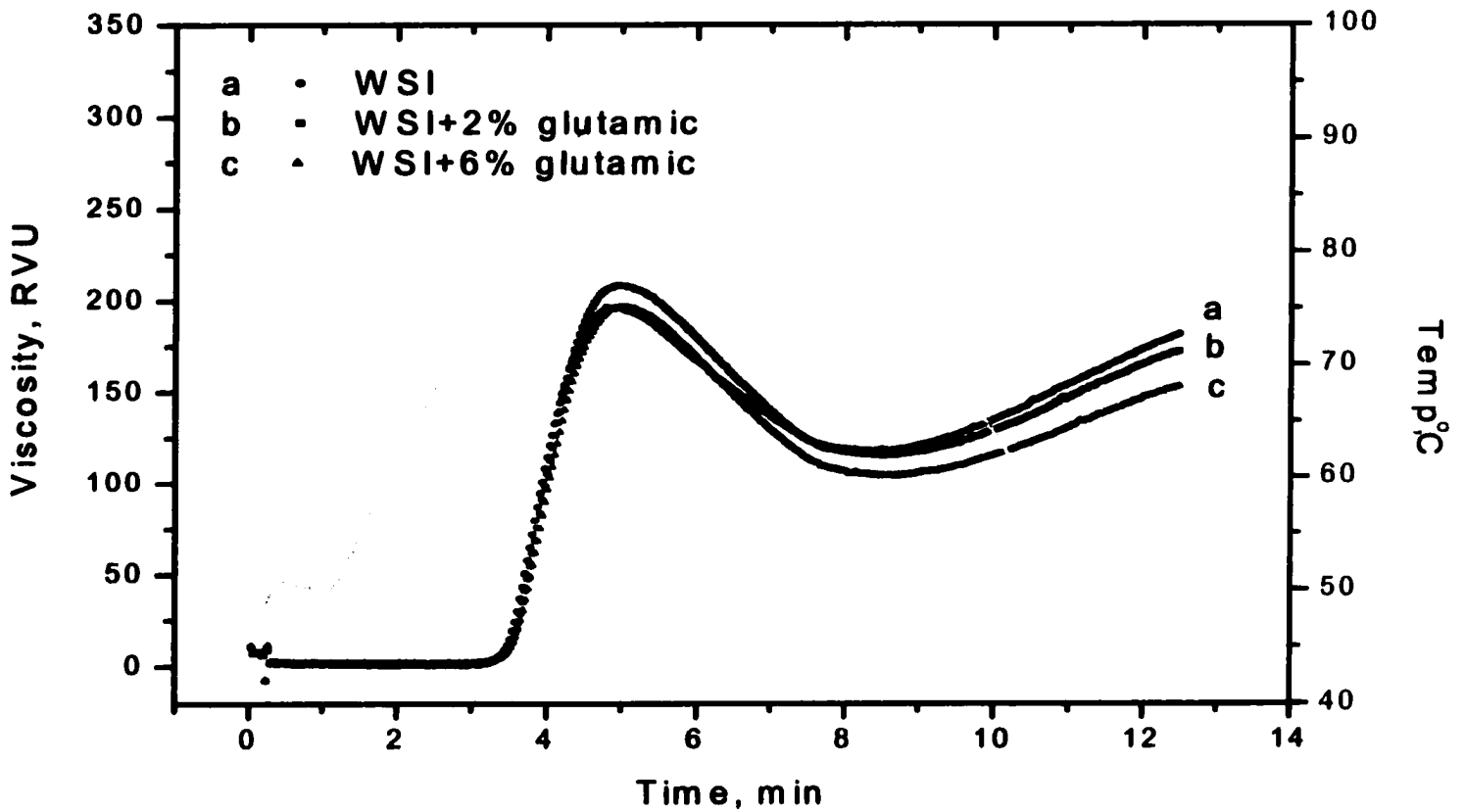


Fig. 3.28. Effect of glutamic acid on pasting properties of white starch isolate (WSI).

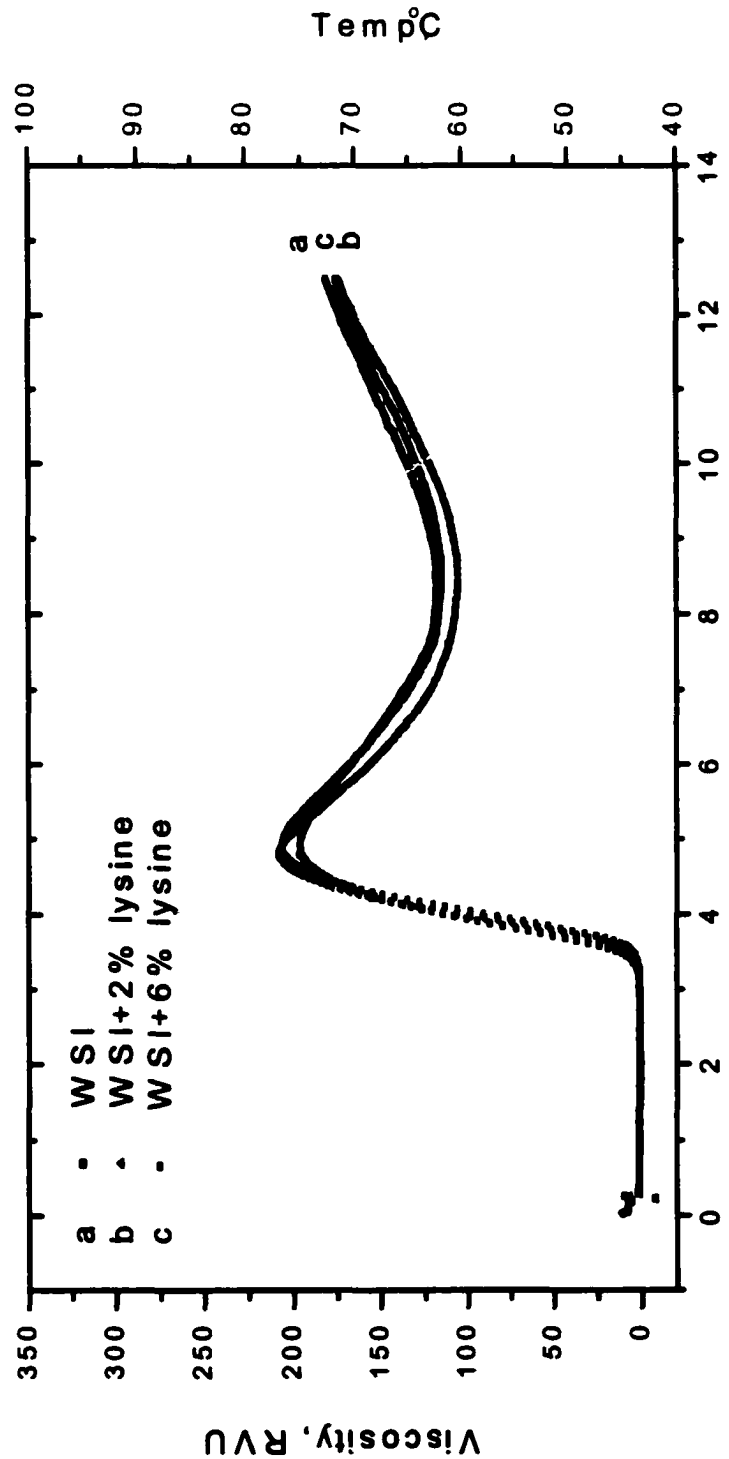


Fig. 3.29. Effect of lysine on pasting properties of white starch isolate (WSI).

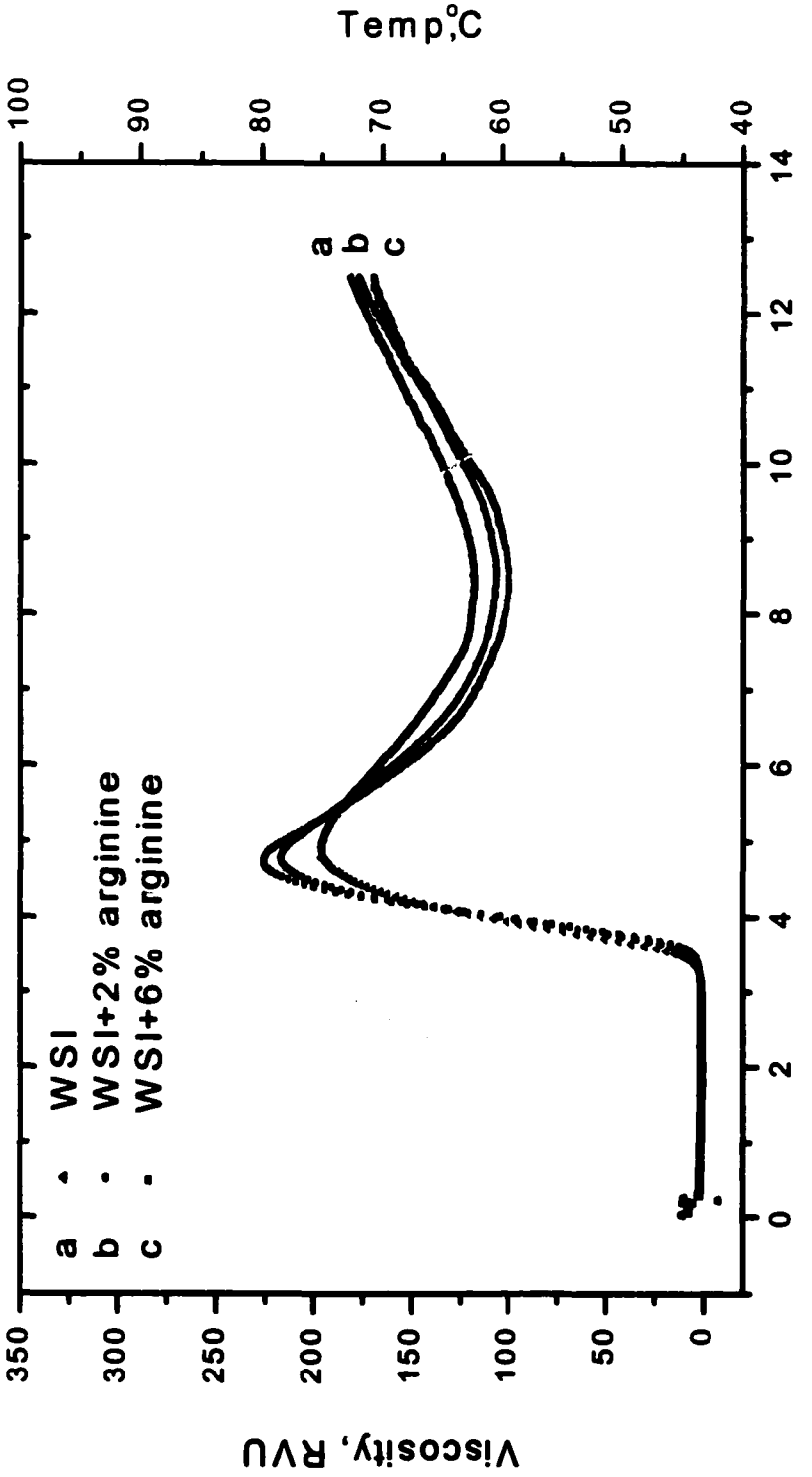


Fig. 3.30. Effect of arginine on pasting properties of white starch isolate (WSI).

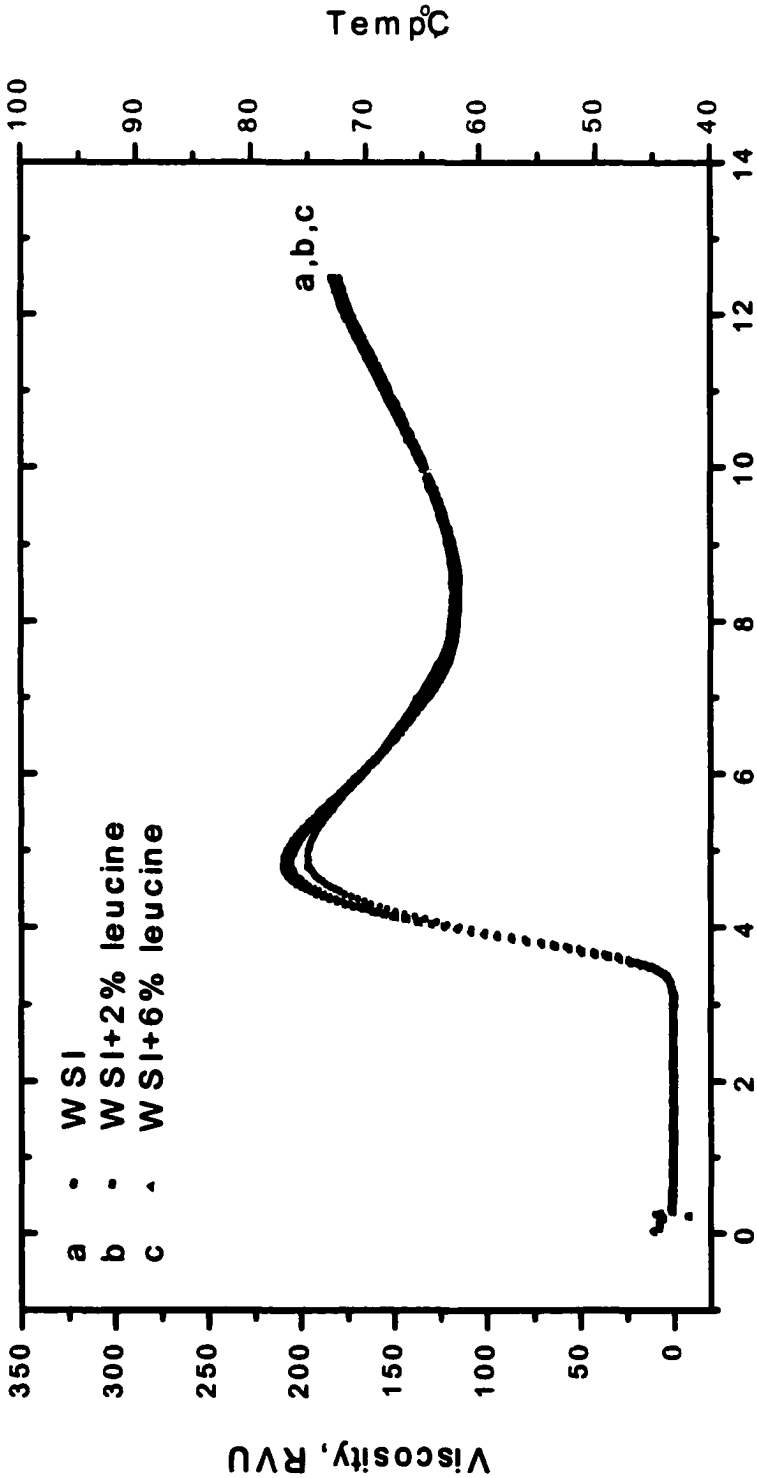


Fig. 3.31. Effect of leucine on pasting properties of white starch isolate (WSI).

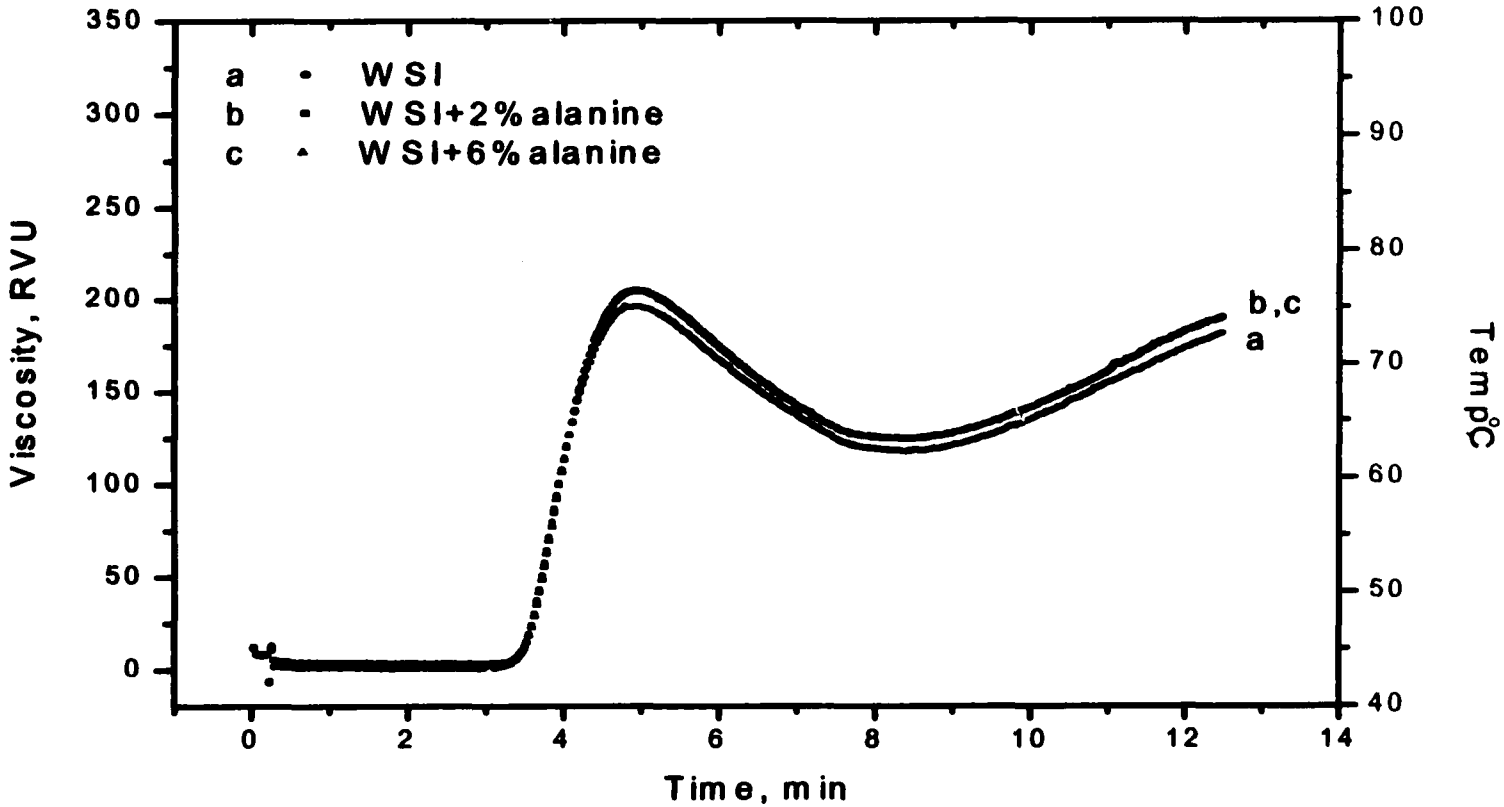


Fig. 3.32. Effect of alanine on pasting properties of white starch isolate (WSI).

Table 3.10. Effect of β-Cyclodextrin on Pasting Properties of White Starch Isolate [1,2,3]

| Sample | Additive[4] (%) | PV | MV | FV | PT | TP | SBK | TSB | BKD |
|---|---|---|---|---|---|---|---|---|---|
| Control | ----- | 197.75[a] | 119.46[a] | 183.625[a] | 77.25[a] | 4.87[a] | -14.13[a] | 64.17[b] | 78.29[c] |
| β-CD | 2 | 201.16[a] | 105.21[b] | 173.55[b] | 77.6[a] | 4.79[a] | -27.61[b] | 68.34[a] | 95.95[b] |
| | 6 | 199.50[a] | 93.15[c] | 157.03[c] | 76.65[a] | 4.70[a] | -42.47[c] | 63.89[b] | 106.35[a] |

[1]PV=Peak Viscosity, MV=Minimum Viscosity, FV=Final Viscosity, PT=Pasting Temperature, TP=Time to Peak, SBK=Set Back. TSB=Total Set Back, BKD=Breakdown;
[2]Units: Viscosity (RVU), Temperature (°C), Time (min).
[3]Different letters within each column indicate values are significantly different at the level of $p \leq 0.05$.
[4]Additive percentage was based on the starch dry weight.

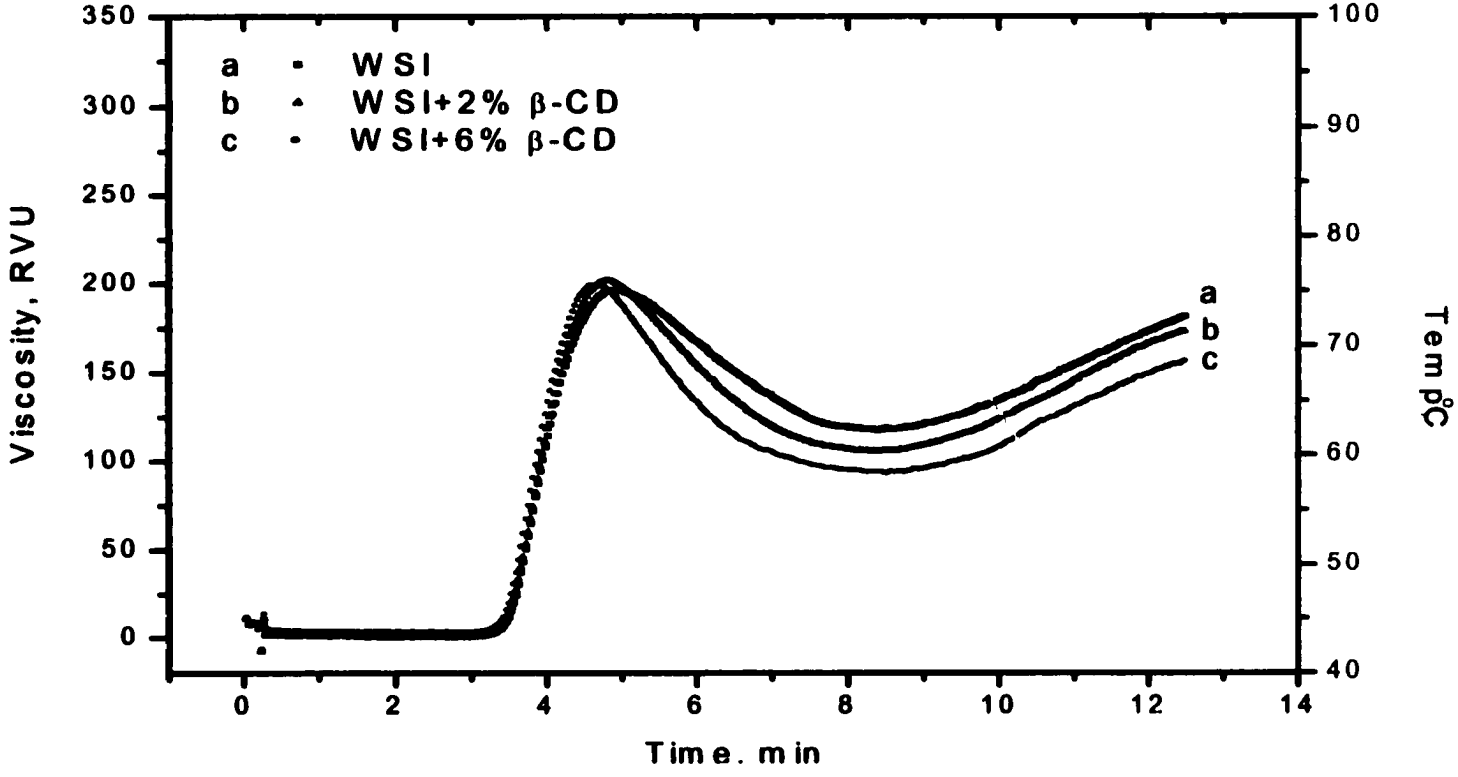


Fig. 3.33. Effect of Beta-Cyclodextrin on pasting properties of white starch isolate (WSI).

This study also showed that amino acids increased the rate of starch swelling but reduced the swelling extent, resulting in lower pasting viscosities and cooking stability. The effect of amino acids on starch pasting property might be related to their amphiphathic characteristics and influenced by the charges that those amino acids carried. Charged amino acids usually showed stronger influence on starch pasting than the neutral amino acids. Positive charged amino acids tend to reduce the pasting temperature and cooking time. Both positive charged and negative charged amino acids reduced the gel viscosity and retrogradation tendency, whereas the breakdown values were increased. Beta-CD had a slight influence on pasting properties of rice starches. The variation of lipids, amino acids, and β-CD influence from commercial starch to white starch isolate might be due to the difference of amylose content and isolation method.

# CHAPTER 4
# EFFECTS OF LIPIDS, AMINO ACIDS, AND BETA-CYCLODEXTRIN ON THERMAL PROPERTIES OF RICE STARCH USING DIFFERENTIAL SCANNING CALORIMETRY

## 4.1. INTRODUCTION

Starch granules swell in the presence of water. The swelling can be reversed upon the removal of the water when the system temperature is not over the starch gelatinization temperature (Hoseney 1986). When the starch granule is heated in excess water over the gelatinization temperature, heat and moisture transfers occur. The granule swells to several times its initial size and results in the crystallite melting, granule components (amylose and /or amylopectin) leaching out, and the loss of birefringence. This gelatinization process usually occurs in cooking or food processing.

The texture of starch-based food products is mainly determined by the gelatinization properties of starch granules (Juliano 1985; Lii et al 1986). The factors that influence gelatinization have been studied for many decades. Those factors include (1) starch varieties, (2) granule size and shape, (3) amylose content, (4) degree of crystallinity and chain length of the amylopectin fraction, (5) the placement and content of starch granule-associated protein and lipid, and (6) the presence of other food additives, such as surfactants, sugars, and salts, etc. (Juliano et al. 1965, 1987; Manginat and Juliano 1980; Hamaker and Griffin 1990, 1993; Tester and Morrison 1990).

Commercial rice starch usually contains trace levels of lipids and proteins (Morrison 1978, 1981; Juliano et al.1965; Juliano 1979, 1982, 1984, 1985). Those lipids could form complex with amylose and result in a remarkable influence on starch thermal properties (Lorenz 1976; Ohashi et al. 1980; Juliano 1984; Morrison et al. 1984; Azudin and Morrison 1986; Morrison and Azudin 1987; Galliard and Bowler

1987; Hibi et al. 1990). Removing starch lipids increases granule swelling and lowers the gelatinization temperature of rice starch (Maningat and Juliano 1980; Tester and Morrison 1990a,b; Champagne et al 1990, Marshall et al 1990; Morrison 1995).

Lipids or surfactants are commonly used in starchy foods formulation to act as texture modifiers. Addition of lipids or surfactants to rice starch is related to the increase of gelatinization temperatures (Maningat and Juliano 1980, Ohashi et al 1980, Chungcharoen and Lund 1987). The starch-lipid interactions have been studied through lipid addition, defatting, and solution-crystallized amylose-lipid complexes formation under model systems (Biliaderis et al 1985; Maningat and Juliano 1980; Tester and Morrison 1990a,b; Champagne et al 1990, Marshall et al 1990). However, further studies are needed to determine the effects of phospholipids on the thermal properties of rice starch.

Cyclodextrins were believed to form inclusion complexes with various hydrophobic compounds including lipids. Beta-CD was reported to increase the swelling power and solubility of wheat starch granules during gelatinization (Kim and Hill 1984). However, the influence of β-CD on rice starch gelatinization has not been documented.

Studies from sorghum and wheat starches suggested that the gelatinization properties of starch are influenced by both endosperm matrix protein and granule associated protein (Chandrashekar and Kirleis 1988, Seguchi 1986). The influence of rice protein on the swelling of rice starch granules had been studied by several investigators (Onate et al 1964, Juliano et al 1965, Cheng 1987, Marshall et al. 1990). Protein content was reported to be negatively correlated with water absorption ratio and

positively correlated with cooking time (Juliano 1965). A decrease of the gelatinization temperature of the flour was observed after protein removal, which indicates that the rice protein has an inhibitory effect on the swelling of rice starch granules (Cheng, 1987; Marshall et al 1990, Yang and Chang 1999). However, the lack of correlation between gelatinization temperature and the protein content in rice or rice starch was also documented (Juliano et al 1964a, Liau 1962).

Hayakawa et al (1980) reported finding pits on the surface of rice starch granules by SEM, which they attributed to the sites where protein bodies are tightly bound to starch granules in the rice endosperm. Adoracion et al (1993) reported that the starch is associated with two protein bodies (prolamin and glutelin), which are both hydrophobic and resist swelling in water at neutral pH. Moreover, protein body II (glutelin) is cross-linked with disulphide bonds (Juliano 1984). Due to those granule associated proteins rice starch extraction is more difficult than that of wheat starch or corn starch (Lumdubwong and Seib 2000). These findings suggest that the type of protein and its binding to starch granules could be an important factor influencing the gelatinization properties of rice starch. Variations in amino acid composition of cereal proteins are well documented (Khan and Bushuk 1979). The observed differences in protein solubility and subunit composition might be related to the differences in amino acid composition (Bushuk 1985). The influence of amino acid structure and type, however, has generally been overlooked, in terms of the physicochemical properties of rice starch.

Starch gelatinization is an endothermic process. Differential scanning calorimetry (DSC) is widely used to characterize the thermal behaviors of rice starch by

measuring the gelatinization temperature and enthalpies (Russell and Juliano 1983; Nakazawa et al 1984a, b; Maurice et al. 1985; Biliaderis et al 1986a,b; Chungcharoen and Lund 1987; Chang and Liu 1988). DSC methods are generally considered to be a sensitive, direct, and dependable, and have been applied to study the thermal characteristics of rice starches under various conditions, including different additives, different moisture levels and heating rates (Nakazawa et al 1984, Biliaderis et al 1986b, Normand and Marshall 1989).

The objectives of this study were: (1) to examine the effects of heating rate and moisture content on thermal characteristics of rice starch, and choose the best test condition for later DSC studies; (2) to determine lipids, amino acids, and β-cyclodextrin effects on gelatinization properties of rice starch. The information obtained may be useful in amino acid supplementation of rice flour and starch modification for special-purpose applications in the food industry.

## 4.2. MATERIALS AND METHODS

### 4.2.1. Materials

Commercial rice starch (S-7260), purchased from Sigma Chemical Co (St. Louis, MO), was used as a control. Three lipids and four amino acids, which showed their significant influences on the pasting properties of rice starch during RVA tests (see Chapter 3), were selected for this study: monopalmitin (MP), lysophosphatidylcholine (LC), lysophosphatidylethanolamine (LE), aspartic acid, glutamic acid, lysine, and arginine. Those lipids and amino acids, along with β-cyclodextrin (β-CD) (C-4767), were purchased from Sigma Chemical Co.(St. Louis, MO).

### 4.2.2. Differential Scanning Calorimetry (DSC)

The DSC experiments were conducted using a DSC Q10 (TA Instruments, USA). Indium was used to calibrate the instrument. To obtain the best testing condition for this specific study, three heating rates (1.0, 5.0, and 10.0 $^{0}$C/min) and three moisture contents (50, 70, and 90%) were examined with commercial rice starch at the temperature range of 20 to 130 °C.

**Step One: Heating Rate Test**

A 6 mg starch sample was weighed into a pre-weighed DSC pan. Then 14 μl of distilled water was measured and transferred to the pan. This prepared sample was approximately 70% water content. The DSC pan was then weighed and sealed using a DSC press. Prepared sample pans were kept at room temperature overnight. Another DSC pan with 20 μl distilled water was used as the reference.

Both sample pan and reference pan were put into the DSC instrument and the hole was covered. The chamber temperature was cooled down and equilibrated at 20 °C using liquid nitrogen. Samples were heated from 20 to 130 °C at different heating rates (1, 5, 10 °C/min) with duplicate runs for each rate.

The starch gelatinization characteristics in a DSC thermogram can be indicated by various temperatures. The onset temperature ($T_o$) and the conclusion temperature ($T_c$) (points where the extrapolated endotherm intersects with the baseline) and the peak temperature ($T_p$) (temperature of maximum differential heat flow) were determined. The amount of energy required for gelatinization ($\Delta H$) was determined by integration of the endotherm, constructing a smooth line from the beginning to the end of the transition. The best heating rate (5 °C/min) was selected for the rest of the DSC tests

based on the DSC thermograms, which provided the best reproducibility of starch thermal characteristics.

**Step Two: Moisture Content Test**

Similarly, 2 mg of starch sample was weighed into a DSC pan with 18 μl of distilled water added, which was approximately a 90% water content. Those sample pans, along with samples, which contain 10 mg starch and 10 μl distilled water (50% moisture), were used to test the moisture content effects with duplicate runs. All samples were heated from 20 to 130 °C at the rate of 5°C/min. Results were analyzed and the best moisture content condition (70% moisture) was selected for the additive effect tests, based on the DSC thermograms.

**Step Three: Additive Solution Preparation**

A 25.7 mg lipid sample was weighed into a 10 ml flask. Distilled water was added up to a total volume of 10 ml. The solutions were then mixed well and let stand for 10 min to equilibrate. A 25.7 mg of β-CD sample was weighed into a 1 ml flask. Distilled water was added up to the volume. The solution was mixed well and let set for 10 min before use.

**Step Four: Additive Effect Test**

A 6 mg starch sample was weighed into a DSC pan. A 14 μl aliquot of prepared solutions was measured and transferred into the pan. Sample pans were sealed and kept overnight for equilibration. Those samples were heated from 20 to 130 °C at the rate of 5 °C/min. DSC thermograms were analyzed and the additive influences were compared.

### 4.2.3. Statistical Analysis

SAS (Statistical Analysis System) software (Version 8.0) was used for data analysis. Analysis of Variance (ANOVA), with Tukey's studentized range (HSD) test, was performed to examine (1) the heating rate, (2) the moisture content, and (3) the additive (lipids, β-cyclodextrin, and amino acids) effects on the gelatinization characteristics ($T_o$, $T_c$, $T_p$, and $\Delta H$) of commercial starch. Type I error was controlled to be less than 0.05.

## 4.3. RESULTS AND DISCUSSION

### 4.3.1. Heating Rate and Moisture Content Effects

**Heating Rate**

Three heating rates were tested (1, 5, 10 $^0$C/min) using 70% moisture content commercial starch (Table 4.1, Fig. 4.1). Increasing the heating rate resulted in greater starch gelatinization (first transition) onset, peak, and completion temperatures. The starch gelatinization shoulder ($T_c$-$T_o$) was also increased when a large heating rate was applied. Some minor reduction in gelatinization enthalpy was also observed when the heating rate was increased from 1 to 10 $^o$C/min. There was no significant change detected in the second transition (amylose-lipid complex) onset, peak, completion temperatures, and enthalpy as well.

Several investigators reported that gelatinization onset temperature decreased as the heating rate was reduced (Nakazawa et al. 1984). The results from this study were consistent with those findings. Studies from Biliaderis et al (1986b) indicated that a slow heating rate might provide a greater opportunity for chain rearrangement in the

Table 4.1. Effect of Heating Rates on Thermal Properties of Commercial Starch [1,2,3]

| Heating Rate (°C/min) | Transition Temperatures and Transition Enthalpies | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | First Transition | | | | Second Transition | | | |
| | $T_o$ | $T_p$ | $T_c$ | $\Delta H$ | $T_o$ | $T_p$ | $T_c$ | $\Delta H$ |
| 1 | 54.3$^c$ | 63.2$^c$ | 71.8$^c$ | 11.3$^a$ | 90.0$^a$ | 97.9$^a$ | 105.7$^a$ | 1.4$^a$ |
| 5 | 57.0$^b$ | 66.1$^b$ | 75.6$^b$ | 10.2$^{ab}$ | 88.5$^a$ | 99.4$^a$ | 107.9$^a$ | 2.1$^a$ |
| 10 | 59.0$^a$ | 68.3$^a$ | 79.6$^a$ | 9.9$^b$ | 93.6$^a$ | 102.3$^a$ | 110.2$^a$ | 2.1$^a$ |

[1] $T_o$, $T_p$, $T_c$, = onset, peak, and completion temperatures, respectively; $\Delta H$=enthalpy.
[2] Different letters within each column indicate values are significantly different at the level of $p \leq 0.05$.
[3]Units: Temperature (°C), Enthalpy (J/g, dry matter); Moisture Content=70%.

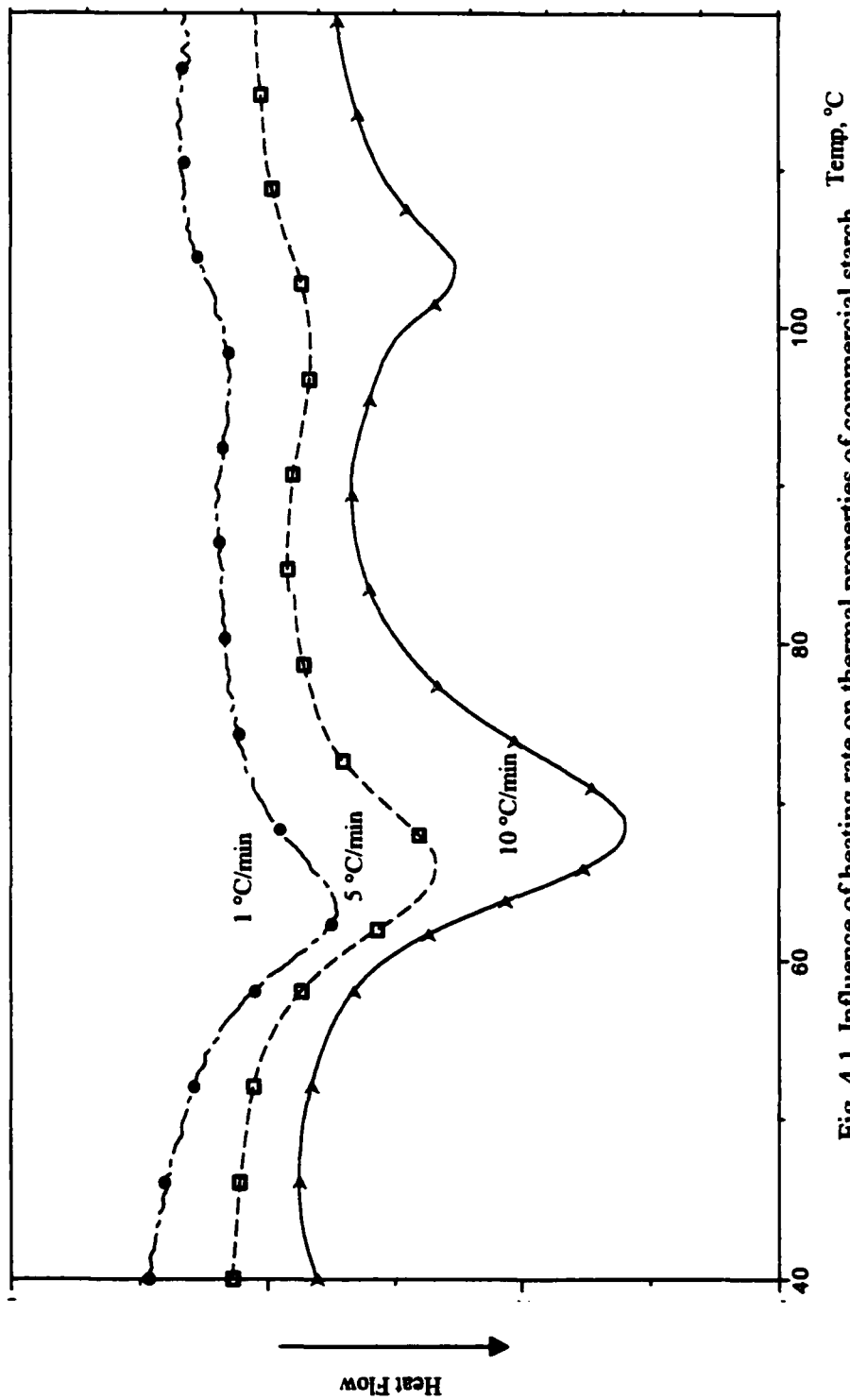


Fig. 4.1. Influence of heating rate on thermal properties of commercial starch

crystallites and thus a smaller fraction might melt at a low temperature. In our study, there were only slight enthalpy changes observed for gelatinization when comparing the high heating rate ($10^0$C/min) with the low one ($1^0$C/min). Compared to 5 °C/min, 1 °C/min heating rate resulted in longer heating time and more fluctuating DSC signals. Therefore, the $5^0$C/min heating rate was chosen for the rest of DSC tests.

**Moisture Content**

As the moisture content decreased from 90% to 50%, the completion temperature of the first transition was greatly increased (from 72.8 to 89.3 °C), resulting in a substantial increase in the gelatinization temperature range ($T_c$-$T_o$) (from 17 to 34°C) (Table 4.2, Fig. 4.2). However, no enthalpy change was observed. The drop in moisture content also affected the second transition, where the peak, onset, and completion temperatures were tremendously increased, while the enthalpy was not affected.

It is generally accepted that starch granules have partially crystalline nature. During the gelatinization process, the softening of the amorphous regions (glass transition) usually occurs before the melting of crystallites. Water acts as a plasticizer of the amorphous parts of the starch granule resulting in the depressed glass transition temperature. Biliaderis et al. (1986b) reported a smaller enthalpy of the first transition with a concomitant development of a second transition at higher temperature when water content became insufficient. Results from this study indicated that 70% was the sufficient moisture content for rice starch gelatinization. Further reduction in the sample size to reach a greater moisture content (such as 90%) might cause higher variance in the result. Thus, the 70% moisture content was chosen for the additive effect test.

Table 4.2. Effect of Moisture Content on Thermal Properties of Commercial Starch [1,2,3]

| Moisture Content (%) | Transition Temperatures and Transition Enthalpies | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | First Transition | | | | Second Transition | | | |
| | $T_o$ | $T_p$ | $T_c$ | $\Delta H$ | $T_o$ | $T_p$ | $T_c$ | $\Delta H$ |
| 50 | 55.0[c] | 66.3[a] | 89.3[a] | 11.1[a] | 98.2[a] | 108.8[a] | 114.9[a] | 1.8[a] |
| 70 | 57.0[a] | 66.1[a] | 75.6[b] | 10.2[a] | 88.5[b] | 99.4[b] | 107.9[b] | 2.1[a] |
| 90 | 55.8[b] | 65.2[b] | 72.8[c] | 10.4[a] | 85.6[c] | 93.7[c] | 100.8[c] | 1.6[a] |

[1] $T_o$, $T_p$, $T_c$, = onset, peak, and completion temperatures, respectively; $\Delta H$=enthalpy.
[2] Different letters within each column indicate values are significantly different at the level of $p \leq 0.05$.
[3] Units: Temperature (°C), Enthalpy (J/g, dry matter); Heating Rate=5 °C/min.

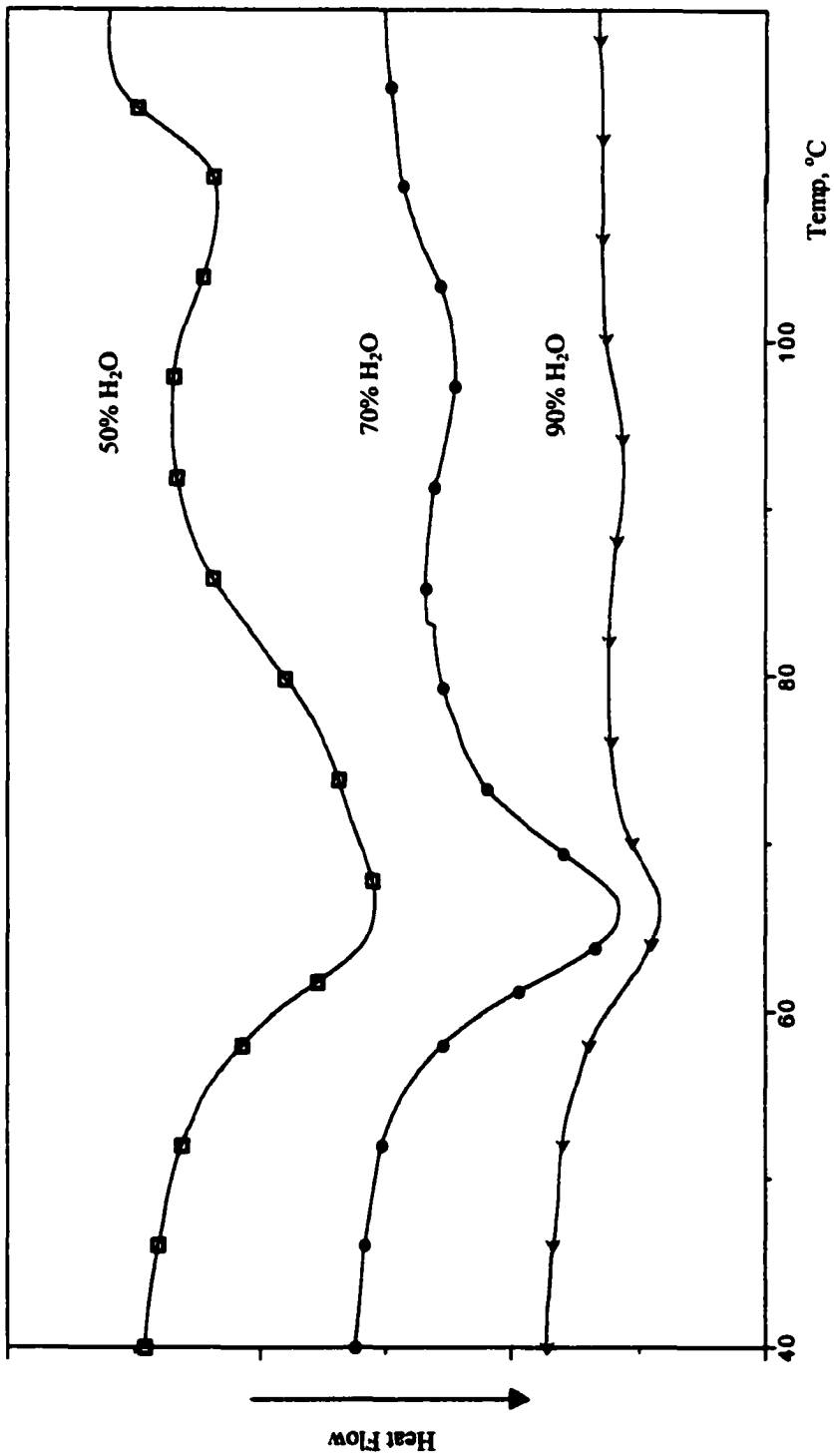


Fig. 4.2. Influence of water content on thermal properties of commercial starch

### 4.3.2. Effect of Lipids on Thermal Properties of Commercial Starch

It is generally accepted that the high temperature transition (95-130°C), which is well above the melting endotherm of starch crystallites at 65-72°C, is assigned to the melting of the amylose-lipid complex (Biliaderis 1991; Szczodrak and Pomernz 1992).

Addition of 0.6% LC and LE led to minor changes on the onset gelatinization temperatures (Table 4.3, Fig. 4.3). Besides that, the presence of LE also caused the peak temperature to increase when compared to the control. However, the completion temperature and transition enthalpy were not influenced by the lipid addition. No significant influence was observed when MP was present. No significant influence on the second transition parameters was observed.

Chungcharoen and Lund (1987) found that the gelatinization temperature was increased when 0.4% glyceryl monostearate was added to the rice starch. Larsson (1980) also reported a delay in the loss of birefringence of potato starch heated with 1% monolaurin. However, they did not find a difference in enthalpy for gelatinization of starch. In this study, addition of lipids did not cause a gelatinization enthalpy change. The presence of LC and LE did increased the onset gelatinization temperature of the control.

### 4.3.3. Effect of Amino Acids on Thermal Properties of Commercial Starch

Compared to the control, the presence of 6% aspartic acid significantly increased the peak and completion temperatures of gelatinization, whereas the enthalpy of the amylose-lipid complex was reduced by 1.4 J/g (Table 4.4, Fig. 4.4). Addition of aspartic acid increased the onset and peak temperatures of the gelatinization by 0.9 and

Table 4.3. Effect of Lipids on Thermal Properties of Commercial Starch [1,2,3]

| Additives (0.6%, starch base) | Transition Temperatures and Transition Enthalpies | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | First Transition (Gelatinization) | | | | Second Transition (Amylose-Lipid Complex) | | | |
| | $T_o$ | $T_p$ | $T_c$ | $\Delta H$ | $T_o$ | $T_p$ | $T_c$ | $\Delta H$ |
| Control | 57.0$^{c}$ | 66.1$^{b}$ | 75.6$^{a}$ | 10.2$^{a}$ | 88.5$^{a}$ | 99.4$^{a}$ | 107.9$^{a}$ | 2.1$^{a}$ |
| MP | 57.3$^{bc}$ | 67.2$^{ab}$ | 76.5$^{a}$ | 11.0$^{a}$ | 88.0$^{a}$ | 97.6$^{a}$ | 107.6$^{a}$ | 2.2$^{a}$ |
| LC | 57.5$^{ab}$ | 67.0$^{ab}$ | 76.3$^{a}$ | 9.5$^{a}$ | 90.9$^{a}$ | 100.4$^{a}$ | 109.8$^{a}$ | 2.3$^{a}$ |
| LE | 58.0$^{a}$ | 67.0$^{a}$ | 75.7$^{a}$ | 9.3$^{a}$ | 88.4$^{a}$ | 99.2$^{a}$ | 107.6$^{a}$ | 2.2$^{a}$ |

[1] $T_o$, $T_p$, $T_c$, = onset, peak, and completion temperatures, respectively; $\Delta H$=enthalpy; MP=monopalmitin, LC=lysophosphatidylcholine, LE=lysophosphatidylethanolamine.

[2] Different letters within each column indicate values are significantly different at the level of $p \leq 0.05$.

[3] Units: Temperature (°C), Enthalpy (J/g, dry matter); Heating Rate=5 °C/min, Moisture Content=70%.

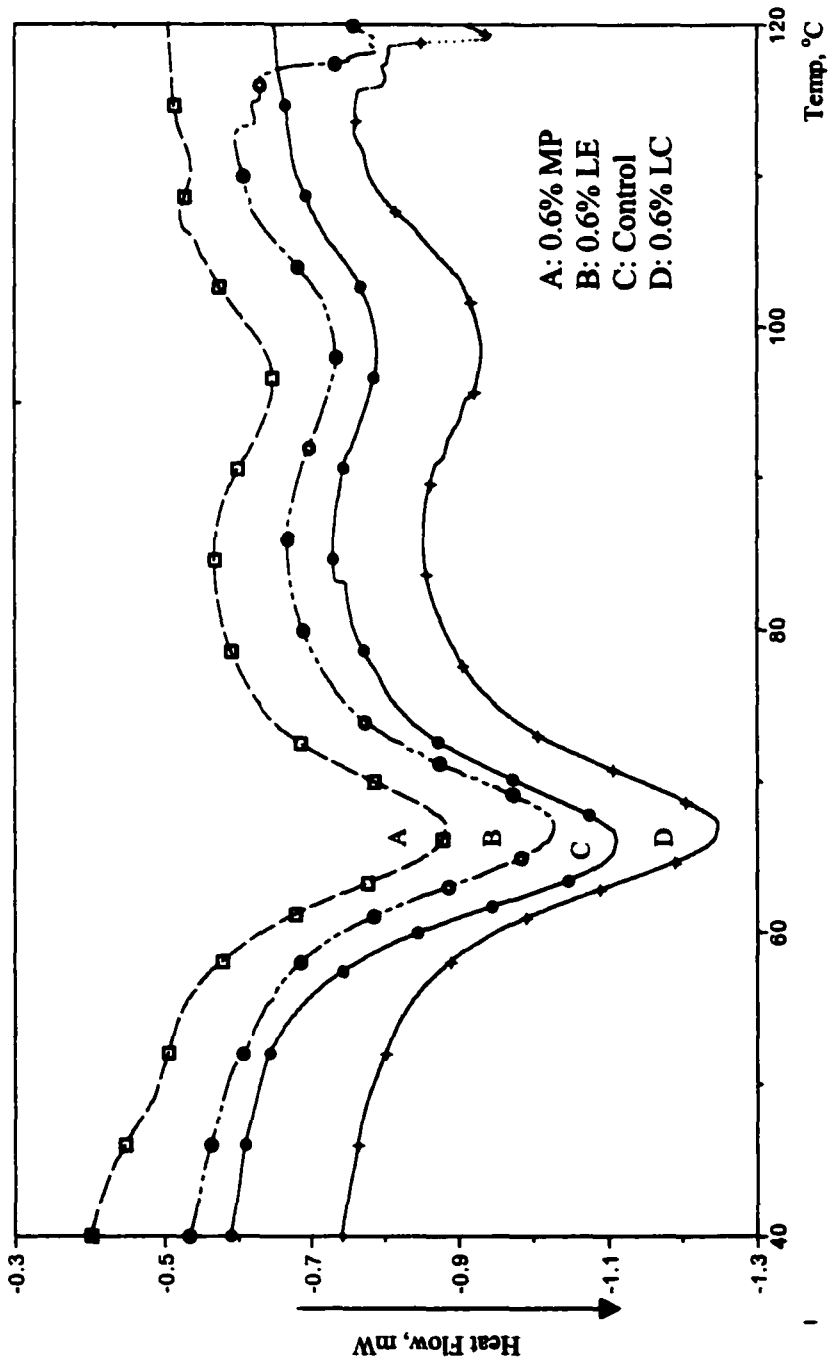


Fig. 4.3. Influence of lipids on thermal properties of commercial starch

Table 4.4. Effect of Amino Acids on Thermal Properties of Commercial Starch [1,2,3]

| Additives (6%, starch base) | Transition Temperatures and Transition Enthalpies | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | First Transition (Gelatinization) | | | | Second Transition (Amylose-Lipid Complex) | | | |
| | $T_o$ | $T_p$ | $T_c$ | $\Delta H$ | $T_o$ | $T_p$ | $T_c$ | $\Delta H$ |
| Control | 57.0$^{c}$ | 66.1$^{c}$ | 75.6$^{b}$ | 10.2$^{a}$ | 88.5$^{b}$ | 99.4$^{a}$ | 107.9$^{a}$ | 2.1$^{a}$ |
| Aspartic | 57.9b$^{c}$ | 67.5$^{b}$ | 78.2$^{a}$ | 9.7$^{a}$ | 94.1$^{ab}$ | 101.3$^{a}$ | 110.0$^{a}$ | 0.7$^{b}$ |
| Glutamic | 58.5b$^{a}$ | 67.4$^{b}$ | 77.4$^{ab}$ | 8.9$^{a}$ | 98.0$^{a}$ | 101.4$^{a}$ | 108.7$^{a}$ | 0.3$^{b}$ |
| Arginine | 58.3b$^{a}$ | 67.7$^{ab}$ | 79.1$^{a}$ | 9.7$^{a}$ | ND | ND | ND | ND |
| Lysine | 58.9$^{a}$ | 68.2$^{a}$ | 79.1$^{a}$ | 10.0$^{a}$ | ND | ND | ND | ND |

[1] $T_o$, $T_p$, $T_c$, = onset, peak, and completion temperatures, respectively; $\Delta H$=enthalpy; ND=none detected.
[2] Different letters within each column indicate values are significantly different at the level of $p \leq 0.05$.
[3]Units: Temperature (°C), Enthalpy (J/g, dry matter); Heating Rate=5 °C/min, Moisture Content=70%.

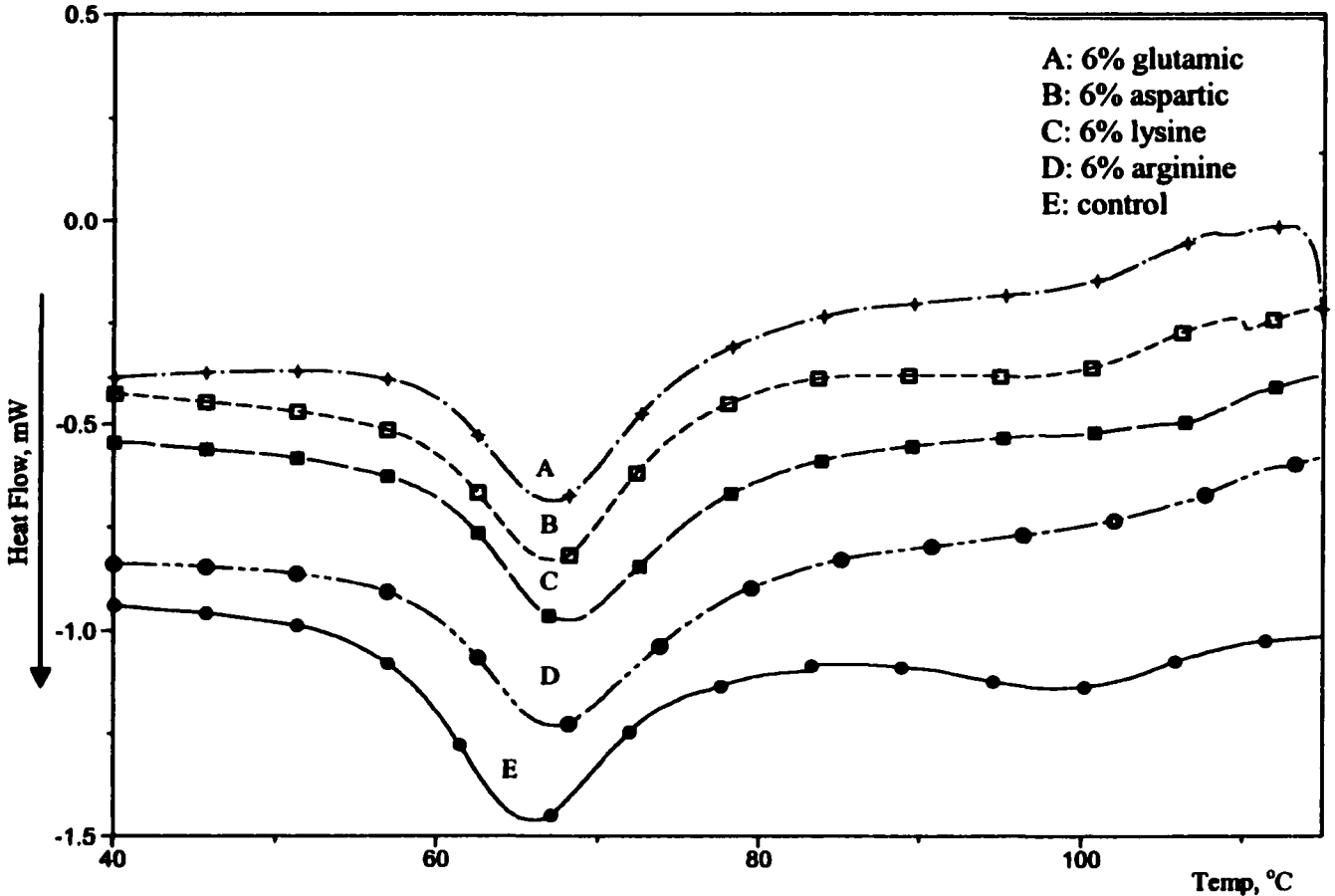


Fig. 4.4. Influence of amino acids on thermal properties of commercial starch

1.4 °C. For the second transition, the presence of glutamic acid significantly increased the onset temperature by 9.5 °C and depressed the enthalpy by 1.8 J/g.

The presence of arginine increased the onset, peak, and completion temperatures of starch gelatinization by 1.3, 1.6, and 3.5 °C, whereas the second transition was not detected. Similarly, the presence of lysine increased the onset, peak, and completion temperatures of starch gelatinization by 1.9, 2.1, and 3.5 °C with the second transition not detected.

Several workers reported that protein removal could decrease the gelatinization temperature of the flour, from which they suggested the inhibitory effect of protein on starch granule swelling (Cheng 1987; Marshall et al. 1990; Yang and Chang 1999). Results from this study indicated that the presence of amino acids increased the gelatinization temperatures and reduced the enthalpy for amylose-lipid complex melting.

**4.3.4. Effect of β-Cyclodextrin on Thermal Properties of Commercial Starch**

Compared to the starch control, the presence of 6% β-CD increased the onset temperature by 0.6°C and depressed the enthalpy of the gelatinization by 1.5 J/g (Table 4.5 and Fig.4.5). However, the peak and completion temperatures of starch gelatinization were not affected. The enthalpy of the second transition (amylose-lipid complex melting) was significantly reduced by the β-CD by 1 J/g.

The influence of β-CD on wheat starch gelatinization had been studied by several investigators, from which they suggested that the formation of β-CD inclusion with lipids could enhance the swelling power and solubility of the granules (Kim and Hill 1984). Results from our studies indicated the formation of β-CD complexes with

**Table 4.5. Effect of β-Cyclodextrin on Thermal Properties of Commercial Starch [1,2,3]**

| Additives (6%, starch base) | Transition Temperatures and Transition Enthalpies | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | First Transition (Gelatinization) | | | | Second Transition (Amylose-Lipid Complex) | | | |
| | $T_o$ | $T_p$ | $T_c$ | ΔH | $T_o$ | $T_p$ | $T_c$ | ΔH |
| Control | 57.0 | 66.1 | 75.6 | 10.2 | 88.5 | 99.4 | 107.9 | 2.1 |
| β-CD | 57.6 | 67.0 | 76.5 | 8.7 | 91.7 | 100.2 | 108.6 | 1.1 |
| Effect [4] | ++ | -- | -- | ++ | -- | -- | -- | ++ |

[1] $T_o$, $T_p$, $T_c$, = onset, peak, and completion temperatures, respectively; ΔH=enthalpy;
[2] Different letters within each column indicate values are significantly different at the level of $p \leq 0.05$.
[3] Units: Temperature (°C), Enthalpy (J/g, dry matter); Heating Rate=5 °C/min, Moisture Content=70%.
[4] "++" = significantly different, "—"=not significantly different (at the level of $p \leq 0.05$).

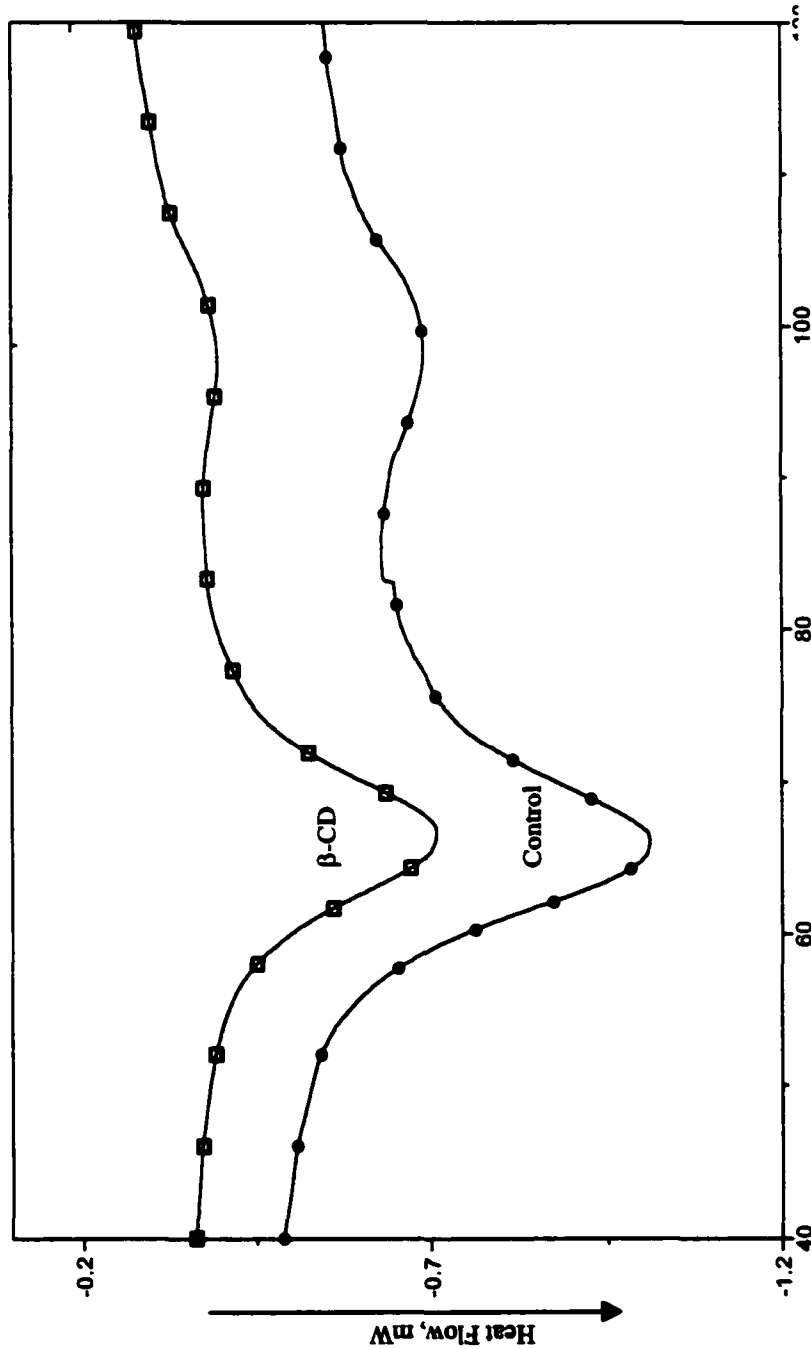


Fig. 4.5. Influence of β-cyclodextrin on thermal properties of commercial starch

lipids, through competition with amylose. Therefore, the enthalpy of the second transition, which was attributed to the formation of amylose-lipid complexes, was reduced. The binding of lipids by β-CD enhanced starch granule swelling and amylose leaching ability, resulting in the reduced gelatinization (first transition) enthalpy.

## 4.4. CONCLUSION

This study showed that increasing heating rate resulted in greater starch gelatinization temperatures (onset, peak, and completion) and wider gelatinization temperature range. Due to the semicrystalline nature of starch granules, a high heating rate might offer a lower chance for amylopectin crystallite chain rearrangement, resulting in a wider gelatinization temperature range. Compared to a system with insufficient water content (<70%), the increase of moisture content greatly depressed the first transition (gelatinization) completion temperature and the second transition (amylose-lipid complex) onset, peak, and completion temperatures.

Addition of lipids only had a minor influence on starch gelatinization. Amino acids inhibited amylose-lipid complex formation, resulting in the reduction of the second transition enthalpy. Beta-CD competed with amylose for lipids, resulting in decreased first and second transition enthalpies.

# CHAPTER 5
# EFFECTS OF LIPIDS, AMINO ACIDS, AND BETA-CYCLODEXTRIN ON RICE STARCH CRYSTALLIZATION USING X-RAY DIFFRACTION (XRD)

## 5.1. INTRODUCTION

Starch retrogradation is the process that occurs when starch molecules reassociate and form an ordered structure during cooling and storage. Junction zone formation is generally considered to be the first step for starch molecules reassociation and crystallization (Miles et al. 1985). Ultimately, under favorable conditions, a crystalline order is formed and a physical phase separation occurs (Atwell et al. 1988). Short-term retrogradation has been attributed to the gelation and crystallization of the amylose fraction, whereas the long-term retrogradation has been attributed to the recrystallization of amylopectin fraction (Eliasson 1985; Miles et al. 1985; Sievert and Wursch 1993; Fredriksson et al. 1998). Starch retrogradation is usually associated with quality defects in food products, such as bread staling and loss of viscosity and precipitation in soups and sauces (Miles et al. 1985; Morris 1994). The rate and extent of starch retrogradation depends on several variables, including (1) botanical source, (2) amylose and amylopectin ratio, (3) temperature, (4) concentration, (5) presence and concentration of other food ingredients (such as lipids and surfactants, sugars, acids, and salts) (Orford et al. 1987; Kalichevsky et al. 1990; Shi and Seib 1992; Eliasson and Ljunger 1988; Eliasson and Gudmundsson 1996; Fredriksson et al. 1998). How this process is affected by interactions between starch and other food components needs to be understood for better quality control of food products.

X-ray diffraction has been used to a large extent to study the crystallinity of starch. Under X-ray diffraction (XRD), cereal starches usually give A patterns (Zobel and Senti 1960; Zobel 1988a, 1988b). V-type crystalline structure is believed to be mainly attributed to the formation of helical complexes of amylose with lipid in gelatinized starch (Hibi et al 1990). However, the change of crystalline structures from V-type to B-type XRD pattern was observed during cold storage, which might indicate that the starch-lipid complexes are metastable and changed to a more stable structure partly characterized by B-type via an amorphous state (Hibi et al 1990).

Normal starches from rice usually contain 0.5-1.3% granule associated lipids. It is generally accepted that starch lipids have a remarkable influence on the behavior of starch in gelatinization and retrogradation processes (Ohashi et al 1980, Juliano 1984, Morrison et al 1984, Azudin and Morrison 1986). Studies from x-ray diffraction (XRD) and differential scanning calorimetry (DSC) have shown the formation of helical complexes of amylose with lipids after starch gelatinization (Kugimiya et al 1980; Zobel et al 1988). Compared with the amorphous starches, those complexes were reported to be more resistant to enzymatic attack and less soluble in water (Gray and Schoch 1962, Hanna and Lelievre 1975, Ohashi et al 1980, Hoover and Hadziyev 1981; Holm et al 1983). Amylose-monoglyceride complex studies showed that long chain, saturated monoglyceride complexes were more resistant to enzymic breakdown than the short chain, unsaturated complexes (Eliasson and Krog 1985). Addition of certain lipids or surfactants may retard the firming and retrogradation of starchy foods (Lagendijk and Pennings 1970; Lin et al 1978; Germani et al 1983; Batres and White 1986; Evans 1986; Eliasson and Ljunger 1988; Krog et al 1989; Chang and Liu 1991).

Retrogradation of nonwaxy starches was reported to form enzyme-resistant starch (RS), which consists of short-chain linear α-glucans (Russell et al 1989). Addition of lipids could result in the reduction of RS formation due to the lipids interaction with amylose chains that were involved in the RS formation (Szczodrak and Pomeranz 1992).

Several investigators reported the protein and starch interaction in baking product manufacturing and storage (Dreese et al 1988; Martin et al 1991a,b; Holm et al 1985; Bjorck et al 1986;Guerrieri et al 1997). They proposed that protein and starch interaction might influence the staling of the final products, the availability of starch to digesting enzymes, and the quality of the final products. Digestion studies showed that surface proteins in the starch granule may act as an obstacle to the access of amylolytic enzymes or may interact with them, resulting in the modification of their surface distribution (Greenwell et al. 1985). Evidence showed that high-protein content rices are generally less tender than low-protein content rices after cooking, which may indicate the influence of protein on starch gelation and reassociation (Onate et al 1964, Juliano et al 1965).

The influences of starch proteins on gelatinization and pasting properties of rice starch have been studied by several researchers (Juliano et al. 1964a; Cheng 1987; Marshall et al. 1990; Hamaker and Griffin 1990; Yang and Chang 1999). Those influences include the reduction of gelatinization temperature, the increase of peak viscosity, and the decrease of peak temperature of the paste. Studies from Hamaker and Griffin (1990) suggested that the hydrolyzed product of rice proteins might interact with rice starch and result in viscosity reduction of rice flour. Studies suggested that protein

and its subunit properties (such as solubility) might be related to their difference in amino acid composition (Bushuk 1985). Further study is needed to exam the influence of amino acid structure and type on the retrogradation properties of rice.

The objectives of this study were: (1) to study the XRD pattern of rice starch and rice flours; (2) to determine the lipid removal and protein removal effects on rice flour XRD patterns; (3) to examine the influence of lipids, amino acids, and β-cyclodextrin on starch granule XRD pattern.

## 5.2. MATERIALS AND METHODS

### 5.2.1. Materials

Commercial rice starch (S-7260), purchased from Sigma Chemical Co (St. Louis, MO), was used as control. Gelatinized samples, which include commercial rice starch, white rice flour, brown rice flour, defatted white flour and brown flour, white and brown starch isolates, were obtained from the Rapid Visco Analyzer (RVA) tests (see Chapter 3) and used in this study. Beta-cyclodextrin, MP, LC, LE, aspartic acid, glutamic acid, lysine, and arginine were used for additive effect study. To limit the number of samples, only the gelatinized samples with 0.6% lipids, 6% β-CD, and 6% amino acids were used.

The gelatinized sample gels, obtained from the RVA tests, were stored at refrigerator temperature for 3 days to accelerate retrogradation. Those gels were then freeze-dried and milled to 100 mesh powders using a rice miller. Samples were hydrated at 75% relative humidity (RH) in a sealed vessel using saturated NaCl before the X-Ray Diffraction (XRD) test.

### 5.2.2. X-Ray Diffraction (XRD)

About 1 g of sample was pressed into a 10x25 mm pellet with a hydraulic press. X-ray diffraction pattern was obtained using a Siemens D5000 X-ray diffraction instrument. X-ray diffractograms were obtained under conditions of 40 KV, 30 mA, with the scanning angle 2 θ set from $2^0$ to $36^0$ at a scanning rate of 0.6°/min. Relative crystallinity (RC) of the starch was determined by the method of Hermans and Weidinger (1948), as described by Nara et al (1978), i.e., the area of the crystalline fraction ($a_c$) is divided by the diffraction area for a 100% crystalline substance ($A_c$). In this study, the area of the crystalline fraction in raw commercial starch XRD pattern was used as the value of Ac (Dragsdorf and Varriano-marston, 1980). X-ray patterns were designated according to the d-spacings and intensities given by Zobel (1988a,b). The diffraction patterns were recorded and compared.

## 5.3. RESULTS AND DISCUSSION

### 5.3.1. Effects of Lipids and Protein Removal on XRD Pattern of Rice Flours

Gelatinized white flour showed a strong peak at 4.4 Å and a weak peak at 6.8 Å, indicating a V-pattern crystallite structure formation between the amylose and the lipids present in white flour (Fig. 5.1). Lipid-removal led to the decrease in intensity of the 4.4 Å peak and the destruction of the 6.8 Å peak. In the mean time, another peak formed at 5.2 Å and the RC was reduced by 8%. The removal of protein from defatted flour caused the total destruction of the 4.4 Å peak and the development of 5.2 Å peak. The RC was reduced for 11% when compared to the white flour control.

The gelatinized brown flour showed a strong peak at 4.4 Å and a weak peak at 5.2 Å (Fig. 5.2). However, defatting of brown flour did not affect the 4.4 Å peak, but

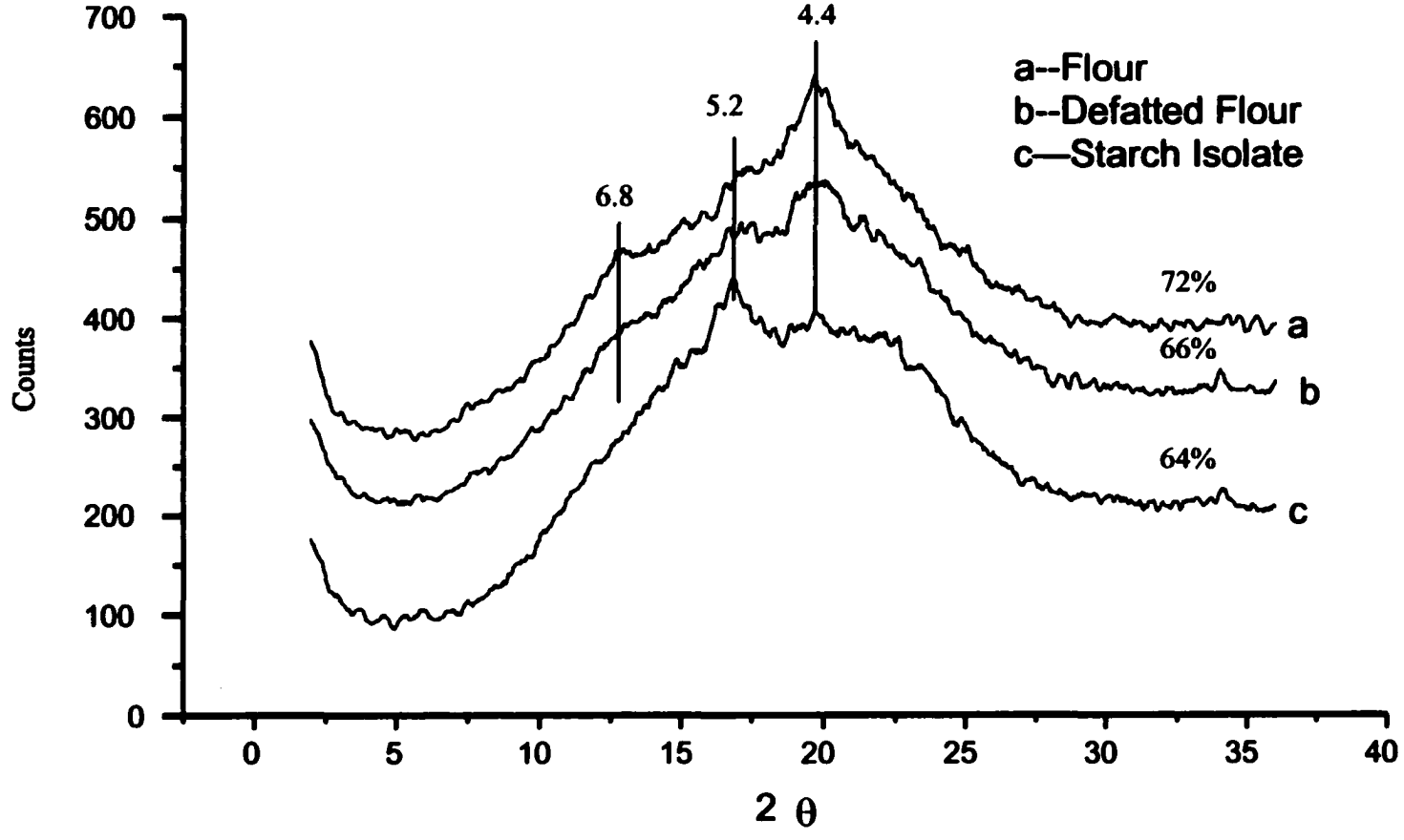


**Fig. 5.1. Influence of defatting and protein removal on X-ray Diffraction pattern of white flour (Samples a, b, and c were gelatinized and stored at the refrigerator for 3 days)**

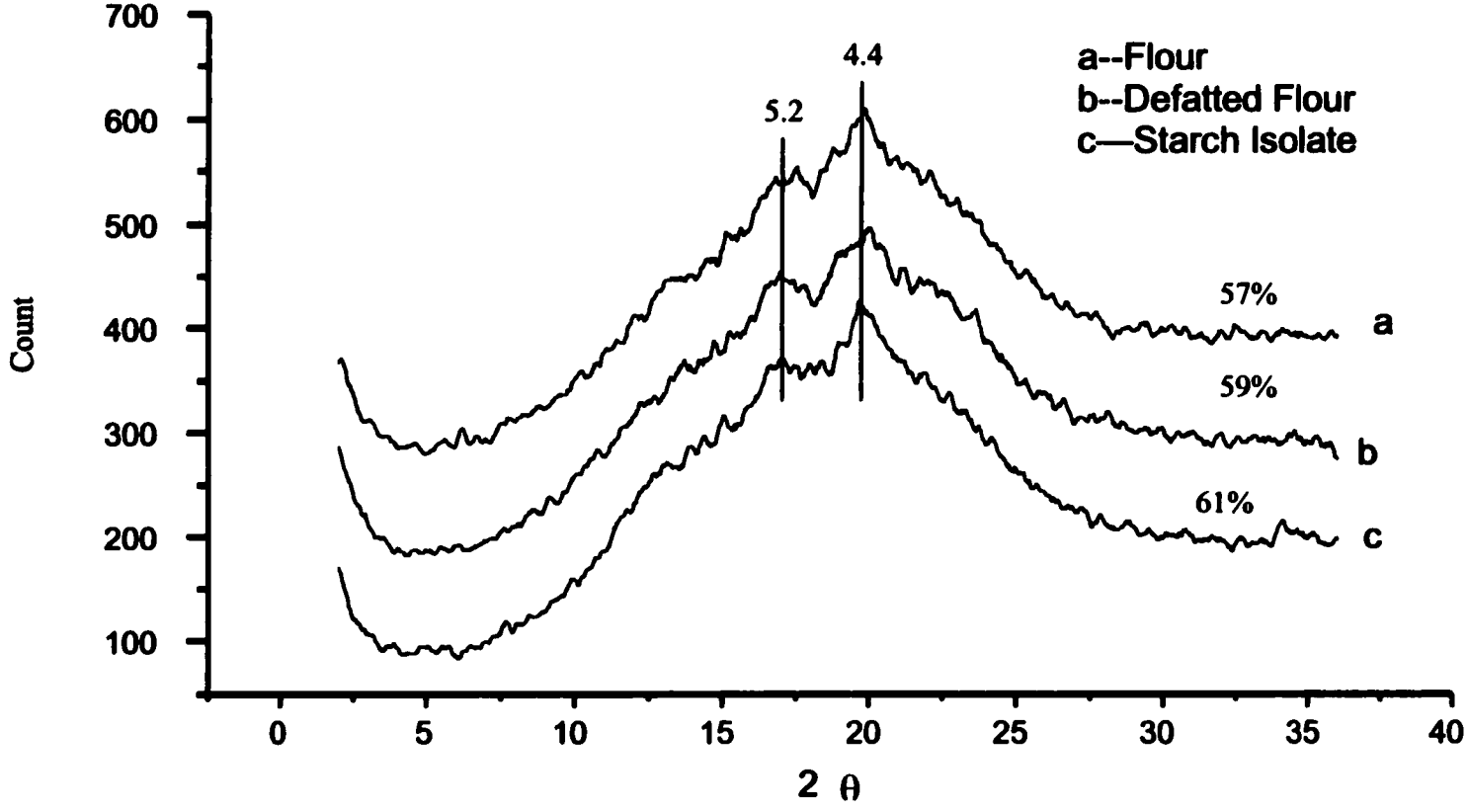


**Fig. 5.2. Influence of defatting and protein removal on X-ray Diffraction pattern of brown flour (Samples a, b, and c were gelatinized and stored at the refrigerator for 3 days)**

induced the further development of the 5.2 Å peak. The RC was slightly increased (3%). After protein removal, the peak at 5.2 Å was weakened, whereas the 4.4 Å peak remained and the RC was increased for another 3%.

### 5.3.2. Effect of Lipids, Amino Acids, and β-Cyclodextrin on Commercial Starch XRD Pattern

The crystalline nature of raw and gelatinized commercial starches was investigated by X-ray diffraction (Fig. 5.3). In raw starch, A-type X-ray diffraction patterns characterized by clear diffraction peaks around 2θ of 3.8, 5.2, and 5.8 Å were seen. The A-pattern was generally regarded as cereal starch crystal forms (Zobel and Senti 1960; Zobel 1988a, b), resulting from the formation of double helical structures of the amylopectin fraction (Jenkins et al. 1993). During starch gelatinization, the double helices of amylopectin were destroyed, whereas part of the free lipids present in the cereal starches formed a helical inclusion complex with the amylose molecules (Eliasson 1986, Zobel 1988b). Thus, the gelatinized cereal starch was granted the V-type X-ray diffraction pattern, which was characterized by peaks around 2θ of 4.4, 6.8, and 12 Å. In this study, gelatinization resulted in a 47% reduction of the RC, and the disappearance of the peaks at 6.8 and 12 Å. The X-ray pattern changed to a strong peak at 4.4 Angstroms.

Compared to the gelatinized commercial starch (control), the presence of 0.6% MP increased the intensity of 4.4 Å peak and gave rise to the development of another peak at 6.8 Å, resulting in a more evident V-type crystalline structure (Fig. 5.4). The RC was also increased by 104%. Similarly, the addition of LC and LE resulted in an increase of the RC by 83% and 104%, respectively, with the X-ray pattern changed to a more evident V-type crystalline structure. Results from this study indicated that addition

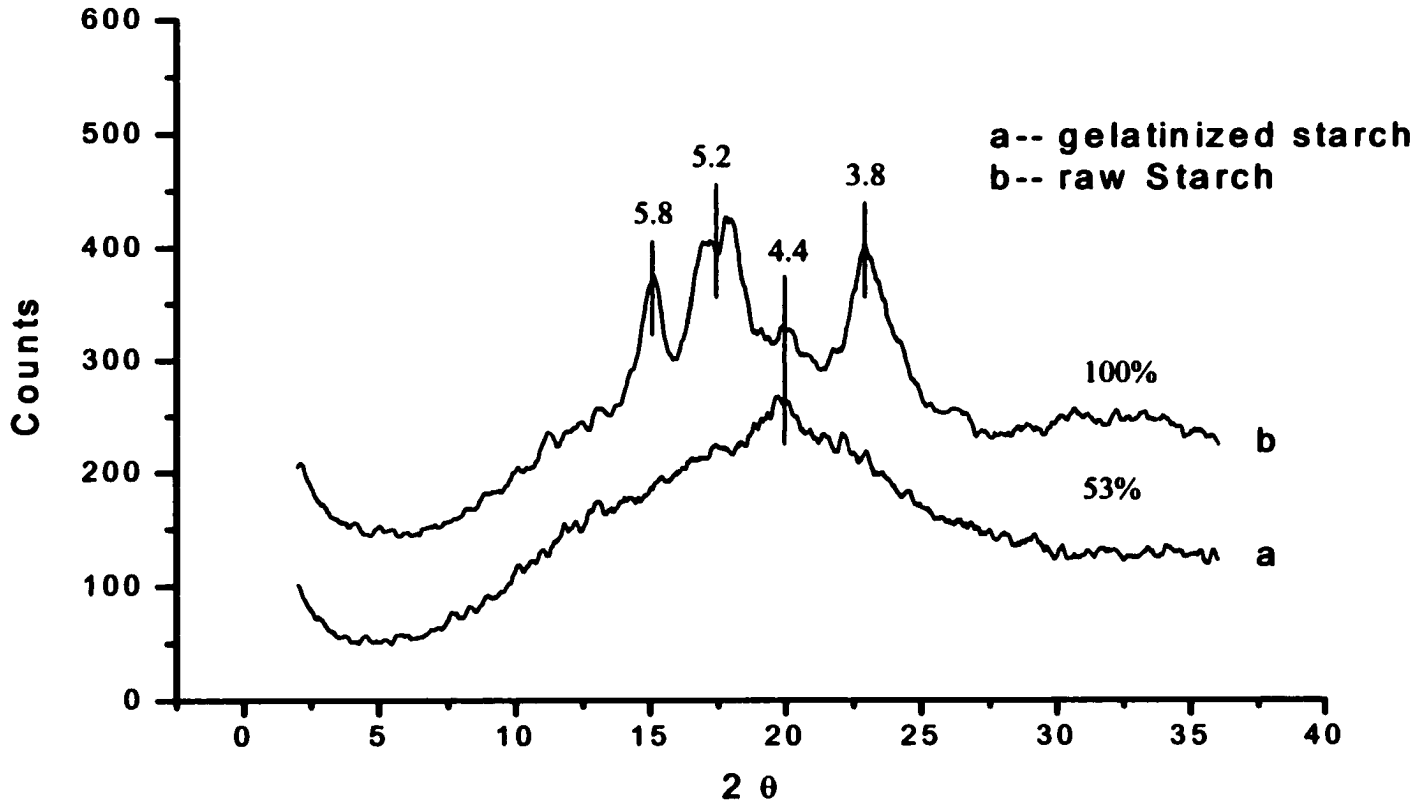


Fig. 5.3. Influence of gelatinization on XRD pattern of commercial starch

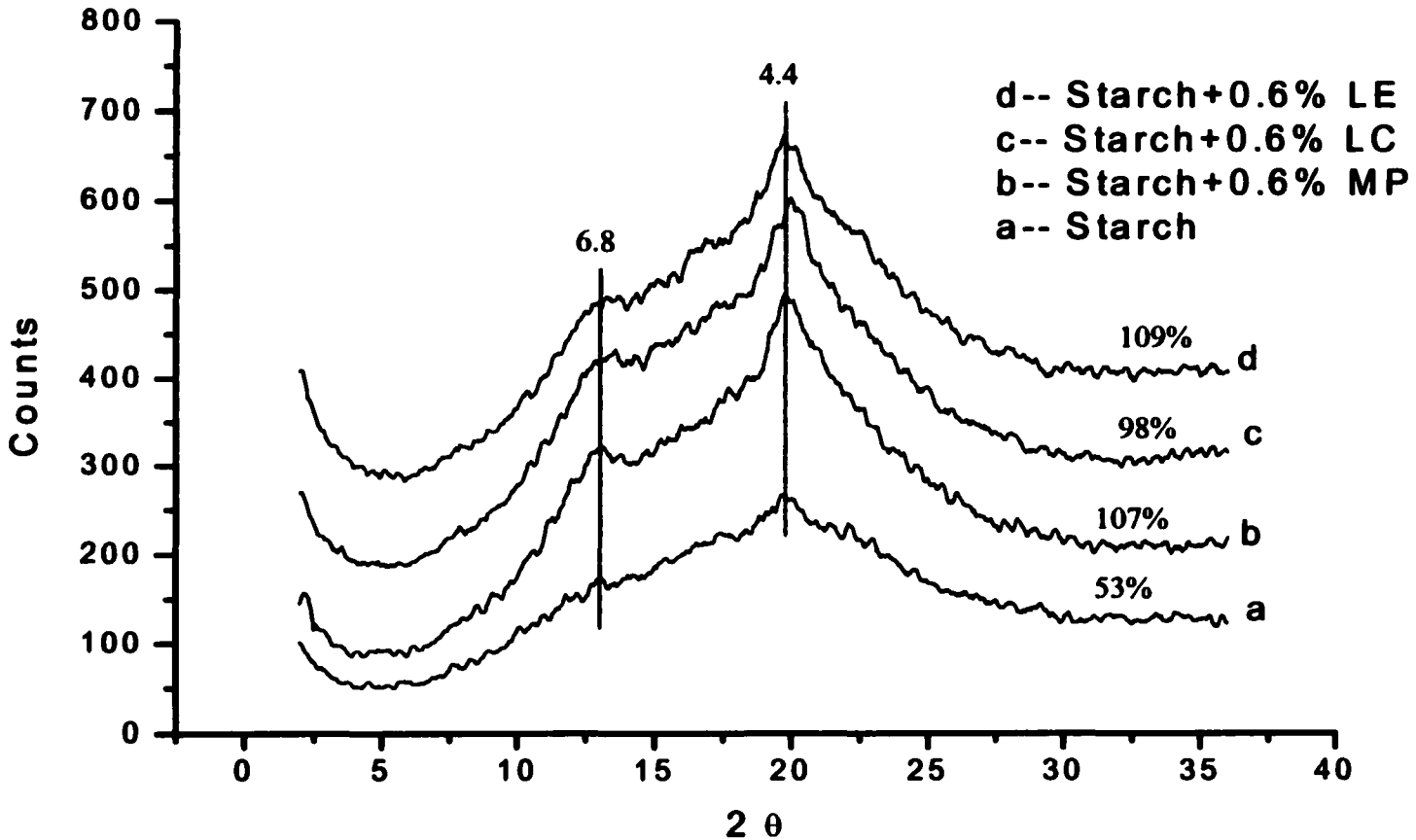


**Fig. 5.4. Influence of lipids on XRD pattern of commercial starch (all samples were gelatinized and stored at the refrigerator for 3 days)**

of lipids led to the development of a V-type X-ray pattern , which was attributed to the enhanced lipid-amylose complexes formation (Kugimiya et al. 1980; Mercier et al. 1980; Zobel 1988b).

The presence of 6% aspartic acid decreased the RC by 3% but increased the 4.4 Å peak intensity and induced the development of another two peaks at 3.8 and 3.4 Å (Fig. 5.5). Addition of 6% arginine resulted in a 7% RC increase while the X-ray pattern was not changed. Both glutamic acid and lysine enhanced the 4.4 Å peak and caused the RC increase for 81% and 79% respectively.

Compared to the control, the presence of 6% β-CD led to the development of a weak peak at 6.8 Å and increased the intensity of the 4.4 Å peak, indicating a weak V pattern was formed (Fig. 5.6). The RC was also increased by 83%. The effect of β-CD on starch crystalline structure has not been documented. The result from our study provided some evidence about the β-CD influence on rice starch X-ray diffraction pattern.

### 5.3.3. Effect of Lipids, Amino Acids, and β-CD on White Starch Isolate XRD Pattern

Compared to the gelatinized white starch isolate (control), the presence of 0.6% MP increased the intensity of 4.4 Å peak (Fig. 5.7). The RC was also increased by 13%. Addition of 0.6% LC increased the RC by 3% and enhanced the 4.4 Å peak. However, the presence of 0.6% LE caused a decrease of the RC for 3% and the increase of the 4.4 Å peak intensity.

The presence of 6% aspartic acid increased the RC by 34%, enhanced the 4.4 Å peak, and induced the development of another peak at 3.4 Å (Fig. 5.8). Addition of 6%

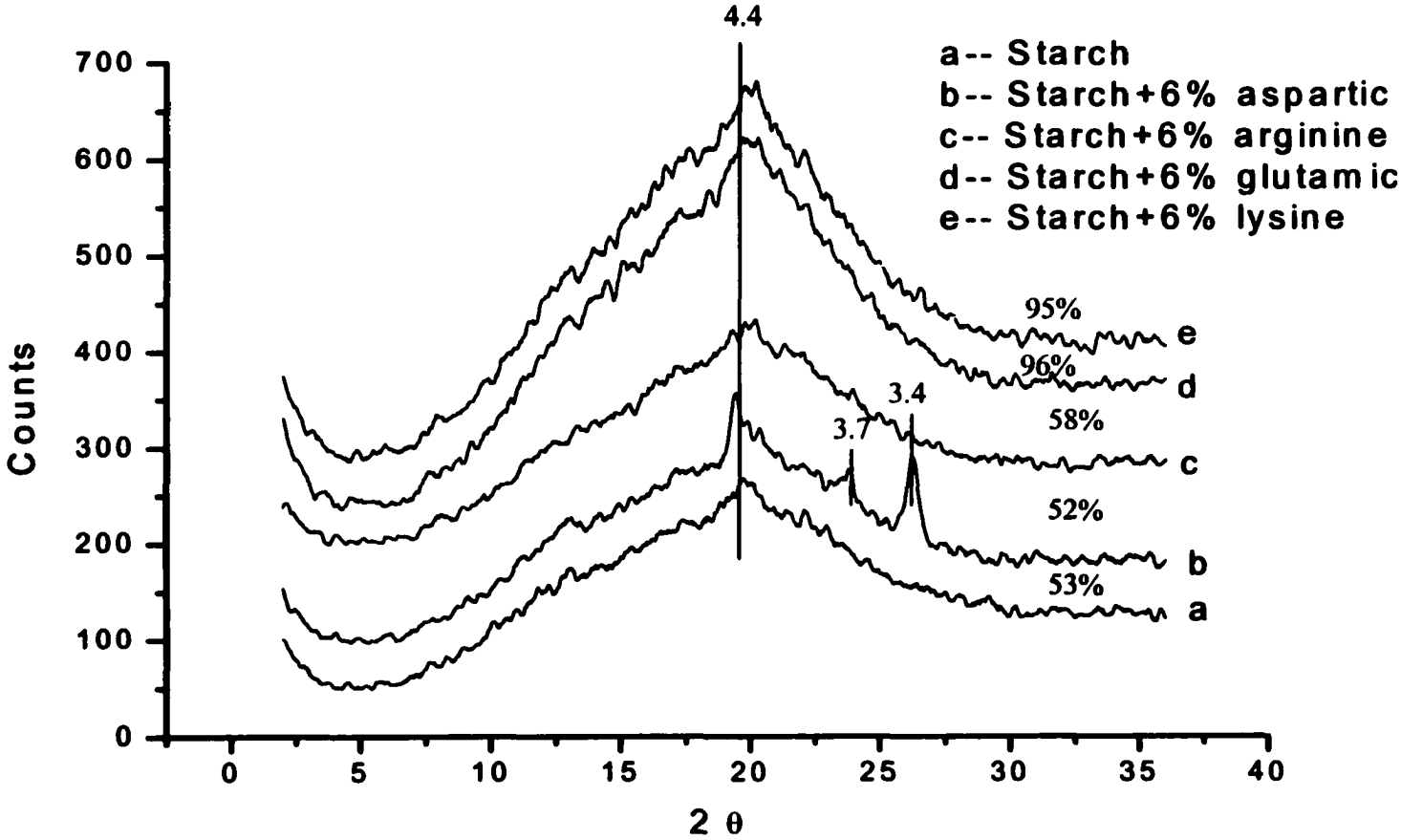


**Fig. 5.5. Influence of amino acids on XRD pattern of commercial starch (all samples were gelatinized and stored at the refrigerator for 3 days)**

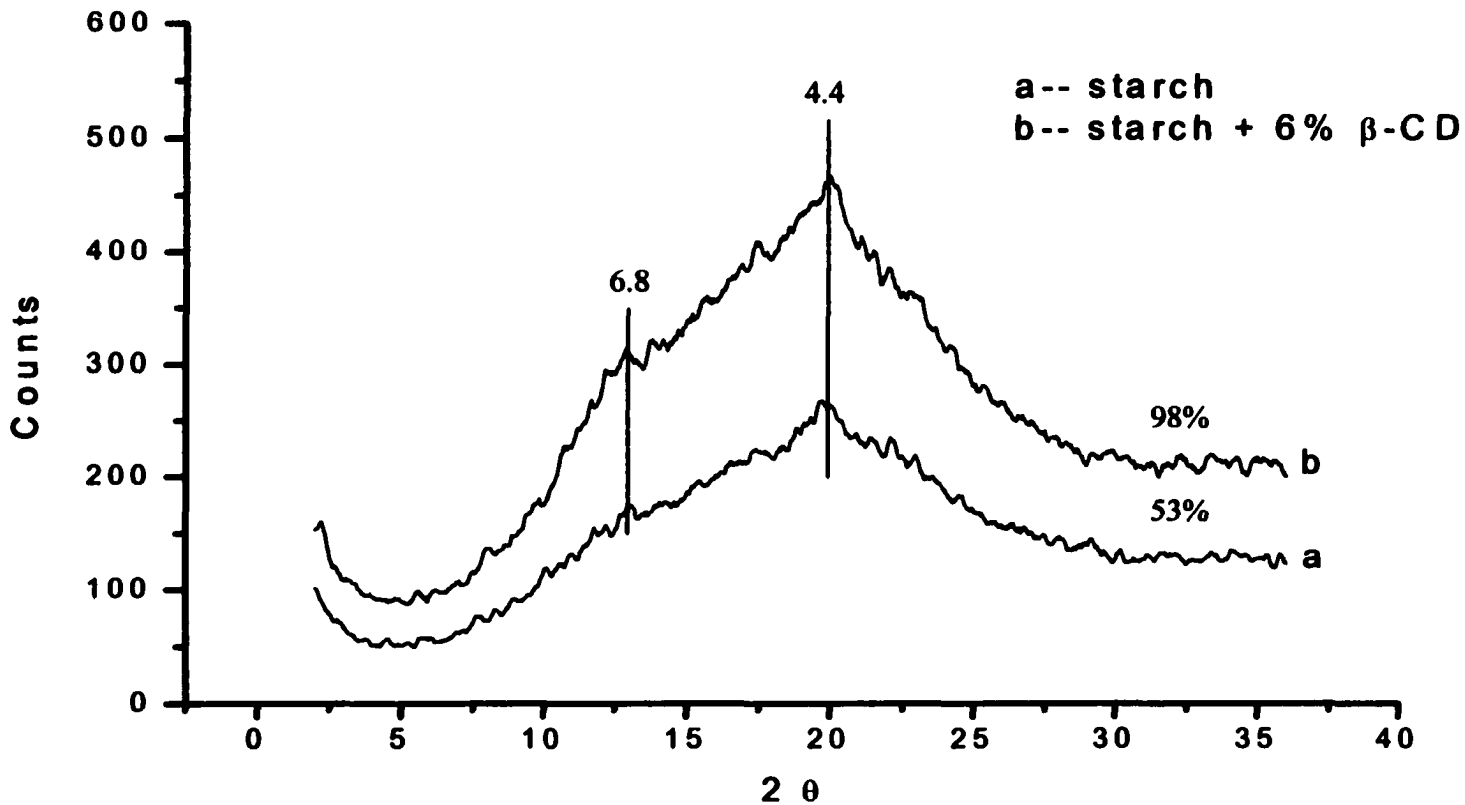


**Fig. 5.6. Influence of β-CD on XRD pattern of commercial starch (all samples were gelatinized and stored at the refrigerator for 3 days)**

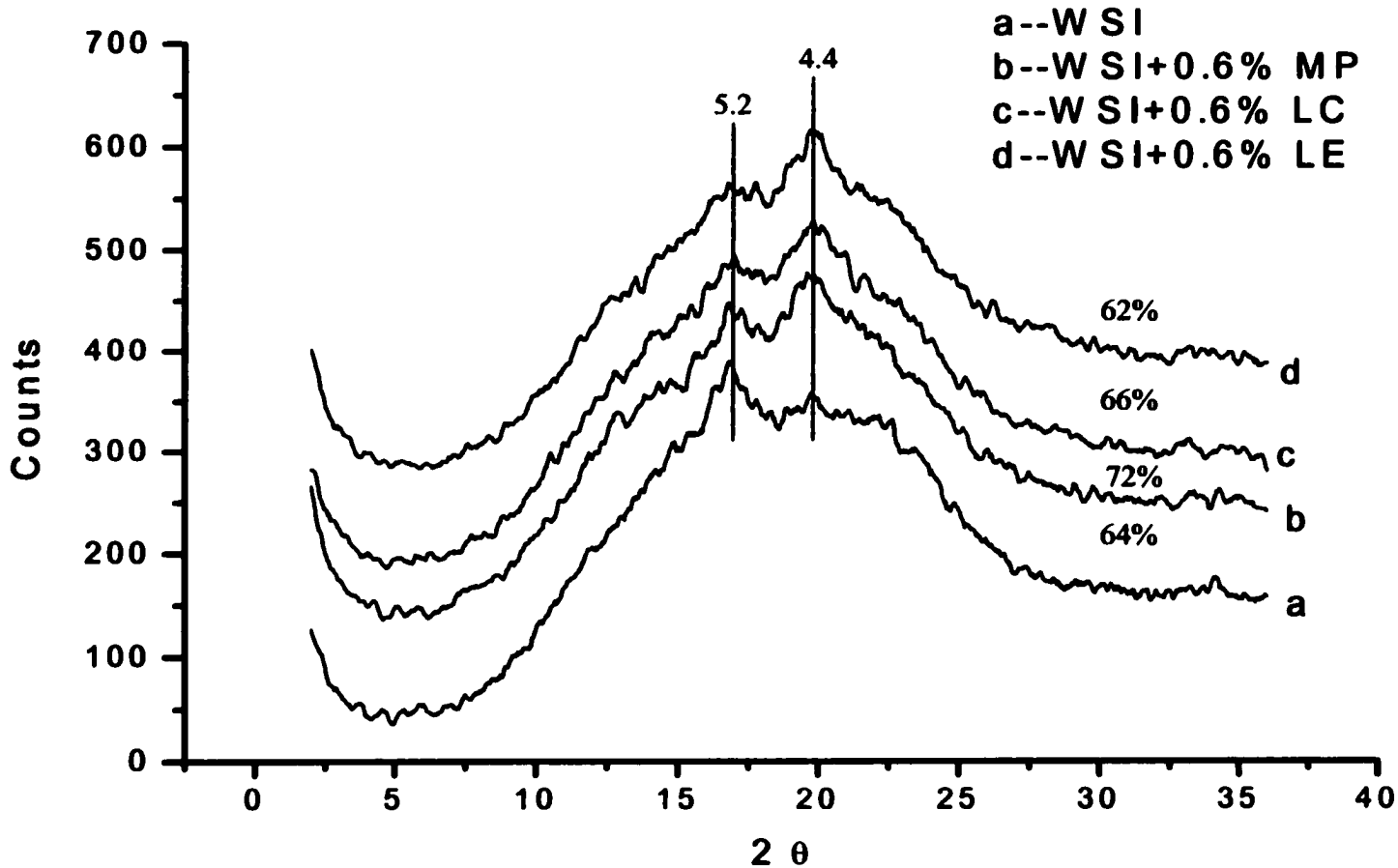


**Fig. 5.7. Influence of lipids on XRD pattern of white starch isolate (WSI) (all samples were gelatinized and stored at the refrigerator for 3 days)**

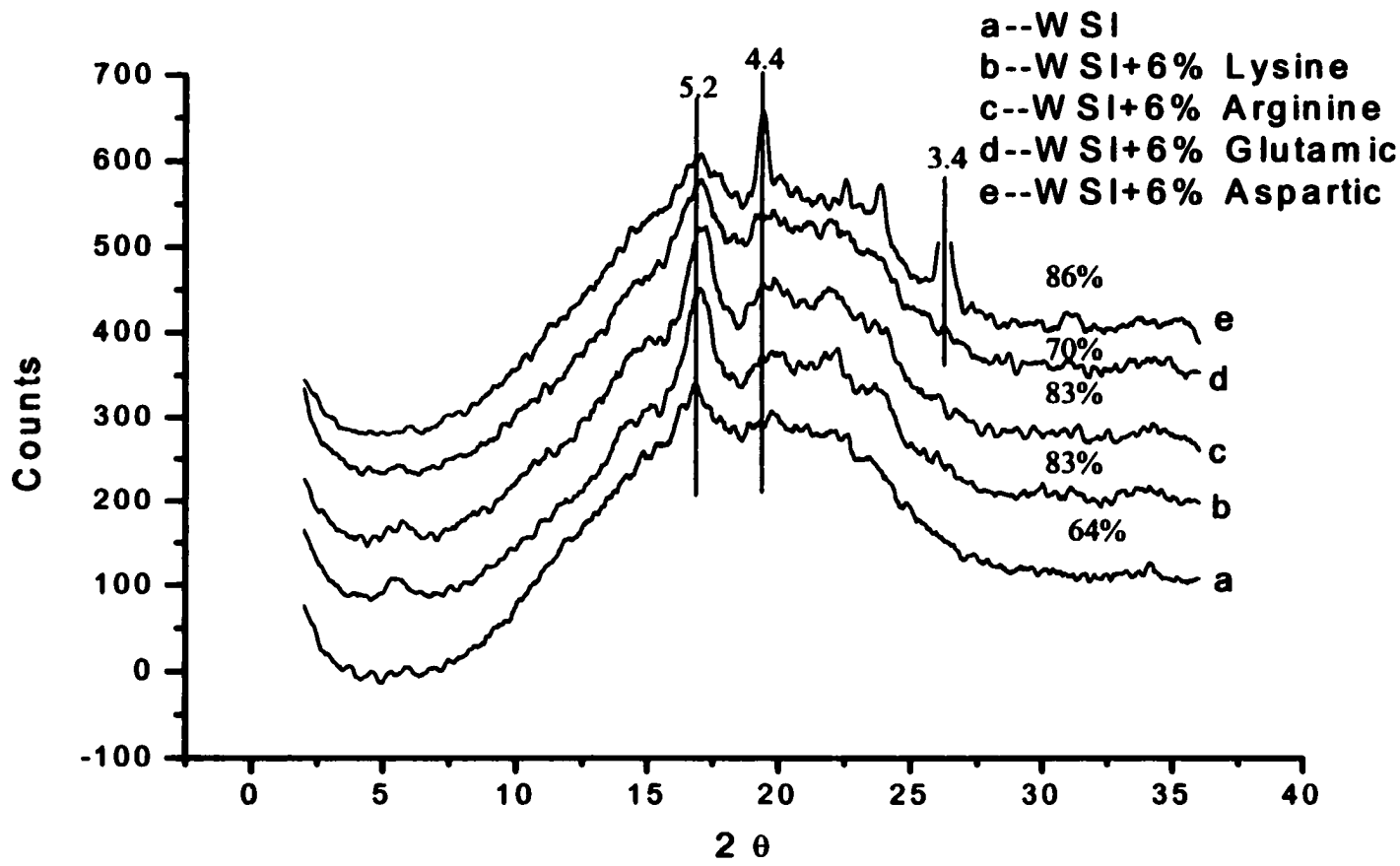


**Fig. 5.8. Influence of amino acids on XRD pattern of white starch isolate (WSI) (all samples were gelatinized and stored at the refrigerator for 3 days)**

glutamic acid resulted in a 9% RC increase while the 4.4 Å peak was enhanced, with no change in the 5.2 Å peak. Similarly, addition of arginine and lysine enhanced the 4.4 Å peak and increased the RC for 30%, while enhancing the peak at 5.2 Å greatly. Compared to the control, the presence of 6% β-CD weakened the 5.2 Å peak and reduced the RC by 67% (Fig. 5.9).

## 5.4. CONCLUSION

The gelatinization process destroyed the double helix structure of starch granules, resulting in an amorphous starch. Addition of lipids to commercial starch induced the development of a new peak at 6.8 Å and increased the intensity of the 4.4 Å peak. The enhanced V pattern may be due to the formation of amylose-lipid complexes. Defatting destroyed the 6.8 Å peak and decreased the intensity of 4.4 Å peak of the white flour, which indicated the destruction of the V-pattern. Addition of aspartic acid, glutamic acid, and lysine to commercial starch increased the 4.4 Å peak. However, protein removal from defatted white flour caused the destruction of the 4.4 Å peak. Those results indicated that the 4.4 Å peak might be related to the content of protein or amino acids residues in the starch. The presence of β-CD competed with amylose for lipids, resulting in less amount of amylose-lipid complex formation in the retrograded gel. The enhanced V-pattern of the β-CD added gel may be due to the complex formation of β-CD and lipids. Further research is needed to explore the β-CD and lipid complex crystalline structure.

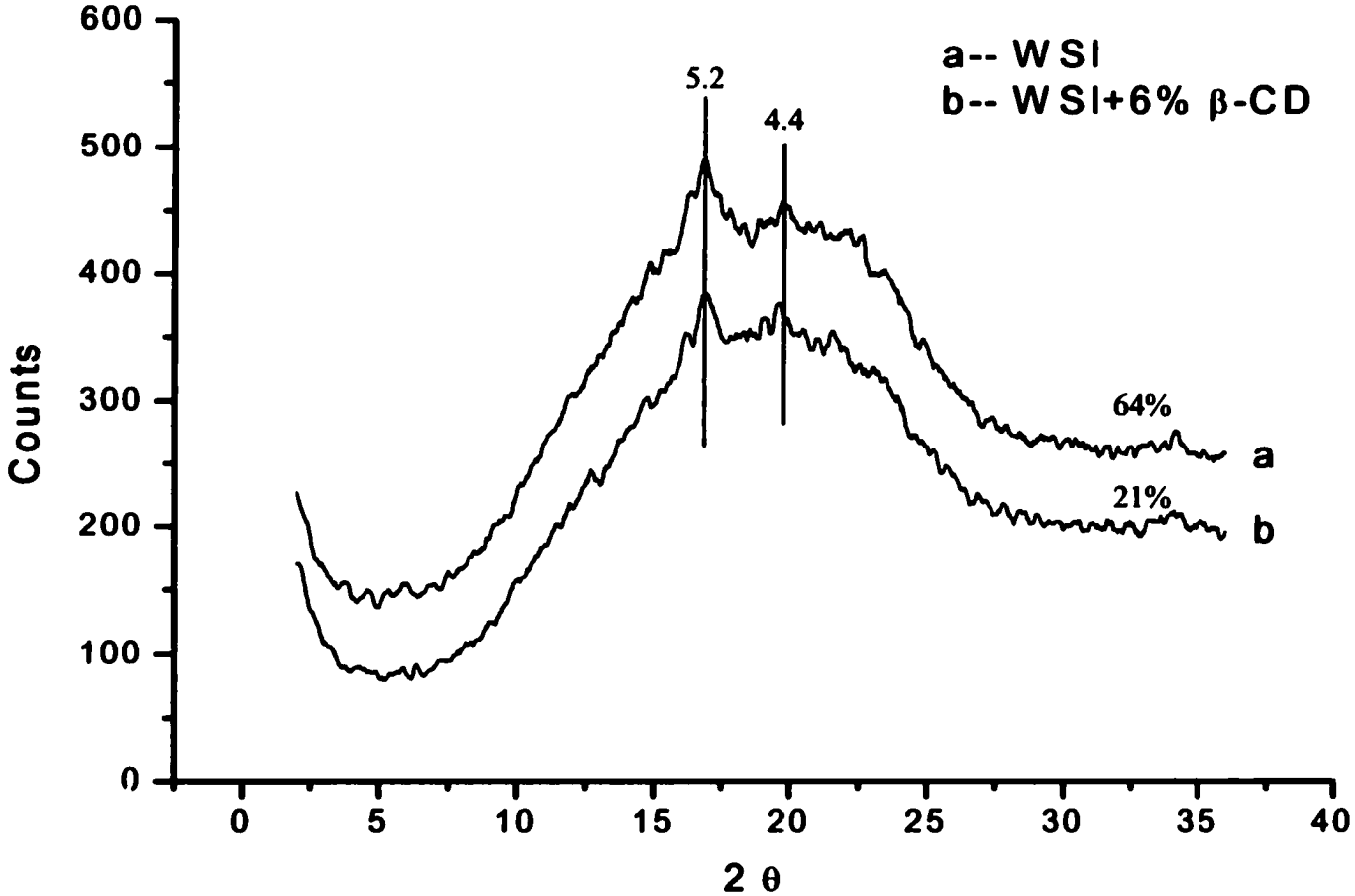


**Fig. 5.9. Influence of β-CD on XRD pattern of white starch isolate (WSI) (all samples were gelatinized and stored at the refrigerator for 3 days)**

# CHAPTER 6
# GENERAL CONCLUSIONS AND RECOMMENDATIONS

It is generally accepted that monoglycerides interact with starch and exhibit anti-firming properties in baking products. Results from this study indicated that, besides monopalmitin, lysophosphatidylcholine (LC) and lysophosphatidylethanolamine (LE) also have strong starch complexing ability. Addition of those lipids delayed starch granule swelling, increased the swelling extent, and enhanced the retrogradation tendency. The presence of lipids also enhanced the V-pattern under XRD. Compared to the control, the presence of amino acids increased the rate of starch swelling but reduced the swelling extent, resulting in lower pasting viscosities and cooking stability. In addition, arginine and lysine could substantially reduce the pasting temperature, which might indicate a possible application of arginine and lysine in convenience foods (such as microwave-food) for low pasting temperature requirement. Both amino acids and β-CD inhibited the amylose-lipid complex formation, resulting in a smaller enthalpy for amylose-lipid complex melting under DSC. Addition of β-CD enhanced the V-pattern of the retrograded gel while amino acids only increased the 4.4 Å peak in commercial starch.

Scanning Electron Microscopy (SEM) is useful for the characterization of starch granule status in complex food or ingredient systems. Further research is needed to use SEM to explore the explore amino acid and lipid effects on starch granules during gelatinization, pasting, and retrogradation. Retrogradation is the process of starch molecules association and recrystallization during cooling and storage. Those re-constructed crystalline portions can be quantified using DSC. Therefore, in future study, DSC techniques can be used to study the lipids, amino acids, and β-CD effect on starch

retrogradation properties. In this study, β-CD showed the inhibitory effect on amylose-lipid complex formation. Resistant starch is formed by amylose-amylose association. Further research is needed to determine the β-CD effect on resistant starch formation.

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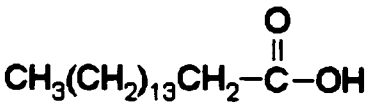
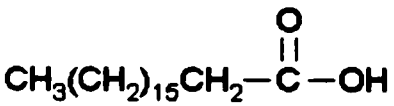
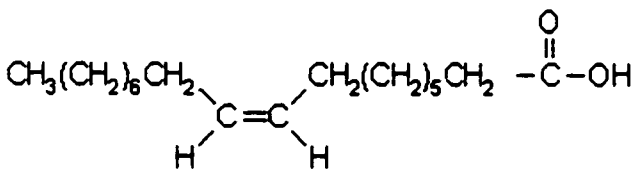
## APPENDIX 1
## LIPIDS MOLECULAR STRUCTURES



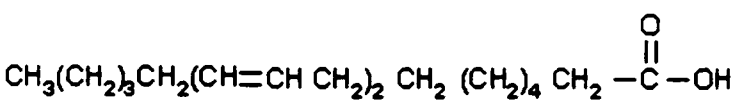
Palmitic Acid (C16:0)



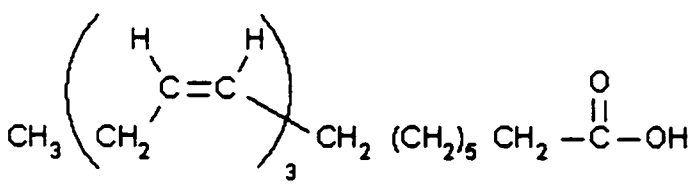
Stearic Acid (C18:0)



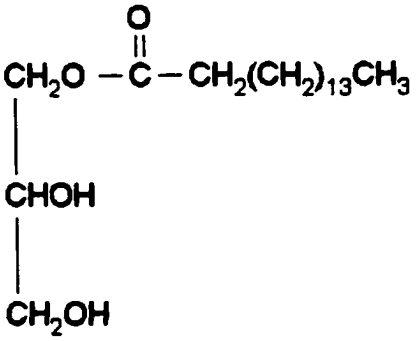
Oleic Acid (C18:1)



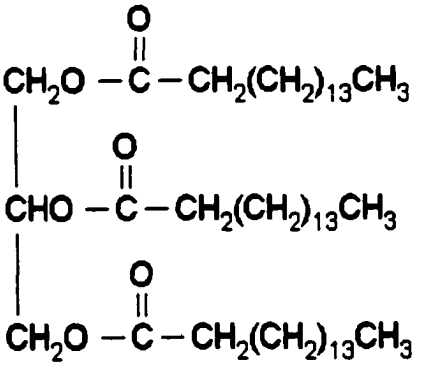
Linoleic Acid (C18:2)



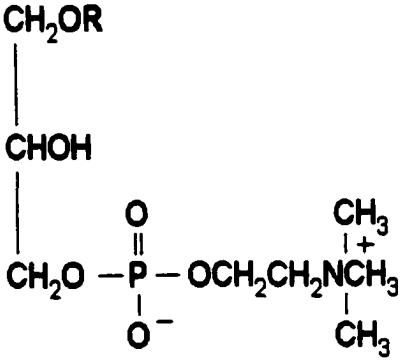
Linolenic Acid (C18:3)



Monopalmitin

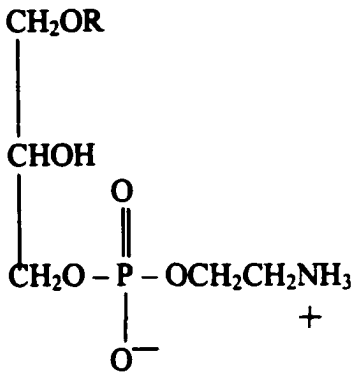


Tripalmitin



**R = Fatty Acid Residue**

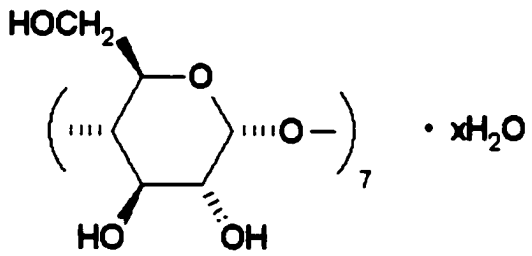
Lysophosphatidylcholine (LC)



R = Fatty Acid Residue

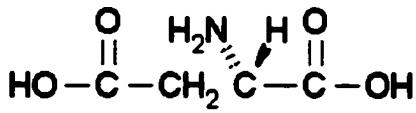
Lysophosphatidylethanolamine (LE)

## APPENDIX 2
## BETA-CYCLODEXTRIN MOLECULAR STRUCTURES

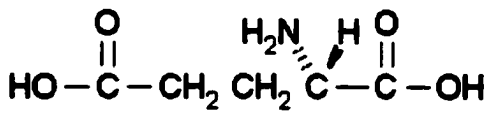



β-Cyclodextrin

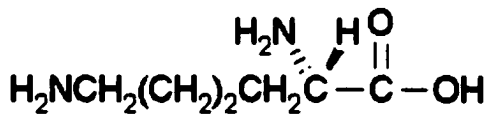
## APPENDIX 3
## AMINO ACIDS MOLECULAR STRUCTURES



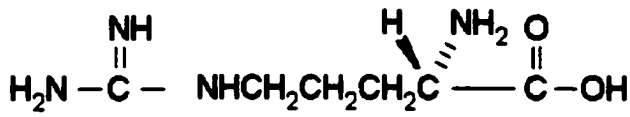
L-Aspartic acid



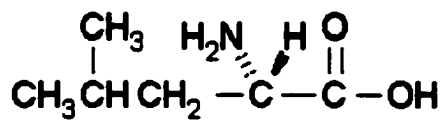
L-Glutamic acid



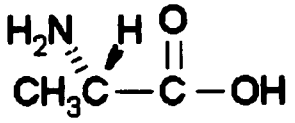
L-Lysine



L-Arginine



L-Leucine



L-Alanine

# VITA

Xiaoming Liang was born September 13, 1967, in Zhejiang Province, the People's Republic of China. He graduated from Zhejiang Fisheries Institute with a bachelor of science degree in food science and engineering in June, 1989. He worked in Zhejiang Wenling Canning Food Corporation as a food engineer from 1989 to 1991. In August 1991, he entered Shanghai Fisheries University for full-time graduate study in the Department of Food Science and Technology, where he received his master of science degree in July 1994. After receiving his master's degree, Mr. Liang worked as a food engineer and international business specialist in the Department of International Trade at Shanghai Fisheries General Corporation from 1994 until 1997. In January 1998, he enrolled in the graduate school of Louisiana State University and pursued his doctoral studies in the Department of Food Science. In January 2000, he entered the Department of Experimental Statistics for a master's degree in applied statistics. He received his master of applied statistics degree in August 2001. Mr. Liang is a candidate for the degree of doctor of philosophy in food science, which he will receive in December 2001. He is a member of the Institute of Food Technologists, American Statistical Association, and Chinese Fisheries Society.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

**Candidate:** Xiaoming Liang

**Major Field:** Food Science

**Title of Dissertation:** Effects of Lipids, Amino Acids, and Beta-Cyclodextrin on Gelatinization, Pasting, and Retrogradation Properties of Rice Starch.

**Approved:**

Major Professor and Chairman

Dean of the Graduate School

**EXAMINING COMMITTEE:**

**Date of Examination:**

October 3, 2001