2013

# Effects of Amino Acids and Fatty Acids on Rice Starch Properties: Thermal, Pasting, Resistant Starch and Structural Characterization

Yu Jiang
*Louisiana State University and Agricultural and Mechanical College*

# EFFECTS OF AMINO ACIDS AND FATTY ACIDS ON RICE STARCH PROPERTIES: THERMAL, PASTING, RESISTANT STARCH AND STRUCTURAL CHARACTERIZATION

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Food Science

by
Yu Jiang
B.S. China Agricultural University, 2007
December 2013

# ACKNOWLEDGEMENTS

In completion of my Ph.D study, I would like to express my great gratitude to many people hereby.

First and most, I would like to thank my major professor Dr. Joan King for providing me this pleasant study experience in LSU. The detailed guidance and encouragement she gave me keep me moving forward in my studies, research and extra-curricular activities. Most importantly, her trust in me and many of my decisions trained me as a critical thinker and a good scientist.

A special thank goes to Dr. Witoon Prinyawiwatkul for imparting valuable knowledge on statistics and product development. He supported me in getting involved in food projects, which were of great wealth for my future career. I would like to thank Dr. Subramaniam Sathivel for being on my committee, guiding me use of rheometer and arranging for his students to help me. I would also like to extend my gratitude to Dr. Raymond Schneider for offering me valuable research suggestions during my general exam.

I wish to thank my parents for their undivided support. I would also extend my appreciation to my friends and colleagues. Dr. Alfredo Prudent is a very good friend and is most helpful when technical problems came to me; Rosaly Manaois, a previous student in the lab, is always there for my questions. Ms. Terri Gilmer encouraged and helped me with preparation of my defense paperwork. My friends in physics and biology are excellent inspiration for me to go after scientific research.

Finally, to all our food science students, faculty and staff in LSU, and to whoever assisted my experiment, thanks for the friendship and your help.

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## LIST OF ABBREVIATIONS

FA, fatty acid

Palm, palmitic acid.

St, stearic acid

La, linoleic acid

Lys, lysine

Asp, aspartic acid.

Cys, cysteine

RVA: rapid visco-analyzer

cP: centipoise (viscosity unit)

MV, minimum viscosity

BKD, breakdown

FV, final viscosity

Ptemp, pasting temperature

Ptime, peak time

TSB, total setback

RS, resistant starch

SDS, slowly digestible starch

TDF, total dietary fiber

DSC: differential scanning calorimetry

XRD: X-ray diffraction

# ABSTRACT

In this study, the effects of fatty acids and amino acids on rice starch were determined in aspects of pasting properties, thermal characteristic, starch digestibility and crystallinity structure.

Results from viscosity analysis showed significantly low peak viscosity when rice starch was combined with 1.0% stearic acid and 6% lysine without any pH adjustment; within 0.6%-1.0% of stearic acid and 6% of lysine addition, there was a linear regression relationship between peak viscosity and the level of stearic acid. Similar pasting curves were found in rice starch with 1.0% stearic acid and 6% glutamine, or 6% cysteine, or 6% glycine in a starch solution of pH 10. Yet the inhibited pasting viscosity was not found in corn starch with the same additive.

The thermal, retrogradation, digestibility, and X-ray diffraction of RVA treated rice starch samples were selectively assayed. When stored 10 days under refrigeration, starches with both fatty acids and lysine added were found to have lower retrogradation (13.3%) than starches with fatty acids added only and starches without additives (41.4%).

With regard to RS content, no pronounced difference was found between starch with additives and without additives except cysteine; however, SDS assay observed more slowly digestible starch when fatty acids were present in the sample, due to amylose-lipid complexes with less order crystalline structure. Both X-ray diffraction and DSC scans of RVA treated starches showed elevated amounts of amylose-lipid complexes when both lysine and fatty acids were present, compared to addition of fatty acids alone. Interaction between starch, fatty acids and amino acids was confirmed, within which amino acids that are negatively charged and stearic acid were indispensible for inhibiting rice starch swelling.

Because the effect of inhibiting starch swelling and pasting is similar to properties of cross-linked starch, application of fatty acids and amino acids in this study in altering starch properties offers big market potential for clean label starch as a food ingredient, such as thickening agent in a pudding, a soup or a sauce.

# CHAPTER 1
# INTRODUCTION

Starch is the main source of carbohydrate for human nutrition. It is mainly collected from cereal grains, tuber and root of many crops. Starches from different plant sources exhibit different size, amylose/amylopectin ratio, granule organization and granule surface compounds, which also result in different thermal, pasting and other physicochemical properties.

For the modern food industry, native starches have many undesirable properties for food processing. A typical challenge is a narrow peak viscosity range of starch paste; continuous heating causes ruptures of starch granules and rapid decrease in paste viscosity, which is accelerated by shear and acid. Starch susceptibility to rigorous process conditions makes it hard to be used for products of good quality. Also, the shelf life of starch-based food is largely dependent on starch retrogradation, a process where cooked starch becomes firm and losses water because of re-association of starch molecules.

The starch industry invented many modified starches to overcome the shortcomings of native starch and to customize the starch properties. According to different treatments, starch can be modified using physical, chemical, enzymatic and genetic methods (Kaur et al. 2012). For starch resistance to overcooking, crosslinking is often used, by linking molecules together through reaction with crosslinking agents, typically sodium trimetaphosphate (STMP) and sodium tripolyphosphate (STPP), phosphorus oxychloride and epichlorohydrin (Wattanachant et al. 2003, Koo et al. 2010).

However, for food application, there is a demand for achieving substantial change of starch properties without use of chemical reagents. While many studies have been done on how native or added proteins change starch properties, reports on effects of amino acids altering starch functional properties is limited and varies on starch of different botanical sources (Liang et al. 2002, Ito et al. 2006b, Liang and King 2003, Lockwood et al. 2008, Ito et al. 2011). Charged amino acids, such as lysine and monosodium glutamate, resulted in inhibited peak viscosity and collapse of potato starch granules at pH 7 under retort treatment (Ito et al. 2006a). Combined with ozone treatment, rice starch with lysine added reduced the viscosity of the starch paste, and decreased pasting time significantly, indicating a less viscous and easy cooking starch (An et al. 2009). While lysine was found to depress starch breakdown for orange-fleshed sweet potato starch and white-fleshed sweet potato starch, however, it was also related to a higher breakdown value in rice starch, compared to starch with no additive (Manaois 2009). Different roles of aspartic acid and lysine additives were found in sweet potato in changing pasting stability (Lockwood et al.

2008). The above research indicates at least two variables are involved in effects of amino acids in starch pasting, either different R groups in amino acids or starch from various botanical sources.

Lipids, such as free fatty acids, mono-,di- and tri-glycerides, have been used in food applications for different purposes. A main function of lipid in starchy food is to retard firming and staling, which is related to inhibited starch retrogradation, for example, monoglyceride and sodium stearoyl lactylate. Lipids additives of different types give various performances for starch retrogradation tendency and formation of amylose-lipid complexes (Liang 2001).

As a result, both amino acids and lipids were important in modifying starch pasting, which are related to their texture and mechanical susceptibility. Besides, they are common food ingredients that are simple yet essential. However, no studies have been conducted on interaction among a three-component system of starch, amino acids and fatty acids. Besides starch pasting, other information is also important in understanding the interaction among those three and finding potential use of rice starches with additives. Rapid visco-analyzer (RVA) mimics the starch processing conditions with programmed heating, cooling and shearing; differential scanning calorimetry (DSC) records thermal properties presented by samples under heating or cooling and helps find new polymers; X-ray diffraction (XRD) shows the degree of crystallinity of polymers with their characteristic peak for certain d-spacing values; yield of resistant starch provides direct evidence regarding the health benefit of starch with additives.

This research was divided into three chapters. The objectives of chapter 3 were 1) to determine the effects of fatty acids and amino acids on pasting properties of rice starch and corn starch by RVA; 2) to compare the effects of both fatty acids and amino acids and effects of fatty acid or amino acid alone in both starches; and 3) to determine the complex indexes of selective fatty acids and amino acid additives in rice starch and corn starch.

The objectives of chapter 4 were 1) to determine retrogradation tendency of rice starch with selective additives using DSC and syneresis methods; 2) to check the amylose-lipid complex peak by DSC for rice starch with selective additives; and 3) to examine the starch digestibility by yield of resistant starch and slowly digestible starch.

The objectives of chapter 5 were 1) to observe selective samples morphology under microscope; 2) to study the XRD pattern of selective samples and its crystallinity; and 3) To discuss the possible hypothesis to explain the role of inhibiting starch swelling for some additives and to test the hypothesis using other additives.

In the end, chapter 6 provides general conclusions for the study and recommendations for future study.

## 1.1 REFERENCES


An, H. and J. King (2009). "Using ozonation and amino acids to change pasting properties of rice starch." Journal of food science **74**(3): C278-C283.

Ito, A., M. Hattori, T. Yoshida and K. Takahashi (2006a). "Contribution of the net charge to the regulatory effects of amino acids and Œμ-poly (L-lysine) on the gelatinization behavior of potato starch granules." Bioscience, biotechnology, and biochemistry **70**(1): 76-85.

Ito, A., M. Hattori, T. Yoshida, A. Watanabe, R. Sato and K. Takahashi (2006b). "Regulatory effect of amino acids on the pasting behavior of potato starch is attributable to its binding to the starch chain." Journal of agricultural and food chemistry **54**(26): 10191-10196.

Ito, A., M. Hattori, T. Yoshida, K. Yoshimura and K. Takahashi (2011). "Contribution of Charged Amino Acids to Improving the Degraded Viscosit of Potato Starch Paste by a Retort Treatment and during Storage." Journal of Applied Glycoscience(0): 1104150017.

Kaur, B., F. Ariffin, R. Bhat and A. A. Karim (2012). "Progress in starch modification in the last decade." Food Hydrocolloids **26**(2): 398-404.

Koo, S. H., K. Y. Lee and H. G. Lee (2010). "Effect of cross-linking on the physicochemical and physiological properties of corn starch." Food Hydrocolloids **24**(6): 619-625.

Liang, X. (2001). Effects of lipids, amino acids, and beta-cyclodextrin on gelatinization, pasting, and retrogradation properties of rice starch Ph.D dissertation, Louisiana State University.

Liang, X. and J. King (2003). "Pasting and crystalline property differences of commercial and isolated rice starch with added amino acids." Journal of food science **68**(3): 832-838.

Liang, X., J. M. King and F. F. Shih (2002). "Pasting property differences of commercial and isolated rice starch with added lipids and beta-cyclodextrin." Cereal chemistry **79**(6): 812-818.

Lockwood, S., J. King and D. Labonte (2008). "Altering pasting characteristics of sweet potato starches through amino acid additives." Journal of food science **73**(5): C373-C377.

Manaois, R. V. (2009). Modification of Rice Starch Properties by Addition of Amino Acids at Various PH Levels Master thesis, Louisiana State University.

Wattanachant, S., K. Muhammad, D. Mat Hashim and R. A. Rahman (2003). "Effect of crosslinking reagents and hydroxypropylation levels on dual-modified sago starch properties." Food Chemistry **80**(4): 463-471.

# CHAPTER 2
# LITERATURE REVIEW

This section reviewed related literature on starch in food applications, including the roles of starch in the food industry, physical and chemical structures of starch, change of starch by some temperature treatments, starch interaction with other compounds, like lipid, amino acids and protein, and modified starch commonly found as a food ingredient.

## 2.1 STARCH AND RICE STARCH

### 2.1.1 Starch in food industry

The history of humans eating starchy food can be tracked back to the beginning of civilization. Starch from different botanical sources, including seeds, tuber and roots was heated with or without water for basic food needs. Although wheat, corn and rice are three main sources of starch with almost equal amounts of production, different cereals and starches are favored in different regions of the world. Corn is indigenous to the Americas and was later popularized by many American starch companies as the only important cereal crop. Americas produce almost half of the corn in the world (49.6%); Asia contribute the biggest percentage of rice (90.4%) and wheat (45.1%) production to the world in the year 2011(retrieved from http://faostat3.fao.org/home/index.html#VISUALIZE_TOP_20).

Before the emergence of the food industry, starch was initially applied in textile, color printing and paper industries. Dextrin production opened up the first chapter of starch application in the food industry in 19$^{th}$ century. Besides development of specialty starch or modified starch, other products such as sweeteners, ethanol, polyol and organic acids can also be derived from starch. In food, starch functions as thickener, gels, water binder, stabilizer, glazer, duster, coating, fat replacer and others. Examples can be found in pudding, lemon pie, cheese product, salad dressing and jelly gum for gelling or short or mediated texture; battered fried crisp food and candy for coating; beverage, soup, and baby food for thickening and stabilizing.

To understand the function of starch in food manufacturing, it is necessary to study the change of starch under pasting, cooling and storage by measurement of viscosity, opacity, thermal transition, texture, and structure change. Modified starches are therefore developed for desirable product appearance, texture and storage stability, such as controlled degree of starch disruption during processing under heating, acid and shearing and to reach good freeze-thaw ability/retrogradation rate. However, while many modified starches are based on chemical modification, it is necessary to find a new method to achieve those desirable functional changes using material that we are more

familiar with, other than chemical reagents. Macronutrients, such as protein, amino acids and lipids are ideal materials to try.

### 2.1.2 Chemical structure of starch

Starch is a larger polymer of glucose units. According to types of glycosidic linkage, starch is divided into amylose and amylopectin in principle (Table 2.1 and Figure 2.1). Amylose has an essentially linear chain structure consisting of α–(1→4)–glucopyranosyl units. The degree of polymerization on average is estimated table around 1500 to 1600 and the molecular weight is $1.6 \times 10^5$ to $1 \times 10^6$ (Zobel 1988, Hizukuri et al. 1989).

Table 2.1 Comparison between properties of amylose and amylopectin. Adapted from Shannon (1984)

| Properties | Amylose | Amylopectin |
|---|---|---|
| Structure | Essential linear | Branched (4-5% branch) point) |
| Degree of polymerization | 1500-6000 | $3\times10^5$-$3\times10^6$ |
| Molecular weight | $1.6 \times 10^5$- $1 \times 10^6$ | $5\times10^7$-$5\times10^8$ |
| Average chain length | 100-10,000 (thousand are common) | 20-30 |
| Color with iodine | Dark blue | Brownish red |
| λmax of iodine complex | ~650nm | ~540nm |
| Solubility in water | Variable | Soluble |

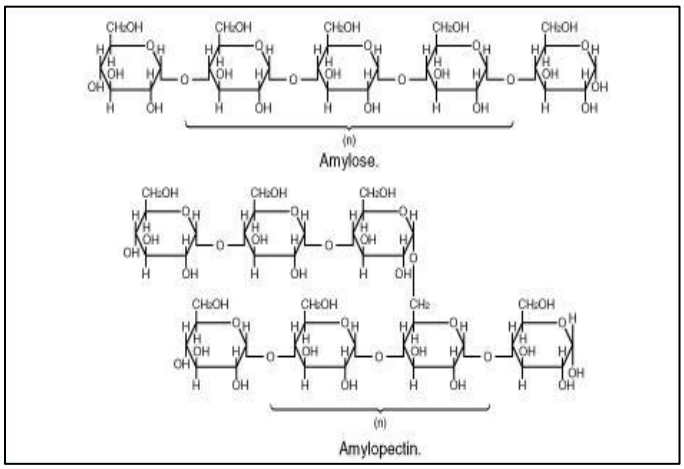


Figure 2.1 Chemical structure of amylose and amylopectin (from http://www.nichidene.com/eng-approach.htm)

Amylopectin has a highly branched structure, which is made up of shorter chains of α–(1→4)–glucopyranosyl units branch-linked by α–(1→6)–glycosidic bonding, which occupies 4-6% of the total linkages (French 1984). The molecular weight of amylopectin is much bigger than amylose, which ranges from $10^6$ to $10^8$ daltons (Zobel 1988). Due to branched glucose units, the polymer chain is classified as short chains (12<DP<20), long chains (30<DP<45) and very long chains (DP>60). Amylopectin in raw starch is made of clusters, whose chains were further named as A-, B- and C-chains (Figure 2.2). A-chain refers to those external chains that attach to a B or C chain but don't carry any other chain. B-chain is attached to other B or C chains using its reducing end and carries A or B chains. The C chain is the only chain that contains a reducing end in one amylopectin (Peat et al. 1952).

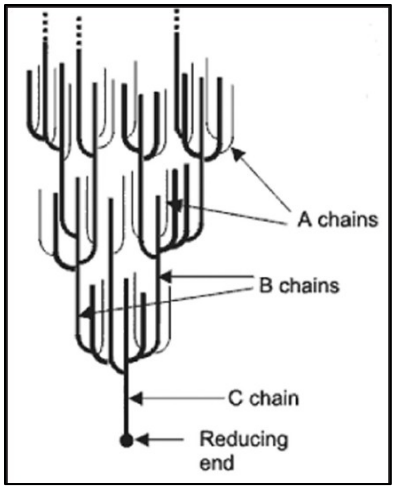


Figure 2.2 Chains of amylopectin. A-chains do not carry any other chains; B-chains connect to one or more A chains and/or B chains; C-chain has the reducing end. From Eliasson et al. (2004)

There is also an intermediate material most notably seen in high-amylose starch. It has an intermediate structure between amylose and amylopectin, which means similar branched structures but lower molecular weights than amylopectin (Baba et al. 1984). Discovered during fractionation of amylose and amylopectin, the existence of intermediate component in starch varies, ranging from 5-7% in normal maize starch to 10% in wheat starch (Shannon 1984).

Native starch exists in the form of starch granules, which are packed with amorphous amylose and amylopectin in semi-crystalline rings. Most native starch has 20-30% amylose, yet this percentage is subject to botanical sources, growth condition, harvesting time and determination methods (Morrison et al. 1987, McGrance et al. 1998, Singh et al. 2003). For example, based on studies on most available cultivars, the amylose content ranges from 20% to 36%

for corn starch; from 18% to 23% for potato starch; from 21% to 35% for sorghum starch; from 17% to 29% for wheat starch; from 11% to 26% for rice starch; from 34% to 37% for pea starch (Shannon 1984).

Commercially bred starch such as waxy starch is essentially free of amylose; high amylose starch refers to those of which amylose content is bigger than 50%. Because of the unique chemical structure and arrangement of amylose and amylopectin within starch granule, the amylose content and starch chains were widely studied concerning the formation of resistant starch, starch thermal properties and retrogradation.

**2.1.3 Physical structure of starch granule**

Native starch exists in the form of starch granules. Starch from different plant origins has their unique starch granule size. Rice starch is usually within 3-8 μm; wheat is 1-45 μm; corn is 5-30μm; and potato starch has the largest granule (5-100 μm). Different morphology is also found in starch granules, including spherical, polygonal, oval, truncated and irregular (Taggart 2004).

Amylose and amylopectin in starch are packed in starch in a semi-crystalline way, and therefore starch displays distinctive maltese-cross (birefringence) under microscopy with polarized light at room temperature. Under microscopic observation, like SEM or TEM, starch reveals a series of concentric rings within a granule, or called growth ring (Jenkins et al. 1993, Atkin et al. 1998). The growth rings are made up of semi-crystalline layers and amorphous layers alternating with each other. Within semi-crystalline layer are crystalline lamellae and amorphous lamellae. The crystalline lamellae are formed by amylopectin side chain cluster, which is of 6nm length on average; the amorphous lamellae are the branching zone of around 4nm length (Gallant et al. 1997). The amorphous regions between crystalline clusters are occupied by amylose. While amylopectin molecule contributes to formation of crystalline lamellae due to its double helix form and cluster arrangement, crystallinity doesn't dominate starch granule structure, ranging from 15-45% according to X-ray diffraction (Zobel 1988).

Investigation of starch crystallinity is widely studied by wide-angle X-ray diffraction (WAXD). There are three types of X-ray diffraction patterns for native starch molecules, named as A, B and C types (Imberty et al. 1991). In general, cereal starch, such as corn, rice and wheat starch, has A-type pattern; tuber starch, such as potato starch and banana starch, belongs to B-type; C-type is mostly found in legumes, which is a mixture of A and B-type. In legume starch, A type dominates the outer margin while B type is formed in the center of granule (Buléon et al. 1998). Both A and B types are built from double helices using side chains of amylopectin. A-type has monoclinic unit cells; and there are 8 water molecules in one cell. B type has hexagonal unit cells; but there are 36 water molecules in one cell,

making it more spacious (Donald 2004). It is suggested that the polymorphism of starch is largely determined by the chain length of amylopectin; other common factors for crystallization, such as temperature, concentration and presence of other solutes can be applied to change the starch crystalline structure (Hizukuri et al. 1983).

Although native raw amylose has a random coil structure in starch granule, it tends to form single-helical structure by including complexing agents, such as fatty acids, glycerol monostearate, and 1-butanol. Some amylose-lipid complexes are found to form V-type diffraction peaks in X-ray diffractogram. It is reported that 6 glucose units formulated one turn in the single-helical amylose-lipid complex (Imberty et al. 1991). Most cereals contain 0.2-1.0% lipid in their raw starches (Jiang et al. 2010), and the existence of V-polymorph of amylose-lipid complex in raw starch, such as maize, oat, barley, rice and wheat starch, is also well justified by C-CP/MAS-NMR (Biliaderis 2009). In most cases, V-polymorph is well observed in heat-moisture treatment of starch, such as autoclaving, gelatinization and extrusion cooking, due to increased mobility of amylose chains to complex with ligands (Biliaderis 2009).

**2.1.4 Physical and structural transition of starch on heating, cooling and freezing**

As being mentioned before, starch granules have a semi-crystalline structure, showing birefringence and a maltese cross under microscopy. At room temperature, native starch is insoluble in cold water. Upon heating, starch granules swell in water solution; water starts to penetrate into starch granule and builds hydrogen bonds with starch chains. Continuous heating destroys the crystalline regions of starch granule and causes significant swelling. The point when molecular order in starch granules collapses, or when there is loss of birefringence, is referred as gelatinization. It is accompanied by irreversible starch swelling, starch thickening and loss of opacity. Starch gelatinization corresponds to an endothermic transition. By differential scanning calorimetry (DSC), it is known that the difference between concluding temperature and onset temperature during this transition can be as wide as 10-15°C (Biliaderis 2009).

After reaching its gelatinization temperature, starch swells very fast. Amylose leaches out from swollen starch and forms starch gel. The loss of amylose finally leads to starch granule collapse and therefore the viscosity of starch solution increases considerably, giving rises to starch paste. Once the rate of starch collapse equals that of starch swelling, starch paste reaches its maximum viscosity (peak viscosity).

A Rapid Visco Analyzer (RVA) is a typical instrument used to measure viscosity change during this process (Figure 2.3). Peak viscosity indicates starch swelling capacity or thickening power, which is an important parameter

to estimate starch product texture. Upon continuous heating, significant disruption of starch granules cause gel viscosity to decrease, until it reaches minimum viscosity under programmed temperature control. The difference between peak viscosity and minimum viscosity is called breakdown, which tells stability of starch gel during cooking, or vulnerability of starch gel to being disrupted. Other factors, such as shearing or acidic conditions can also accelerate starch granule collapse and breakdown.

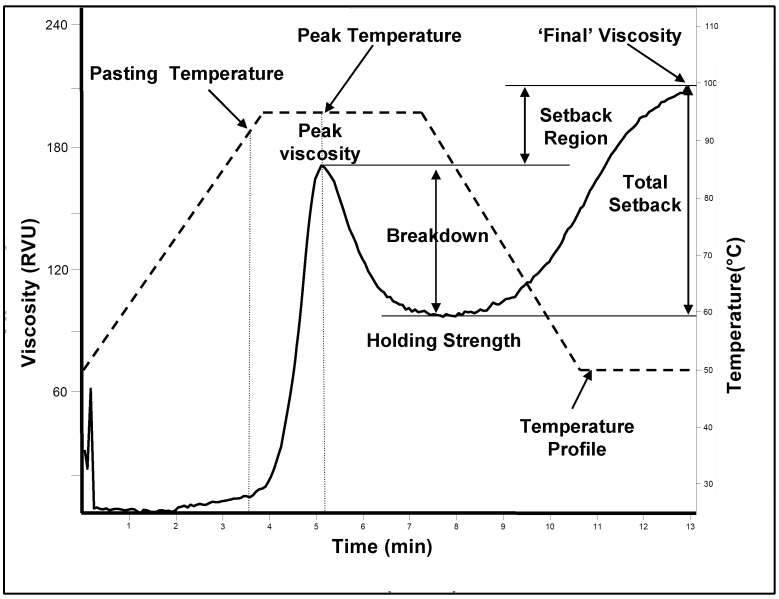


Figure 2.3 Typical RVA curve of starch gel. Adapted from Zaidul et al. (2007)

Cooling down starch paste increases its viscosity due to reassociation of starch gel and starch retrogradation (Figure 2.4). Amylose chains gradually pack into double helices due to hydrogen bonding between glucose units. This is reflected as total setback in RVA curves, which equals to the difference between final viscosity and minimum viscosity. Amylose with less than 110 degree of polymerization (DP) favors the precipitation of retrograded starch, while amylose of higher DP may prefer to form amylose gel (Gidley et al. 1989). Starch gel becomes turbid and firm upon cooling. While retrogradation is initiated by amylose double helices, recrystallization of amylopectin is the reason for starch gel stiffness during long time storage. Retrograded amylose is known to be more thermally stable than retrograded amylopectin at melting temperatures from 130 to 170°C (Biliaderis 2009). Beside amylose properties and amylose amount, the rate of retrogradation is also subject to the storage temperature and starch concentration (Orford et al. 1987); between 5-35°C, the higher the temperature is, the lower the rate of retrogradation is; increasing starch retrogradation is closely related to the thermal reversible amylopectin retrogradation (Imberty et al. 1991, Lu et al. 1997).

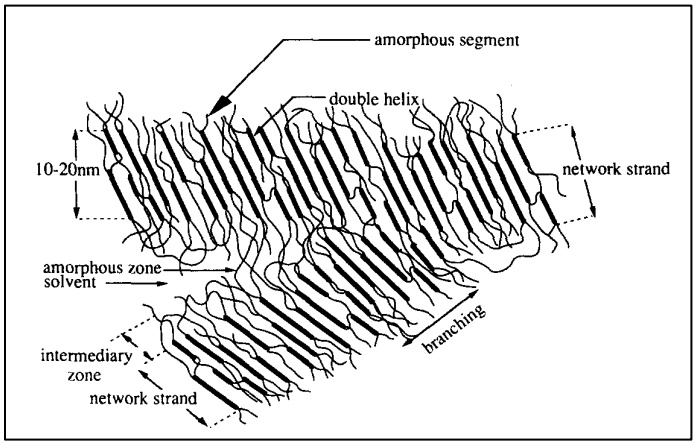


Figure 2.4 Amylose reassociation. From Eliasson et al (2004)

Starch retrogradation is considered as the main reason for bread staling or undesirable firming of other starchy food (Kulp et al. 1981, Seow et al. 1988). While amylose retrogradation can take place within only a few hours, amylopectin retrogradation lasts for days or even weeks. Therefore, native starch with high retrogradation rate is not suitable to produce frozen food due to retrogradation, such as wheat and rice starch. However, high retrogradation may be a desirable attribute for products that require crispy structure and low stickiness, such as breakfast cereal. Moreover, retrograded starch was discovered as type 3 resistant starch, that is, starch resistant to be digested by amylases in human bodies, due to the rigid crystalline structure of retrograded starch. In order to increase yield of resistant starch, cycles of autoclaving and cooling is often applied to raw starch to get more retrograded starch, especially high-amylose starch. For food application, specific types of starch may be selected based on their characteristics (Table 2.2).

Table 2.2 Gelatinization and pasting properties of native starches. Adapted from Biliaderis (2009)

| | Potato | Corn | Wheat | Tapioca | Waxy Maize | Rice |
|---|---|---|---|---|---|---|
| Gelatinization temp range | 58-68 | 62-72 | 58-64 | 63-72 | 63-72 | 68-78 |
| Brabender peak viscosity (BU) | 2900 | 700 | 250 | 1200 | 1100 | 500 |
| Swelling power at 95$^{O}$C | 1150 | 24 | 21 | 71 | 64 | 19 |
| Cold paste texture | stringy | congealing | short | long | long | short |
| Paste clarity | translucent | opaque | opaque | translucent | translucent | opaque |
| Resistance to shear | medium,low | medium | medium | low | low | medium |
| Retrogradation rate | medium | high | high | low | very low | high |

### 2.1.5 Enzymatic digestion of starch

There are many enzymes that digest starch, such as alpha-amylase, beta-amylase, isoamylase and pulluanase. Concerning human digestion, starch breaks down partially in the mouth and small intestines by pancreatic α-amylase, dextrinase, amyloglucosidase, α- glycosidase, and maltase, most of which are embedded in the brush border of the intestinal wall (Sang et al. 2006). Adverse factors that impede starch hydrolysis in the human body mainly are the physical structure of starch, such as crystallinity or starch-embedded matrix, and branching pattern. For example, alpha-amylase cannot breakdown alpha 1,6 glycosidic linkage.

Based on different digestion rates, starch is divided into rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS). Both RDS and SDS are digested completely in the human small intestine, but SDS is digested slower than RDS, ranging among 20-120 min of enzymatic hydrolysis (Englyst et al. 1992). Resistant starch is a special starch that has no digestibility in the human body. Unlike regular starch that can be hydrolyzed by human enzymes, resistant starch escapes the digestion in small intestine and is fermented in the colon. As a result, resistant starch slows down the glycemic response and reduce the risk of colon cancer (Yue et al. 1998). The reduced calories compared to regular starch also make it an ideal diet for diabetes and weight control-consumers.

Englyst further subdivided RS into four categories (Englyst et al. 1992). RS1 is physically inaccessible starch, like partially milled grains, seeds, and legumes. RS2 has intact granules that are resistant to enzyme digestion due to its special crystalline structure and amorphous area in the granule, examples include raw potato and raw banana. RS3 refers to retrograded amylose, which is produced in staled bread, cooked and cooled potatoes and breakfast cereal. RS4 is chemical modified starch. Common principles are esterification and crosslinking starch molecules, such as hydroxypropyl starches, acetate starches, phosphate starches and citrate starches (Xie et al. 2004). The reason for decreased enzyme digestibly of RS4 is probably due to the failure of derivatizing groups to form the enzyme-substrate complex steric hindrance (Xie et al. 2006).

Common practice for production of RS3 involves annealing and heat-moisture treatment (Thompson 2000). While both methods are hydrothermal, annealing happens at temperatures lower than the starch gelatinization temperature but higher than the starch glass transition temperature. Heat-moisture method treats starch at low moisture level (<35% w/w) for long time periods at temperatures higher than gelatinization temperature, usually between 84–120 °C. These methods give starch chains more mobility and therefore a chance to perfect crystallinity and decrease enzyme digestibility (Chung et al. 2009). Partial acid hydrolysis can be applied to increase starch chain

mobility and build a more ordered structure (Brumovsky et al. 2001). Similarly, the principle of RS3 production is to yield a more ordered amylose structure by retrogradation. Autoclaving and cooling cycles are used to gelatinize starch with excess water and to promote nucleation and propagation of amylose crystals.

### 2.1.6 Rice starch

Rice is a staple food in most Asian countries. Its production has reached second highest after corn among grains (from http://faostat.fao.org/site/567/DesktopDefault.aspx#ancor). As an indispensible part in local culture and cuisine, rice has been grown in Louisiana for years, of which production is just behind Arkansas and California. Most rice grown in Louisiana is long grain that when rice cooked is separate, firm and fluffy (Bao et al. 2004).

Rice starch plays important role for pharmaceutical, food and other material applications. One advantage of rice over wheat or other cereal in food applications is due to its hypoallergenic property; because rice has bland taste and is gluten free, it is the only starch source for baby food. More application can be found in snacks, breakfast cereals, noodles and candies (Marshall et al. 1994)

Rice starch has the smallest granule size among all cereal grains, which ranges from 3 to 8 μm. The granule has a polygonal shape and usually exists in cluster between 20-60 individual granules (Champagne 1996). As a cereal starch, rice starch has an A type crystalline X-ray diffraction pattern in general, although B-type exists in some high amylose mutants (Yano et al. 1985).

Amylose in rice starch has a few α-1,6 branch points. Rice amylose is reported to be slightly branched with 2-5 chains in one amylose molecule on average; its degree of polymerization and average chain length range from 980 to 1110 and from 250 to 370, respectively (Takeda et al. 1986). The apparent amylose content of amylose varies from 0 to 30% in common rice cultivars (Bao et al. 2004).

## 2.2 STARCH INTERACTION WITH OTHER COMPOUNDS

### 2.2.1 Iodine affinity of starch

Amylose and amylopectin has different iodine-binding properties. The iodine affinity is around 19-20% for amylose but less than 1% for amylopectin (Shannon 1984). In pH 4.7 acetate buffer, the amylose-iodine complex is dark blue but amylopectin-iodine shows brownish red. Therefore this property is often used to determine the amount of amylose in starch by the methods of potentiometric iodine titration or spectrometric absorbance using amylose standard to compare with.

However, Montgomery believed that all amylose determination methods based on iodine complex formation measured “apparent amylose”. Iodine affinity for purified amylose could range from 18.5%-20.0%, depending on starch origins (Montgomery, Sexson et al. 1961). The long branched chains within amylopectin also bind iodine, and develop similar dark blue color like amylose; therefore, the apparent amylose content is overestimated, especially in high-amylose starch due to the existence of intermediate components (Banks et al. 1970). For example, the true amylose content in rice starch is reported as being overestimated by iodine binding, according to the SEC separation of amylose coupled to multi-angle laser light scattering (SEC-MALLS)(Ramesh et al. 1999). The true amylose content was measured as 7-11%, which was almost half of the apparent amylose.

**2.2.2 Starch–lipid complex**

Because starch and lipid co-exist in many food items, studies on starch-lipid interaction constitute an important section in cereal chemistry. There is a very small amount of lipid from cereal grain; and most lipid in food presents in the form of emulsifier. Common emulsifiers used in the baking industry are mono-glycerides, di-glycerides, diacetyl tartaric acid ester of mono-glyceride, sodium stearoyl lactylate and sucrose esters (Eliasson et al. 2004). Although the purpose of the emulsifier in food application varies from one to anther, such as prolonging shelf life of bread against staling and reducing stickiness of instant mashed potatoes, explanation on principles of those functions has always been a debate for food scientists (Kulp et al. 1981, Rogers et al. 1988). Fundamental studies are necessary for better understanding and utilization of starch-lipid interaction.

As mentioned before, amylose-lipid complex can be observed as V-type polymorph by wide angle X-ray diffraction. The hydrophobic part of lipid is trapped inside the amylose single helix inside due to their hydrophobic interaction; the head of lipids is outside of the helix. It is postulated that the existence of amylose-lipid complex would interfere with the re-crystallization of amylopectin and therefore retard bread firming, although the existence and effects of amylopectin-lipid complex cannot be excluded (Gudmundsson et al. 1990). Shortening, as a triglyceride, does not form a complex with starch; it reduces bread firming rate in a way different from monoglycerides (Rogers et al. 1988). Using DSC to observe thermal transition, the complex was found to melt at a higher temperature than the gelatinization temperature. The one that melts at about 94 to 100°C is considered as type I amylose-lipid complex while the one that melts at 100-125°C belongs to type II, which has a more ordered/crystallinity structure that can be detected by X-ray diffraction (Jane 2009). The corresponding 2θ values are 11.9, 6.9, 4.6 and 4.0 Å (Zobel et al. 1967).

It has been reported that the stability of amylose-lipid complex varies. At fatty acid chain lengths above 8 to 10, the saturated mono-glyceride results in a higher melting temperature of its amylose complex (Kowblansky 1985, Tufvesson et al. 2003). Also, the higher degree of unsaturation the lipid has, the less stability its complex displays (Eliasson et al. 1985, Tufvesson et al. 2003). Different steric structures of *trans* and *cis* lipids also may result in great differences in its corresponding complex structure stability. Because of the bended structure of *cis*-type lipids, *cis*-unsaturated C18:1 mono-glyceride showed much less stability than its *trans*-counterpart (Kowblansky 1985).

**2.2.3 Starch-protein interaction**

The association between starch and protein has caught people's attention early due to flour application in bakery, such as the formation of dough from wheat gluten and wheat starch. The starch granule surface is an important anchor for proteins during bread dough formation. Conformations of exogenous proteins bound to the wheat starch granule surface appeared to be influence by the native starch granule protein on the granule surface (Ryan et al. 2007).

It is postulated that protein and starch granules are associated by hydrophobic interaction and hydrogen binding. In Ryan's study, wheat starch was striped of its endogenous protein, and exogenous proteins bound to granules at the highest level of 2.5%, yet the binding was not changed with pH (Ryan et al. 2007). However, in real food application starch cannot be totally separately from surface protein. Because of the role of native protein in attracting and holding added protein to starch, factors such as protein shape, size or charge should be considered comprehensively in starch-native protein-exogenous protein association.

By treating starch using reducing agent or protease, proteins were found to be a barrier that inhibit starch swelling at low shear and changing rheological and cooking properties of rice (Hamaker et al. 1990). It also showed an effect of limiting solid leaching during cooking, increasing hardness and decreasing stickiness of cooked rice; disulfide bonds of protein tertiary structure were accountable for this protein barrier function.

Starch, free fatty acid (FFA) and protein are reported to interact with each other, constituted a nano-scale complex and were accountable for changed starch pasting properties. Starch-FFA and protein-FFA complexes were secondary structures resulting from the three-way interaction (Zhang et al. 2003). The negative charged carboxyl group from FFA was the bridge for amylose and protein connection; aliphatic tail of FFA connected to amylose; and the negative charged carboxyl group of FFA could interact with the polyionic protein (Zhang et al. 2010).

### 2.2.4 Starch-amino acids interaction

Proteins are made up of amino acids. While most investigations focused on protein, little was done on the effect of amino acids on altering starch properties. It is known that hydrophobicity of amino acids plays an important role in protein solubility and protein fat binding. Lockwood and King's (2008) study examined the effects of amino acid additives, aspartic acid, leucine, lysine and methionine, on the thermal properties of two potato starches. They found that charged amino acids had greater impact on starch thermal characteristics than neutral ones.

A charged amino acid was likely to effectively stabilize the starch structure and inhibit the collapse of the swollen starch granules during a heat treatment. Similar results were also found in Ito and other's study that charged amino acids like lysine and glutamic acid strongly elevated the gelatinization temperate, reduced the viscosity and swelling of retorted potato starch paste when compared with glycine and alanine (Ito et al. 2006b). Compared to other amino acids, only lysine increased gelatinization temperature of orange-fleshed sweet potato; lysine and aspartic acid addition each resulted in higher gelatinization temperature of the white-fleshed sweet potato (Lockwood et al. 2008). Amino acids in combination with pH treatments can be used to alter thermal and pasting properties of starches (Manaois 2009).

It is not well elucidated as to the mechanism of amino acids effects on starch function change. Because amino acids and ε-poly- (l-lysine) were found to regulate gelatinization, pasting viscosity and swelling of potato starch granule, which followed the change of absolute value of the net charge of those amino acids, it is highly possible that amino acid's effect on starch belongs to certain electrostatic attraction (Ito et al. 2006a). Using a biomolecular interaction analyzer (IAsys) that immobilizes starch, Ito reported the binding of GluNa and Lys to starch chains with increasing concentration while little binding for Gly, Ala, and ε-AC(Ito et al. 2006b). Correspondingly, it was hypothesized that the binding inhibits the interaction between starch and water, leading to reduced swelling of starch granules, increased pasting temperature and decrease peak viscosity and breakdown (Ito et al. 2006b).

Another possibility for amino acid and starch interaction lies in the reducing end group of starch conjugating with amino groups of amino acids, of which principle is similar to the Maillard reaction of forming glycosylamine, although the reaction may be suppressed by the sturdy granule structure of starch (Yang et al. 1998). Potato starch conjugated with poly(lysine) reduced swelling and solubility of starch. The gelatinization temperature of carboxymethyl potato starch (CMS) and corn starch phosphate monoester (PCS) was elevated after lysine

conjugation (Yang et al. 1998). Some studies also suggested that the Millard reaction products resulted in decreased starch digestibility (Yang et al. 1998, Chung et al. 2011).

**2.3 COMMON COMMERCIAL MODIFIED STARCH**

Starch is widely used in food manufacturing for thickening, gelling, as an emulsion stabilizer, encapsulation, water retention, filming, dusting and others. While native starches from different plant origins display different functional properties, most of them suffer from the following disadvantages in food applications, for instance, low shear resistance, low thermal resistance and high tendency of retrogradation. Modified starch refers to starch that can be chemically, physically, genetically or enzymatically modified in order to gain desirable properties, gearing towards specific food applications. Properties like viscoelastic, thermophysical and enzymatic digestion are studied and modified from various aspects, including starch structure, composition, existence of other constituents, processing and storage condition (Table 2.3).

In principle, common chemical modification of starch includes crosslinking and mono-substitution. Crosslinking improves starch stability under acid, shear and heat, since adjacent starch polymers are linked by covalent bonds, which inhibit starch swelling and granule rupture. As a result, crosslinked starch gives less gel strength because of less amylose leaching out. Most used crosslinking agents in the commercial market are phosphoryl chloride, sodium trimetaphosphate (STMP) and adipic acetic mixed anhydride (Mason 2009).

Table 2.3 Properties and application of some modified starch. Adapted from Singh et al. (2007)

| Types | Properties | Food application |
|---|---|---|
| Pregelatinization | Cold water dispersibility | Instant convenience foods |
| Partial acid or enzymatic hydrolysis | Reduce molecular weight; exhibit reduced viscosity, increased retrogradation | Confectionery, batters and food coatings |
| Oxidation /bleaching | Low viscosity, high clarity, and low temperature stability | Batters and breading for coating various food stuffs, in confectionery as binders and film formers, in dairy as texturizers |
| Cross-linking | Higher stability of granules towards swelling, high temperature, high shear and acidic conditions | Texturizers in soups, sauces, gravies, bakery and dairy product |
| Stabilization | Lower gelatinization temperature and retrogradation, lower tendency to form gels and higher paste clarity | Refrigerated and frozen foods, as emulsion stabilizers and for encapsulation |

Monosubstituion or stabilization is starch of which hydroxyl groups are added with blocking substituents so the reassociation of starch polymer is hindered due to failure to form hydrogen bonds among starch chains (Singh et al.

2007). As a result, starch retrogradation, as a quality issue for many starchy foods, is delayed. Improved freeze-thaw and water-holding ability in cold storage makes monosubstituted starch ideal for frozen food or refrigerated food.

Acid modification is the oldest modification. It produces thin-boiling/fluidity starches due to reduced molecular weight of the starch by acid treatment. While viscosity at boiling is lowered, the gel strength of the fluidity starch is not changed (Mason 2009). After cooling, starch forms rigid and opaque gel, allowing the formation of the specific shape. An example of thin-boiling starch application is the production of jelly gum candies, because it requires high gel strength and hot molding.

The most commonly found physical modification in starch is pregelatinized (precooked) starch in instant food. Other modifications included but not limited to heat-moisture, annealing, drying heating, mechanical energy to disrupt starch granule and shortening the starch polymer (Mason 2009). Regarding genetically modification, change of amylose/amylopectin ratio, chain length and structure of amylopectin are most practiced and studied, such as different mutants from waxy corn and high amylose corn.

**2.4 REFERENCES**


Atkin, N. J., R. M. Abeysekera, S. L. Cheng and A. W. Robards (1998). "An experimentally-based predictive model for the separation of amylopectin subunits during starch gelatinization." Carbohydrate polymers **36**(2): 173-192.

Baba, T. and Y. Arai (1984). "Structural characterization of amylopectin and intermediate material in amylomaize starch granules." Agricultural and Biological Chemistry **48**.

Banks, W., C. Greenwood and K. Khan (1970). "Studies on starch-degrading enzymes: Part XII. The initial stages of the action on amylose of the alpha-amylases from B. subtilis, human saliva, malted rye, and porcine pancreas." Carbohydrate Research **12**(1): 79-87.

Bao, J. and C. Bergman (2004). "The functionality of rice starch." Starch in Food: Structure, Function and Applications. A.-C. Eliasson, ed. Woodhead Publishing: Cambridge, UK: 152-184.

Biliaderis, C. (2009). "Structural transitions and related physical properties of starch." Starch: Chemistry and technology: 293-372.

Brumovsky, J. O. and D. B. Thompson (2001). "Production of Boiling-Stable Granular Resistant Starch by Partial Acid Hydrolysis and Hydrothermal Treatments of High-Amylose Maize Starch." Cereal Chemistry Journal **78**(6): 680-689.

Buléon, A., C. Gérard, C. Riekel, R. Vuong and H. Chanzy (1998). "Details of the crystalline ultrastructure of C-starch granules revealed by synchrotron microfocus mapping." Macromolecules **31**(19): 6605-6610.

Champagne, E. (1996). "Rice starch composition and characteristics." Cereal foods world **41**(11): 833-838.

Chung, H.-J., Q. Liu and R. Hoover (2009). "Impact of annealing and heat-moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches." Carbohydrate Polymers **75**(3): 436-447.

Chung, S. Y., S. W. Lee and C. Rhee (2011). "Effects of various maillard reaction products on in vitro starch hydrolysis and blood glucose responses in mice." Starch−Stärke **63**(7): 443-449.

Donald, A. (2004). "Understanding starch structure and functionality." Starch in Food. Structure, Function and Applications. Woodhead Publishing Ltd, Cambridge, UK: 156-184.

Eliasson, A. and N. Krog (1985). "Physical properties of amylose-monoglyceride complexes." Journal of Cereal Science **3**(3): 239-248.

Eliasson, A. and M. Wahlgren (2004). "Starch-lipid interactions and their relevance in food products." Starch in food: structure, function, and applications. Woodhead, Cambridge: 441-454.

Englyst, H. N., S. Kingman and J. Cummings (1992). "Classification and measurement of nutritionally important starch fractions." European Journal of Clinical Nutrition **46**: S33.

French, D. (1984). "Organization of starch granules." Starch: Chemistry and technology **2**: 183-247.

Gallant, D. J., B. Bouchet and P. M. Baldwin (1997). "Microscopy of starch: evidence of a new level of granule organization." Carbohydrate polymers **32**(3): 177-191.

Gidley, M. J. and P. V. Bulpin (1989). "Aggregation of amylose in aqueous systems: the effect of chain length on phase behavior and aggregation kinetics." Macromolecules **22**(1): 341-346.

Gudmundsson, M. and A. Eliasson (1990). "Retrogradation of amylopectin and the effects of amylose and added surfactants/emulsifiers." Carbohydrate Polymers **13**(3): 295-315.

Hamaker, B. R. and V. K. Griffin (1990). "Changing the viscoelastic properties of cooked rice through protein disruption." Cereal Chem **67**(3): 261-264.

Hizukuri, S., T. Kaneko and Y. Takeda (1983). "Measurement of the chain length of amylopectin and its relevance to the origin of crystalline polymorphism of starch granules." Biochimica et Biophysica Acta (BBA)-General Subjects **760**(1): 188-191.

Hizukuri, S., Y. Takeda, N. Maruta and B. O. Juliano (1989). "Molecular structures of rice starch." Carbohydrate Research **189**: 227-235.

Imberty, A., A. Buléon, V. Tran and S. Péerez (1991). "Recent advances in knowledge of starch structure." Starch−Stärke **43**(10): 375-384.

Ito, A., M. Hattori, T. Yoshida and K. Takahashi (2006a). "Contribution of the net charge to the regulatory effects of amino acids and poly (L-lysine) on the gelatinization behavior of potato starch granules." Bioscience, biotechnology, and biochemistry **70**(1): 76-85.

Ito, A., M. Hattori, T. Yoshida, A. Watanabe, R. Sato and K. Takahashi (2006b). "Regulatory effect of amino acids on the pasting behavior of potato starch is attributable to its binding to the starch chain." Journal of agricultural and food chemistry **54**(26): 10191-10196.

Jane, J.-I (2009). Structures of amylose and amylopectin. Starch: chemistry and technology, Academic Press**:** 210.

Jenkins, P. J., R. E. Cameron and A. M. Donald (1993). "A universal feature in the structure of starch granules from different botanical sources." Starch−Stärke **45**(12): 417-420.

Jiang, H., M. Campbell, M. Blanco and J.-L. Jane (2010). "Characterization of maize amylose-extender (ae) mutant starches: Part II. Structures and properties of starch residues remaining after enzymatic hydrolysis at boiling-water temperature." Carbohydrate Polymers **80**(1): 1-12.

Kowblansky, M. (1985). "Calorimetric investigation of inclusion complexes of amylose with long-chain aliphatic compounds containing different functional groups." Macromolecules **18**(9): 1776-1779.

Kulp, K., J. Ponte Jr and B. L. D'Appolonia (1981). "Staling of white pan bread: Fundamental causes." Critical Reviews in Food Science & Nutrition **15**(1): 1-48.

Lockwood, S. and J. M. King (2008). "Amino acid additives effects on sweet potato starch thermal properties." Starch−Stärke **60**(12): 690-695.

Lu, T.-J., J.-I. Jane and P. L. Keeling (1997). "Temperature effect on retrogradation rate and crystalline structure of amylose." Carbohydrate polymers **33**(1): 19-26.

Manaois, R. V. (2009). Modification of Rice Starch Properties by Addition of Amino Acids at Various pH Levels, Master thesis, Louisiana State University,

Marshall, W. E. and J. I. Wadsworth (1994). Rice science and technology, CRC Press.

Mason, W. R. (2009). Starch Uses in Foods. Starch: chemistry and technology, Academic Press**:** 746-794.

McGrance, S. J., H. J. Cornell and C. J. Rix (1998). "A simple and rapid colorimetric method for the determination of amylose in starch products." Starch−Stärke **50**(4): 158-163.

Montgomery, E. M., K. Sexson and F. Senti (1961). "High-Amylose Corn Starch Fractions." Starch-Stärke **13**(6): 215-222.

Morrison, W. and H. Gadan (1987). "The amylose and lipid contents of starch granules in developing wheat endosperm." Journal of Cereal Science **5**(3): 263-275.

Orford, P. D., S. G. Ring, V. Carroll, M. J. Miles and V. J. Morris (1987). "The effect of concentration and botanical source on the gelation and retrogradation of starch." Journal of the Science of Food and Agriculture **39**(2): 169-177.

Peat, S., W. Whelan and G. Thomas (1952). "Evidence of multiple branching in waxy maize starch." J. Chem. Soc **4546**: 4548.

Ramesh, M., J. R. Mitchell, K. Jumel and S. E. Harding (1999). "Amylose content of rice starch." Starch−Stärke **51**(8−9): 311-313.

Rogers, D. E., K. J. Zeleznak, C. Lai and R. Hoseney (1988). "Effect of native lipids, shortening, and bread moisture on bread firming." Cereal chemistry. **65**(5): 398.

Ryan, K. and M. Brewer (2007). "In situ examination of starch granule-soy protein and wheat protein interactions." Food chemistry **104**(2): 619-629.

Sang, Y. and P. A. Seib (2006). "Resistant starches from amylose mutants of corn by simultaneous heat-moisture treatment and phosphorylation." Carbohydrate polymers **63**(2): 167-175.

Seow, C. and K. Thevamalar (1988). "Problems associated with traditional Malaysian starch-based intermediate moisture foods."

Shannon, J. (1984). "Genetics and physiology of starch development." Starch, Chemistry and Technology.

Singh, J., L. Kaur and O. McCarthy (2007). "Factors influencing the physico-chemical, morphological, thermal and rheological properties of some chemically modified starches for food applications—A review." Food Hydrocolloids **21**(1): 1-22.

Singh, N., J. Singh, L. Kaur, N. Singh Sodhi and B. Singh Gill (2003). "Morphological, thermal and rheological properties of starches from different botanical sources." Food Chemistry **81**(2): 219-231.

Taggart, P. (2004). Starch as an ingredient: manufacture and applications. Didalam: Eliasson AC (Ed). Starch In Food: Structure, Function and Applications, Woodhead Publishing Limited. Cambridge.

Takeda, Y., S. Hizukuri and B. O. Juliano (1986). "Purification and structure of amylose from rice starch." Carbohydrate research **148**(2): 299-308.

Thompson, D. B. (2000). "Strategies for the manufacture of resistant starch." Trends in Food Science & Technology **11**(7): 245-253.

Tufvesson, F., M. Wahlgren and A. C. Eliasson (2003). "Formation of Amylose‐Lipid Complexes and Effects of Temperature Treatment. Part 1. Monoglycerides." Starch−Stärke **55**(2): 61-71.

Xie, X. and Q. Liu (2004). "Development of new resistant starch-citrate starch as a functional food ingredient." Starch−Stärke **56**(8): 364-370.

Xie, X., Q. Liu and S. W. Cui (2006). "Studies on the granular structure of resistant starches (type 4) from normal, high amylose and waxy corn starch citrates." Food research international **39**(3): 332-341.

Yang, W., M. Hattori, T. Kawaguchi and K. Takahashi (1998). "Properties of starches conjugated with lysine and poly (lysine) by the Maillard reaction." Journal of agricultural and food chemistry **46**(2): 442-445.

Yano, M., K. Okuno, J. Kawakami, H. Satoh and T. Omura (1985). "High amylose mutants of rice, Oryza sativa L." Theoretical and Applied Genetics **69**(3): 253-257.

Yue, P. and S. Waring (1998). "Resistant starch in food applications." Cereal Foods World **43**.

Zaidul, I. S. M., H. Yamauchi, S.-J. Kim, N. Hashimoto and T. Noda (2007). "RVA study of mixtures of wheat flour and potato starches with different phosphorus contents." Food Chemistry **102**(4): 1105-1111.

Zhang, G., M. Maladen, O. H. Campanella and B. R. Hamaker (2010). "Free fatty acids electronically bridge the self-assembly of a three-component nanocomplex consisting of amylose, protein, and free fatty acids." Journal of Agricultural and Food Chemistry **58**(16): 9164-9170.

Zhang, G., M. D. Maladen and B. R. Hamaker (2003). "Detection of a novel three component complex consisting of starch, protein, and free fatty acids." Journal of agricultural and food chemistry **51**(9): 2801-2805.

Zobel, H. (1988). "Molecules to granules: a comprehensive starch review." Starch−Stärke **40**(2): 44-50.

Zobel, H., A. French and M. Hinkle (1967). "X−Ray diffraction of oriented amylose fibers. II. Structure of V amyloses." Biopolymers **5**(9): 837-845.

# CHAPTER 3
# PASTING PROPERTIES OF STARCH ADDED WITH AMINO ACIDS AND FATTY ACIDS AT DIFFERENT LEVELS

## 3.1 INTRODUCTION

Rice is one of the staple cereals in most Asian countries and is gaining popularity in the United States due to the rising production of snacks, frozen dinners, pudding and candy made from rice. Being hypoallergenic, rice flour is bland and of high protein quality, making it ideal to process gluten-free food.

For most native starches, there is a sharp increase in viscosity when being cooked; however, the starch gel starts loses its viscosity once overcooked, which can also be accelerated by heat, acid and shear. During gelatinization, water penetrates into starch granules and starch undergoes irreversible swelling of its granules, disruption of molecular order within the granule, and lost of its semi-crystalline feature. Because of starch hydration, the viscosity of starch solution increases. Amylose leaches out of starch granules and may interact with compounds in the starch solution. Eventually, starch granules rupture, leading to a very rapid decrease in viscosity, which is termed as breakdown (Biliaderis 2009). The narrow peak viscosity range indicates that natural starch is susceptible to process conditions. From a sensory evaluation point of view, native starch may fail to provide desirable texture for consumers. Modified starch is needed to control peak viscosity, to gain improved tolerance to rigorous processes, for desirable texture and for prolonged stability.

Studies on food additives using natural substances have become popular, because it runs a lower risk of food safety issues than synthetic food additives. Interaction of food components in the existing food system is a good source to find natural food additives for starch modification. Interaction of starch, protein and fatty acids is one typical example in food products. For example, during breadmaking, wheat starch interacts with gluten proteins. It is proposed that the surface proteins of starch granules help gluten-starch interaction (Dreese et al. 1988). Martin et al. (1991) suggested that bread firming could be partly attributed to the entanglements and cross-links between gluten and starch by hydrogen bonds. The protein starch complex, which is formed on the granule surface, may prevent the escape of amylose from the granules and therefore increase the gelatinization temperature of the starch (Olkku et al. 1978). Different model systems were also put forward to explain the association between starch and gluten protein, such as electrostatic force, hydrophobic interaction and surface protein mediated gluten-starch model, although no conclusive results were obtained because of different origins of starch, protein and treatments applied (Lindahl et al. 1986, Eliasson et al. 1990). It is suggested that gliadin and maize amylose formed an aggregate, which interfered

amylogluocosidase hydrolysis of starch most efficiently that other protein, such as gluten and glutenin (Guerrieri et al. 1997)

Proteins are made up of amino acids. While most investigations focused on protein effects on starch-based film making, only a few were done on the effect of amino acids altering starch properties. For example, it is known that hydrophobicity and electrostatic nature of amino acids play important roles in protein structure, solubility and fat binding properties. Lockwood and King's (2003) study examined the effects of amino acid additives, aspartic acid, leucine, lysine and methionine on the thermal properties of two sweet potato starches. They found that charged amino acids had greater impact on starch thermal characteristics than neutral ones. A charged amino acid was likely to effectively stabilize the starch structure and inhibit the collapse of the swollen starch granules during a heat treatment at high temperature. Using carboxymethyl potato starch and corn starch phosphate monoester, Yang (1998) made new starches that were conjugated with lysine at 0.12%-0.68% and poly(lysine) at 2.8%-4.3%. This conjugation is based on the Maillard reaction in which the reducing end of starch was connected to the amino groups of lysine or poly(lysine). Conjugation with peptide was found to be more effective than with amino acids, judged by the performance of treated starch, including restricted swelling, lower solubility, higher gelatinization temperature and lower α-amylase digestibility than control starch. In principle, conjugation increased the stability of starch granule and depressed rearrangement of starch chains after being gelatinized.

By comparing the effects of different types of amino acids additives on potato starch properties, Ito and other's study suggested that charged amino acids like lysine and sodium glutamate strongly elevated the gelatinization temperature, and reduced the viscosity and swelling of potato starch paste in retort treatment (Ito et al. 2006b). Because amino acids in combination with pH treatments can be used to alter thermal and pasting properties of starches (Manaois 2009), it is suggested that net change of amino acids or peptide played important roles in altering starch swelling, indicating an electrostatic interaction between starch and its additives (Ito et al. 2006a).

The application of lipids on starch-based food products is also very common. Shortening and monosaccharides, for example, are used to inhibit bread staling rate. It was widely accepted that besides retrogradation, water loss is one main reason for bread firming. Shortenings keep bread moisture through interaction with lipids rather than starch, since shortening has no effect on firming of defatted bread (Rogers et al. 1988); surfactant inhibits starch retrogradation by complexing with starch, amylose in most cases, so starch recrystallization that gives unfavorable

hard texture is depressed. More mechanism and models were also proposed to explain how the starch-lipid complex play a role in bread firming, although no consistent conclusions were reached (Stampfli et al. 1995).

Most studies of lipid effects on starch properties are involved with amylose-lipid complex. The amylose V complex helix was detected by X-ray diffraction, which has a hydrophobic channel inside for complexing hydrophobic ligands (Eliasson et al. 1985, Putseys et al. 2010). For amylose-lipid complex, it is the aliphatic chain of lipid that is located inside the helix cavity. It is hypothesized that 18-24 glucose units can combine only one lipid molecular that has 14-18 carbon as fatty acids skeleton or tail of monoglycerol, which equals to three turns of single amylose helix (Putseys et al. 2010).

The lipid characteristics are important in formation of lipid-starch complex. The nature of the polar group in the surfactant, whether ionic surfactants or not, is the first fundamental variable in binding starch, indicated by changed gelatinization onset temperature and enthalpy (Villwock et al. 1999). For nonionic emulsifier, monoglyceride (MG) was given special attention as a simple model to study interaction of lipids with starch. Higher complex index was found in MG with saturated fatty acid chain than unsaturated fatty acid chain (Lagendijk et al. 1970, Hahn et al. 1987). Long lipid chain length usually stabilizes the amylose-lipid complex, making an elevated dissociation temperature due to stronger hydrophobic interaction with the amylose helix than lipids with short chain length (Eliasson et al. 1985, Raphaelides et al. 1988). However, this is not always the case. Some studies argued that chain length of 14 was best for complexing with starch (Hoover et al. 1981, Bhatnagar et al. 1994). For example, a study on complex index of rice flour-lipid indicated that free fatty acids with shorter carbon chains like myristic acid was favored over long carbon chains like stearic acid, when the addition level was 1.5%~4.5% of dry starch weight (Kaur et al. 2000).

Through comparison of individual lipids effects on the pasting characteristics of rice starch using RVA, Liang et al. (2002) found some interesting phenomena. Addition of monopalmitin and rice native lipids to commercial rice starch increased both pasting temperature and peak viscosity. Therefore these additives increased energy required for starch pasting and enhanced starch swelling power. Greater total setback (TSB) of starch paste with addition of monopalmitin indicated a high tendency for starch retrogradation and more solubilized amylose released from starch solution. Enhanced V- type amylose pattern caused by formation of amylose-lipid complexes was also found by X-ray diffraction. However, this was in contradiction with the starch anti-firming effect of mono-glyceride. The presence of 0.6% free fatty acids, palmitic acid, oleic acid, linoleic acid, and linolenic acid also increased the TSB.

As a result, more studies should be conducted regarding the relationship between addition of lipids and starch retrogradation.

There is no study on how the combination of amino acids and fatty acid mixtures influences rice starch pasting properties. Hydrophilic amino acids would reside in aqueous environment while hydrophobic amino acids would more likely be found in the lipids area. In the case of starch solution added with lipid, hydrophilic amino acids would be in a starch solution with free amylose leached out from the granule, and hydrophobic amino acid would be close with lipid which may affect formation of starch-lipid complex. Learning how combinations of amino acids and fatty acids affect the pasting and thermal properties of starch will not only help clarify the mechanism of intermolecular interaction for macronutrients in altering starch properties, but also provides a new way to modify starch, e.g. whether amino acids and lipids work in a synergistic or mutual suppression way. The objective of this study was to investigate the effects of combinations of different fatty acids with amino acids on starch pasting properties; complex index measurement was also applied to shed light on the structural transitions and possible mechanisms accounting for these changes during starch pasting.

**3.2 MATERIALS AND METHODS**

All materials and chemicals were purchased from Sigma Chemical (St. Louis, MO), including rice starch, corn starch, fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid) and amino acids (leucine, lysine, aspartic acid, cysteine and tyrosine). Materials for the amylose assay were from a Megazyme kit (Megazyme International Ireland Ltd.Co. Wicklow, Ireland).

**3.2.1 Chemical composition analysis of commercial rice starch and corn starch**

The moisture content was determined by moisture loss using a convection oven to dry samples, based on AOAC 925.10 (2005). The oven was preheated at 130$^{O}$C for 1 hr. The aluminum dish was labeled, placed into the oven for 1hr and then cooled down in a desiccator for 2min in order to reach constant weight. The dish was then weighed as $W_{dish}$. Two grams of prepared starch sample (precise to 0.001gram) were placed in the aluminum dish and then weighed ($W_{dish+wet\ sample}$). After being heated for 1hr, the sample dishes were taken out and cooled down in a desiccator to room temperature (approximately10min). The sample dishes were weighed. The dishes were again put into the oven for half an hour, cooled down and weight to see if the weights were constant or not. If not, the dishes were placed into the oven again for continuous heating until constant weights were reached ($W_{dish+dry\ sample}$). The moisture content was calculated as $MC\%=(W_{dish+wet\ sample}-W_{dish+dry\ sample})/(W_{dish+wet\ sample}-W_{dish})*100\%$

Protein content was measured based on the Dumas combustion method with nitrogen to protein conversion factor of 5.17 for rice starch (Mosse 1990). A modified Soxhlet extraction method (AACC 30-25) was applied to determine crude fat in the starch sample. A sample of 100g of starch was transferred into a 15cm–high extraction thimble, and then covered with cotton and put into a Soxhlet extraction tube. A 500ml flask with 300ml of petroleum ether was connected with an Allihn condenser and Soxhlet extraction tube. Then, the flask was placed in a water bath with temperature setting of 45˚C. The condenser was then connected to a cooling system with a coolant temperature setting at 5℃. A vacuum pump was connected with a Soxhlet extraction tube to help solvent evaporate. The petroleum ether extraction was done for 24 hours. Then the petroleum ether solution was condensed by rotary evaporation to 1/5 of its volume and dried by nitrogen until a constant weight was reached. The weight of residual oil was the percentage of fat content in 100g of starch sample.

For the amylose content assay, amylose was separated from amylopectin by ConA-amylopectin precipitation, which followed the Megazyme Amylose-Amylopectin assay procedure (Megazyme International, Wicklow, Ireland) with the reagent and buffers provided by the assay kit (K-AMYL). The detailed procedure can be found in product brochure. All the above assays were done in duplicate.

**3.2.2 Standard rapid viscosity analysis of rice starch and corn starch**

Fatty acids of two addition levels (0.6% and 1.0%) and 6% amino acids (both on a dry basis) were selected to check their effects on pasting properties of commercial rice starch, based on a study by Liang and King (2003) and Liang et al (2002). Later, different levels of stearic acid (0.2%, 0.4%, 0.6%, 0.8% and 1.0%) and lysine (2%, 4% and 6%) combinations were applied to maximize their effects on certain desirable pasting properties of commercial rice starch. Meanwhile, in order to see the effect of starch types towards those additives, both corn starch and rice starch were tested.

A Rapid Visco Analyzer (RVA-4, Newport Scientific, Warrieword, Austrilia) was used to mimic heating and shearing of starch samples during industrial processing by time, temperature and shear speed control; and apparent viscosity was recorded. Distilled water ~20ml was first added into an RVA canister; fatty acids additives were weighed in the canister. Starch 2.65g (db) and amino acids were measured by weigh boats and transferred to the canister; starch and amino acids residue in the weigh boats were washed by less than 5ml of distilled water and transferred into the canister as well. The total weight of starch, water and additives were 28g.

AACCI Method 61-02.01 was followed for the controlled heat-hold-cool temperature cycle in the mixture. Each sample was held at 50$^{o}$C for 10sec, with the stirring speed of the spindle set at 960rpm. The speed was then reduced to 160rpm while the temperature started increasing at a rate of 12$^{o}$C/min until 95$^{o}$C. The holding time for 95$^{o}$C was 2.5min. Then the canister was cooled to 50$^{o}$C at a rate of -12$^{o}$C/min. During the whole heating and cooling process, the stirring speed was kept at 160rpm. The following parameters were recorded: The pasting temperature (PT), peak viscosity (PV), minimum viscosity (MV), final viscosity (FV), and peak time (PTime). Total setback (TSB) and breakdown (BD) were calculated as TSB=FV-MV; BD=PV-MV. All treatments were done in triplicate.

The gelatinized starch paste was either used instantly for complex index measurement or was quickly frozen at -80°C and later freeze-dried. Dried samples were ground with a 0.5 mm screen in the Cyclone Sample Mill (Udy Corp., Port Collins, CO).

**3.2.3 Complex index measurement**

Following the method of Tang and Coperland (2007), starch paste 5.0 g was taken from RVA canister into capped tube right after RVA treatment of starches with or without additives. Twenty ml of distilled water at 50°C was added into the tube followed by 2 min of vortexing. The mixture was kept at 50°C in a water bath less than 2hrs, in order to prevent starch retrogradation. One ml of the above solution was taken out and centrifuged at 10,000 rpm for 10min. 50 µl supernatant was transferred to a test tube and diluted by 7.5 ml distilled water. 1ml iodine solution (2.0% KI and 1.3% of $I_2$ in distilled water) was added into the test tube as well with slight shaking. The bluish solution was measured for its absorbance at 690nm. Starch paste without any additives was used as reference for complex index calculation of starch-lipid treatment; starch paste with lysine treatment was used as reference for complex index calculation of starch-lipid-lysine calculation. All starch pastes were from RVA treatments in duplicate; the iodine binding step was also done in duplicate. Complex index was calculated as following:

$CI_{\text{starch-lipid}} = (Abs_{\text{starch}} - Abs_{\text{starch-lipid}})/Abs_{\text{starch}} * 100$

$CI_{\text{starch-lipid-lysine}} = (Abs_{\text{starch-lysine}} - Abs_{\text{starch-lipid-lysine}})/Abs_{\text{starch-lysine}} * 100$

**3.2.4 Statistical analysis**

Statistical software SAS (v.9.0) was used for data analysis. RVA data were analyzed by ANOVA with Tukey's studentized range (HSD) to test the effects of additives, including amino acids and fatty acid addition individually or combined. Triplicate samples were used and the significance level was set at $P \leq 0.05$.

## 3.3 RESULTS AND DISCUSSION

### 3.3.1 Chemical composition analysis of commercial rice and corn starch

Commercial rice starch from Sigma had 11.7% moisture, 0.20% lipid, 0.70% protein. There was 24.9% amylose based on dry rice starch weight. Commercial corn starch from Sigma had 12.5% moisture, 0.3% lipid, 0.22% protein. There was 27.2% amylose based on dry corn starch weight.

There are many amylose content determination methods, however, not every method is accurate even they are of high repeatability. Many factors, such as starch structure, choice of standard and sample preparation affect the result. For example, traditional colorimetric determination compares the absorbance at 620nm of amylose-lipid complex between sample starch and standard starch at pH 4.5-4.8 in acetate buffer, based on the linear relationship between absorbance and amylose content. Our previous result showed that colorimetric determination of amylose at 620nm usually gave a higher amylose content than being labeled. As a result, the commercial amylose assay kit from Megazyme was selected to avoid this dispute, because it measured both amylose and amylopectin contents rather than application of pure amylose as a standard.

### 3.3.2 Altering pasting properties of rice starch with added amino acid and fatty acid

The heating and cooling cycles in RVA mimic the processing of starch. When starch solution is heated to its pasting temperature (PT), its viscosity rises rapidly and starts pasting. Peak viscosity (PV) reflects the granule swelling ability after enough hydration. Long time heating damages starch granules; once granule collapse dominates in the solution rather than granule swelling, starch paste viscosity decreases to a minimum viscosity (MV). The difference between PV and MV is breakdown (BKD), indicating starch process stability, which is also subject to heating, shearing and pH. Once starch paste cools down, the viscosity will recover due to re-association of dispersed starch molecules, forming three dimensional gel network or intermolecular junction zone. Total setback (TSB) is the difference between final viscosity (FV) and MV. Since formation of junction zone is followed by recrystallization and retrogradation, TSB is considered to reflect starch retrogradation tendency (Bao et al. 2004).

Great difference was found between saturated fatty acid and unsaturated fatty acid addition in terms of stability of starch paste (Figure 3.1 and Figure 3.2). Saturated fatty acids, such as palmitic acid and stearic acid inhibited starch breakdown, as opposed to increased breakdown after addition of unsaturated fatty acids of linoleic and linolenic acids at both 0.6% and 1.0% levels. Also, peak time and pasting time of saturated fatty acids were delayed, whereas no change of peak time was found for linoleic and linolenic acids. Oleic acid, which has only one double

bond, showed intermediate effects between saturated fatty acids and highly unsaturated fatty acids on breakdown and peak time values. This is explained by starch-lipid complexation, formed either at the surface or inside the granule, which prevents leaching of soluble starch components (Hoover et al. 1981, Lauro et al. 2000). Correspondingly, starch pasting was depressed, which was shown by prolonged pasting time. The decreased breakdown was considered to be due to the granule stabilizing effect of lipids or amylose-lipid complex. The reason why only saturated fatty acids demonstrated this effect is due to stronger and more stable complexes formed for starch with saturated fatty acid than with unsaturated fatty acid (Lagendijk et al. 1970, Hahn et al. 1987). However, addition of 0.6 and 1.0% fatty acids did not change the swelling degree of rich starch much as indicated by peak viscosity. While many previous studies demonstrated that starch-lipid interaction decreased swelling capacity, current data can be explained as insufficient complex formation for swelling restriction by RVA heating cycle applied (Biliaderis et al. 1991, Lauro et al. 2000, Mira et al. 2007).

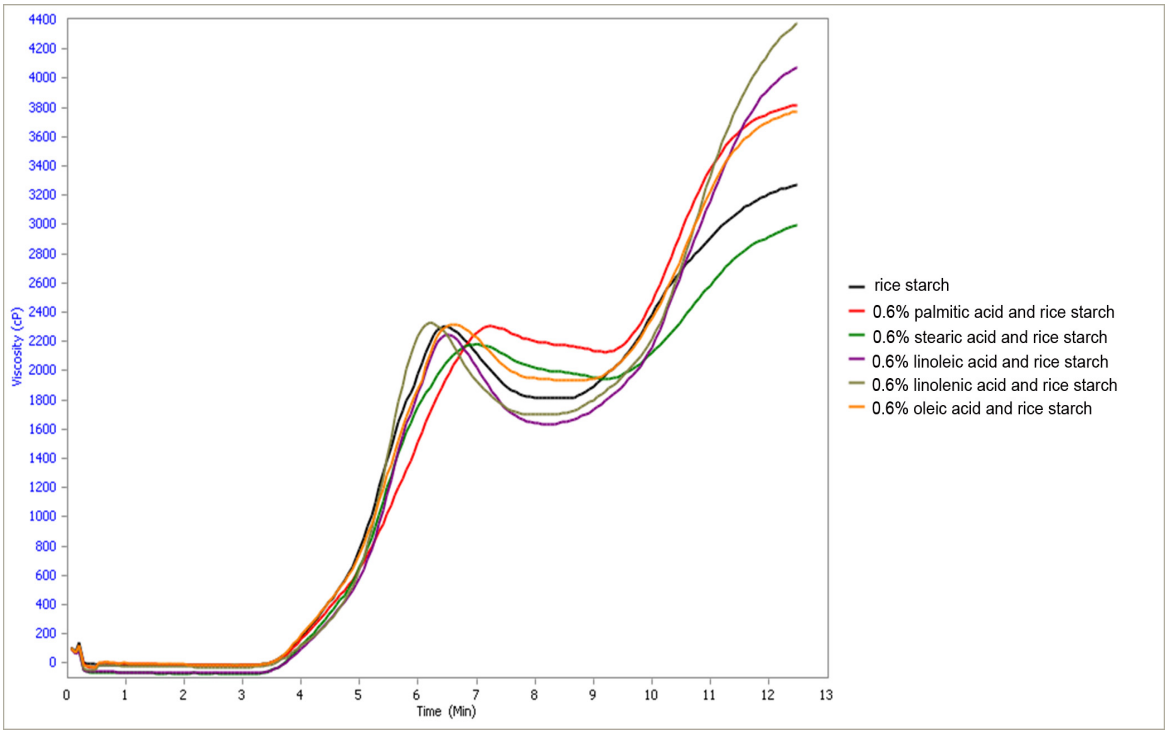


Figure 3.1 RVA curves of rice starch added with fatty acids at 0.6%

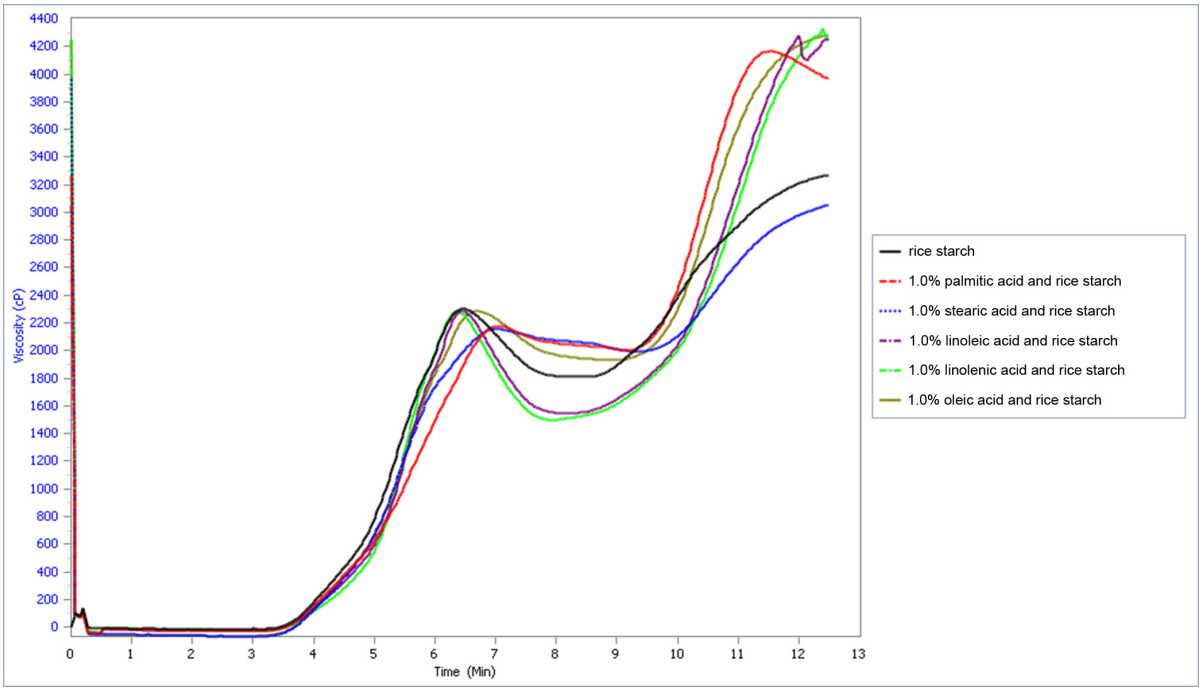


Figure 3.2 RVA curves of rice starch added with fatty acids at 1.0%

Except stearic acid, all other fatty acids increased FV and TSB significantly (Table 3.1). Those findings above were consistent with Liang et al. (2002) on pasting properties change of rice starch with added fatty acids on 0.2% and 0.6% levels. Stearic acid seems to be an obstacle for amylose re-associating, meaning lower retrogradation potential. It also imparted lower peak viscosity than other fatty acid additives. Those phenomena afforded us a special attention and further investigation should be conducted on stearic acid-rice starch interaction.

Five representative amino acids were selected for studying their effects on commercial rice starch (Table 3.2 and Figure 3.3). Previous studies demonstrated that neutral amino acids had minimal effects on the pasting properties of starch, but charged amino acids and proteins with disulfide bonds would more likely to alter starch pasting (Hamaker et al. 1993, Ito et al. 2006a, Liang et al. 2003). The data in this study showed similar patterns of change in that effects of leucine and tyrosine on starch pasting were very limited (Table 3.2). Compared to the control, cysteine and charged amino acids, aspartic acid and lysine, caused sharp drops of viscosity during continuous heating, as indicated by higher breakdown than original rice starch. This was inconsistent with potato starch pasting, which showed lower breakdown after addition of charged amino acids compared to their control, indicating a more stabilized starch granule (Ito et al. 2006b). However, stabilizing effect of aspartic acid and cysteine could also be found before reaching their peak viscosity, since both gave prolonged pasting time and higher pasting temperature (Table 3.2)

Table 3.1 Effects of fatty acids on pasting properties of commercial rice starch [a,b,c]

| %[b] | FA | Peak (cp) | MV (cp) | BKD (cp) | FV (cp) | Ptime (min) | PT (°C) | TSB (cp) |
|---|---|---|---|---|---|---|---|---|
| 0 | Control | 2307.33±18.58a | 1807.67±6.81c | 499.67±25.38c | 3281.67±16.26c | 6.49±0.03c | 82.68±0.49a | 1474±22.61c |
| 0.6 | Palmitic | 2317±21.93a | 2114±11.53a | 203±23.90e | 3851.33±40.67b | 7.16±0.10a | 82.63±0.89a | 1737.33±47.35b |
| 0.6 | Stearic | 2207.33±23.71b | 1972±24.27b | 235.33±13.01de | 3037.33±63.22d | 7±0a | 82.68±0.41a | 1065.33±46.92d |
| 0.6 | Oleic | 2320±6.24a | 1987.67±64.27b | 332.33±67.17d | 3773.33±66.01b | 6.69±0.1b | 82.38±0.03a | 1785.67±52.6b |
| 0.6 | Linoleic | 2273.33±42.15ab | 1644±24.58d | 629.33±18.34b | 4178.33±104.57a | 6.53±0bc | 83.93±0.03a | 2534.33±86.32a |
| 0.6 | Linolenic | 2299.67±54.78a | 1543±34e | 756.67±53.53a | 4231.67±109.74a | 6.42±0.04c | 85.77±3.93a | 2688.67±141.17a |
| | | | | | | | | |
| 0 | Control | 2307.33±18.58a | 1807.67±6.81c | 499.67±25.38b | 3281.67±16.26c | 6.49±0.03c | 82.68±0.49a | 1474±22.61d |
| 1.0 | Palmitic | 2224.67±42.57ab | 2055.33±48.44a | 169.33±7.37d | 3900.67±58.39b | 7.07±0.07a | 82.35±0.09a | 1845.33±106.36c |
| 1.0 | Stearic | 2195±46.18b | 2008.33±32.72ab | 186.67±19.73d | 3088.33±79.03d | 7.07±0.07a | 82.37±0.03a | 1080±46.36e |
| 1.0 | Oleic | 2280.67±7.57ab | 1948.33±46.49b | 332.33±54.05c | 4253.67±45.65a | 6.74±0.12b | 82.38±0.1a | 2305.33±39.93b |
| 1.0 | Linoleic | 2267±32.91ab | 1533±22.52d | 734±10.39a | 4206.33±53.59a | 6.49±0.03c | 83.73±0.97a | 2673.33±34.95a |
| 1.0 | Linolenic | 2302.67±26.84a | 1489.33±39.72d | 813.33±64.24a | 4167.33±79.94a | 6.4±0.07c | 87.37±4.36a | 2678±103.26a |

Table 3.2 Effects of amino acids on pasting properties of commercial rice starch [a,b,c]

| %[b] | Amino | PV(cp) | MV(cp) | BKD (cp) | FV(cp) | PTime(min) | PT(°C) | TSB(cp) |
|---|---|---|---|---|---|---|---|---|
| 0.0 | Control | 2307.33±18.58c | 1807.67±6.81b | 499.67±25.38d | 3281.67±16.26a | 6.49±0.03b | 82.68±0.49c | 1474±22.61bc |
| 6.0 | Asp | 2460±41.15b | 1490.67±22.72e | 969.33±56.86a | 2480.33±18.01c | 6.53±0.12ab | 90.28±0.78a | 989.67±29.4d |
| 6.0 | Cys | 2750±21.52a | 2038.33±23.12a | 711.67±9.87b | 3077±26.21b | 6.67±0a | 86.12±2.37b | 1038.67±6.81d |
| 6.0 | Leu | 2323±22.65c | 1751±17.09bc | 572±5.57cd | 3289±20.07a | 6.47±0b | 82.98±0.46c | 1538±10.44b |
| 6.0 | Lys | 2402±23.07b | 1654.33±30.92d | 747.67±11.59b | 3069.33±32.25b | 5.45±0.04c | 81.63±0.08c | 1415±4c |
| 6.0 | Try | 2307.67±36.56c | 1699±52.85cd | 608.67±18.72c | 3329±71.08a | 6.4±0.07b | 82.35±0.05c | 1630±48.28a |

a. FA, fatty acid; PV, peak viscosity; MV, minimum viscosity; BKD, breakdown; FV, final viscosity; PT, pasting temperature; Ptime, peak time; TSB, total setback
b. All levels are based on starch dry weight
c. Values followed by the same letter in the same column in the same subtable are not significantly different ($P > 0.05$)

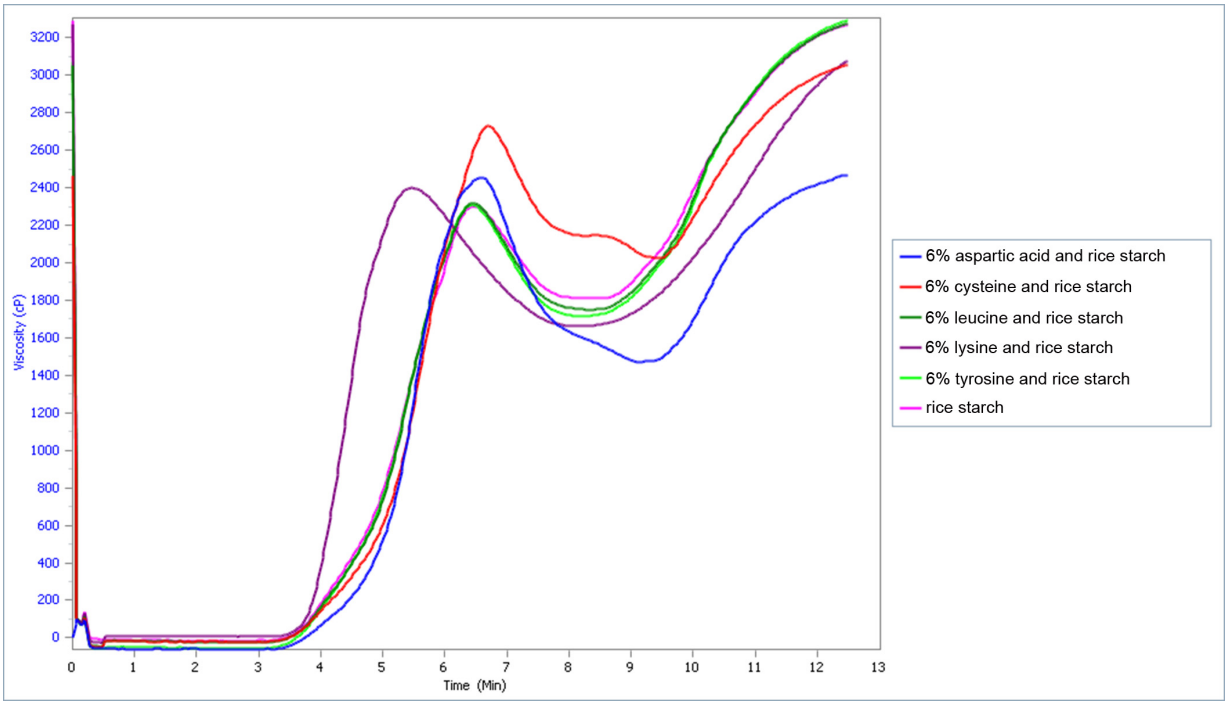


Figure 3.3 RVA curves of rice starch added with 6.0% amino acids

A noteworthy thing different from Ito's study was that addition of those amino acids into starch solution in this study did not require pH adjustment. Six percent aspartic acid water solution was acidic (pH 3.0) and 6% lysine water solution (pH 10.0) was basic. It was highly possible that under acid condition caused by aspartic acid, starch went through slight acid hydrolysis, since preliminary study showed low viscosity cause by acidic hydrolysis when hydrochloric acid was used to prepare starch solution (pH 3.0) for RVA test. Therefore aspartic acid added starch paste became soft and long after its maximum hydration; and viscosity of starch paste recovered slowly, giving a lower TSB value than control starch.

Because the pH of 6% lysine water solution (pH 10.0) is higher than its pI value of 9.74, lysine is slightly negatively charged in 6% lysine water solution (Figure 3.4). Instead of stabilizing, lysine increased starch pasting rate, since all other starch started to paste at the same time, but the peak time of starch paste decreased almost 1min. This result was also found in other similar studies as well (Liang 2001, Manaois 2009).

Maillard browning is an important flavoring/color inducing reaction in food chemistry. During food processing, an amino-carbonyl reaction happens through glycolsylamine formation. In our study, lysine added starch turned apparently yellow after the RVA heating cycle, which can be considered as the amino group that conjugated with the reducing end group of starch by the Maillard reaction. Similar phenomena were also found in potato starch or

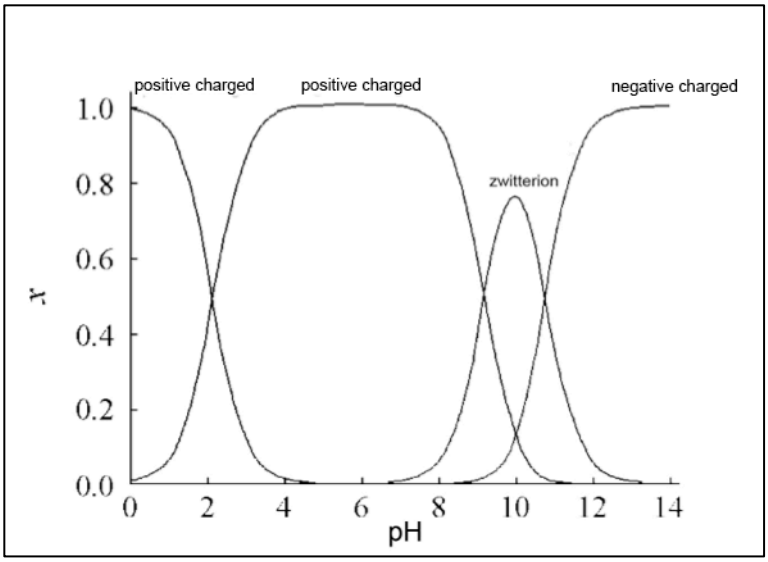

Figure 3.4 Lysine charge at various pH conditions. From Shusheng Zhang (2005)

modified potato starch (Yang et al. 1998). If we correlate this Maillard reaction with rapid increase of starch paste viscosity caused by addition of lysine, there is a possibility that Maillard reaction promote flexibility of starch molecules by leaching out from the granule and react with lysine. With a rapid rate of amylose leaching out from the starch granule, the viscosity of starch paste grew faster until it reached to its peak viscosity. In general, while heating provides the initial energy for starch to gelatinize, lysine is hypothesized to accelerate melting of semi-crystalline starch structure in order to yield free starch molecules.

For starch with added cysteine, although it had similar pasting rate as control starch, it swelled longer than the former. The peak viscosity was therefore higher than other starches. But this swelled starch granule was not stable, as it broke down quickly when continuous heating was applied. So for, no previous studies have been done on starch with added cysteine. Instead, much work highlighted the disulfide bonding within protein that linked two cysteine molecules (Figure 3.5). For example, when cysteine in rice flour protein was reduced to cysteine, rice flour was found swell more and with higher breakdown; it was explained by the removal effect of disulfide bonding in protein in control starch which restricted starch swelling (Hamaker et al. 1993). While the reason for these changed pasting properties of rice starch is unclear, it is possible that disulfide bonding, which may link cysteine to rice surface protein, plays a role in changing starch properties. The fact that cysteine is used as oxygen scavenger indicated cysteine may be oxidized under current RVA condition (Talwalkar et al. 2004).

The combination of fatty acids and amino acids was expected to show a different effect than adding fatty acids and amino acids separately. Indeed, for most fatty acids and amino acids added together, peak viscosity, MV, FV,

Ptime and TSB demonstrated combined effect of their individual fatty acid or amino acid addition. For example, addition of both 0.6% palmitic acid and 6% aspartic acid showed similar or intermediate pasting curves of 0.6% palmitic acid added starch and 6% aspartic acid added starch.

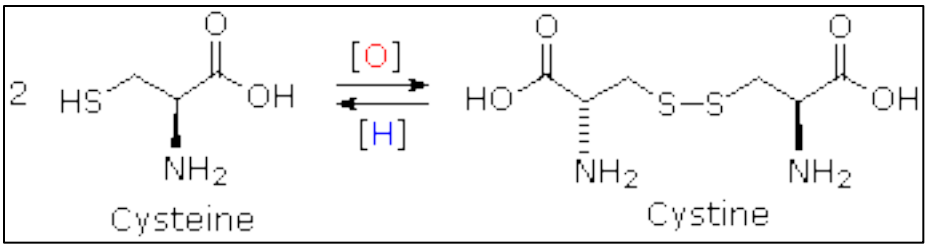


Figure 3.5: Formation of disulfide bond by cysteine
From http://www.biog1445.org/demo/01/proteinstructure.html

Special attention has been given to starch with added stearic acid/lysine combination and oleic acid/lysine combination because these two lowered starch breakdown synergistically, comparing to other combinations (Figure 3.6, Figure 3.7, Figure 3.8, Figure 3.9 and Table 3.3). Addition of 0.6% stearic acid/6.0% lysine lowered starch BKD from 400.67cP for control to 111.67cP; and BKD values for 0.6% stearic acid added and 6% lysine added starches were 235.33 and 747.67cP. Addition of 1.0% stearic acid/6% lysine reduced starch BKD to an almost unnoticeable level; and BKD values for 1.0 % stearic acid added and 6% lysine added starches were 187.67 and 747.67cP. Addition of 0.6% oleic acid/6% lysine reduced starch BKD from 500cP to 163cP; and BKD values for 0.6% oleic acid added and 6% lysine added starches were 332.33 and 747.67 cP. Addition of 1.0% oleic acid /6% lysine reduced starch BKD to 97cP; and BKD values for 0.6% oleic acid added and 6% lysine added starches were 332.33 and 747.67cP.

Also, notable increase of pasting temperature and delayed peak time was found in the above stearic acid/lysine and oleic acid/lysine combinations (Table 3.3). While rice starch control started to paste at 82.68°C, starch added with 0.6% oleic acid/ 6% lysine did not start to paste until 88.72°C and peak time was 6.93min. Starch with added 0.6% oleic acid and 6% lysine alone started to paste at 82.38°C and 81.63°C and their peak times were only 6.69 min and 5.45 min. Starch with added 1.0% oleic acid/ 6% lysine did not start to paste until 93.98°C and peak time was 7.58 min. Starch with added 0.6% oleic acid and 6% lysine alone started to paste at 82.38°C and 81.63°C and their peak times were only 6.74min and 5.45min. Starch added with 0.6% stearic acid/ 6% lysine didn't start to paste until 93.78°C and peak time was 8.00 min; starch with added 0.6% oleic acid and 6% lysine alone started to paste at 82.68°C and 81.63°C and their peak times were only 7.00min and 5.45min. Starch with added 0.6% stearic acid/ 6%

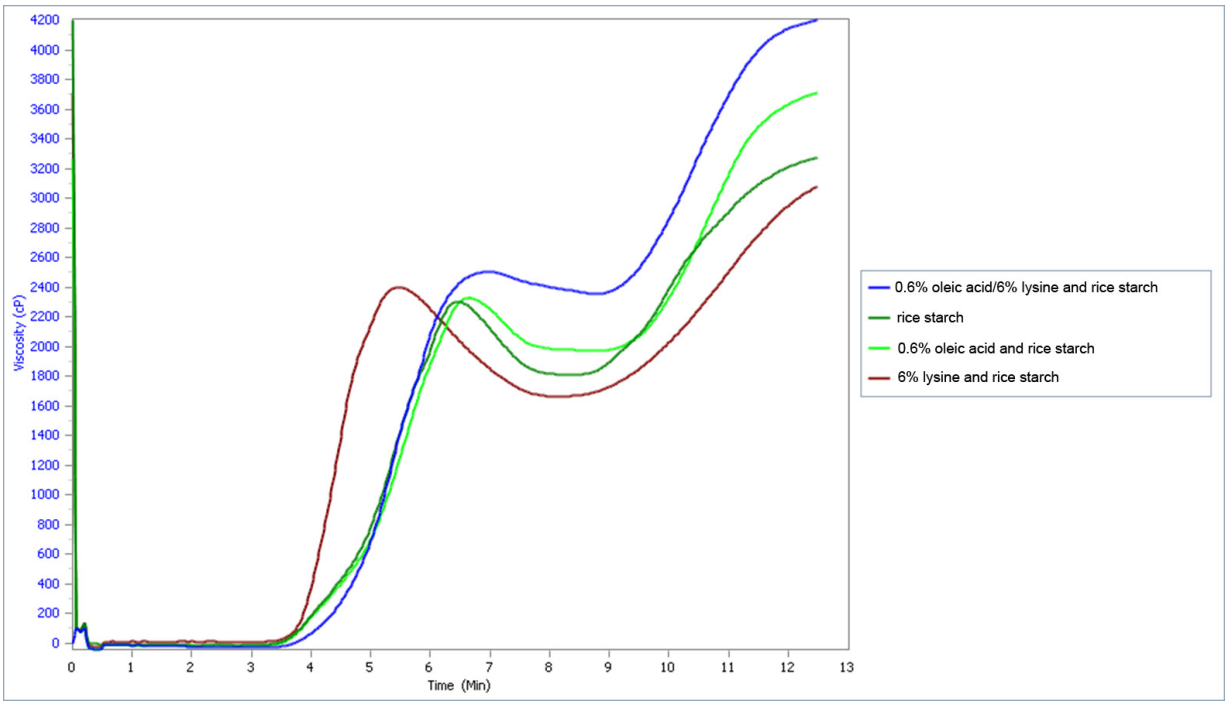


Figure 3.6 RVA curves of rice starch added with 0.6% oleic acid and 6.0% lysine

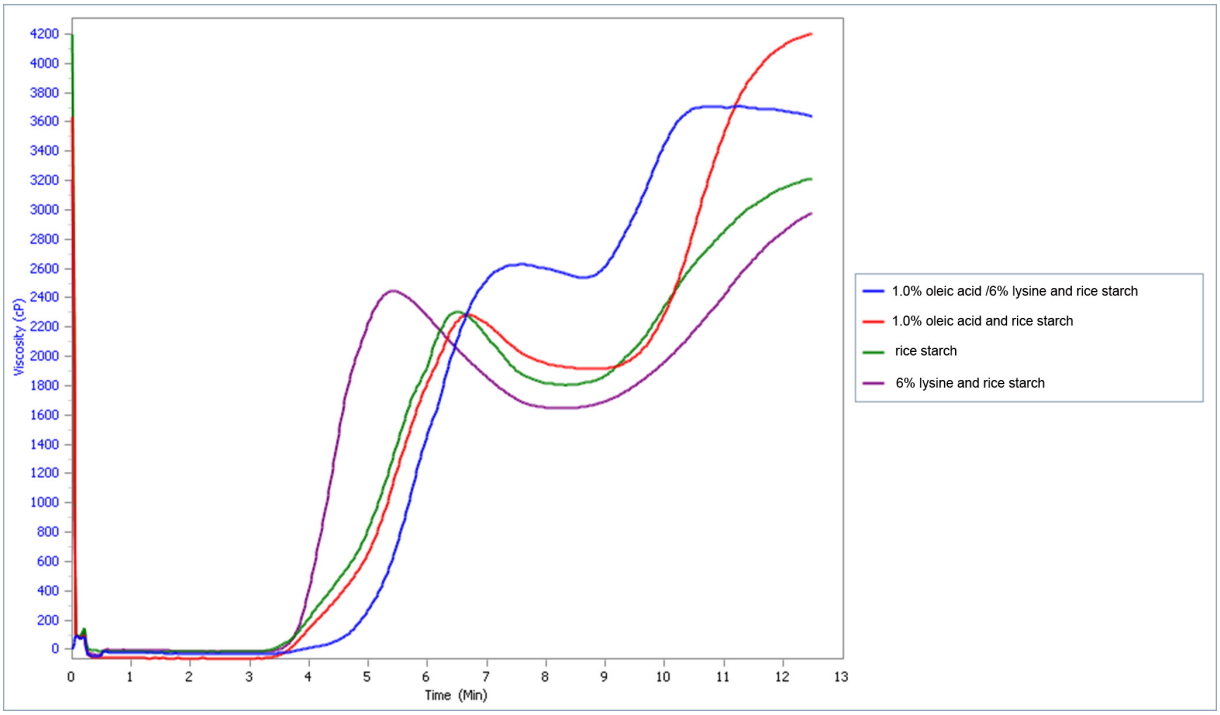


Figure 3.7 RVA curves of rice starch added with 1.0% oleic acid and 6.0% lysine

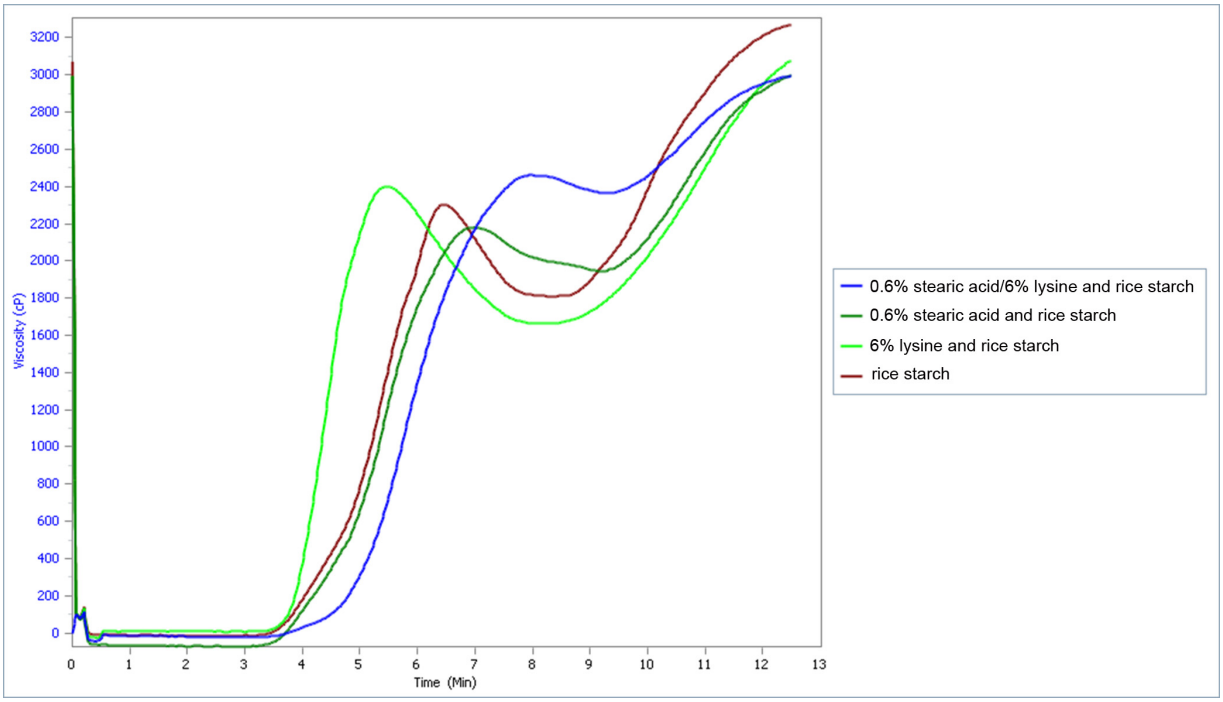


Figure 3.8 RVA curves of rice starch added with 0.6% stearic acid and 6.0% lysine

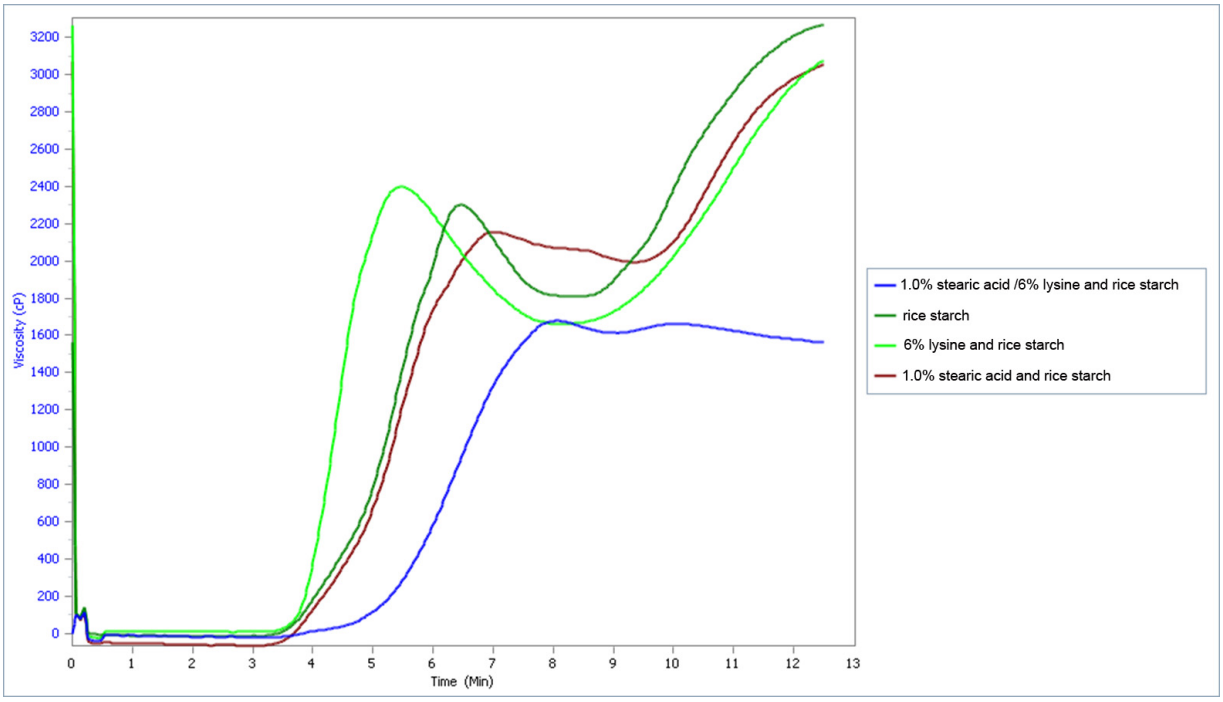


Figure 3.9 RVA curves of rice starch added with 1.0% stearic acid and 6.0% lysine

Table 3.3 Effects of different fatty acid and amino acid combinations on pasting properties of commercial rice starch, compared to added fatty acids or amino acids individually [a,b,c]

| FA | FA (%)[b] | Amino acids (6.0%) | Peak (cp) | MV (cp) | BKD (cp) | FV (cp) | Ptime (min) | Ptemp (°C) | TSK (cp) |
|---|---|---|---|---|---|---|---|---|---|
| N/A | 0 | N/A | 2307.33±18.58b | 1807.67±6.81b | 499.67±25.38c | 3281.67±16.26b | 6.49±0.03b | 82.68±0.49b | 1474±22.61b |
| Palmitic | 0.6 | N/A | 2317±21.93b | 2114±11.53a | 203±23.9d | 3851.33±40.67a | 7.16±0.1a | 82.63±0.89b | 1737.33±47.35a |
| N/A | 0 | Asp | 2460±41.15a | 1490.67±22.72c | 969.33±56.86a | 2480.33±18.01d | 6.53±0.12b | 90.28±0.78a | 989.67±29.4d |
| Palmitic | 0.6 | Asp | 2229±15.13c | 1437±35.04c | 792±25.51b | 2591.67±30.66c | 6.49±0.03b | 90.27±0.03a | 1154.67±8.62c |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58b | 1807.67±6.81b | 499.67±25.38c | 3281.67±16.26b | 6.49±0.03b | 82.68±0.49b | 1474±22.61b |
| Palmitic | 1 | N/A | 2224.67±42.57b | 2055.33±48.44a | 169.33±7.37d | 3900.67±58.39a | 7.07±0.07a | 82.35±0.09b | 1845.33±106.36a |
| N/A | 0 | Asp | 2460±41.15a | 1490.67±22.72c | 969.33±56.86a | 2480.33±18.01d | 6.53±0.12b | 90.28±0.78a | 989.67±29.4c |
| Palmitic | 1 | Asp | 2227.33±64.17b | 1450.33±89.19c | 777±33.42b | 2778.67±98.65c | 6.44±0.08b | 88.65±2.79a | 1328.33±18.23b |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58b | 1807.67±6.81b | 499.67±25.38c | 3281.67±16.26c | 6.49±0.03b | 82.68±0.49a | 1474±22.61b |
| Palmitic | 0.6 | N/A | 2317±21.93b | 2114±11.53a | 203±23.9d | 3851.33±40.67b | 7.16±0.1a | 82.63±0.89a | 1737.33±47.35b |
| N/A | 0 | Lys | 2402±23.07ab | 1654.33±30.92c | 747.67±11.59a | 3069.33±32.25c | 5.45±0.04d | 81.63±0.08a | 1415±4b |
| Palmitic | 0.6 | Lys | 2526.33±99.14a | 1833±111.22b | 693.33±15.31a | 5129.67±195.77a | 6.07±0c | 82.97±0.5a | 3296.67±265.51a |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58bc | 1807.67±6.81b | 499.67±25.38c | 3281.67±16.26bc | 6.49±0.03b | 82.68±0.49b | 1474±22.61b |
| Palmitic | 1 | N/A | 2224.67±42.57c | 2055.33±48.44a | 169.33±7.37d | 3900.67±58.39b | 7.07±0.07a | 82.35±0.09b | 1845.33±106.36b |
| N/A | 0 | Lys | 2402±23.07b | 1654.33±30.92c | 747.67±11.59a | 3069.33±32.25c | 5.45±0.04d | 81.63±0.08b | 1415±4b |
| Palmitic | 1 | Lys | 2524±61.65a | 1834.67±59.14b | 689.33±31.09b | 4880.67±587.14a | 6.31±0.03c | 88.2±3.64a | 3046±645.92a |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58c | 1807.67±6.81d | 499.67±25.38b | 3281.67±16.26b | 6.49±0.03d | 82.68±0.49a | 1474±22.61b |
| Palmitic | 0.6 | N/A | 2317±21.93c | 2114±11.53a | 203±23.9c | 3851.33±40.67a | 7.16±0.1a | 82.63±0.89a | 1737.33±47.35a |
| N/A | 0 | Cys | 2750±21.52a | 2038.33±23.12b | 711.67±9.87a | 3077±26.21c | 6.67±0c | 86.12±2.37a | 1038.67±6.81c |
| Palmitic | 0.6 | Cys | 2412±15.13b | 1884.33±14.36c | 527.67±25.97b | 3351.67±45.54b | 6.82±0.04b | 86.05±2.97a | 1467.33±31.18b |

(Table 3.3 continued)

| FA | FA (%)[b] | Amino acids (6.0%) | Peak (cp) | MV (cp) | BKD (cp) | FV (cp) | Ptime (min) | Ptemp (°C) | TSK (cp) |
|---|---|---|---|---|---|---|---|---|---|
| N/A | 0 | N/A | 2307.33±18.58b | 1807.67±6.81c | 499.67±25.38b | 3281.67±16.26b | 6.49±0.03d | 82.68±0.49a | 1474±22.61c |
| Palmitic | 1 | N/A | 2224.67±42.57c | 2055.33±48.44a | 169.33±7.37d | 3900.67±58.39a | 7.07±0.07a | 82.35±0.09a | 1845.33±106.36b |
| N/A | 0 | Cys | 2750±21.52a | 2038.33±23.12a | 711.67±9.87a | 3077±26.21c | 6.67±0c | 86.12±2.37a | 1038.67±6.81d |
| Palmitic | 1 | Cys | 2272.67±19.86bc | 1941.33±23.59b | 331.33±31.82c | 3964.33±82.62a | 6.78±0.04b | 85.27±2.01a | 2023±65.34a |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58b | 1807.67±6.81b | 499.67±25.38c | 3281.67±16.26a | 6.49±0.03b | 82.68±0.49b | 1474±22.61a |
| Stearic | 0.6 | N/A | 2207.33±23.71c | 1972±24.27a | 235.33±13.01d | 3037.33±63.22b | 7±0a | 82.68±0.41b | 1065.33±46.92b |
| N/A | 0 | Asp | 2460±41.15a | 1490.67±22.72c | 969.33±56.86a | 2480.33±18.01c | 6.53±0.12b | 90.28±0.78a | 989.67±29.4b |
| Stearic | 0.6 | Asp | 2270.33±24.21bc | 1444±54.69c | 826.33±52.77b | 2240.67±37.29d | 6.45±0.04b | 90.28±0.03a | 796.67±37.9c |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58b | 1807.67±6.81b | 499.67±25.38b | 3281.67±16.26a | 6.49±0.03bc | 82.68±0.49b | 1474±22.61a |
| Stearic | 1 | N/A | 2195±46.18b | 2008.33±32.72a | 186.67±19.73c | 3088.33±79.03b | 7.07±0.07a | 82.37±0.03b | 1080±46.36b |
| N/A | 0 | Asp | 2460±41.15a | 1490.67±22.72c | 969.33±56.86a | 2480.33±18.01c | 6.53±0.12b | 90.28±0.78a | 989.67±29.4b |
| Stearic | 1 | Asp | 2285.33±71.06b | 1452.67±34.08c | 832.67±105a | 2274.67±36.02d | 6.27±0.12c | 89.68±0.45a | 822.00±68.09c |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58b | 1807.67±6.81c | 499.67±25.38b | 3281.67±16.26a | 6.49±0.03c | 82.68±0.49b | 1474±22.61a |
| Stearic | 0.6 | N/A | 2207.33±23.71c | 1972±24.27b | 235.33±13.01c | 3037.33±63.22b | 7±0b | 82.68±0.41b | 1065.33±46.92b |
| N/A | 0 | Lys | 2402±23.07a | 1654.33±30.92d | 747.67±11.59a | 3069.33±32.25b | 5.45±0.04d | 81.63±0.08b | 1415±4a |
| Stearic | 0.6 | Lys | 2462.67±32.53a | 2351±37.24a | 111.67±13.05d | 2968±83.62b | 8±0a | 93.78±0.49a | 617±46.49c |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58b | 1807.67±6.81b | 499.67±25.38b | 3281.67±16.26a | 6.49±0.03c | 82.68±0.49b | 1474±22.61a |
| Stearic | 1 | N/A | 2195±46.18b | 2008.33±32.72a | 186.67±19.73c | 3088.33±79.03b | 7.07±0.07b | 82.37±0.03b | 1080±46.36b |
| N/A | 0 | Lys | 2402±23.07a | 1654.33±30.92c | 747.67±11.59a | 3069.33±32.25c | 5.45±0.04d | 81.63±0.08c | 1415±4a |
| Stearic | 1 | Lys | 1231.67±36.36c | n/a* | n/a* | 1101.33±46.31d | 7.82±0.1a | 95.08±0.06a | n/a* |

(Table 3.3 continued)

| FA | FA (%)[b] | Amino acids (6.0%) | Peak (cp) | MV (cp) | BKD (cp) | FV (cp) | Ptime (min) | Ptemp (°C) | TSK (cp) |
|---|---|---|---|---|---|---|---|---|---|
| N/A | 0 | N/A | 2307.33±18.58c | 1807.67±6.81b | 499.67±25.38c | 3281.67±16.26a | 6.49±0.03c | 82.68±0.49b | 1474±22.61a |
| Stearic | 0.6 | N/A | 2207.33±23.71d | 1972±24.27a | 235.33±13.01d | 3037.33±63.22b | 7±0a | 82.68±0.41b | 1065.33±46.92b |
| N/A | 0 | Cys | 2750±21.52a | 2038.33±23.12a | 711.67±9.87a | 3077±26.21b | 6.67±0b | 86.12±2.37a | 1038.67±6.81b |
| Stearic | 0.6 | Cys | 2486.33±7.64b | 1866.33±42.85b | 620±39.15b | 2772.33±20.23c | 6.73±0.07b | 83.45±0.87ab | 906±30.35c |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58b | 1807.67±6.81c | 499.67±25.38b | 3281.67±16.26a | 6.49±0.03c | 82.68±0.49a | 1474±22.61a |
| Stearic | 1 | N/A | 2195±46.18c | 2008.33±32.72a | 186.67±19.73c | 3088.33±79.03b | 7.07±0.07a | 82.37±0.03a | 1080±46.36d |
| N/A | 0 | Cys | 2750±21.52a | 2038.33±23.12a | 711.67±9.87a | 3077±26.21b | 6.67±0b | 86.12±2.37a | 1038.67±6.81bc |
| Stearic | 1 | Cys | 2348.67±25.11b | 1919.33±20.6b | 429.33±42.74b | 2881±61.22c | 6.67±0.07b | 86.87±3.22a | 961.67±50.34c |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58b | 1807.67±6.81a | 499.67±25.38c | 3281.67±16.26b | 6.49±0.03a | 82.68±0.49b | 1474±22.61b |
| Linoleic | 0.6 | N/A | 2273.33±42.15b | 1644±24.58b | 629.33±18.34b | 4178.33±104.57a | 6.53±0a | 83.93±0.03b | 2534.33±86.32a |
| N/A | 0 | Asp | 2460±41.15a | 1490.67±22.72c | 969.33±56.86a | 2480.33±18.01c | 6.53±0.12a | 90.28±0.78a | 989.67±29.4c |
| Linoleic | 0.6 | Asp | 2268.33±83.28b | 1219±25.36d | 1049.33±57.95a | 2607.33±91.66c | 6.4±0a | 89.77±0.92a | 1388.33±69.29b |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58b | 1807.67±6.81a | 499.67±25.38c | 3281.67±16.26b | 6.49±0.03a | 82.68±0.49b | 1474±22.61b |
| Linoleic | 1 | N/A | 2267±32.91bc | 1533±22.52b | 734±10.39b | 4206.33±53.59a | 6.49±0.03a | 83.73±0.97b | 2673.33±34.95a |
| N/A | 0 | Asp | 2460±41.15a | 1490.67±22.72b | 969.33±56.86a | 2480.33±18.01d | 6.53±0.12a | 90.28±0.78a | 989.67±29.4c |
| Linoleic | 1 | Asp | 2197±9.54c | 1209±22c | 988±19.97a | 2607.33±21.78c | 6.42±0.04a | 90.3±0.05a | 1398.33±43.39b |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58b | 1807.67±6.81a | 499.67±25.38d | 3281.67±16.26c | 6.49±0.03a | 82.68±0.49b | 1474±22.61c |
| Linoleic | 0.6 | N/A | 2273.33±42.15b | 1644±24.58b | 629.33±18.34c | 4178.33±104.57b | 6.53±0a | 83.93±0.03a | 2534.33±86.32b |
| N/A | 0 | Lys | 2402±23.07a | 1654.33±30.92b | 747.67±11.59b | 3069.33±32.25c | 5.45±0.04c | 81.63±0.08c | 1415±4c |
| Linoleic | 0.6 | Lys | 2332±31.43ab | 1405±56.45c | 927±45.92a | 4537.67±224.23a | 5.71±0.03b | 83.98±0.03a | 3132.67±171.21a |

(Table 3.3 continued)

| FA | FA (%)[b] | Amino acids (6.0%) | Peak (cp) | MV (cp) | BKD (cp) | FV (cp) | Ptime (min) | Ptemp (°C) | TSK (cp) |
|---|---|---|---|---|---|---|---|---|---|
| N/A | 0 | N/A | 2307.33±18.58b | 1807.67±6.81a | 499.67±25.38c | 3281.67±16.26b | 6.49±0.03a | 82.68±0.49bc | 1474±22.61b |
| Linoleic | 1 | N/A | 2267±32.91b | 1533±22.52c | 734±10.39b | 4206.33±53.59a | 6.49±0.03a | 83.73±0.97b | 2673.33±34.95a |
| N/A | 0 | Lys | 2402±23.07a | 1654.33±30.92b | 747.67±11.59b | 3069.33±32.25b | 5.45±0.04c | 81.63±0.08c | 1415±4b |
| Linoleic | 1 | Lys | 2264.33±11.5b | 1113.33±29.37d | 1151±19.16a | 4108±453.57a | 5.67±0.07b | 85.55±0.05a | 2994.67±426.94a |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58c | 1807.67±6.81b | 499.67±25.38d | 3281.67±16.26c | 6.49±0.03c | 82.68±0.49b | 1474±22.61c |
| Linoleic | 0.6 | N/A | 2273.33±42.15c | 1644±24.58c | 629.33±18.34c | 4178.33±104.57a | 6.53±0bc | 83.93±0.03b | 2534.33±86.32a |
| N/A | 0 | Cys | 2750±21.52a | 2038.33±23.12a | 711.67±9.87b | 3077±26.21d | 6.67±0a | 86.12±2.37ab | 1038.67±6.81d |
| Linoleic | 0.6 | Cys | 2628±65.21b | 1754.33±69.08b | 873.67±33.08a | 3646±45.13b | 6.62±0.08ab | 88.2±1.99a | 1891.67±43b |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58c | 1807.67±6.81b | 499.67±25.38c | 3281.67±16.26c | 6.49±0.03b | 82.68±0.49b | 1474±22.61c |
| Linoleic | 1 | N/A | 2267±32.91c | 1533±22.52d | 734±10.39b | 4206.33±53.59a | 6.49±0.03b | 83.73±0.97b | 2673.33±34.95a |
| N/A | 0 | Cys | 2750±21.52a | 2038.33±23.12a | 711.67±9.87b | 3077±26.21d | 6.67±0a | 86.12±2.37b | 1038.67±6.81d |
| Linoleic | 1 | Cys | 2418.33±28.75b | 1633.67±15.37c | 784.67±13.65a | 3964.67±44.16b | 6.62±0.04a | 89.72±0.46a | 2331±55.65b |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58c | 1807.67±6.81b | 499.67±25.38c | 3281.67±16.26c | 6.49±0.03b | 82.68±0.49b | 1474±22.61c |
| Linolenic | 0.6 | N/A | 2267±32.91c | 1533±22.52d | 734±10.39b | 4206.33±53.59a | 6.49±0.03b | 83.73±0.97b | 2673.33±34.95a |
| N/A | 0 | Asp | 2750±21.52a | 2038.33±23.12a | 711.67±9.87b | 3077±26.21d | 6.67±0a | 86.12±2.37b | 1038.67±6.81d |
| Linolenic | 0.6 | Asp | 2418.33±28.75b | 1633.67±15.37c | 784.67±13.65a | 3964.67±44.16b | 6.62±0.04a | 89.72±0.46a | 2331±55.65b |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58b | 1807.67±6.81a | 499.67±25.38d | 3281.67±16.26b | 6.49±0.03ab | 82.68±0.49b | 1474±22.61c |
| Linolenic | 1 | N/A | 2302.67±26.84b | 1489.33±39.72b | 813.33±64.24c | 4167.33±79.94a | 6.4±0.07ab | 87.37±4.36ab | 2678±103.26a |
| N/A | 0 | Asp | 2460±41.15a | 1490.67±22.72b | 969.33±56.86b | 2480.33±18.01d | 6.53±0.12a | 90.28±0.78a | 989.67±29.4d |
| Linolenic | 1 | Asp | 2330±49.33b | 1225.33±43.1c | 1104.67±19.66a | 2898.33±94.88c | 6.33±0b | 89.52±1.36a | 1673±66.09b |

(Table 3.3 continued)

| FA | FA (%)[b] | Amino acids (6.0%) | Peak (cp) | MV (cp) | BKD (cp) | FV (cp) | Ptime (min) | Ptemp (°C) | TSK (cp) |
|---|---|---|---|---|---|---|---|---|---|
| N/A | 0 | N/A | 2307.33±18.58b | 1807.67±6.81a | 499.67±25.38c | 3281.67±16.26b | 6.49±0.03a | 82.68±0.49a | 1474±22.61b |
| Linolenic | 0.6 | N/A | 2299.67±54.78b | 1543±34c | 756.67±53.53b | 4231.67±109.74a | 6.42±0.04a | 85.77±3.93a | 2688.67±141.17a |
| N/A | 0 | Lys | 2402±23.07ab | 1654.33±30.92b | 747.67±11.59b | 3069.33±32.25b | 5.45±0.04b | 81.63±0.08a | 1415±4b |
| Linolenic | 0.6 | Lys | 2456.67±84.83a | 1340±16.09d | 1116.67±71.06a | 4043±148.07a | 5.45±0.04b | 81.9±0.48a | 2703±143.7a |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58c | 1807.67±6.81a | 499.67±25.38c | 3281.67±16.26b | 6.49±0.03a | 82.68±0.49a | 1474±22.61c |
| Linolenic | 1 | N/A | 2302.67±26.84c | 1489.33±39.72c | 813.33±64.24b | 4167.33±79.94a | 6.4±0.07a | 87.37±4.36a | 2678±103.26b |
| N/A | 0 | Lys | 2402±23.07b | 1654.33±30.92b | 747.67±11.59b | 3069.33±32.25b | 5.45±0.04b | 81.63±0.08a | 1415±4c |
| Linolenic | 1 | Lys | 2479.67±6.43a | 1126.33±34.53d | 1353.33±40.67a | 4175.67±263.34a | 5.35±0.04b | 82.37±0.08a | 3049.33±244.13a |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58c | 1807.67±6.81b | 499.67±25.38c | 3281.67±16.26c | 6.49±0.03bc | 82.68±0.49a | 1474±22.61c |
| Linolenic | 0.6 | N/A | 2299.67±54.78c | 1543±34d | 756.67±53.53b | 4231.67±109.74a | 6.42±0.04c | 85.77±3.93a | 2688.67±141.17a |
| N/A | 0 | Cys | 2750±21.52a | 2038.33±23.12a | 711.67±9.87b | 3077±26.21c | 6.67±0a | 86.12±2.37a | 1038.67±6.81d |
| Linolenic | 0.6 | Cys | 2574.33±48.21b | 1678.33±54.85c | 896±51.39a | 3898.33±107.92b | 6.6±0.07ab | 84.97±4.58a | 2220±162.2b |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58c | 1807.67±6.81b | 499.67±25.38c | 3281.67±16.26c | 6.49±0.03b | 82.68±0.49a | 1474±22.61b |
| Linolenic | 1 | N/A | 2302.67±26.84c | 1489.33±39.72d | 813.33±64.24ab | 4167.33±79.94b | 6.4±0.07b | 87.37±4.36a | 2678±103.26a |
| N/A | 0 | Cys | 2750±21.52a | 2038.33±23.12a | 711.67±9.87b | 3077±26.21d | 6.67±0a | 86.12±2.37a | 1038.67±6.81c |
| Linolenic | 1 | Cys | 2511±85b | 1601.33±70.71c | 909.67±73.66a | 4349.67±76.54a | 6.49±0.08b | 84.72±4.14a | 2748.33±110.23a |
| | | | | | | | | | |
| N/A | 0 | N/A | 2307.33±18.58c | 1807.67±6.81c | 499.67±25.38b | 3281.67±16.26c | 6.49±0.03c | 82.68±0.49b | 1474±22.61b |
| Oleic | 0.6 | N/A | 2320±6.24c | 1987.67±64.27b | 332.33±67.17c | 3773.33±66.01b | 6.69±0.1b | 82.38±0.03b | 1785.67±52.6a |
| N/A | 0 | Lys | 2402±23.07b | 1654.33±30.92d | 747.67±11.59a | 3069.33±32.25d | 5.45±0.04d | 81.63±0.08b | 1415±4b |
| Oleic | 0.6 | Lys | 2532±51.97a | 2369±42.53a | 163±14.42d | 4242.33±112.1a | 6.93±0a | 88.72±1.4a | 1873.33±69.64a |

(Table 3.3 continued)

| FA | FA (%)[b] | Amino acids (6.0%) | Peak (cp) | MV (cp) | BKD (cp) | FV (cp) | Ptime (min) | Ptemp (°C) | TSK (cp) |
|---|---|---|---|---|---|---|---|---|---|
| N/A | 0 | N/A | 2307.33±18.58c | 1807.67±6.81c | 499.67±25.38b | 3281.67±16.26c | 6.49±0.03b | 82.68±0.49b | 1474±22.61b |
| Oleic | 1 | N/A | 2280.67±7.57c | 1948.33±46.49b | 332.33±54.05c | 4253.67±45.65a | 6.74±0.12b | 82.38±0.1b | 2305.33±39.93a |
| N/A | 0 | Lys | 2402±23.07b | 1654.33±30.92d | 747.67±11.59a | 3069.33±32.25d | 5.45±0.04c | 81.63±0.08b | 1415±4b |
| Oleic | 1 | Lys | 2623.67±49.22a | 2526.33±56.41a | 97.33±8.5d | 3650.67±50.2b | 7.58±0.17a | 93.98±0.88a | 1124.33±106.61c |

* n/a indicates the value did not exist because of changed shape of RVA curve

a. FA, fatty acid; MV, minimum viscosity; BKD, breakdown; FV, final viscosity; Ptemp, pasting temperature; Ptime, peak time; TSB, total setback; N/A in FA and amino acid means no added FA or amino acid.
b. The levels are based on starch dry weight.
c. Values followed by the same letter in the same column in the same subtable are not significantly different ($P > 0.05$)

lysine did not start to paste until 94.97°C and peak time was 8.05 min. Starch added with 0.6% oleic acid and 6% lysine alone started to paste at 82.37°C and 81.63°C and their peak times were only 7.07min and 5.45min.

The above data demonstrated that there must be a certain mechanism that inhibits starch from pasting and rupture of starch granule under continuous heating, when those additives were added into starch. Moreover, starch with added 1.0% stearic and 6% lysine was associated with low peak viscosity (1661.67cP), compared to its original starch (2307.33cP). This indicated that the stabilizing effect of additives was very strong, and either water penetration into starch granule or starch hydration was depressed.

These results are very exciting because usually the function of starch stabilization is achieved by cross-linked starch and especially chemically cross-linked starch, such as sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), epichlorohydrin (EPI) and phosphoryl chloride ($POCl_3$) (Koo et al. 2010). Generally, ester linkages are formed between the hydroxyl groups of starch molecules and carboxyl groups in chemical agents. As a result, crosslinking stabilizes starch granules by restricting swelling, so they are resistant to overcooking and other variations in processing conditions, such as temperature, acid and shear. In the study of this chapter, starches with added stearic/lysine and oleic/lysine showed inhibited pasting and were resistant to continuous cooking, which is very similar to properties of cross-linked starch; but unlike popular cross-linked starch, it utilized amino acids and fatty acids as additives without much pretreatment, making them ideal for clean label starch and providing nutritious starch as food ingredients.

**3.3.3 Effects of fatty acid and amino acid on corn starch pasting**

Addition of fatty acids or amino acids to corn starch showed limited degree of modification to its original starch, in terms of pasting properties (Table 3.4 and Table 3.5). Similar to rice starch, addition of palmitic acid lowered starch breakdown; but stearic acid gave higher final viscosity and higher breakdown that the control, so was linoleic acid. In general, all these changes were very small and no difference was found for stearic acid (saturated fatty acid) and linoleic (unsaturated fatty acid) addition except TSK value.

No significant changes were found with added leucine and tyrosine for both starches. Like rice starch, added aspartic acid reduced starch viscosity in total, whether it was minimum viscosity or final viscosity. Lysine addition promoted starch breakdown, lowered pasting temperature and elevated starch pasting rate. However, while addition of 6% cysteine affected rice starch with increased peak viscosity by 19% (from 2307cP to 2750cP), no significant

Table 3.4 Effects of fatty acids on pasting properties of corn starch [a,b,c]

| %[b] | FA | Peak (cp) | MV (cp) | BKD (cp) | FV( cp) | Ptime( min) | PT (°C) | TSB (cp) |
|---|---|---|---|---|---|---|---|---|
| 0 | N/A | 2808.33±40.38a | 1790.33±21.59b | 1018±32.51a | 2694.33±76.79b | 5.31±0.04a | 77.63±0.03a | 1676.33±54.5c |
| 0.6 | Palmitic | 2802.33±20.01a | 1891±15a | 911.33±17.62b | 2620.67±45.63b | 5.31±0.04a | 78.08±0.46a | 1709.33±28.04bc |
| 0.6 | Stearic | 2809±54.81a | 1718.33±22.01c | 1090.67±41.55a | 2896±78.89a | 5.31±0.04a | 78.12±0.36a | 1805.33±37.45b |
| 0.6 | Linoleic | 2806±34.6a | 1730.33±25.01c | 1075.67±46.46a | 3007.67±71.01a | 5.33±0a | 78.42±0.8a | 1932±50.09a |
| 0.6 | Linolenic | 2808.33±40.38a | 1790.33±21.59b | 1018±32.51a | 2694.33±76.79b | 5.31±0.04a | 77.63±0.03a | 1676.33±54.5c |
| | | | | | | | | |
| 0 | N/A | 2808.33±40.38a | 1790.33±21.59b | 1018±32.51b | 2694.33±76.79c | 5.31±0.04a | 77.63±0.03a | 1676.33±54.5c |
| 1.0 | Palmitic | 2789±24.43a | 1898.33±54.28a | 890.67±30.29c | 2614.67±33.13c | 5.33±0a | 77.85±0.52a | 1724±59.09c |
| 1.0 | Stearic | 2791.33±30.92a | 1731.67±24.5bc | 1059.67±9.07ab | 2923.67±26.95b | 5.36±0.04a | 77.82±0.43a | 1864±24.25b |
| 1.0 | Linoleic | 2781.67±39.8a | 1668.33±31.26c | 1113.33±13.05a | 3162.67±60.72a | 5.33±0a | 78.38±0.1a | 2049.33±61.33a |
| 1.0 | Linolenic | 2808.33±40.38a | 1790.33±21.59b | 1018±32.51b | 2694.33±76.79c | 5.31±0.04a | 77.63±0.03a | 1676.33±54.5c |

Table3.5 Effects of amino acids on pasting properties of corn starch [a,b,c]

| %[b] | Amino acids | PV (cp) | MV (cp) | BKD (cp) | FV (cp) | PTime (min) | PT (°C) | TSB (cp) |
|---|---|---|---|---|---|---|---|---|
| 0.0 | N/A | 2808.33±40.38bc | 1790.33±21.59a | 1018±32.51c | 2694.33±76.79ab | 5.31±0.04a | 77.63±0.03a | 1676.33±54.5a |
| 6.0 | Asp | 2762±44.19c | 1440.67±29.19b | 1321.33±18.34b | 2234±37.75c | 5.24±0.04a | 77.32±0.36a | 912.67±22.28b |
| 6.0 | Cys | 2869.67±5.86b | 1825.33±54.45a | 1044.33±59.21c | 2665±25.63b | 5.27±0a | 76.78±0.03a | 1620.67±36.12a |
| 6.0 | Leu | 2840±39bc | 1770.67±19.73a | 1069.33±19.66c | 2731.67±34.67ab | 5.31±0.03a | 77.07±0.51a | 1662.33±18.88a |
| 6.0 | Lys | 3355.33±81.94a | 1419.67±5.51b | 1935.67±78.21a | 2847±105.51a | 4.18±0.04b | 74.55±0.09b | 911.33±172.53b |
| 6.0 | Try | 2890±28.16bc | 1794.67±64a | 1095.33±36.91c | 2757±14.73ab | 5.29±0.03a | 77.07±0.46a | 1661.67±49.05a |

a. FA, fatty acid; PV, peak viscosity; MV, minimum viscosity; BKD, breakdown; FV, final viscosity; Ptemp, pasting temperature; PT, peak time; TSB, total setback. N/A in amino acid means no added amino acid.
b. All levels are based on starch dry weight
c. Values followed by the same letter in the same column in the same subtable are not significantly different ($P > 0.05$)

change were found for corn starch with added cysteine (2808cP and 2869cP). This discrepancy is caused by different sources of starch used for the RVA test, which may be attributed to protein location and protein constitution on the surface of rice starch granules and corn starch granules, since surface compounds of starch granules were the main reasons that caused various pasting properties of starch, even those that have the same gelatinization temperature (Debet et al. 2006).

Combinations of amino acid and fatty acid showed mediated effects between those of amino acid and fatty acid tested separately. No special pasting properties were found for stearic acid/lysine added corn starch (Table 3.6). This indicated that the unique function of stearic acid/lysine additives as starch pasting inhibitor applies to rice starch only.

**3.3.4 Comparing effects of stearic acid and lysine combination at different concentrations in rice starch**

Due to the low breakdown and low peak viscosity found for rice starch with added stearic acid (0.6 and 1.0%) and lysine (6%), stearic acid and lysine combination was again checked for their influence on pasting properties at different concentrations (stearic acid at 0.4%, 0.6%, 0.8% and 1.0% levels; lysine at 2%, 4% and 6% levels). All combinations showed lower breakdown than rice starch control (Table 3.7). As the level of stearic acid increased, the peak viscosity and total setback of starch paste decreased; the shape of RVA curve become more and more flat and independent of temperature change (Figure 3.10). Addition of stearic acid 1.0% and lysine 6% caused the rice starch RVA curve to be too flat to tell peak and trough viscosity; the time to peak was not recognized during heating at 95$^{o}$C, but approximately 1min after cooling started. The viscosity was then kept at a certain level, which was considered as “peak viscosity”. At the end, the viscosity decreased slightly. Total setback therefore cannot be calculated from the pasting curve (Figure3.11).

It seems that change of peak viscosity is largely dependent on the level of stearic acid, while the presence of lysine is strictly necessary. When lysine was kept at 6%, extra addition of stearic acid at 0.4%, 0.6%, 0.8% and 1.0 % gave average peak viscosities of 2506.33cP, 2198.67cP, 1685.67cP and 1231.67cP, separately. Based on these data, the peak viscosity (Y, in cp) and stearic acid level (X, percentage in dry starch by weight) were found to follow a linear relationship of

$$Y = -2168.5X + 3423.5 \ (R^2 = 0.991)$$

The corresponding RVA curve and peak viscosity values were displayed in Figure 3.11 and Table 3.7. Also, peak time values for all the above treatments were significantly delayed than control.

Table 3.6 Effects of stearic acid and lysine on pasting properties of corn starch [a,b,c]

| FA | FA% | Amino acid (6%) | Peak (cp) | MV (cp) | BKD (cp) | FV( cp) | Ptime( min) | PT (°C) | TSB (cp) |
|---|---|---|---|---|---|---|---|---|---|
| N/A | 0 | N/A | 2808.33±40.38b | 1790.33±21.59b | 1018±32.51b | 2694.33±76.79bc | 5.31±0.04a | 77.63±0.03a | 904±64.55c |
| Stearic acid | 0.6 | N/A | 2821.67±29.67b | 1892.67±46.14a | 929±16.52b | 2609±70.15c | 5.31±0.04a | 77.6±0.73a | 716.33±24.01c |
| N/A | 0 | Lys | 3355.33±81.94a | 1419.67±5.51c | 1935.67±78.21a | 2847±105.51b | 4.18±0.04c | 74.55±0.09b | 1427.33±105.7b |
| Stearic acid | 0.6 | Lys | 2808.33±40.38b | 1790.33±21.59b | 1018±32.51b | 2694.33±76.79bc | 5.31±0.04a | 74.97±0.49b | 3403.67±74.33a |
| | | | | | | | | | |
| N/A | 0 | N/A | 2808.33±40.38b | 1790.33±21.59a | 1018±32.51c | 2694.33±76.79b | 5.31±0.04b | 77.63±0.03a | 904±64.55c |
| Stearic acid | 1.0 | N/A | 2750±19.52b | 1842.33±43.15a | 907.67±26.27c | 2512.33±29.26b | 5.31±0.04b | 77.32±0.45a | 670±27.22c |
| N/A | 0 | Lys | 3355.33±81.94a | 1419.67±5.51c | 1935.67±78.21a | 2847±105.51b | 4.18±0.04c | 74.55±0.09c | 1427.33±105.7b |
| Stearic acid | 1.0 | Lys | 2875.67±68.06b | 1653±31.1b | 1222.67±92.45b | 5157.67±258.35a | 5.56±0.08a | 75.75±0.39b | 3504.67±278.68a |

a. FA, fatty acid; PV, peak viscosity; MV, minimum viscosity; BKD, breakdown; FV, final viscosity; Ptemp, pasting temperature; PT, peak time; TSB, total setback. N/A in FA or amino acid means no added FA or amino acid.
b. All levels are based on starch dry weight
c. Values followed by the same letter in the same column in the same subtable are not significantly different ($P > 0.05$)

Table 3.7: Effects of stearic acid and lysine combination at different concentration on pasting properties of commercial rice starch [a,b,c]

| Stearic acid (%) | Lysine (%) | Peak (cp) | MV (cp) | BKD (cp) | FV (cp) | Ptime (min) | Ptemp (°C) | TSK (cp) |
|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 2372.33±58.48 | 2131.67±68.04 | 240.67±51.73 | 3112±36.59 | 6.87±0.07 | 83.15±2.17 | 980.33±60.34 |
| | | | | | | | | |
| 0.4 | 2.0 | 2517.33±56.52a | 2424.33±48.79a | 93±12.17a | 3425.67±95.34a | 7.58±0.1b | 88.92±1.22b | 1001.33±55.77a |
| | 4.0 | 2507.67±46.54a | 2431±45.21a | 76.67±2.08ab | 3458.67±25.72a | 7.82±0.14ab | 89.22±0.94ab | 1027.67±70.44a |
| | 6.0 | 2506.33±31.26a | 2435.33±30.86a | 71±8.19b | 3331.67±69.5a | 7.96±0.08a | 91.28±0.45a | 896.33±48.64a |
| | | | | | | | | |
| 0.6 | 2.0 | 2427.33±36.2a | 2329.33±29.74a | 98±10.39a | 2698.33±36.56a | 8.07±0.07a | 94.05±0.43a | 369±20.52a |
| | 4.0 | 2296±47.82b | 2200±39.66b | 96±8.72a | 2604.67±23.12b | 8.04±0.04a | 94.02±0.49a | 404.67±49.7a |
| | 6.0 | 2198.67±15.5c | 2119±7c | 79.67±8.5a | 2555.33±14.22b | 8.09±0.1a | 94.58±0.49a | 436.33±20.31a |
| | | | | | | | | |
| 0.8 | 2.0 | 1920.33±135.08 | 1837.67±126.71a | 82.67±14.57a | 1859±133.01a | 8.13±0a | 94.93±0.08a | 21.33±8.5b |
| | 4.0 | 1799±60.31a | 1751.67±55.59a | 47.33±10.41b | 1853.33±70.16a | 8.07±0.18a | 94.93±0.03a | 101.67±14.74a |
| | 6.0 | 1685.67±69.97a | 1648±69.29a | 37.67±3.06b | 1743.33±115.21a | 8±0.18a | 94.85±0.09a | 95.33±51.08ab |
| | | | | | | | | |
| 1.0 | 2.0 | 1553.33±63.97a | n/a* | n/a* | 1388±59.63a | 8.13±0.18a | 95.05±0a | n/a* |
| | 4.0 | 1404.67±104.01 | n/a* | n/a* | 1273.33±151.8ab | 8.07±0.12a | 95±0a | n/a* |
| | 6.0 | 1231.67±36.36a | n/a* | n/a* | 1101.33±46.31b | 7.82±0.1a | 95.08±0.06a | n/a* |

* n/a indicated the value did not exist because of changed shape of RVA curve

a. FA: fatty acid; MV, minimum viscosity; BKD, breakdown; FV, final viscosity; Ptemp, pasting temperature; Ptime, peak time; TSB, total setback

b. The levels are based on starch dry weight

c. Values followed by the same letter in the same column in the same subtable are not significantly different ($P > 0.05$)

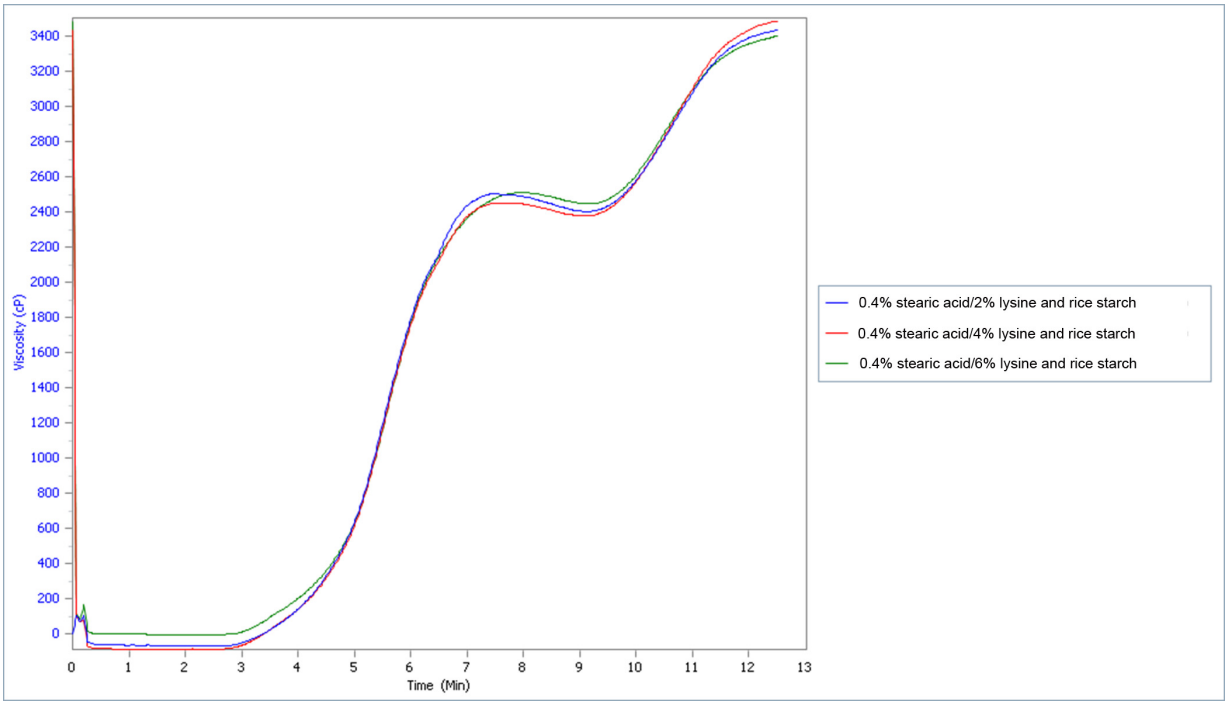


Figure 3.10 RVA curves of rice starch with added 0.4% stearic acid and 2.0%, 4.0%, 6.0% lysine

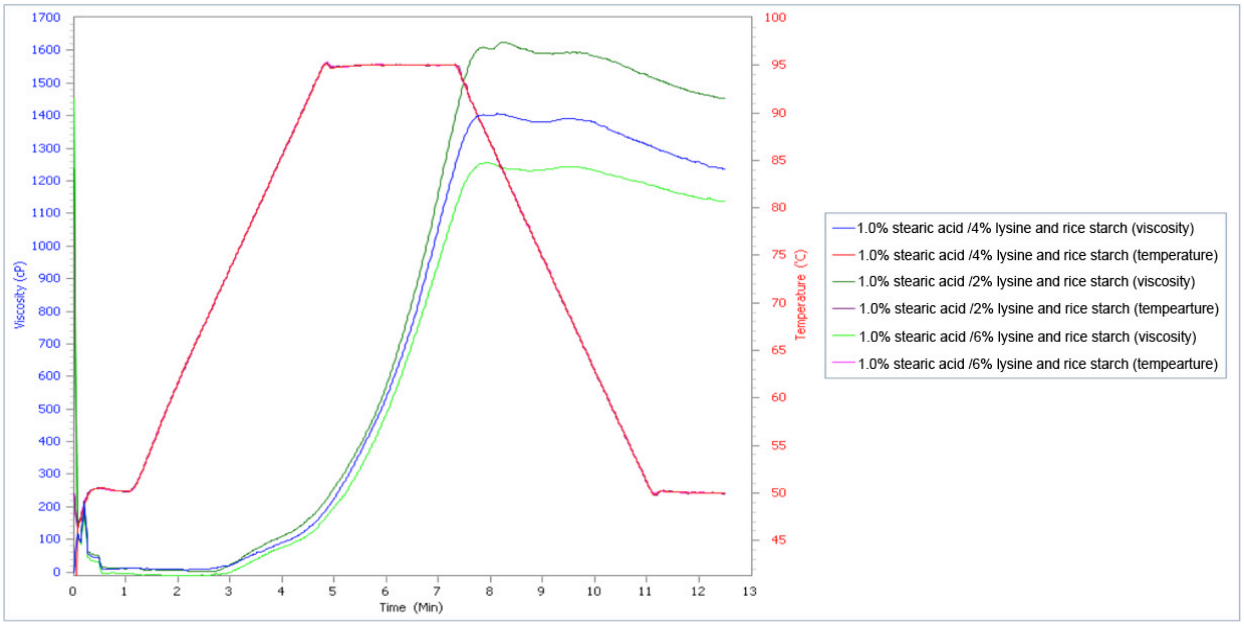


Figure 3.11 RVA curves of rice starch with added 1.0% stearic acid and 2.0%, 4.0%, 6.0% lysine

Compared to change caused by various stearic acid concentrations, the level of lysine did not change the overall shape of the pasting curve very much. As shown in Figure 3.10, when stearic acid was kept at 0.4%, extra addition of lysine at 2%, 4% and 6% did not make much difference in starch pasting properties. The same happened when stearic acid levels were 0.6% and 0.8%. However, when stearic acid level was as high as 1.0%, lysine level started to affect the peak viscosity and final viscosity, as significant difference were found between addition of stearic /lysine 6% and stearic 1.0/lysine 2% (Figure 3.11).

The information above demonstrated that the mechanism that inhibits starch from pasting and rupture of starch granules under continuous heating was subject to additive concentration. Ito et al (2006a) considered the stabilizing effect of lysine as the result of electrostatic interaction, and there was a linear relationship between peak viscosity of potato starch and the absolute value of the net charge of lysine. Since both lysine and stearic acid were required for restricting pasting, a possible explanation is that a starch complex was formed when lysine and stearic acid were added to starch under the RVA treatment. At low levels of stearic acid (0.4%-0.8%), 2% lysine was enough to form that complex, so no significant difference were found between additions of 2% lysine and 6% lysine. At high level of stearic acid (1.0%), 2% lysine was insufficient to form complex with stearic acid while 6% lysine was enough, resulting in a significant difference in peak viscosity between stearic 1.0/lysine 6% and stearic 1.0/lysine 2% added rice starch.

**3.3.5 Complex index measurement**

The complex index is used to measure the amount of amylose capable of complexing with lipid in the presence of iodine as a competitor. Because rice starch paste after RVA treatment gave high turbidity, the starch solution had to be centrifuged before iodine binding. While the complex index was for measuring the amylose-lipid binding, in this study, however, it was thought that the reduced amount of amylose iodine binding should be also understood as the result of amylose being prohibited from leaching out from starch granule. Therefore amylose cannot be dissolved in the supernatant after centrifugation and therefore cannot bind iodine. The decreased amylose solubility can be attributed to amylose-lipid complex or decreased starch solubility.

Within the range of 0.6% to 1.0% stearic acid, the complex index grew progressively with increased levels of fatty acid for both rice starch and corn starch (Table 3.8 and Table 3.9). By adding both lysine and fatty acids to starch, the complex index changed differently. In corn starch, it is apparent that lysine promoted amylose-lipid complex when fatty acids were present (Table3.9). Increased complex index was observed after addition of

Table 3.8: Complex index after addition of fatty acids and lysine for rice starch

| FA | Level (%) | CI (No lysine) | CI (6% lysine addition) |
|---|---|---|---|
| Palmtic acid | 0.6 | 71.6 ±2.35 | 70.5±1.54 |
| | 1.0 | 91.4±1.47 | 84.2±6.34 |
| Stearic acid | 0.6 | 31.5±2.38 | 80.8±3.32 |
| | 1.0 | 52.4±1.70 | 90.8±5.34 |
| Oleic acid | 0.6 | 45.5±3.90 | 68.5±3.83 |
| | 1.0 | 70.9±2.20 | 81.8±3.80 |
| Linoleic acid | 0.6 | 68.9±3.61 | 59.6±3.50 |
| | 1.0 | 86.0±5.32 | 82.5±4.10 |
| Linolenic acid | 0.6 | 52.8±2.33 | 49.0±2.48 |
| | 1.0 | 83.6±5.42 | 85.2±3.50 |

Table 3.9: Complex index after addition of fatty acids and lysine for corn starch

| FA | Level (%) | CI (No lysine) | CI (6% lysine addition) |
|---|---|---|---|
| Palmtic acid | 0.6 | 26.2±2.46 | 58.8±2.34 |
| | 1 | 27.6±0.90 | 86.8±3.03 |
| Stearic acid | 0.6 | 23.7±1.81 | 46.3±2.71 |
| | 1 | 28.5±2.23 | 62.4±4.10 |
| Oleic acid | 0.6 | 19.4±1.38 | 45.3±2.37 |
| | 1 | 25.2±1.32 | 73.2±3.51 |
| Linoleic acid | 0.6 | 17.0±2.00 | 50.4±3.01 |
| | 1 | 20.8±3.21 | 75.8±4.22 |
| Linolenic acid | 0.6 | 17.0±1.10 | 50.4±2.14 |
| | 1 | 20.8±1.35 | 75.8±2.43 |

lysine/stearic acid and lysine/stearic acid to rice starch, compared to addition of stearic acid and stearic acid alone. The complex index for 0.6% and 1.0% stearic acid were 31.5% and 52.4%; addition of 6% lysine raised them to 80.8% and 90.8%, respectively. The complex index for 0.6% and 1.0% oleic acid were 45.5% and 70.9%, addition of 6% lysine promoted them to 68.5% and 81.8%, respectively.

However, for linoleic and linolenic acid, the role of lysine as increasing starch-lipid index was not that obvious for rice starch (Table 3.8). While this determination may actually reflect the amount of amylose-lipid, one other factor should not be neglected that may cause errors of complex index determination (Figure 3.12). First, high complex index can be caused by high viscosity of a starch gel sample. After RVA treatment, some starch gels were

of high viscosity and became firmer after removal from RVA canister; the starch gel could not be diluted or dissolved by water completely if homogenization measure was not taken. After centrifugation, some starch gel along with amylose would precipitate, leading to a lower amount of amylose in the supernatant. So instead of complex with lipid, the reason that the amylose level was low in the supernatant is because amylose precipitated as a gel. This leads to higher amylose-lipid complex content than its real value. Indeed, after RVA treatment, the starch gel became stiff and hard to dissolve in 50$^{o}$C distilled water; this was especially the cases for starch with added linoleic and linolenic acid, which gave a final viscosity of more than 4000cP, even with extra addition of lysine. Therefore, there is a high possibility that the starch-lipid complex index of rice starch with added linoleic and linolenic acids were not correctly measured (Fig 3.12).

Finally, from the above data, we can postulate that the existence of lysine promoted formation of amylose-lipid complex, if complex index truly reflects the amylose-lipid binding intensity and is measured correctly. Meanwhile, addition of aspartic acid and cysteine to fatty acids did not change the complex index of starch samples significantly. Previous literature suggests that a helical V structure should be found in amylose-lipid complex by X-ray diffraction. Therefore it is necessary to figure out the X-ray diffraction pattern for those samples to validate the function of lysine in promoting formation of the complex (see chapter 5).

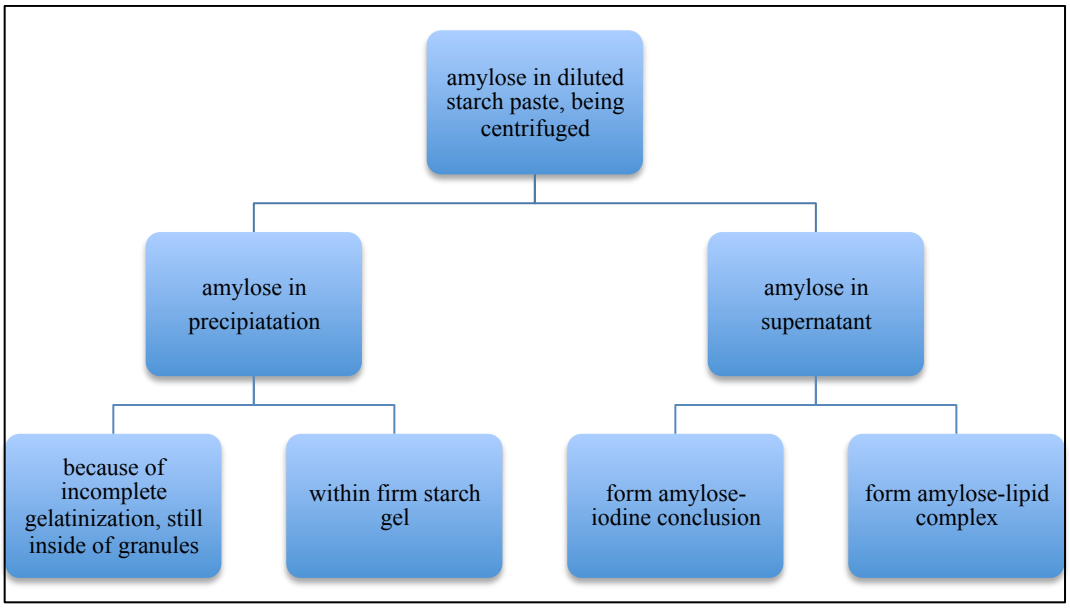


Figure 3.12 Four forms of amylose existence when complex-index assay was conducted

## 3.4 CONCLUSION

In this chapter, the pasting properties of rice starch and corn starch with added fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid) and amino acids (leucine, aspartic acid, lysine, cysteine and tyrosine) were studied using RVA test. Significant low peak viscosity was found when rice starch was added with 1.0% stearic acid and 6% lysine; within 0.6%-1.0% of stearic acid and 6% of lysine addition, there was a linear regression relationship between peak viscosity and the level of stearic acid. Compared to rice starch, corn starch showed less change of pasting properties when fatty acids /amino acids were present, and added 1.0% stearic acid and 6% lysine did not lower its peak viscosity as rice starch did. Complex index measurement indicated that lysine promoted the formation of amylose-lipid complex when fatty acids were present. Further study regarding retrogradation, structure of rice starch with additives and pH adjustment are presented in chapter 4 and chapter 5.

## 3.5 REFERENCES


Bao, J. and C. J. Bergman (2004). Starch in food: Structure, function and applications, CRC PressI Llc.

Bhatnagar, S. and M. A. Hanna (1994). "Amylose-lipid complex formation during single-screw extrusion of various corn starches." Cereal Chemistry 71(6): 582-586.

Biliaderis, C. (2009). "Structural transitions and related physical properties of starch." Starch: Chemistry and technology: 293-372.

Biliaderis, C. G. and J. R. Tonogai (1991). "Influence of lipids on the thermal and mechanical properties of concentrated starch gels." Journal of Agricultural and Food Chemistry 39(5): 833-840.

Debet, M. R. and M. J. Gidley (2006). "Three classes of starch granule swelling: Influence of surface proteins and lipids." Carbohydrate Polymers **64**(3): 452-465.

Dreese, P., J. Faubion and R. Hoseney (1988). "Dynamic rheological properties of flour, gluten, and gluten-starch doughs. I. Temperature-dependent changes during heating." Cereal Chem 65(4): 348-353.

Eliasson, A.-C. and N. Krog (1985). "Physical properties of amylose-monoglyceride complexes." Journal of Cereal Science 3(3): 239-248.

Eliasson, A.-C. and E. Tjerneld (1990). "Adsorption of wheat proteins on wheat starch granules." Cereal chemistry 67(4): 366-372.

Guerrieri, N., L. Eynard, V. Lavelli and P. Cerletti (1997). "Interactions of protein and starch studied through amyloglucosidase action." Cereal chemistry 74(6): 846-850.

Hahn, D. and L. Hood (1987). "Factors influencing corn starch-lipid complexing." Cereal chemistry 64.

Hamaker, B. R. and V. K. Griffin (1993). "Effect of disulfide bond-containing protein on rice starch gelatinization and pasting." Cereal Chemistry 70.

Hoover, R. and D. Hadziyev (1981). "Characterization of potato starch and its monoglyceride complexes." Starch-Stärke 33(9): 290-300.

Ito, A., M. Hattori, T. Yoshida and K. Takahashi (2006a). "Contribution of the net charge to the regulatory effects of amino acids and poly (L-lysine) on the gelatinization behavior of potato starch granules." Bioscience, biotechnology, and biochemistry 70(1): 76-85.

Ito, A., M. Hattori, T. Yoshida, A. Watanabe, R. Sato and K. Takahashi (2006b). "Regulatory effect of amino acids on the pasting behavior of potato starch is attributable to its binding to the starch chain." Journal of agricultural and food chemistry 54(26): 10191-10196.

Kaur, K. and N. Singh (2000). "Amylose-lipid complex formation during cooking of rice flour." Food chemistry 71(4): 511-517.

Koo, S. H., K. Y. Lee and H. G. Lee (2010). "Effect of cross-linking on the physicochemical and physiological properties of corn starch." Food Hydrocolloids 24(6): 619-625.

Lagendijk, J. and H. Pennings (1970). "Relation between complex formation of starch with monoglycerides and the firmness of bread." Cereal Sci. Today 15(354-356): 365.

Lauro, M., K. Poutanen and P. Forssell (2000). "Effect of partial gelatinization and lipid addition on α-amylolysis of barley starch granules." Cereal chemistry 77(5): 595-601.

Liang, X. (2001). Effects of lipids, amino acids, and beta-cyclodextrin on gelatinization, pasting, and retrogradation properties of rice starch Ph.D dissertation, Louisiana State University.

Liang, X. and J. King (2003). "Pasting and crystalline property differences of commercial and isolated rice starch with added amino acids." Journal of food science 68(3): 832-838.

Liang, X., J. M. King and F. F. Shih (2002). "Pasting property differences of commercial and isolated rice starch with added lipids and beta-cyclodextrin." Cereal chemistry 79(6): 812-818.

Lindahl, L. and A. C. Eliasson (1986). "Effects of wheat proteins on the viscoelastic properties of starch gels." Journal of the Science of Food and Agriculture 37(11): 1125-1132.

Manaois, R. V. (2009). Modification of Rice Starch Properties by Addition of Amino Acids at Various pH Levels, Master thesis, Louisiana State University.

Martin, M., K. Zeleznak and R. Hoseney (1991). "A mechanism of bread firming. I. Role of starch swelling." Cereal chemistry. 68(5): 498.

Mira, I., K. Persson and V. K. Villwock (2007). "On the effect of surface active agents and their structure on the temperature-induced changes of normal and waxy wheat starch in aqueous suspension. Part I. Pasting and calorimetric studies." Carbohydrate polymers 68(4): 665-678.

Mosse, J. (1990). "Nitrogen-to-protein conversion factor for ten cereals and six legumes or oilseeds. A reappraisal of its definition and determination. Variation according to species and to seed protein content." Journal of Agricultural and Food Chemistry 38(1): 18-24.

Olkku, J. and C. K. Rha (1978). "Gelatinisation of starch and wheat flour starch —A review." Food Chemistry 3(4): 293-317.

Putseys, J., L. Lamberts and J. Delcour (2010). "Amylose-inclusion complexes: Formation, identity and physico-chemical properties." Journal of Cereal Science 51(3): 238-247.

Raphaelides, S. and J. Karkalas (1988). "Thermal dissociation of amylose-fatty acid complexes." Carbohydrate Research 172(1): 65-82.

Rogers, D. E., K. J. Zeleznak, C. Lai and R. Hoseney (1988). "Effect of native lipids, shortening, and bread moisture on bread firming." Cereal chemistry. 65(5): 398.

Stampfli, L. and B. Nersten (1995). "Emulsifiers in bread making." Food Chemistry 52(4): 353-360.

Shusheng Zhang, Y. X., Shiduo Zhao (2005). "Difference between pI and pH of amino acid solutions." University Chemistry **20**(5): 47-51.

Talwalkar, A. and K. Kailasapathy (2004). "The role of oxygen in the viability of probiotic bacteria with reference to L. acidophilus and Bifidobacterium spp." Current issues in intestinal microbiology 5(1): 1-8.

Tang, M. C. and L. Copeland (2007). "Analysis of complexes between lipids and wheat starch." Carbohydrate polymers **67**(1): 80-85.

Villwock, V. K., A. C. Eliasson, J. Silverio and J. N. BeMiller (1999). "Starch-Lipid Interactions in Common, Waxy, ae du, and ae su2 Maize Starches Examined by Differential Scanning Calorimetry 1." Cereal chemistry 76(2): 292-298.

Yang, W., M. Hattori, T. Kawaguchi and K. Takahashi (1998). "Properties of starches conjugated with lysine and poly (lysine) by the Maillard reaction." Journal of agricultural and food chemistry 46(2): 442-445.

# CHAPTER 4
# CHARACTERIZATION OF STARCH WITH ADDED AMINO ACID AND FATTY ACIDS (I): THERMAL PROPERTY, RETROGRADATION AND STARCH DIGESTIBILITY

## 4.1 INTRODUCTION

Starch is digested by several amylolytic enzymes in human body, including pancreatic α-amylase and intestinal brush border glucoamylases, maltase-glucoamylase and sucrose-isomaltase (Nichols et al. 2003). Recent nutrition concerns for food calories and glycemic index has brought starch into people's attention. According to the digestion rate, there are three groups of starch: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst et al. 1992). Especially, resistant starch is a starch that is resistant to digestion by those enzymes in the small intestine and passes into colon where fermentation happens by natural micro-flora to products of short chain fatty acids. Using Englyst's starch digestion method *in vitro*, RDS represents starch that is digested within 20 min; SDS belongs to starch digested between 20 and 120 min; and the remaining is RS. Because of their slow digestion characteristic, SDS and RS were considered to lower level of postprandial glucose response in blood as compared to a regular starch (Englyst et al. 2003). This indicates their health benefits in reducing risks of common chronic diseases such as diabetes, obesity and cardiovascular disease because of weakened glucose stress and related regulatory system (Ludwig 2002).

Conventionally, RS is classified into four types (Englyst et al. 1992). Type I is a physically inaccessible starch, such as partly milled grains and seeds, inside of which starch is entrapped by the cellular matrix. Type II is native starch granule such as raw potato and banana starch. Because of its special granule structure, starch exists in a tight arrangement (B-type semi-crystallinity), resulting in limited accessibility of the enzyme to find starch substrate. Type III is retrograded starch, which is developed by cooling of gelatinized starch. Once amylose leaches out from its granule after gelatinization, it tends to re-associate with each other, forms crystallized structure, and therefore inhibits enzymatic breakdown. The retrograded starch is common in most moist-heated food, such as bread and corn flakes; type IV is chemically modified starch. Chemical bonds other than α -(1,4) or α -(1,6) can be developed between starches, especially cross-linked starch by chemical agents such as sodium tripolyphosphate (STPP) and trimetaphophate (STMP) (Woo et al. 2002).

Many factors have been studied on their roles in influencing *in vitro* digestibility of starch, such as the botanical source of starch, starch granule size, molecular arrangement of starch components, amylose/amylopectin

ratio, degree of crystallinity, type of crystalline polymorphic (A, B or C) form, amylose–lipid complexes, and granule porosity (Hoover and Zhou 2003). Among these factors, what is of most interests are the effects of amylose-lipid complexes and starch retrogradation on starch digestibility.

In the presence of ligands such as fatty acids and lipid, amylose from gelatized starch goes through conformation change and complexes ligands as single, left handed helices, with its internal cavity holding ligands inside (Zobel 1988). Intermolecular forces such as hydrophobic interaction favors hydrophobic compound to be inside of its helical cavity and therefore the hydrocarbon portion of the lipid is inside, while the hydrophilic part in a mono-/di-glyceride is outside. Intermolecular forces such as Van der Waals force and hydrogen bonds between the helical turns stabilize this formation (Putseys et al. 2010).

According to its preparation methods and structure, there are two types of amylose complexes. The first type refers to complex that was reported to be formed at or below 90°C and the second types was developed at temperatures higher than 90°C (Putseys et al. 2010). For the first type, low temperature leads to a high nucleation rate and low level of crystallinity. This complex melts between 95°C and 105°C (Biliaderis et al. 1986, Biliaderis et al. 1990, Karkalas et al. 1995). On the contrary, if the nucleation rate is low, which happens when the mixture of amylose and ligand is heated to 90°C or higher, the crystalline region tends to propagate slowly to form highly ordered amylose complexes, which dissociate above 110°C (Biliaderis et al. 1986). An even more thermostable amylose complex can be developed by more annealing and recrystallination cycles, which melts at even high temperature, between 110-125°C (Seneviratne et al. 1991, Karkalas et al. 1995).

Decreased digestibility of amylose-lipid complexes has been observed in many studies; and their hydrolysis products by porcine pancreatic α-amylase are mainly maltose, maltotriose and maltotetraose (Jane et al. 1984). For example, a 33% decrease in digestibility was achieved with an emulsifier containing 18 carbons (decaglyceryl monostearate) for nonwaxy rice, while short-chain saturated and unsaturated emulsifiers did not lower the digestibility that much (Guraya et al. 1997). When potato starch went through constant heating in the presence of glycerol monopalmitin and then digested by porcine pancreatic α-amylase, its hydrolysis rate was significantly slower than the non-complexed starch for the same treatment (Tufvesson et al. 2001). Barley starch with added lysophospholipid during partial gelatinization (54°C for 3 hr) was correlated with inhibited granule swelling and decreased rate of α-amylolysis (Lauro et al. 2000).

Although amylose-lipid complexes are often considered as a type IV resistant starch, their performance on enzyme breakdown varies under different crystalline structure, types of lipid and starch contained, way of preparation, and others. Biliaderis and Seneviratne (1990) observed that starch-lipid complexes have different crystallinities and complexes with greater crystallinity were more resistant to enzymatic degradation. But with long digestion time and high enzyme (α-amylase) level, the crystalline form of the complexes was fully digested at last. Moreover, starch-lipid complexes may impede the formation of type III RS, leading to loss of total RS instead. Using barley starch, Szczodrak and Pomeranz (1991) decreased RS yield when starch was pretreated with emulsifier, comparing to its yield without addition of emulsifier. Free amylose leaching out from granule is supposed to get involved in RS formation, but the appearance of lipid probably takes away amylose and leads to a suppressed level of retrograded amylose. Similar competition between amylose retrogradation and formation of amylose-lipid complexes were also demonstrated elsewhere (Eerlingen et al. 1994).

Starch retrogradation is an important and widely accepted mechanism that contributes to the formation of SDS or RS. However, in most instances, starch retrogradation is accompanied with product defects, especially with increased rigidity, opacity and separation between solid and liquid phases, such as bread firming and soup precipitation. By freezing/thawing cycles, starchy product exhibits different chewiness, consistency and stickiness than the first cooked.

Many methods were put forward to study starch retrogradation due to its significance on changed digestibility and shelf life estimation. Some emulsifiers are known to inhibit starch retrogradation. For example, a typical anti-staling reagent in bread includes mono and di-glycerides. It is suggested that the addition of lipid impacts starch retrogradation in two possible ways (Putseys et al. 2010). First, the amylose-lipid complex competes with amylose re-association. Since re-associated amylose double helices serve as nuclei for starch recrystallization, amylose-lipid complexes therefore inhibit starch recrystallization and retrogradation. Another explanation is that lipid may complex with the outer branches of amylopectin to inhibit recrystallization of amylopectin, which is the reason for starch-based product quality defects under long time refrigerated storage.

In this study, because fatty acids were added to starch along with other ingredients, it is very meaningful to study the effects of those additives on starch retrogradation and formation of SDS and RS. No method has been identified as a standard for measurement starch retrogradation due to its complexity and therefore thermal analysis

was selected as the primary method, with syneresis record as a secondary measurement (Karim et al. 2000). RS levels were also assayed with two methods for comparison.

## 4.2 MATERIALS AND METHODS

### 4.2.1 Thermal properties of selected RVA treated samples

Starches after RVA treatment were freeze-dried and analyzed for their thermal properties using a differential scanning calorimeter (DSC) (TA Q100, TA Instruments, Newcastle, DE) in duplicate. Targeted starch samples were those with added lysine/stearic acid combination at two concentration levels; starch with added cysteine/stearic acid and aspartic acid/stearic acid combinations were also selected as samples in order to figure out the difference between lysine and other amino acids (see Table 4.1)

Ten mg of starch was weighed into a steel DSC pan. Then 20mg of distilled water was well dispersed into the starch using pipette tips. The pan was sealed and stored at room temperature overnight for starch hydration. The pans were heated in the DSC from 15 °C to 140 °C at a rate of 5 °C /min. Another pan containing 20 ml distilled water was used as a reference. The thermal transition parameters, including enthalpy (J/g), onset temperature and peak temperature were determined using Universal Analysis 2000 (TA Q100, TA Instruments, Newcastle, DE). All tests were done in duplicate.

### 4.2.2 Retrogradation of selected RVA treated samples by DSC method

After the above starch (from 4.2.1) was cooled down to room temperature, they were kept in a refrigerator at 4 ± 1 °C. After 10 days' of storage, those starch pans were taken out and left at room temperature for 2 hrs for the analysis. The same heating conditions of DSC as in step 4.2.1 were again applied to those starch pans. The samples were heated from 15°C to 140 °C at a rate of 5°C /min.

The degree of starch retrogradation was calculated as % retrogradation = 100 ×ΔH of dissociation of retrograded starch/ΔH of starch gelatinization, where ΔH is the enthalpy change of the thermal transition. Outliers were removed and the result was calculated from at least duplicate.

### 4.2.3 Retrogradation of selected RVA treated samples by syneresis measurement

Selected starch samples, including starch with added stearic acid at 1%, lysine 6%, their combinations and a control without additives, were dissolved in distilled water at 10% (w/v) with gently stirring for 1 hr to form homogenous gels at room temperature. These gels were distributed into small petri-dishes with approximated 15 grams gel of per dish and covered. The weight of those gels with the whole set of petri dish was recorded to 4

decimal places (0.0001g); then the dishes were placed into a refrigerator at 4°C for storage. Each treatment were prepared in quadricate. The weight (to 0.0001g) of the petri dishes was recorded everyday for two weeks by wiping away water droplet on the side and cover of the dishes. Syneresis of starch gel was calculated as the percentage of gel moisture loss divided by the original weight of the gel.

**4.2.4 Resistant starch assay**

Resistant starch yield of all RVA treated rice starch samples was assayed by both the TDF (total dietary fiber) method and Megazyme (enzyme-chemical) method. Besides reagents and enzymes provided by the commercial kits, all other reagents were of analytical grade.

4.2.4.1 TDF method

The total dietary fiber kit was from Sigma (TDF-100A), which is adapted from Official Methods of Analysis of AOAC International, 16th Edition, Volume II, Section 45.4.07, Method 985.29 (1997). All RVA treated rice starch samples and RS control (52.5% dwb resistant starch) were assayed for their resistant starch content.

Two hundred mg of starch sample was weighed into a 125-mL Erlenmeyer flask and dispersed with 20 mL phosphate buffer (pH6, 0.08 M) by shaking. Heat stable α-amylase (68,300 U/mL, 0.5mL) was added and well mixed. The Erlenmeyer flask was sealed with aluminum foil and incubated into a 95°C water bath for 15 min with agitating every 5 min. After being cooled down to room temperature, the starch solution was adjusted for its pH to 7.5±0.2 by 0.275N aqueous NaOH solution (around 4 ml). Freshly prepared protease 0.02mL (P3910) (50mg/mL protease in phosphate buffer) were added into the solution with agitating. The mixture was then incubated at 60°C in shaking water bath for 30 min. Aqueous HCl solution (0.325 N) was then used to adjust its pH to 4.3±0.2. The mixture was at last decomposed by the addition of 0.02 mL of amyloglucosidase (10,863 U/mL; A9913) with 30 min incubation at 60°C in a shaking water bath.

After digestion, the residue starch in solution was precipitated overnight (>10hr) by the addition of 40 mL of absolute ethanol. The insoluble portion was collected with a Büchner funnel with Whatman #5 filter paper. The insoluble residue was washed twice by 15 ml of absolute ethanol and 10 ml of acetone. The residue was dried on the filter paper in an oven at 40°C overnight.

The yield of resistant starch was determined as: Resistant starch (%) = residue weight (g) ÷100% (dry weight basis) sample weight (g).

4.2.4.2 Megazyme method

A Megazyme resistant starch kit (K-RSTAR, Megazyme International Ireland Ltd., Co. Wicklow, Ireland) was used for resistant starch determination in an enzyme-chemical way, according to AOAC Method 2002.02 and AACC Method 32-40. All RVA treated rice starches and RS control (52.5% dwb resistant starch) were assayed for resistant starch content. To start with, a starch sample (100±5mg) was weighed into centrifuge tubes. Pancreatic α-amylase(3 Ceralpha Units/mg, 10 mg/mL, 4.0 mL) containing AMG (3 U/mL) was added to the tube with gentle mixing. The tube was then tightly capped, attached horizontally in a shaking water bath and aligned in the direction of motion. It was incubated at 37°C with continuous shaking (200 strokes/min) for exactly 16 hr. Then it was removed from the water bath, uncapped, and 4.0 ml of absolute ethanol were added. The starch in the tube was allowed to precipitate for at least 15min. The tube was then centrifuged at 1,500 x g (approx. 3,000 rpm) for 10 min (non-capped). The supernatant was decanted and collected in a 100 mL volumetric flask; the pellet was re-dispersed again in 2 mL of 50% ethanol and agitated using a vortex mixer. A further 6 mL of 50% ethanol was added. The re-suspended starch solution was again centrifuged at 1,500 x g (approx. 3,000 rpm) for 10 min (non-capped). The supernatant was again collected in the same 100 mL volumetric flask as the first collection; and the pellet was again re-suspended in a total of 8 ml of 50% ethanol as previous. Later, the tube was centrifuged for the third time with supernatant collection in the same 100 mL volumetric flask. Excess liquid of the residue pellet/starch was removed of the inverting the tube on absorbent paper.

Later, the residue starch was suspended in 2 mL of 2 M KOH. A magnetic stirrer bar (5 x 15 mm) was added to each tube; and the tubes were stirred for about 20 min in an ice/water bath over a magnetic stirrer. Then, 8 mL of sodium acetate buffer (1.2M pH3.8) were added to each tube with stirring on a magnetic stirrer. Then 0.1 mL AMG (3300 U/mL) was added immediately with magnetic stirring. The tube was capped, put into a water bath at 50°C for 30min and mixed intermittently by flipping over the tube.

The tube was then centrifuged at 1,500 x g for 10 min with a stir bar inside. For RVA starch sample, which contained less than 10% RS, a 0.1ml aliquot (in duplicate) of the supernatants was transferred into glass tubes, to which later was added 3.0 mL of GOPOD reagent. After mixing in a vortex mixer, the solution was incubated in 50°C water bath for 20 min. For RS control (52.5% RS), the supernatant of the tube was diluted by distilled water in a 100-mL volumetric flask, than a 0.1 ml aliquot was transferred into a glass tube and reacted with 3.0 mL GOPOD reagent under incubation as in the previous step. After 20 min, the absorbances of the solutions in the tubes were

measured at 510 nm against the reagent blank. The reagent blank was prepared by mixing 0.1 mL of 0.1 M sodium acetate buffer (pH 4.5) and 3.0 mL of GOPOD reagent. Meanwhile, glucose standards were prepared by mixing 0.1 mL of glucose (1 mg/ mL), which was provided in the kit, with GOPOD reagent for absorbance reading.

The percentage of RS was calculated using the following formula:

i. For samples, RS (g/100 g sample) = A × F × (10.3/0.1) × (1/1000) × (100/W) × (162/180) = A × F/W × 9.27

ii. For Resistant Starch Control (>10% RS), RS (g/100 g sample) = A ×F × (100/0.1) × (1/1000) × (100/W) × (162/180) = A ×F/W ×90

A= average absorbance (reaction) read against the reagent blank at 510 nm;

F = conversion factor from absorbance to micrograms (the absorbance obtained for 100 μg of glucose in the GOPOD reaction is determined, and F = 100 (μg of glucose) divided by the GOPOD absorbance for this 100 μg of glucose)

100/0.1 = volume adjustment (0.1 mL taken from 100 mL)

1/1000= conversion from micrograms to milligrams

W = dry weight of sample analyzed

100/W = factor to present starch as a percentage of test portion weight

162/180 = factor to convert from free glucose, as determined, to anhydro-glucose as occurs in starch

10.3/0.1 = volume adjustment (0.1 mL taken from 10.3 mL) for test portion containing 0-10% RS where the incubation solution is not diluted and the final volume is 10.3 mL.

**4.2.5 Determination of slowly digestible starch (SDS)**

Content of slowly digestible starch was assayed by modification of Englyst's method (Englyst et al. 1992). A sample of 0.8 g was weighed into a 50 ml polypropylene centrifuge tube to the nearest 0.1 mg. Fifty mg of guar gum, 5 glass balls (⌀1.5cm) and 20 ml 0.1M pH 5.2 acetate buffer were added, then the samples were mixed in a vortex mixer and incubated at a 37°C in a water bath for at least 5 min. In the mean time, 100 ml of enzyme solution was prepared by mixing 1.5 g of AMG (1000AG/g, Bio-Cat), 200 mg of invertase (300U/mg, Sigma), 10 ml of distilled water, and 90 ml of pancreatin solution together. The pancreatin solution was prepared 1hr before the assay by dissolving 3 g of pancreatin (8x, Sigma) in 20ml distilled water. Only supernatant was kept after centrifugation. Six batches of pancreatin were used for preparation of 90ml pancreatin solution.

Five ml of enzyme solution was added into the above centrifuge tube that contained starch sample, gum, glass balls and acetate buffer. All tubes were capped and incubated in a 37 °C shaking water bath by horizontally shaking at 200 rpm/min. After 20 min, 0.5 ml of sample was transferred into a tube labeled G20 that contained 20nml 66 % ethanol and mixed well. Immediately the sample tubes were placed back into the 37 °C shaking water bath at 200 rpm/min for a further 100 min (total time of 120 min). A second 0.5 ml sample was removed and placed into another labeled tube (G120) that contained 20 ml 66 % ethanol and mixed well.

Both G20 and G120 tubes stand overnight and were centrifuged for 2 min at 1500 rpm. 100 µl clear supernatant was removed for glucose determination using reagents from the Megazyme kit (K-RSTAR, Megazyme). Three ml of GOPOD regents were added into the clear supernatant. In the meantime, 100µl of glucose standard solution (1.0 mg/mL) was also prepared for the assay. After incubating the solution at 50°C for 20 min, its absorbance at 510 nm against the reagent blank were obtained. So the glucose content in G20 and G120 portions were calculated as,

$$\text{Glucose (mg)} = \frac{\Delta A \text{ sample}}{\Delta A \text{ glucose standard}} * C * Vt * \frac{Vh}{Vs}$$

ΔA sample = absorbance of test solution

ΔA glucose standard = absorbance of standard solution

C = concentration of standard, which is 1mg glucose/ml;

Vt= total volume of final glucose test solution, which is 20ml

Vh= total volume of the hydrolysate in the centrifuge tube from which the subsample was taken for glucose determination, which is 25ml

Vs =volume of the supernatant for glucose assay, which is 0.5ml

The calculation of slow digestible starch is therefore calculated as

$$SDS = (G120 - G20) * 0.9/Wt$$

Wt =weight (in mg) of starch sample taken for analysis

0.9= factor to convert from free glucose, as determined, to anhydro-glucose in starch

**4.3 RESULTS AND DISCUSSION**

**4.3.1 Thermal Property and retrogradation of prepared starch by DSC**

From the DSC scan, the gelatinization peak of raw rice starch was at 75.17 ± 0.45 °C, with an enthalpy of 12.06± 0.62 J/g. DSC scans of RVA treated rice starch with or without additives showed there was no un-gelatinized

starch left due to the lack of a gelatinization peak, indicating that RVA heating treatment provided enough energy to destroy the semi-crystalline structure of rice starch granule (Figure 4.1, Figure 4.2, Figure 4.3, Figure 4.4, Figure 4.5, Figure 4.6, Figure 4.7, Figure 4.8, Figure 4.9, and Figure 4.10). For starch with added stearic acid 1.0%/cysteine 6% and stearic acid 1.0%/aspartic acid6% (Figure 4.5), a very small peak at 70 °C appeared, suggesting the existence of stearic acid residue that has a melting point of 70 °C.

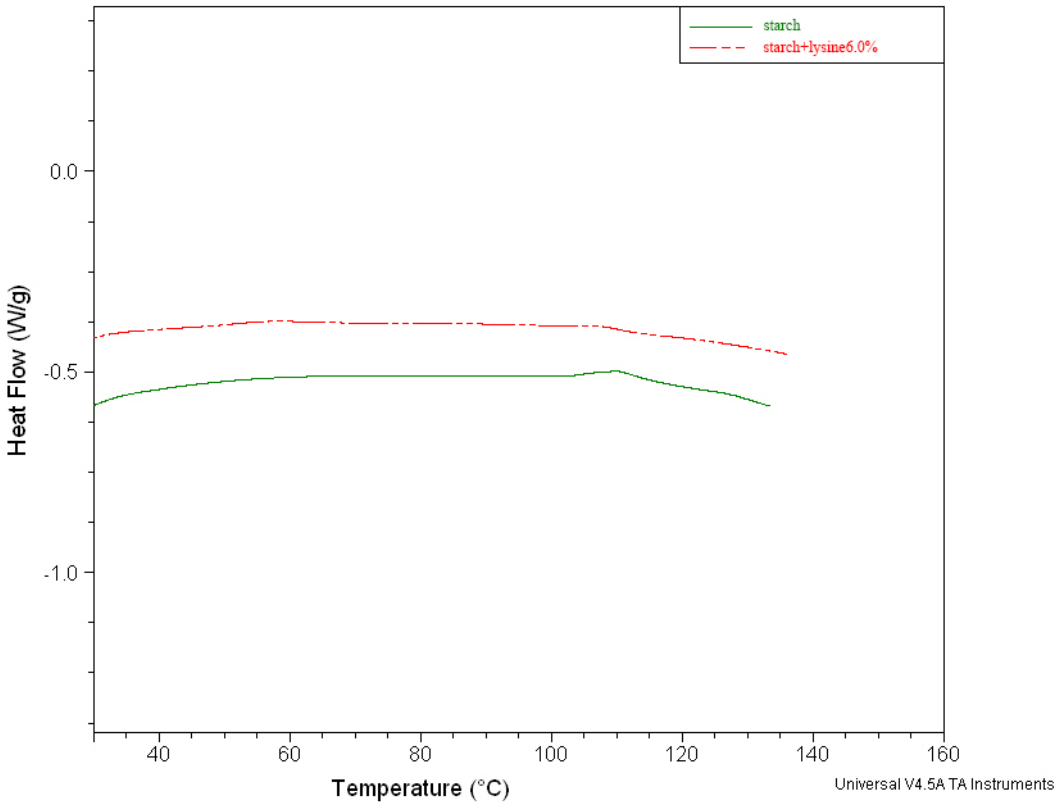


Figure 4.1 Thermal curve of rice starch with added lysine 6.0% (RVA treated)

Previous literature on amylose-lipid characteristics suggested the DSC peak from 93°C to 120°C was a consequence of amylose-lipid complex melting; complex form I had a peak temperature between 93.0°C and 97.9°C and complex form II between 115.8 °C and 120.7 °C (Tufvesson et al, 2001). As shown in Table 4.1 and Table 4.2, starch complexed with all fatty acids, including palmitic acid, stearic acid and linoleic acid. However, unlike most reports discovering two forms of amylose-lipid complex, complexes in this study were between form I and form II, even after 10 days of refrigerated storage. This may be because most studies on amylose-lipid complexes were based on preparation of amylose and lipid mixture, whereas in our study rice amylose was not isolated from rice starch. In that case, amylose and amylopectin were both presented in freeze-dried sample in a disordered way; amylose-lipid

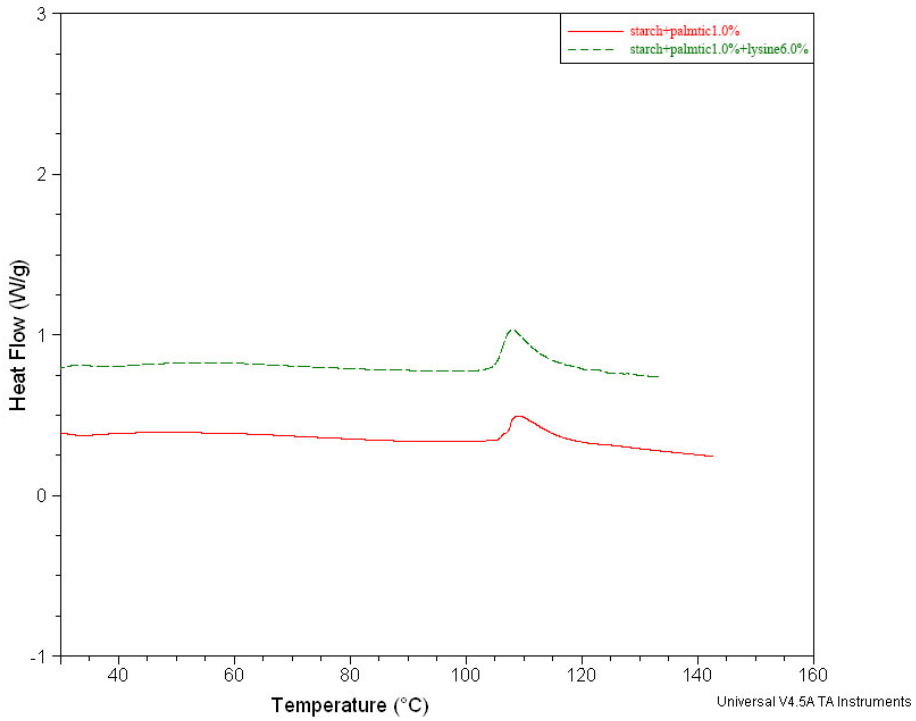


Figure 4.2 Thermal curve of rice starch with added palmtic acid 1.0% and lysine 6.0% (RVA treated)

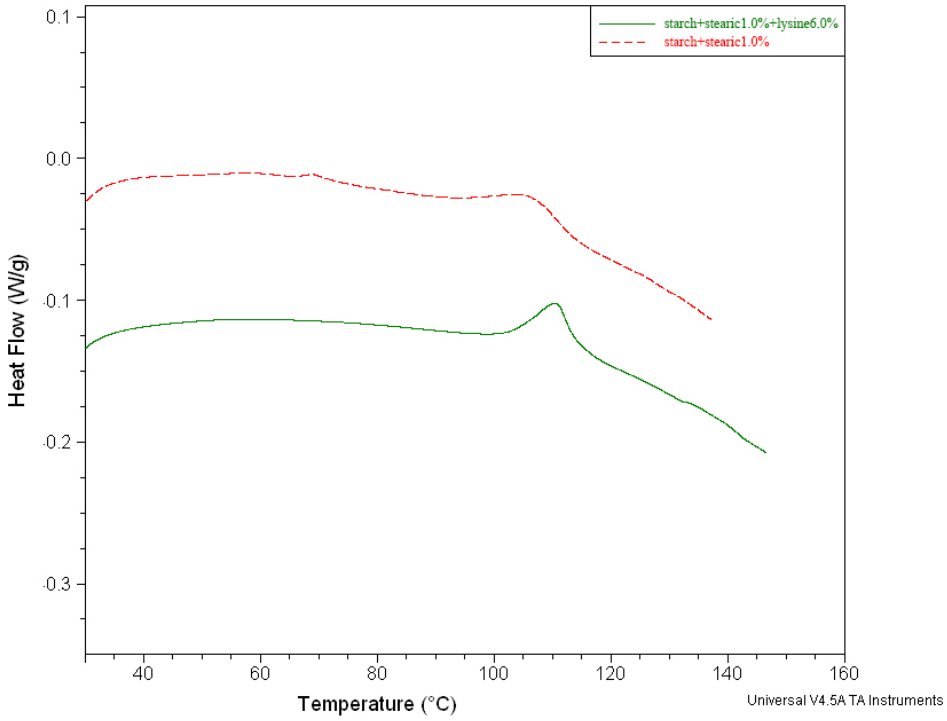


Figure 4.3 Thermal curve of rice starch with added stearic acid 1.0% and lysine 6.0% (RVA treated)

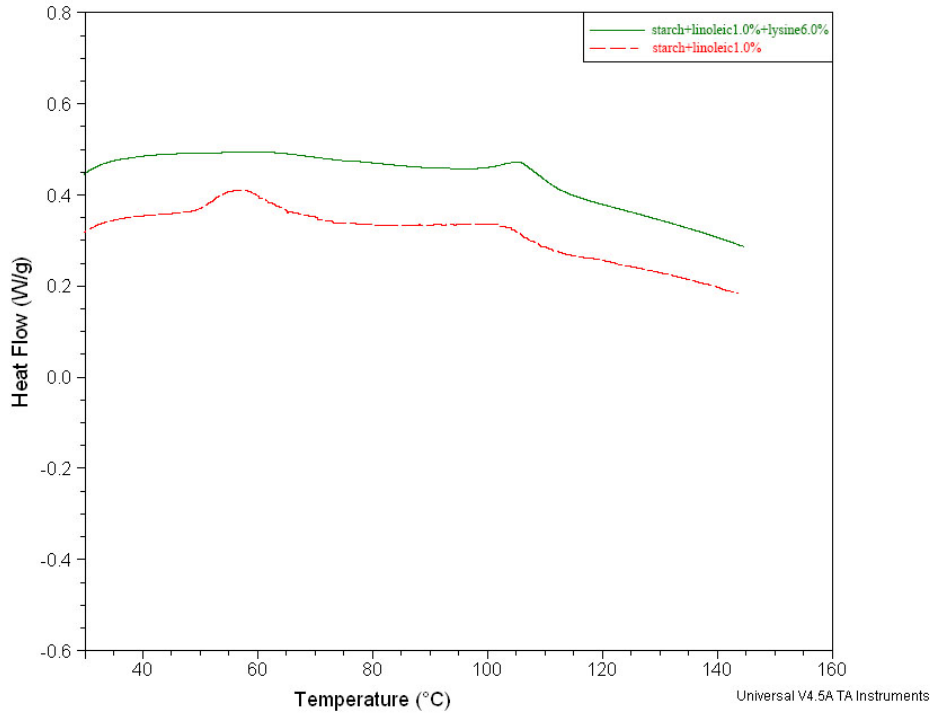


Figure 4.4: Thermal curve of rice starch with added linoleic acid 1.0% and lysine 6.0% (RVA treated). The peak at ~60 °C in starch with added linoleic acid 1.0% is gelatinization peak

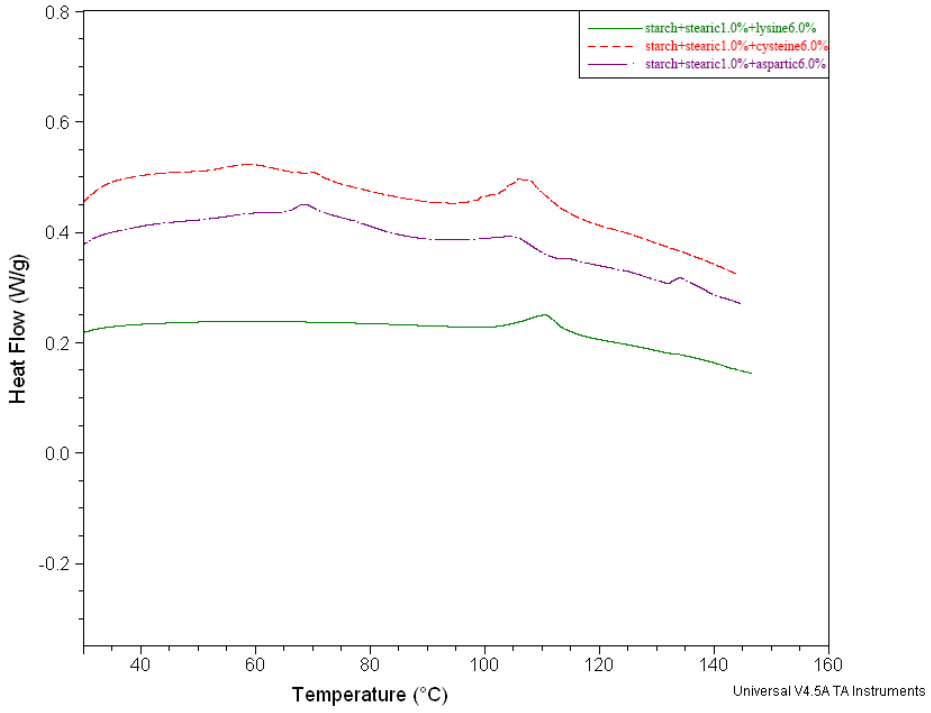


Figure 4.5: Thermal curve of rice starch with added stearic acid 1.0% and amino acids 6.0% (RVA treated)

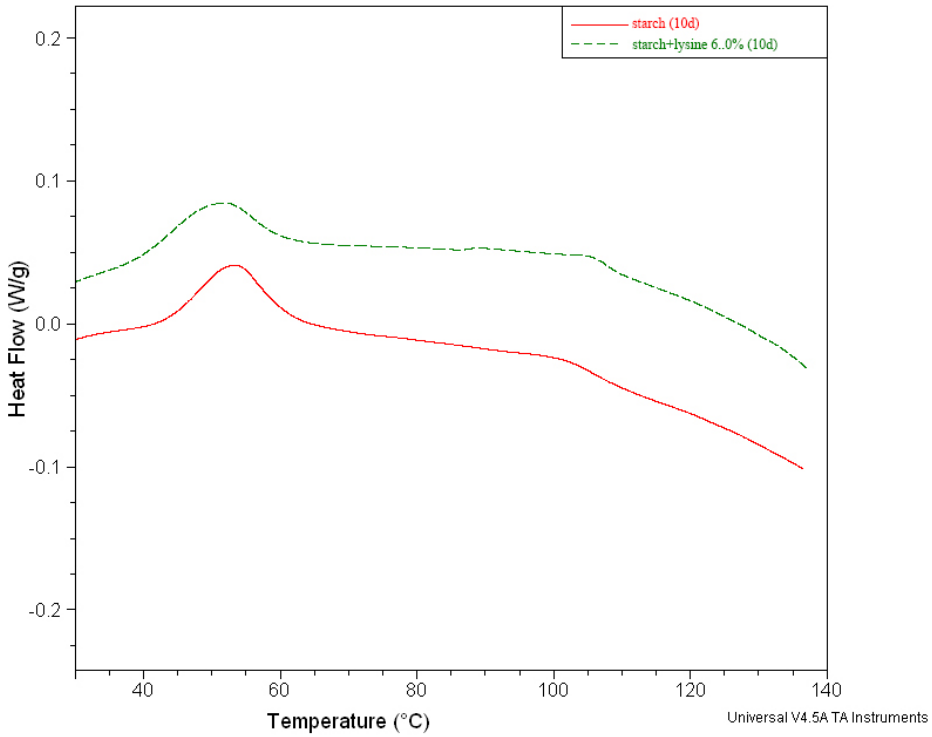


Figure 4.6 Thermal curve of rice starch with added lysine 6.0% after ten days refrigeration storage (RVA treated)

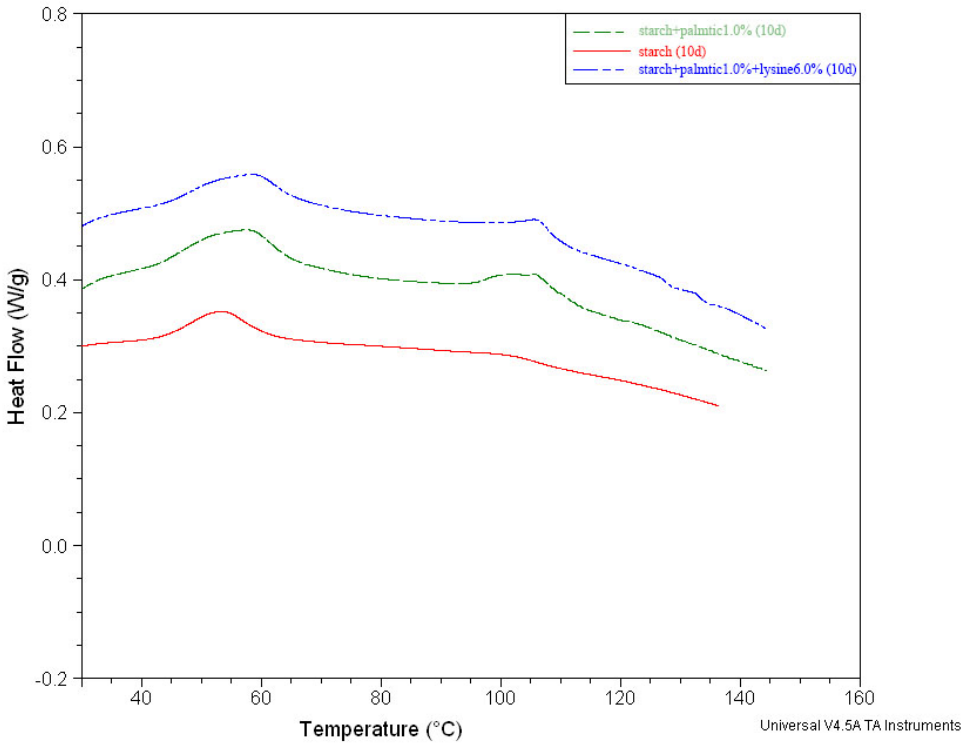


Figure 4.7 Thermal curve of rice starch with added palmtic acid 1.0% and lysine 6.0% after ten days refrigeration storage (RVA treated)

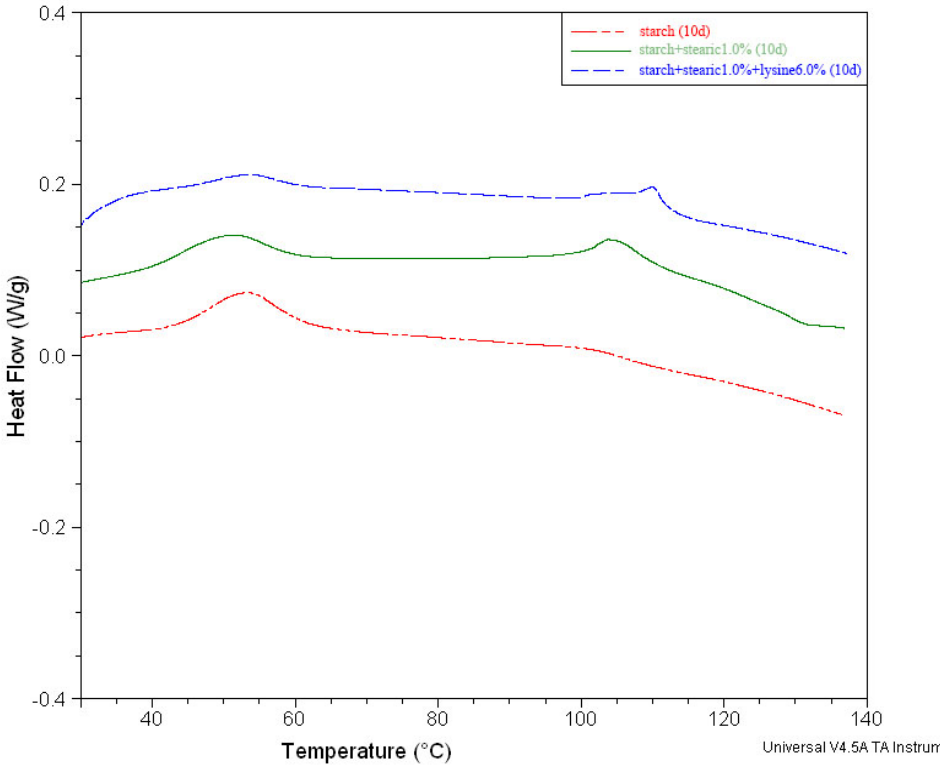


Figure 4.8 Thermal curve of rice starch with added stearic acid 1.0% and lysine 6.0% after ten days refrigeration storage (RVA treated)

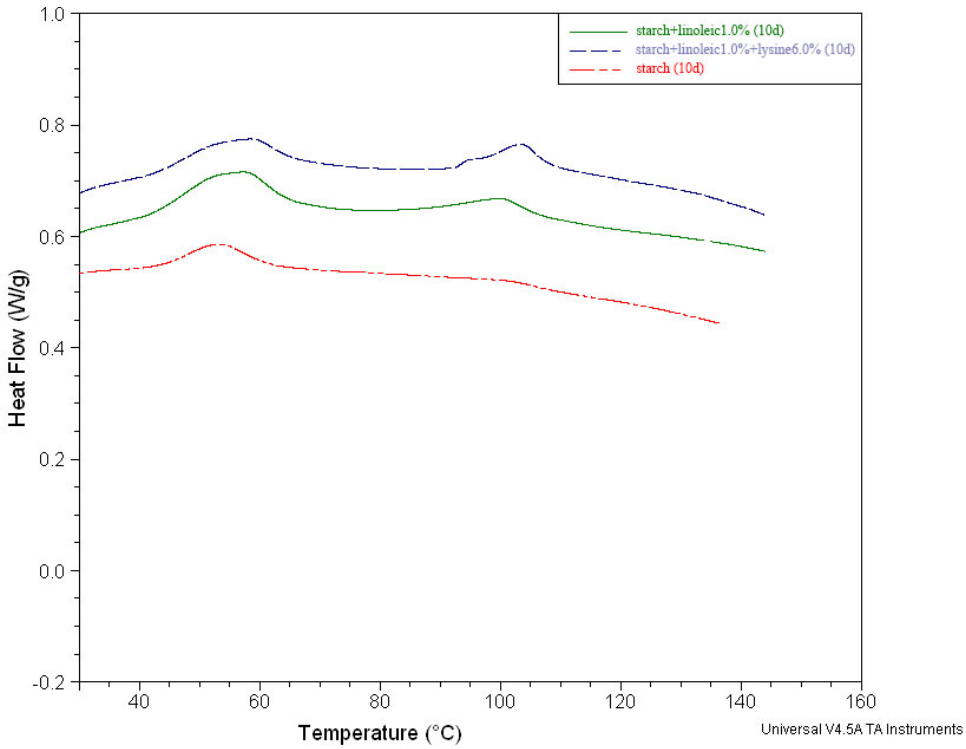


Figure 4.9 Thermal curve of rice starch with added linoleic acid 1.0% and lysine 6.0% after ten days refrigeration storage (RVA treated)

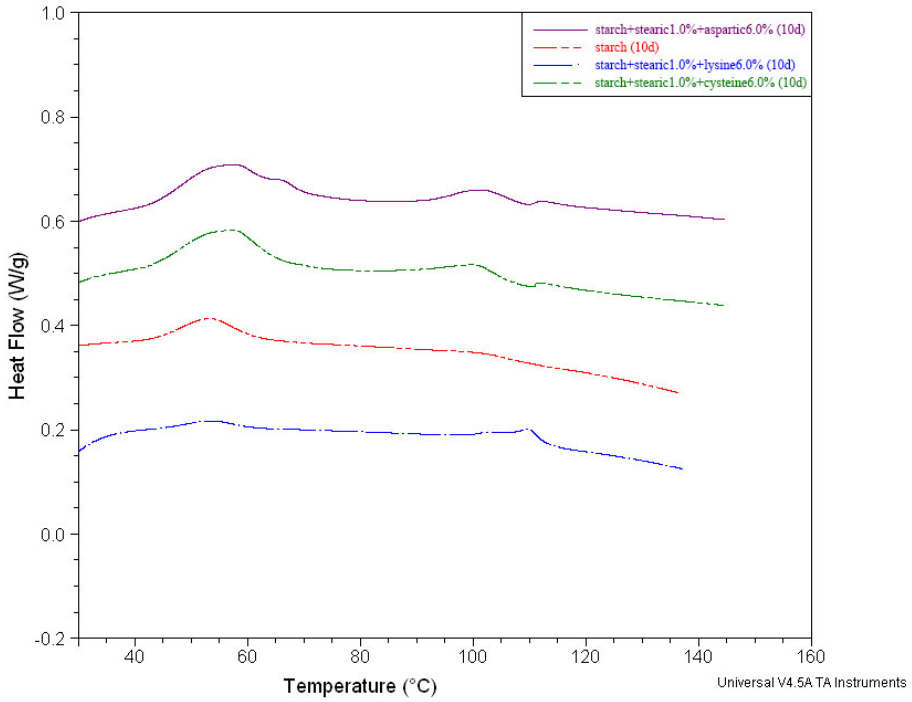


Figure 4.10 Thermal curve of rice starch with added stearic acid 1.0% and amino acids 6.0% after ten days refrigeration storage (RVA treated)

complex formation was therefore limited by the amount of amylose that can be utilized. RVA heating may not result in sufficient amount of crystalline structure of amylose-lipid complex (form I).

Judged by the value of enthalpy (Table 4.1), the amount of complex formed by RVA treatment and fatty acid additives was in the order of: palmitic acid/lysine > palmitic acid > stearic acid/lysine > stearic acid > linoleic acid/lysine > stearic acid/cysteine > stearic acid/aspartic acid > linoleic acid. The result was based on duplicates at least and any outlier values were removed; and frequent occurrence of outliers was also found in another study on DSC parameters of starch with added fatty acids (Tufvesson et al. 2001).

According to the peak melting temperature of the complexes, the DSC results were consistent with a finding that has been validated in many studies, that fatty acids of same chain length has reduced transition temperatures of their complexes with higher unsaturation (Tufvesson et al. 2001). The heat stability of amylose C18 lipid complex decreased with existence of two double bonds, from a peak temperature of 108°C in amylose stearic acid complex to 102°C in amylose linoleic acid complex. As to the influence of the chain length of fatty acids, although there are reports concluding that the complex stability increases with chain length, water solubility of fatty acids should be served as another reference; and longer chain length fatty acid has lower solubility and low accessibility for amylose

Table 4.1 DSC parameters of selected RVA treated samples*

| Sample (RVA treated) | Starch-lipid complex form | | | Other endothermic peak(s) | | | |
|---|---|---|---|---|---|---|---|
| | To (°C) | Tp (°C) | ΔH (J/g) | To (°C) | Tp (°C) | ΔH (J/g) | Peak form |
| Control rice starch | 102.41 | 110.11 | 0.77 | n/a | n/a | n/a | n/a |
| Lys6% added | n/a | n/a | n/a | n/a | n/a | n/a | n/a |
| St1.0% added | 97.37 | 107.79 | 2.07 | n/a | n/a | n/a | n/a |
| St1.0%/lys6% added | 103.58 | 110.45 | 2.18 | n/a | n/a | n/a | n/a |
| St1.0%/asp6% added | 98.82 | 105.92 | 1.35 | 66.29 | 68.84 | 0.26 | Stearic acid melt |
| St1.0%/cys6% added | 99.41 | 106.53 | 1.95 | 51.38 | 58.84 | 0.61 | Retrogradation |
| | | | | 67.84 | 70.24 | 0.12 | Stearic acid melt |
| Palm1.0% added | 104.63 | 108.30 | 5.03 | n/a | n/a | n/a | n/a |
| Palm1.0%/lys6% added | 105.48 | 108.97 | 8.34 | n/a | n/a | n/a | n/a |
| La1.0% added | 94.13 | 102.00 | 1.34 | 49.29 | 57.65 | 3.76 | Retrogradation |
| La1.0%/lys6% added | 100.35 | 105.46 | 1.97 | n/a | n/a | n/a | n/a |

* control starch is starch without any additive. n/a means no value observed. Palm stands for palmitic acid. St stands for stearic acid. La stands for linoleic acid. Lys means lysine. Asp means aspartic acid. Cys means cysteine

to complex with. It is therefore reasonably to attribute a more stable starch palmitic acid complex over starch stearic acid complex to the higher solubility of palmitic acid than stearic acid in hot water.

It is interesting to find that the combination of fatty acid and lysine (stearic acid/lysine, palmitic acid/lysine and linoleic acid) showed either higher melting temperature or higher enthalpy than fatty acids alone (stearic acid, palmitic and linoleic acid) added to rice starch. This indicates that the presence of lysine promotes more fatty acids to combined with starch or formation of more ordered amylose-lipid complex (higher melting temperature).

Retrogradation peak was found in samples after being stored for 10 days under refrigeration. It is widely accepted that starch retrogradation under long time storage is caused by amylopectin crystallization, which can be measured by DSC in a temperature range of 40-100°C (Eliasson et al. 1988, Sievert et al. 1989, Eerlingen et al. 1994). In this study, the temperature of this peak ranged from 51.1°C to 58.8°C, which was around 20 °C less than the gelatinization temperature of raw rice starch, indicating a less ordered and less perfect starch structure than the native starch granules. The presence of lysine has strikingly influenced enthalpy of the retrogradation peak in starch with added stearic acid. Clearly, it gave a lower retrogradation peak than the control starch, starch with added lysine only, and starch with added stearic acid only. Its peak enthalpy occupied only 13.27% of that from the gelatinization enthalpy of raw starch, while RVA treated control starch without any additives had a retrogradation percentage of 41.38%. This showed that decreased starch retrogradation for rice starch with added lysine and stearic acid.

Starch retrogradation is composed of two phases. In the early phase, gelatinized amylose leaches out of the starch granule and forms starch gel. This prepared crystal nuclei for further amylose crystallization, which usually takes less than 1 day (Biliaderis 1992). This explains why starch with high amylose content usually retrogrades

faster. In the late phase, amylopectin recrystallization dominates starch retrogradation. On one hand, fine structure of amylopectin, such as chain length with different degrees of polymerization and branch types affects amylopectin retrogradation (Vandeputte et al. 2003, Matalanis et al. 2009); on the other hand, high amylose fraction may promote amylopectin retrogradation, by possible mechanism of using amylose as a nuclei and co-crystallizes with amylopectin (Gudmundsson et al. 1990).

In the present study, reduced retrogradation by addition of stearic acid and lysine can be explained by limited availability of free amylose in starch solution. As was discussed in the previous chapter, starch with added stearic acid and lysine showed significantly lower peak viscosity, indicating that granule rupture or amylose leaching out was suppressed. Correspondingly, it is believed that limited amylose mobility within the starch granule is a primary reason restraining amylose retrogradation in starch with added stearic acid and lysine

After ten days cooling storage, the amylose-lipid complex peaks were found in DSC curves of starch samples with added fatty acids (Table 4.2). However, they were not as obvious as those on the first day. This suggested that the amylose-lipid complexes were not stable. Similar results were also observed by X-ray diffraction, due to the crystalline structure shift from V-type to B-type of starch during cold storage, indicating that the amylose-lipid complexes are metastable and are liable to a more stable B-type crystalline starch (Hibi et.al, 1990).

Table 4.2 Retrogradation of selected RVA treated samples after 10days refrigeration storage*

| Sample (RVA heated) | Starch-lipid complex form | | | Retrogradation peak | | | |
|---|---|---|---|---|---|---|---|
| | $T_o$ (°C) | $T_p$ (°C) | ΔH (J/g) | $T_o$ (°C) | $T_p$ (°C) | ΔH (J/g) | Percentage % |
| Control rice starch | n/a | n/a | n/a | 43.84 | 53.34 | 4.99 | 41.38 |
| Lys6% added | n/a | n/a | n/a | 40.08 | 51.43 | 5.28 | 43.78 |
| St1.0% added | 102.38 | 104.10 | 2.16 | 40.56 | 51.10 | 4.56 | 37.81 |
| St1.0%/lys6% added | 100.46 | 110.13 | 2.08 | 45.24 | 53.75 | 1.60 | 13.27 |
| St1.0%/asp6% added | 93.43 | 101.29 | 1.36 | 44.49 | 57.34 | 5.59 | 46.35 |
| St1.0%/cys6% added | 93.23 | 101.63 | 1.61 | 42.91 | 57.18 | 6.73 | 55.80 |
| Palm1.0% added | 96.84 | 105.47 | 2.08 | 42.62 | 56.97 | 5.01 | 41.54 |
| Palm1.0%/lys6% added | 97.51 | 106.28 | 1.99 | 43.9 | 58.74 | 4.41 | 36.57 |
| La1.0% added | 95.14 | 101.59 | 0.99 | 44.54 | 56.01 | 5.44 | 45.11 |
| La1.0%/lys6% added | 95.17 | 103.87 | 1.99 | 43.09 | 57.88 | 4.86 | 40.30 |

* Control starch is starch without any additive. Palm stands for palmitic acid. St stands for stearic acid. La stands for linoleic acid. Lys means lysine. Asp means aspartic acid. Cys means cysteine

### 4.3.2 Retrogradation of selected RVA treated samples by syneresis measurement

In either the academic or industrial world, many methods are developed to measure starch retrogradation. This is not only because of its significance in starch research and application, but also due to lack of an absolute method for accurate retrogradation measurement. Due to different understanding of detailed starch retrogradation principle, methods such as rheological analysis, X-ray diffraction, thermal analysis, nuclear magnetic resonance (NMR) and syneresis can be found in most publications but sometimes give inconsistent result interpretation (Karim et al. 2000). Therefore it is necessary to list more than one method for studying starch retrogradation.

Syneresis is a simple measurement of starch retrogradation. When starch recrystallizes, excess water will be expelled from starch gel network and usually a small amount of water will weep out on the surface of starch gel. Previous experience for measuring freeze-thaw stability in the lab did not work out well, presenting low repeatability and high difference between replicates (low precision), which may have resulted from uncontrollable factors such as freezing temperature and rate. The result is also sensitive to centrifuge force applied for liquid and gel separation(Karim et al. 2000). Therefore the original syneresis method based on freeze thaw cycles was not used, but refrigeration-thaw measurement was used.

Table 4.3 Weight (%) of starch gel remaining after being refrigerated, compared to the first day weight

| Days | Control | Lysine 6.0% | Stearic 1.0% | Stearic 1.0% lysine 6.0% |
|---|---|---|---|---|
| 1 | 100.00% | 100.00% | 100.00% | 100.00% |
| 2 | 97.98% | 97.47% | 97.67% | 98.56% |
| 4 | 95.63% | 95.00% | 95.81% | 97.20% |
| 5 | 94.06% | 93.75% | 94.83% | 96.20% |
| 6 | 92.70% | 92.83% | 94.02% | 94.74% |
| 7 | 90.93% | 91.63% | 93.09% | 93.50% |
| 8 | 87.50% | 88.05% | 89.98% | 90.59% |
| 10 | 84.07% | 84.44% | 86.82% | 87.27% |
| 11 | 80.88% | 81.45% | 83.83% | 85.58% |
| 12 | 77.82% | 77.85% | 80.62% | 84.11% |
| 13 | 75.81% | 76.21% | 79.07% | 82.78% |
| 14 | 74.38% | 74.73% | 77.77% | 80.75% |
| 15 | 71.82% | 72.36% | 75.22% | 77.85% |

Selected starch samples, including control, lysine added, stearic acid added, and stearic acid/lysine added were prepared at 10% for direct syneresis measurement. Consistent to thermal analysis, starch with added stearic acid 1.0% /lysine 6% gave the lowest syneresis, that was, 80.5% of its original gel weight after 15 days of refrigerated storage, compared to 74.7% original weight for starch gel without additives (Table 4.3). This also showed the function of lysine and stearic acid combination in controlling starch retrogradation.

**4.3.3 Resistant starch (RS) assay**

Inconsistent results from different methods for resistant starch assay have been found by previous researchers and other publications (Manaois 2009, Perera et al. 2010). Current popular methods include Englyst's method (Englyst et al., 1992), total dietary fiber (TDF) method and AOAC 2002.02/AACC 32–40 method (AOAC, 2005); the latter is incorporated into a commercial resistant starch assay kit from Megazyme (K-RSTAR) and is considered as a modification of Englyst's method. The method disagreement is mainly because of differences with regard to sample preparation, enzymes used and the experimental conditions to mimic the gastrointestinal digestion of starch. A main concern of these methods lies in sample preparation. For example, it is pointed out that Englyst's method is based on digestion of raw starch while in real life starch has to be cooked for consumption; resistant starch content of raw starch without cooking can be misleading (Li et al. 2008). As a result, total dietary fiber assay is put forward as a more desirable method for resistant starch determination, because it requires boiling starch for 30min for thermal stable α-amylase hydrolysis (Shin et al. 2004).

In the present study, both the Megazyme kit and total dietary fiber kit were applied to measure RS content and the results can be found in Table 4.4. A RS control containing 52.5% RS provided by the Megazyme kit was used to check precision of the assays. It turned out 30 min starch gelatinization in the TDF method indeed affected RS content of the above RS control, which gave only 17.0% RS content, whereas using the Megazyme kit method, the same RS control gave a value of 53.2%. This demonstrates that starch gelatinization reduced RS fractions, probably RSI and RSII, because heating will help RSI starch out of its cellular matrix and destroy B type crystalline structure in RSII. In this study, however, because starch samples were pre-gelatinized, starch gelatinization in the TDF method should not be a factor causing discrepancies between RS content measured by the TDF and Megazyme methods.

A review of the RS assay using the Megazyme method revealed that starch samples with added fatty acids and amino acids did not increase their RS content much comparing to starch without any additives and most values were

less than 1%. Because Megazyme claims its method is applicable to samples that contain more than 2% RS, the result from Megazyme may not provide a reliable conclusion when RS content was less than 2%. However, rice starch with amino acids additives formed a certain amount of RS during the RVA heating cycle. According to the Megazyme assay, Asp, Lys and Cys elevated RS content from 0.59% in control to 2.19%, 2.44% and 2.71%.

Using the TDF assay, starch gave higher RS content than the Megazyme method in general. A previous researcher in the lab also reported similar results in that RS values for pre-gelatinized starch obtained from Megazyme were lower than other methods (Manaois 2009). One factor that may be accountable for this conclusion lies in the general low RS content in the starch samples. The step of centrifuging starch hydrolytes to separate soluble starch and resistant starch fractions could not be well performed, because the precipitated resistant starch was very loose and did not form a solid pellet, making it easy to be washed away, especially when the Megazyme method requires at least 3 ethanol rinsings. While ethanol washing may help separate the RS out, if a large amount of RS exists in a starch sample, for example, 52.5% RS control; for starch samples with small amount of RS, consistent washing will comprise the accuracy and precision of the result instead.

In the TDF assay, although duplicates were done for each treatment, it was still hard to get good repeatability, due to calculation based on weight difference of filter paper and filter paper is very sensitive to air moisture (Table 4.3). Generally, no significant change of RS content was found before and after addition of those additives except for cysteine, which introduced RS of 8.88%, compared to the RS content of 3.19% for starch without any additives. Even after an extra addition of fatty acids, including palmitic acid, stearic acid and oleic acid, starches containing cysteine still gave improved RS content. As mentioned in Chapter 3, cysteine is very susceptible to oxidation to form disulfide bonds with other cysteine-containing protein. It is therefore postulated that the increased amount of RS is related to the interaction of cysteine with residual surface protein of rice starch. Based on the role of disulfide bonding of protein in restricting starch swelling (Hamaker et al. 1993, Puncha-arnon et al. 2013), formation of aggregates among proteins by disulfide bonds may interfere with amyloglucosidase hydrolysis of starch due partly to inaccessibility of the active site of amyloglucosidase.

Amylose-lipid complexed starch is often considered to be a special type of resistant starch because of its high dissolution temperature and resistance to amylase hydrolysis (Jane et al. 1984, Kaur et al. 2000). However, no increased amount of RS in rice starch after addition of fatty acids was found in the present study. There are many factors attributable to the result. For example, starch gelatinization itself during preparation of amylose-lipid

Table 4.4 RS yield of starch samples added with fatty acids and amino acids [a, b]

| Treatment # | Amino acid additive (6%) | Fatty acid additive (0.6% and 1.0%) | | Resistant starch yield (%) | |
|---|---|---|---|---|---|
| | Type | Type | Percentage | Megazyme method | TDF method |
| 1 | N/A | N/A | N/A | 0.55±0.07ghi | 3.19±1.55de |
| 2 | Asp | 0 | 0 | 2.23±0.29abc | 3.96±1.22cde |
| 3 | Lys | 0 | 0 | 2.44±0.91ab | 4.19±1.12cde |
| 4 | Cys | 0 | 0 | 2.71±0.21a | 8.88±0.63a |
| 5 | N/A | Palmitic | 0.6 | 0.38±0.12ghi | 3.11±0.83de |
| 6 | N/A | Stearic | 0.6 | 0.83±0.18ghi | 2.1±0.43e |
| 7 | N/A | Oleic | 0.6 | 0.36±0.06ghi | 2.3±0.97e |
| 8 | N/A | Linoleic | 0.6 | 0.39±0.09ghi | 2.26±0.91e |
| 9 | N/A | Palmitic | 1.0 | 0.47±0.06ghi | 3.31±1.75cde |
| 10 | N/A | Stearic | 1.0 | 0.34±0.05hi | 3.83±0.39cde |
| 11 | N/A | Oleic | 1.0 | 0.41±0.12ghi | 3.36±1.79cde |
| 12 | N/A | Linoleic | 1.0 | 0.36±0.1hi | 4±0.78cde |
| 13 | Lys | Palmitic | 0.6 | 0.57±0.18ghi | 2.75±0.59de |
| 14 | Lys | Stearic | 0.6 | 0.39±0.08ghi | 3.97±0.72cde |
| 15 | Lys | Oleic | 0.6 | 0.56±0.39ghi | 3.34±0.51cde |
| 16 | Lys | Linoleic | 0.6 | 0.45±0.07ghi | 3.77±1.36cde |
| 17 | Lys | Palmitic | 1.0 | 0.42±0.14ghi | 4.88±0.56bcde |
| 18 | Lys | Stearic | 1.0 | 0.48±0.14ghi | 3.48±0.34cde |
| 19 | Lys | Oleic | 1.0 | 0.4±0.19ghi | 3.8±0.97cde |
| 20 | Lys | Linoleic | 1.0 | 0.51±0.18ghi | 4.03±1.72cde |
| 21 | Asp | Palmitic | 0.6 | 0.8±0.26ghi | 3.12±0.91de |
| 22 | Asp | Stearic | 0.6 | 2.15±0.16abc | 6.39±1.33abc |
| 23 | Asp | Oleic | 0.6 | 1.68±0.24 | 4.52±0.9bcde |
| 24 | Asp | Linoleic | 0.6 | 0.77±0.09ghi | 3.02±0.94de |
| 25 | Asp | Palmitic | 1.0 | 0.99±0.13efgh | 3.73±1.7cde |
| 26 | Asp | Stearic | 1.0 | 1.78±0.21bcd | 5.65±1.19bcd |
| 27 | Asp | Oleic | 1.0 | 1.58±0.37cdef | 3.56±0.59cde |
| 28 | Asp | Linoleic | 1.0 | 0.85±0.24fghi | 3.53±1.2cde |
| 29 | Cys | Palmitic | 0.6 | 0.65±0.16ghi | 5.43±0.65bcd |
| 30 | Cys | Stearic | 0.6 | 2.17±0.26abc | 7.43±0.8ab |
| 31 | Cys | Oleic | 0.6 | 1.1±0.72efdg | 4.38±1.33bcde |
| 32 | Cys | Linoleic | 0.6 | 0.38±0.16ghi | 5.5±1.26bcd |
| 33 | Cys | Palmitic | 1.0 | 0.79±0.32ghi | 4.33±1.84bcde |
| 34 | Cys | Stearic | 1.0 | 1.05±0.11defgh | 5.69±1.08 |
| 35 | Cys | Oleic | 1.0 | 0.78±0.2ghi | 3.78±1.47cde |
| 36 | Cys | Linoleic | 1.0 | 0.55±0.07i | 3.83±0.2bcde |

a. All additive levels are based on starch dry weight. N/A indicated no additive. Control starch is starch without additives and RVA treated.

b. Values followed by the same letter in the same column are not significantly different (P >0.05)

complex is a process that destroys any possible tightly packed resistant starch fraction; formation of amylose-lipid complex is too low to influence RS content. According to the dissociation temperature from the DSC curve, there are two forms of amylose-lipid complex; complex form I had a peak temperature between 93.0$^{o}$C and 97.9$^{o}$C and complex form II between 115.8 $^{o}$C and 120.7 $^{o}$C. The amylose-lipid complex in the study is the less ordered form I, which may not contributed to formation of RS.

**4.3.4 Determination of slowly digestible starch (SDS)**

In order to figure out how amylose-lipid complex influences the starch digestibility performance, slow digestible starch content was also assayed on rice starch with added fatty acids and combinations of fatty acids/amino acids (Table 4.5).

The original SDS content in RVA treated rice starch was 8.31%. Addition of lysine lowered its SDS content to 2.42% only. This may be caused by complete destruction of starch granules after addition of lysine to rice starch, according to its high peak viscosity and early peak time in the RVA curve (Chapter 3). This physical damage made it more accessible to digestive enzymes and therefore it had low SDS content.

Addition of fatty acids alone or with additional lysine increased the SDS content of rice starch. After addition of palmitic acid, stearic acid, oleic acid and linoleic acid, SDS contents of RVA treated rice starch control have risen to

Table 4.5 SDS yield of selective starch samples added with fatty acids and amino acids*

| Amino acid additive (6%) | Fatty acid additive (1%) | SDS yield (%) |
|---|---|---|
| N/A | N/A | 8.31±3.29g |
| Lysine | N/A | 2.42±0.29i |
| N/A | Palmtic acid | 27.24±1.34a |
| N/A | Stearic acid | 13.27±2.78d |
| N/A | Oleic acid | 17.31±2.50c |
| N/A | Linoleic acid | 18.82±3.62bc |
| Lysine | Palmtic acid | 7.40±2.67h |
| Lysine | Stearic acid | 9.18±1.30f |
| Lysine | Oleic acid | 8.78±3.27g |
| Lysine | Linoleic acid | 10.37±3.97e |

* Values followed by the same letter in the same column are not significantly different (P >0.05); N/A indicates no additive; SDS means slowly digestible starch.

27.24±1.34%, 13.27±2.78%, 17.31±2.50% and 18.82±3.62%. This is in agreement with studies reporting that amylose-lipid complex inhibited α-amylolysis of starch. Seneviratne et al.(1991) found that the rate of α-

amylolysis of amylose-monostearate complex was inversely related to its degree of crystallinity. Because RVA treated rice starch with added fatty acids showed thermal properties of form I amylose-lipid complexes, which have a peak temperature below 110 $^{o}$C and is less ordered than form II amylose-lipid complex, it is easy to correlate its existence to SDS instead of RS.

## 4.4 CONCLUSION

The thermal, retrogradation and amylase digestibility of RVA treated rice starch samples were selectively assayed and characterized. When stored 10 days under refrigeration, starches with both added fatty acids and lysine were found to have lower retrogradation than starches with added fatty acids and starches without additives. DSC scans of RVA treated starches showed elevated amounts of amylose-lipid complexes when both lysine and fatty acids were present, compared to addition of fatty acids alone.

With regard to RS content, no pronounced difference was found between starch with additives and without additives except cysteine; however, SDS assay observed more slowly digestible starch for when fatty acids were present in the sample, due to amylose-lipid complexes with less order crystalline structure.

## 4.5 REFERENCES


Biliaderis, C. G. and H. Seneviratne (1990). "On the supermolecular structure and metastability of glycerol monostearate-amylose complex." Carbohydrate Polymers **13**(2): 185-206.

Biliaderis, C. G. (1992). "Structures and phase transitions of starch in food systems." Food Technology **46**(6): 98-109.

Biliaderis, C. G., C. M. Page and T. J. Maurice (1986). "On the multiple melting transitions of starch/monoglyceride systems." Food Chemistry **22**(4): 279-295.

Eerlingen, R., G. Cillen and J. Delcour (1994). "Enzyme-resistant starch. 4. Effect of endogenous lipids and added sodium dodecyl-sulfate on formation of resistant starch." Cereal chemistry **71**(2): 170-177.

Eerlingen, R., H. Jacobs and J. Delcour (1994). "Enzyme-resistant starch. 5. Effect of retrogradation of waxy maize starch on enzyme susceptibility." Cereal Chemistry **71**(4): 351-355.

Eliasson, A., H. Finstad and G. Ljunger (1988). "A Study of Starch - Lipid Interactions for Some Native and Modified Maize Starches." Starch−Stärke **40**(3): 95-100.

Englyst, H. N., S. Kingman and J. Cummings (1992). "Classification and measurement of nutritionally important starch fractions." European Journal of Clinical Nutrition **46**: S33.

Englyst, K. N., S. Vinoy, H. N. Englyst and V. Lang (2003). "Glycaemic index of cereal products explained by their content of rapidly and slowly available glucose." British Journal of Nutrition **89**(3): 329-340.

Gudmundsson, M. and Eliasson, A. (1990). "Retrogradation of amylopectin and the effects of amylose and added surfactants/emulsifiers." Carbohydrate Polymers **13**(3): 295-315.

Guraya, H. S., R. S. Kadan and E. T. Champagne (1997). "Effect of rice starch-lipid complexes on in vitro digestibility, complexing index, and viscosity." Cereal Chemistry **74**(5): 561-565.

Hamaker, B. R. and V. K. Griffin (1993). "Effect of disulfide bond-containing protein on rice starch gelatinization and pasting." Cereal Chemistry **70**.

Hibi, Y., S. Kitamura and T. Kuge (1990). "Effect of lipids on the retrogradation of cooked rice." Cereal Chem **67**(1): 7-10.
Hoover, R. and Y. Zhou (2003). "In vitro and in vivo hydrolysis of legume starches by α-amylase and resistant starch formation in legumes—a review." Carbohydrate Polymers **54**(4): 401-417.

Jane, J.-L. and J. F. Robyt (1984). "Structure studies of amylose-V complexes and retro-graded amylose by action of alpha amylases, and a new method for preparing amylodextrins." Carbohydrate Research **132**(1): 105-118.

Karim, A. A., M. Norziah and C. Seow (2000). "Methods for the study of starch retrogradation." Food Chemistry **71**(1): 9-36.

Karkalas, J., S. Ma, W. R. Morrison and R. A. Pethrick (1995). "Some factors determining the thermal properties of amylose inclusion complexes with fatty acids." Carbohydrate Research **268**(2): 233-247.

Kaur, K. and N. Singh (2000). "Amylose-lipid complex formation during cooking of rice flour." Food chemistry **71**(4): 511-517.

Lauro, M., K. Poutanen and P. Forssell (2000). "Effect of partial gelatinization and lipid addition on α-amylolysis of barley starch granules." Cereal chemistry **77**(5): 595-601.

Li, L., H. Jiang, M. Campbell, M. Blanco and J.-l. Jane (2008). "Characterization of maize amylose-extender (ae) mutant starches. Part I: Relationship between resistant starch contents and molecular structures." Carbohydrate polymers **74**(3): 396-404.

Ludwig, D. S. (2002). "The glycemic index." JAMA: The Journal of the american medical association **287**(18): 2414-2423.

Manaois, R. V. (2009). Modification of rice starch properties by addition of amino acids at various pH levels, Master thesis, Louisiana State University.

Matalanis, A., O. Campanella and B. Hamaker (2009). "Storage retrogradation behavior of sorghum, maize and rice starch pastes related to amylopectin fine structure." Journal of Cereal Science **50**(1): 74-81.

Nichols, B. L., S. Avery, P. Sen, D. M. Swallow, D. Hahn and E. Sterchi (2003). "The maltase-glucoamylase gene: common ancestry to sucrase-isomaltase with complementary starch digestion activities." Proceedings of the National Academy of Sciences **100**(3): 1432-1437.

Perera, A., V. Meda and R. T. Tyler (2010). "Resistant starch: A review of analytical protocols for determining resistant starch and of factors affecting the resistant starch content of foods." Food Research International **43**(8): 1959-1974.

Puncha-arnon, S. and D. Uttapap (2013). "Rice starch vs. rice flour: Differences in their properties when modified by heat–moisture treatment." Carbohydrate Polymers **91**(1): 85-91.

Putseys, J., L. Lamberts and J. Delcour (2010). "Amylose-inclusion complexes: Formation, identity and physico-chemical properties." Journal of Cereal Science **51**(3): 238-247.

Seneviratne, H. and C. Biliaderis (1991). "Action of α-amylases on amylose-lipid complex superstructures." Journal of Cereal Science **13**(2): 129-143.

Shin, M., J. Song and P. A. Seib (2004). "In vitro Digestibility of Cross‐Linked Starches‐RS4." Starch−Stärke **56**(10): 478-483.

Sievert, D. and Y. Pomeranz (1989). "Enzyme-resistant starch. I. Characterization and evaluation by enzymatic, thermoanalytical, and microscopic methods." Cereal Chem **66**(4): 342-347.

Szczodrak, J. and Y. Pomeranz (1991). "Starch and enzyme-resistant starch from high-amylose barley." Cereal Chem **68**(6): 589-596.

Tufvesson, F., V. Skrabanja and Eliasson, A. (2001). "Digestibility of starch systems containing amylose-glycerol monopalmitin complexes." Lebensmittel-Wissenschaft und-Technologie **34**(3): 131-139.

Vandeputte, G., R. Vermeylen, J. Geeroms and J. Delcour (2003). "Rice starches. III. Structural aspects provide insight in amylopectin retrogradation properties and gel texture." Journal of Cereal Science **38**(1): 61-68.

Woo, K. and P. Seib (2002). "Cross-linked resistant starch: preparation and properties 1." Cereal Chemistry **79**(6): 819-825.

Zobel, H. (1988). "Starch crystal transformations and their industrial importance." Starch−Stärke **40**(1): 1-7.

# CHAPTER 5
# CHARACTERIZATION OF STARCH WITH ADDITIVE AFTER HEATING TREATMENT (II): MORPHOLOGY, X-RAY DIFFRACTION AND POSSIBLE MECHANISM

## 5.1 INTRODUCTION

Upon heating, starch granules in solution starts swelling accompanied with the lost of granule birefringence. For regular starch, continuous heating causes amylose to leach out from the starch granules and dissolve in water. If a granule maintains its integrity in the state of being swollen and gelatinized, it is considered as a granule "ghost" (Obanni and BeMiller 1995). The ghost fails to manifest its structural organization, bringing in particulate dispersion of starch. Cross-linked starch is one good example that keeps its ghost integrity upon a long time heating, so the granule will not dissolve; the viscoelastic properties of the solution are different from regular starch solution as well (Debet and Gidley 2007).

As observed in chapter 3, rice starch with certain additives exhibited low peak viscosity during RVA treatment. In order to figure out whether the reduced peak viscosity was caused by "ghost" granule or starch hydrolysis, it was necessary to observe the starch morphology after being RVA treated.

X-ray diffraction was applied to dig into the molecular structure of those RVA treated samples for better understanding of the mechanism of inhibited viscosity for rice starch with additives. It is known that raw starches of different botanical sources and modifications give different X-ray diffraction patterns. In principle, cereal starches give an A pattern; tuber starches yield a B pattern; certain legume and root starches give a C pattern; and V pattern refers to amylose-lipid complex existing either in native raw starches or starches with added ligands; whereas high amylose starches usually don't follow the above rule (Zobel 1988). Although all native starches have helices in their molecular chains, XRD of A pattern is more compact, with a double helix filling the central, open space; XRD of B pattern starch consists of double helices filled with water inside (Zobel 1988). V-complex has a single helical structure composed of amylose and its ligands, including aliphatic alcohols and fatty acids, certain surfactants and iodine. It is hydrophilic outside and has a hydrophobic cavity inside for ligand to complex with (Krog 1971). In XRD curves, the 4.4 Å line (d-spacing) first showed up when V-complexes were formed, and then 12 Å and 6.8 Å (Table 5.1).

Table 5.1 D-spacing for raw starches of different XRD patterns

| Source of starches | Pattern | D-spacing (Angstrom, Å) |
|---|---|---|
| Cereal | A | Strong at 5.8, 5.2, 3.8 |
| Tuber and retrograded starch | B | Medium at 15.8-16.0; broad medium at 5.9; strong at 5.2; medium doublet at 4.0 and 3.7 |
| Amylose-lipid complex | V | Strong at 4.4; medium at 12.0, 6.8 |

V-complex is common in heat-moisture treated starches when lipid additives are present. A typical application is emulsifier in bread, which retards bread firming and improves its shelf life by formation of amylose-lipid V complex. Similarly, starch with a high percentage of amylose-lipid complexes was reported to have higher gelatinization temperature, less granule swelling, less shear in the gelled state and less setback in general (Zobel 1988).

Stearic acid has been observed as a main factor for inhibiting pasting viscosity of rice starch with added stearic acid and lysine in chapter 3. Debet and Gidley (2007) proposed that starch surface proteins and lipids were restricting factors for granule swelling during heating. However, it is unclear how these components interact with each other and what chemical bonds are formed. Previous report on lysine's stabilizing effect of starch granules illustrated the conjugation of carboxymethyl group in starch molecules with lysine by amide-carbonyl bonding (Yang, et al. 1998). If that was the same reaction that happened in this study, covalent bonds should be found through an increased amount of lysine found in the starch samples. On the other hand, a hypothesis on cross-linking among amylose or long branches of amylopectin in swollen granules was put forward as origin of the "starch ghost", although the interaction could not be detected due to limited detection ability of DSC or $^{13}C$ NMR (Debet and Gidley 2007).

The objective of chapter 5 was to reveal possible mechanisms that lead to inhibited starch pasting of rice starch with additives. Besides microscopy observation and X-ray diffraction of starch samples with selective additives, amino acids other than lysine were added in the reaction system to see their influence in starch pasting. Determination of nitrogen was done to test the existence of covalent bonds that may link lysine and starch together. Formation of complexes from starch, fatty acids and amino acids was discussed based on their chemical structures, influence on starch pasting, retrogradation, and crystalline structure.

## 5.2 MATERIALS AND METHODS

### 5.2.1 Microscope observation

Four types of 3% starch solutions (20ml) were prepared. The starch samples were from RVA and freeze-dried rice starch control, starch with 6% lysine added, starch with 1.0% stearic acid added, and starch with 6% lysine and 1.0% stearic acid added (chapter 3). The starch solutions were stirred for 2 hrs by magnetic stir bars. Then half of the starch solutions was transferred into other vials and heated at 90°C for 20 min to check their heating stability. All starch solutions, before or after heating, were stained by 2% $I_2$-KI solution (0.2 g $I_2$ and 2 g KI in 100 ml distilled water). The stained samples were observed by differential interference contrast microscopy (Leica DM RXA2) and photographed by PCO Sensicam.

### 5.2.2 X-ray diffraction

RVA and freeze-dried starches were prepared as described in chapter 3, including A-rice starch+lysine 6.0%, B-rice starch+stearic acid 1.0%+lysine 6.0%, C-rice starch+stearic acid 0.4%+lysine 4.0%, D-rice starch+stearic acid 1.0%, E-rice starch+ stearic 0.4%, F-rice starch+palmitic acid 1.0%+lysine 6.0%, G-rice starch+linoleic acid 1.0%+lysine 6.0 %, H-raw rice starch, I-rice starch RVA pregelatinized, J-rice starch+palmitic acid 1.0%, K-rice starch+linoleic acid 1.0%, L-rice starch+stearic acid 1.0%+aspartic acid 6.0% and M rice starch+stearic acid 1.0%+ cysteine 6.0%. They were conditioned for their moisture contents by saturated NaCl solution for a week. The samples were deposited onto aluminum sample holders. X-ray diffraction was monitored by Siemen D5000 X-ray diffraction instrument with CuKa radiation. The following conditions were applied: 45KV, 40mA, scanning angle 2Θ from 5 ° to 40° at a scanning rate of 0.4°. Jade 7.0 software was used to analyze their relative crystallinity (RC), characteristic peak and peak intensity. Specifically, RC was relative to raw rice starch (H), that is, the crystalline area from treated starch samples divided by the crystalline area from raw rice starch. The crystalline fraction from the XRD diffractogram was the sum of each peak area, meaning area between each peak and a tail-to-tail baseline; the amorphous fraction was not considered for RC calculation; its value was the area between a tail-to-tail baseline of each peak to a flat horizon baseline (Hayakawa et al. 1997).

### 5.2.3 Influence of added glycine and stearic acid to rice starch pasting with or without pH 10 adjustment

Previous experiments showed inhibited starch swelling and pasting when 1.0% stearic acid and 6.0% lysine were added. In order to check the role that amino acids played in the change of starch pasting properties and whether it is related with ionic nature of amino acids, glycine was first selected to replace lysine for effects on starch RVA

performance. Starches with added glycine 6.0% and stearic acid 1.0% were tested with and without pH adjustment. NaOH was used to adjust the pH of the starch solution to pH 10 before RVA treatment. The same RVA treatment as chapter 3 was conducted. The following parameters were recorded: The pasting temperature (PT), peak viscosity (PV), minimum viscosity (MV), final viscosity (FV), and peak time (PTime). Total setback (TSB) and breakdown (BKD) were calculated as TSB=FV-MV; BKD=PV-MV. All treatments were done in triplicate.

### 5.2.4 Determination of nitrogen

After RVA treatment, selective sample gels were washed by distilled water of five times the volume. After addition of water, a starch gel turned into liquid of low viscosity. It was then vortexed to get more homogeneous starch dissolution and centrifuged at 3000 rpm. The same procedure was done five times to wash away any possible amino acids or protein attached to starch molecules. For the last centrifuge, the supernatant was pipeted away and the precipitate was freeze-dried. The nitrogen contents of freeze-dried samples were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Samples for determination include starches with added lysine 6.0%; with added stearic acid 1.0% and lysine 4.0%; with added stearic 1.0% and lysine 2.0%; with added glycine 6.0%; and with added stearic acid 1.0% and glycine 6.0%. Each treatment was done in duplicate.

## 5.3 RESULTS AND DISCUSSION

### 5.3.1 Microcopy observation

From Figure 5.1 and Figure 5.2, all samples lost birefringence and were stained deep by iodine. Amylose leaching out resulted in lumpy and fuzzy images after staining. Starch granules were swollen and deformed. Figure 5.1 showed how starches with additives were in different degrees of rupture after RVA heating treatment. In rice starch control (D1), swollen starch fragments can be observed, indicating rupture of starch granules and development of starch gels. These starch fragments became even more cloudy in rice starch with added 6.0 % lysine (D2), suggesting more amylose leached out and was stained by iodine. This was consistent with the result of escalated breakdown of starch with lysine added during the RVA test caused by rapid starch granule rupture. In rice starch with 1.0% stearic acid (D3) added, the starch fragment has shapes of starch granules with more clarity than starch with added 6.0% lysine. It can be explained by a lower degree of starch granule rupture. In starch with 1.0% stearic acid and 6.0% lysine (D4) added, intact swollen starch granules were obviously found; some starch granules even moved around during the observation. This serves as strong evidence that by the same other treatments,

addition of both 1.0% stearic acid and 6.0% lysine can inhibit starch pasting by keeping swollen starch granules structure from rupturing.

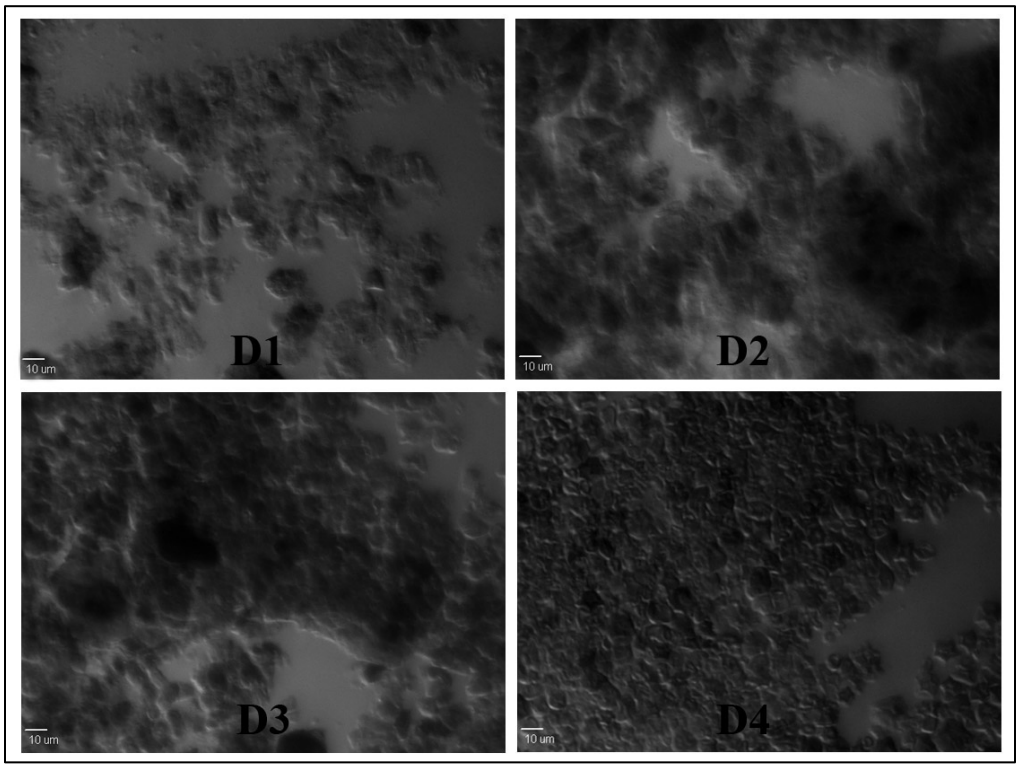


Figure 5.1 Starch solutions (without heating) under microscope observation. All were pre-gelatized starches by RVA heating and were dissoved in cold water. D1 was rice starch control (freeze-dried); D2 was rice starch with added 6% lysine (freeze-dried); D3 was rice starch with added 1.0% stearic acid (freeze-dried); D4 was rice starch with added both 6% lysine and 1.0% stearic acid (freeze-dried).

To see how stable the treated starch granule was, the above starch solutions were heated at 90°C for 20 min and observed for their shapes (Figure 5.2). Rice starch heated (H1) and rice starch heated with either lysine or stearic acid (H2 and H3) added displayed more amylose leaching, giving more blurred and fuzzier starch granule shape than unheated ones, as heating provides more energy to let starch swell and paste. Strikingly, the shape of rice starch granule with both stearic acid 1.0% and lysine 6.0% (H4) added remained intact. It suggested that this starch sample is heat-resistant and there must be certain forces preventing starch from further swelling and pasting. The reduced peak viscosity found in starch with 1.0% stearic acid and 6.0% lysine added was caused by inhibited starch swelling, rather than starch hydrolysis.

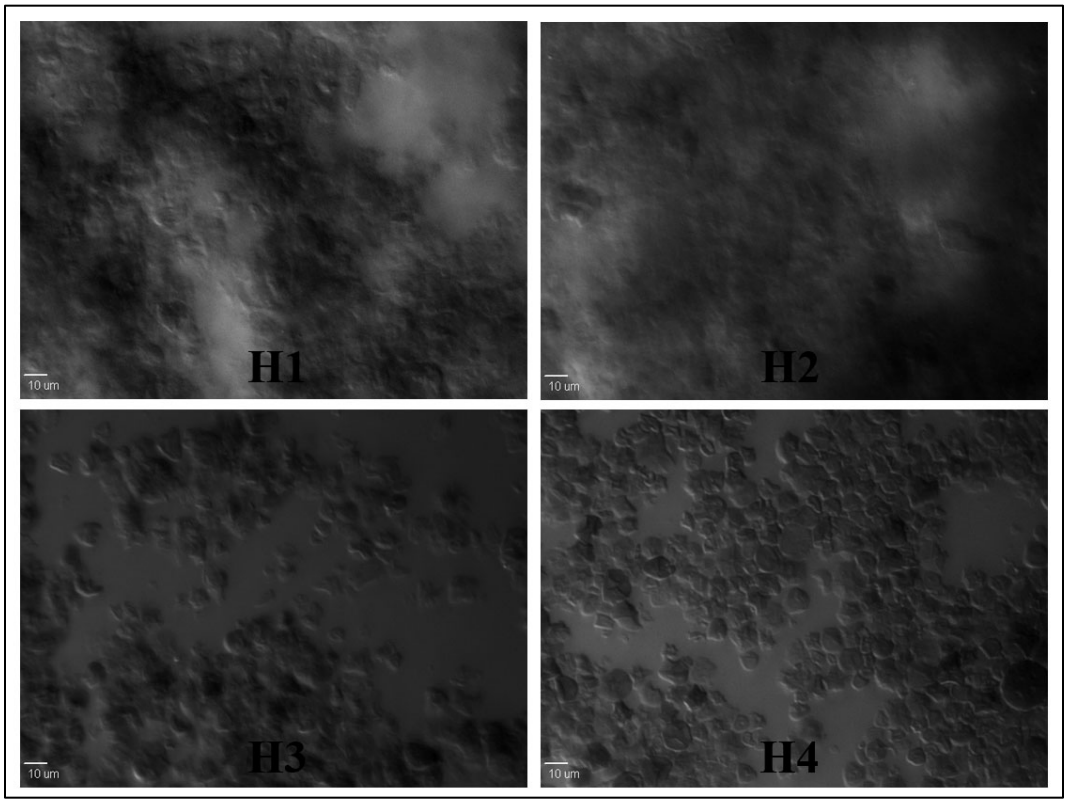


Figure 5.2 Starch solutions (with heating) under microscope observation. All were pre-gelatized starches by RVA heating and were dissoved in hot water (90°C for 20min). H1 was rice starch control (heated); H2 was rice starch with added 6% lysine (heated); H3 was rice starch with added 1.0% stearic acid (heated); H4 was rice starch with added both 6% lysine and 1.0% stearic acid (heated).

### 5.3.2 X-ray diffraction

Raw rice starch showed characteristic peaks for A type crystallinity and was assumed as 100% crystallinity (Table 5.3). Pregelatinzied rice starch obtained from RVA treatment showed a strong peak at 2 Θ of 19.8° and a weak peak at 12.7°, which corresponds to d-spacing of 4.4 Å and 6.8 Å (Figure 5.4). It thus validates a V-pattern crystalline structure formed between amylose and lipids in rice starch (Bhatnagar and Hanna 1994, Godet et al. 1995). The disappearance of all characteristic peaks of A-type cereal starch illustrated complete gelatinization of starch sample after RVA treatment, which agrees with its DSC curve of a missing gelatinization peak.

Addition of lysine caused disappearance of the 6.8 Å peak (12.7°) in RVA treated rice starch (Figure 5.5). This can be explained by more complete starch granule rupture promoted by lysine, as its RVA curve manifested increased starch breakdown; the starch-lipid complex in native starch granule may also be impaired by RVA heating.

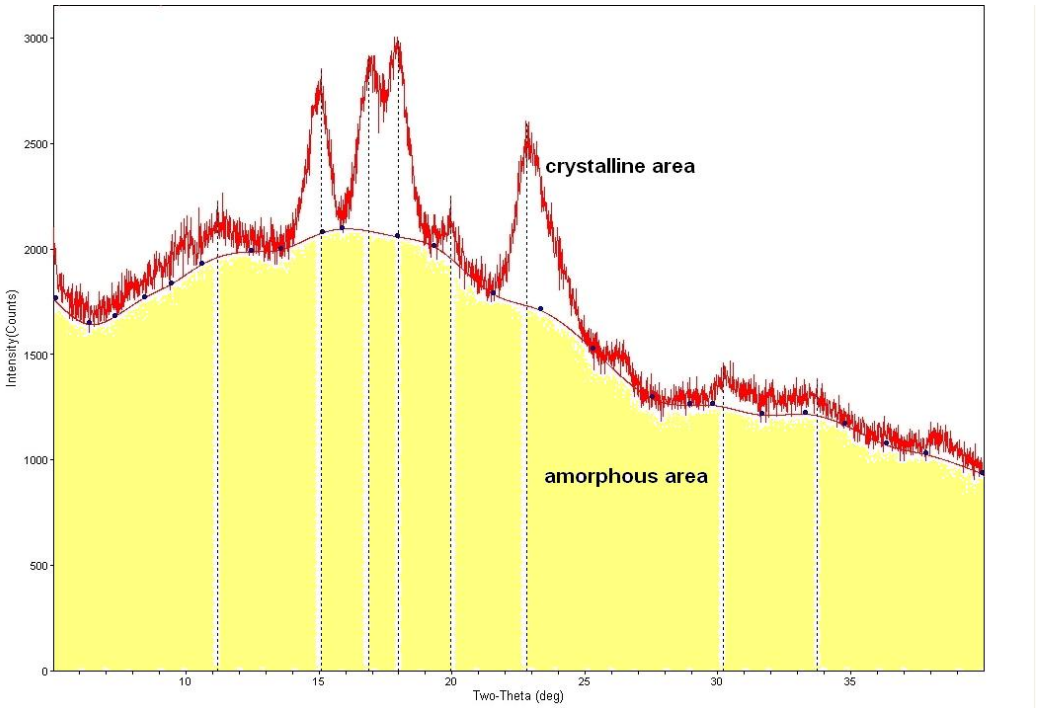


Figure 5.3 XRD pattern of raw rice starch and calculation of crystalline area

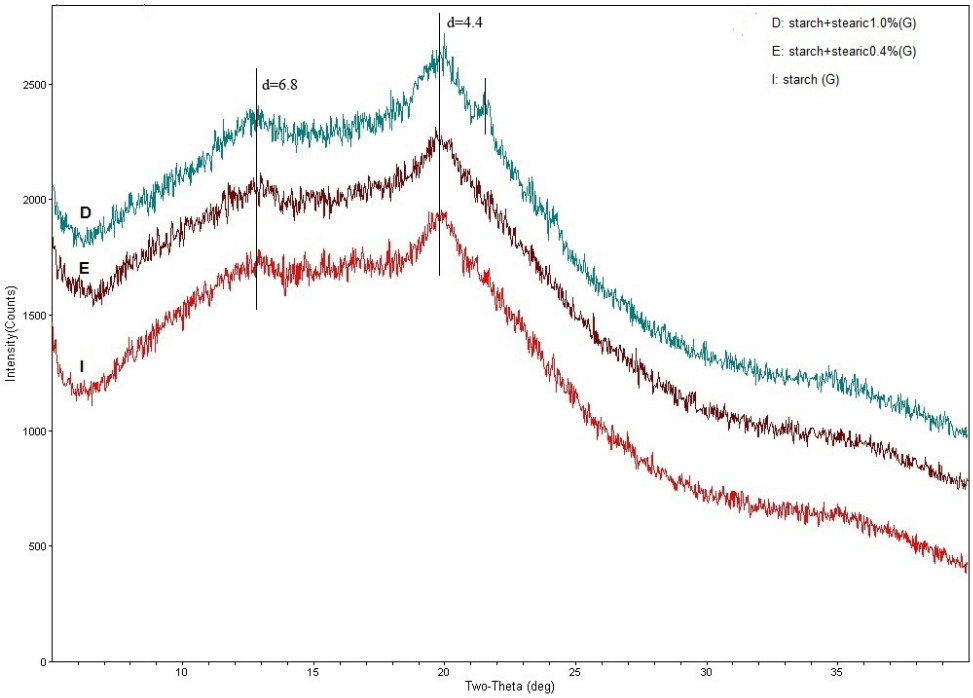


Figure 5.4 XRD patterns for starches with added 0.4% and 1.0% stearic acid. Note extra peak for stearic acid aggregate at 2Θ of 21.5° was found when the concentration of stearic acid reached to 1.0%.

Addition of palmtic acid, stearic acid, and linoleic acid gave the same amylose-lipid V complex and their RC increased more or less, compared to RVA treated starch without additive (Table 5.3). For addition of palmtic acid 1.0%, both RC and the intensity of peak at 6.8 Å (corresponds to 12.7°) were strengthened obviously (Figure 5.6). Correspondingly, this is explained by an increased amount of amylose-lipid V complex by the addition of fatty acids, which is also observed in its DSC curve. For addition of stearic acid, the higher the concentration of stearic acid added, the larger the RC obtained. Interestingly, extra peaks for stearic acid addition at 2 Θ of 21.5° were found (Figure 5.4), which is identified as the crystalline pattern of stearic acid aggregates (Tang and Copeland 2007). When Tang and Copeland did their experiment on starch lipid complexes, they used RVA pastes from wheat starch with added stearic acid. They found that instead of complexing with starch, lipid with low water solubility tend to self-associate when its concentration was above certain level. The XRD pattern in this study reflects the similar phenomenon that should be taken into caution when studying starch with added fatty acids of high concentration. The critical concentration may vary with starches of different sources or types. Addition of linoleic acid at 1.0%, not only increased V complex peak intensity at 4.4 Å was observed, but a B type starch pattern at 5.2 Å (correspond to 17.0°) also appeared. This suggests the existence of retrograded starch, which is also consistent with its retrogradation peak in DSC curve (chapter 4). This may be cause by inappropriate freezing storage of starch, because temperature fluctuation and slow freezing rate would also contribute to formation of retrograded starch (Lu et al. 1997).

Addition of both fatty acids and lysine increased the amount of amylose-lipid V complexes, compared to addition of fatty acids alone (Figure 5.5, Figure 5.6 and Figure 5.7). The function of lysine in promoting the formation of amylose-lipid complex is also validated in chapter 4 by DSC analysis when escalated enthalpy for melting amylose-lipid complex was observed, including amylose with stearic acid, palmitic acid and linoleic acid. Furthermore, without pH adjustment, lysine seems to be the only amino acid that promotes the formation of amylose-lipid complexes, compared to the addition of aspartic acid and cysteine for starch XRD pattern (Figure 5.8, Table 5.2). It is not clear whether it is caused by the special amine group of lysine related reaction or electronic force by pH of lysine solution. For further study, it is necessary to check the effects of other amino acids under pH adjustment in pasting viscosity and amylose-lipid complex formation.

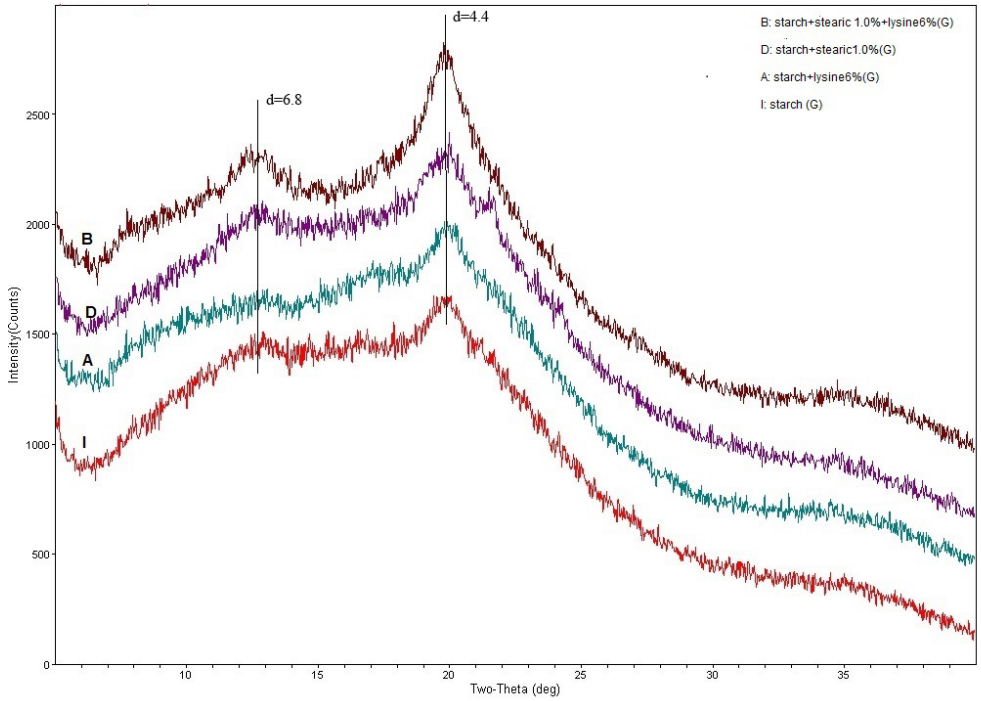


Figure 5.5 XRD patterns for starches with added 1.0% stearic acid and 6.0% lysine. The combination of stearic acid and lysine promoted the formation of amylose-lipid V complex.

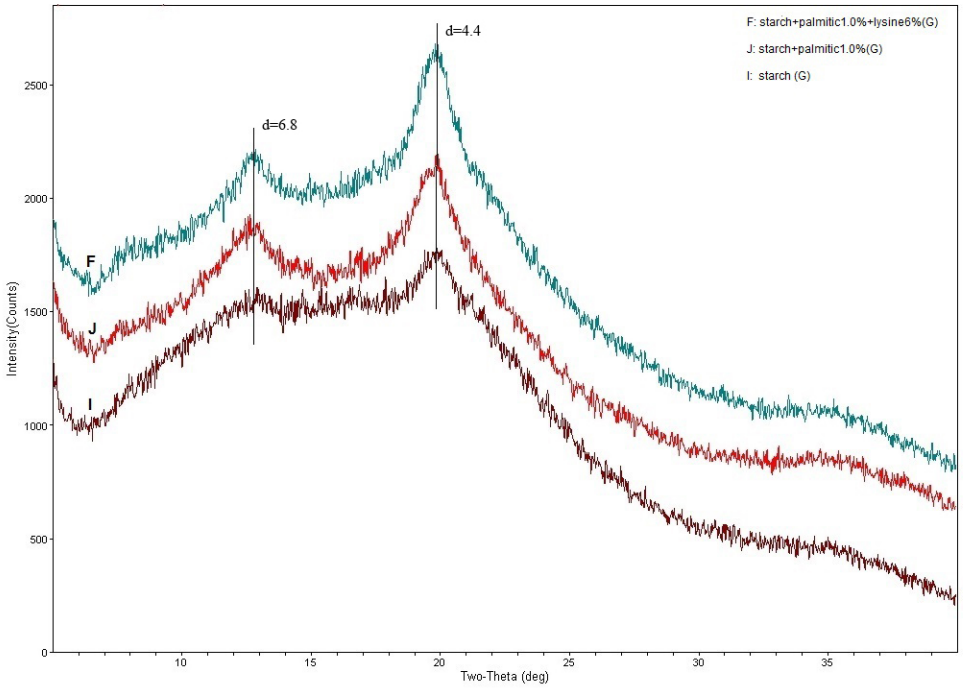


Figure 5.6 XRD patterns for starches with added 1.0% palmtic acid and 6.0% lysine. The combination of palmitic acid and lysine promoted the formation of amylose-lipid V complex.

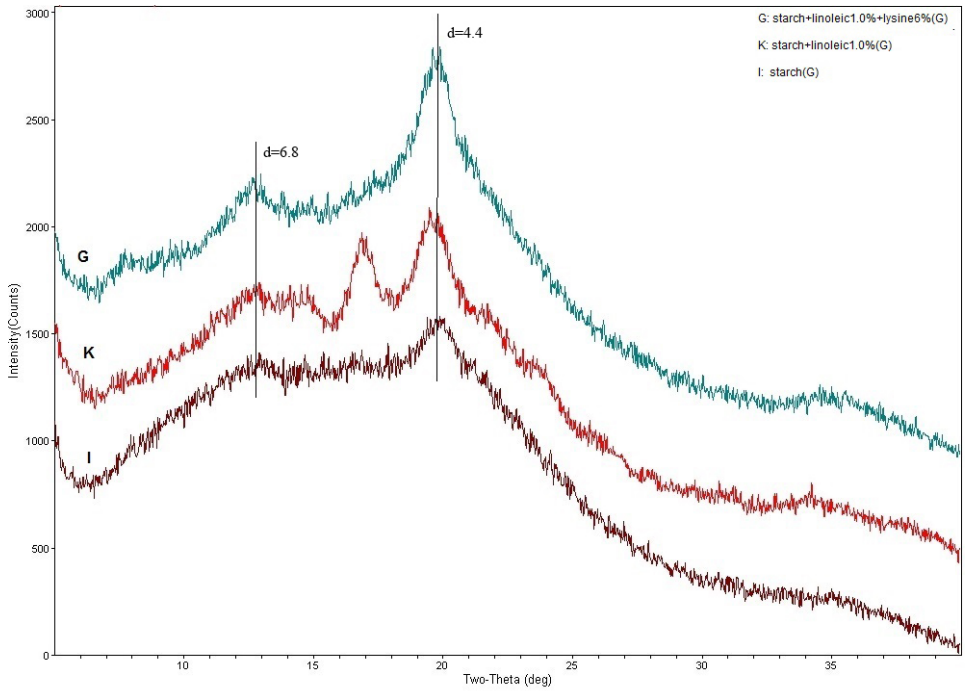


Figure 5.7 XRD patterns for starches with added 1.0% linoleic acid and 6.0% lysine. The combination of linoleic acid and lysine promoted the formation of amylose-lipid V complex. For added linoleic acid alone, the peak at 2Θ of 17° (d=5.2 Å) was caused by retrograded starch.

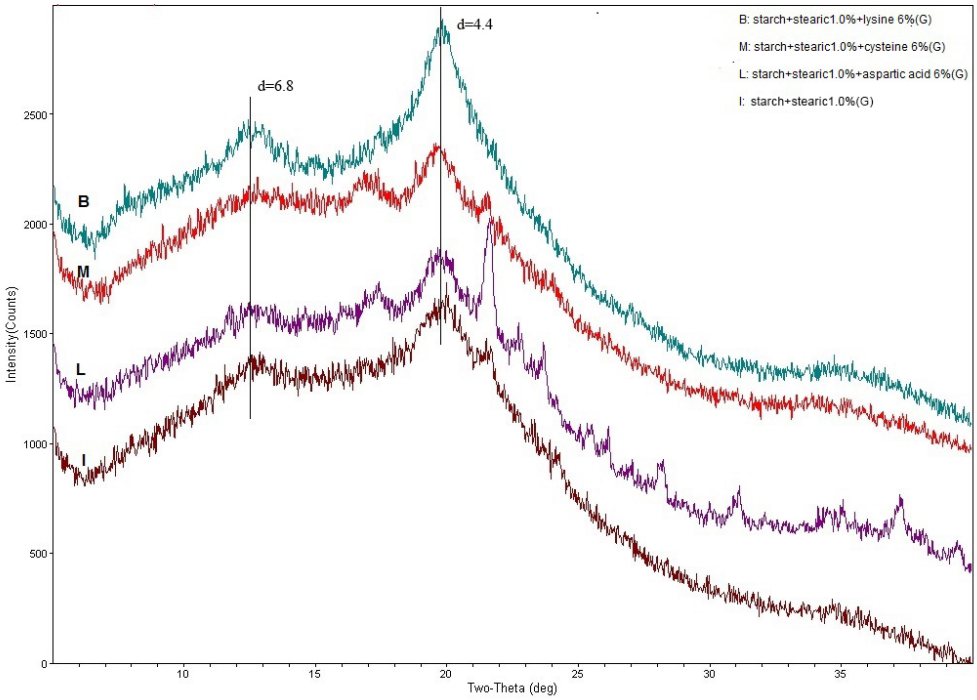


Figure 5.8 XRD patterns for starches with added 1.0% stearic acid and 6.0% amino acids. The combination of stearic acid and lysine promoted the formation of amylose-lipid V complex. Note for added stearic acid and aspartic acid, the curve noise was high; extra peaks for stearic acid aggregate at 2Θ of 21.5° were found when cysteine and aspartic acid were present.

Table 5.2 Relative crystallinity (RC) of starch samples by XRD

| # | Additives (RVA treated, unless specified) | Area of crystallinity | Relative crystallinity (RC) |
|---|---|---|---|
| A | Lysine 6.0% | 102928 | 22.47% |
| B | Stearic acid 1.0%+lysine 6.0% | 212330 | 46.35% |
| C | Stearic acid 0.4%+lysine 4.0% | 158154 | 34.52% |
| D | Stearic acid 1.0% | 130374 | 28.46% |
| E | Stearic acid 0.4% | 118476 | 25.86% |
| F | Palmitic acid 1.0%+lysine 6.0% | 201685 | 44.02% |
| G | Linoleic acid 1.0%+lysine 6.0% | 211727 | 46.22% |
| H | N/A (raw rice starch) | 458132 | 100.00% |
| I | N/A | 123589 | 26.98% |
| G | Palmitic acid 1.0% | 165024 | 36.02% |
| K | Linoleic acid 1.0% | 129478 | 28.26% |
| L | Stearic acid 1.0%+aspartic acid 6.0% | 120708 | 26.35% |
| M | Stearic acid 1.0%+cysteine 6.0% | 121722 | 26.57% |

### 5.3.3 Possible mechanism: influence of added glycine/other amino acids and stearic acid to rice starch pasting at neutral and basic pH

Previous experiments showed the role of lysine in inhibiting starch pasting when stearic acid was present. To investigate the mechanism, different amino acids additives were tested to check their RVA performance. Glycine as a polar non-charged amino acid was added into rice starch solution along with stearic acid for RVA preparation, with or without pH adjustment. Without pH adjustment, the glycine solution had a pH of 6.4; with NaOH pH adjustment, the glycine solution had a pH of 10.

Similar to lysine, addition of both 6.0% glycine and 1.0% stearic acid at pH10 showed inhibited starch pasting, compared to the addition of 6.0% glycine at pH 10 or 1.0% stearic acid at pH10, separately (Figure 5.9 and Table 5.3). The trough of its RVA curve disappeared and its peak viscosity was only 10.6% of its starch control (without additives). Meanwhile, its time to reach peak viscosity was postponed for another 1.5 min (from 6.75 min to 7.89min), compared to its starch control (without additives).

To see if the above phenomena happened for lysine and glycine only, other types of amino acids were also checked. Surprisingly, it turned out that at pH 10, not only lysine, but glycine, cysteine and glutamine all demonstrated inhibited starch pasting and at degrees even higher than that of lysine (Figure 5.10). Meanwhile, addition of 6.0% glycine and 1.0% stearic acid without pH adjustment was also prepared for starch RVA testing, which failed to demonstrate the function of inhibiting starch pasting. Therefore, pH is a key factor contributing to the interaction among fatty acid, starch and amino acids.

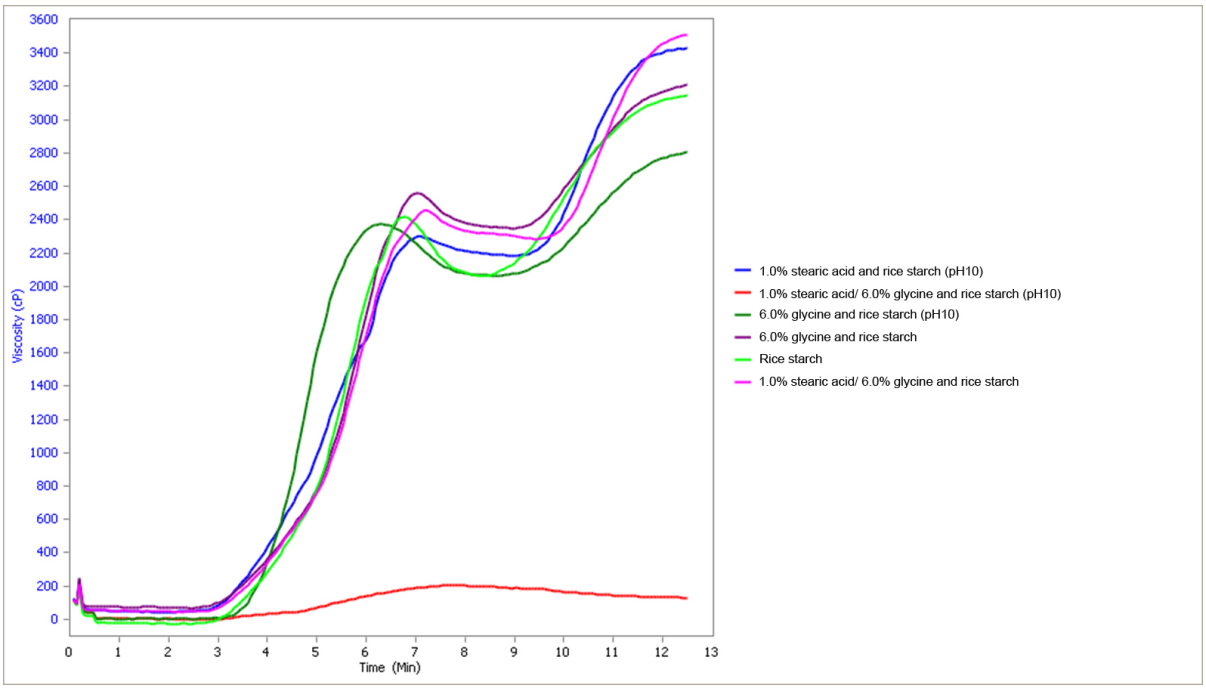


Figure 5.9 RVA curves of rice starch with added 1.0 % stearic acid and 6% glycine, with or without pH adjustment

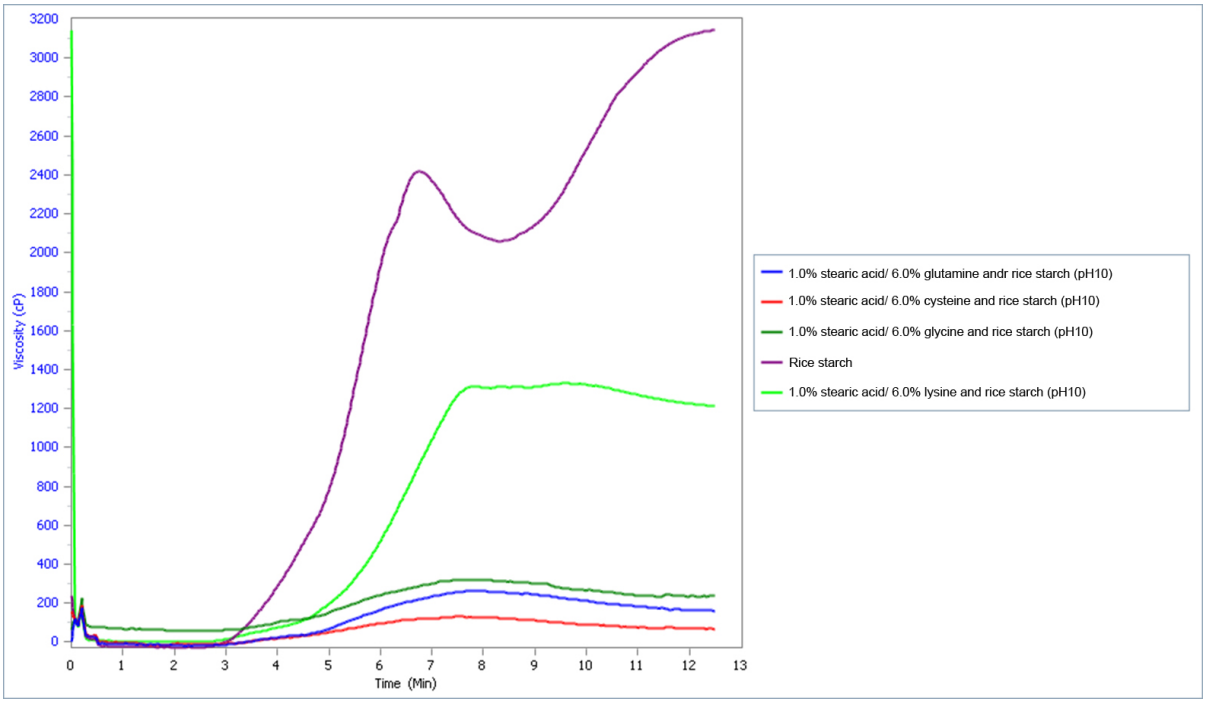


Figure 5.10 RVA curves of rice starch added with 1.0 % stearic acid and 6% amino acids, with NaOH adjustment at pH10

Table 5.3 Effects of added stearic acid (at 1.0%) and glycine (at 6.0%) on pasting properties of rice starch[d]

| pH | Additives | PV (cp)[a] | MV (cp)[a] | BKD (cp)[a] | FV (cp)[a] | Ptime (min)[a] | Ptemp (°C)[a] | TSB (cp)[a] |
|---|---|---|---|---|---|---|---|---|
| 10 | N/A(control) | 2389±56.47a | 2046.33±29.94b | 342.67±26.58a | 3121.33±49.22b | 6.75±0.04b | 84.73±2.09a | 1075±19.29a |
| | Stearic 1.0%[b] | 2302.33±14.64a | 2154.33±24.09a | 148±28.62b | 3418.67±26.27a | 7.04±0.02b | 80.27±0.2a | 1264.33±27.54b |
| | Gly 6.0%[b] | 2379.33±32.72a | 2093±30.51ab | 286.33±53.35a | 2882.33±134.86c | 6.33±0.18c | 80.48±0.51b | 789.33±109.45c |
| | Stearic1.0%+Gly6%[b] | 253±61.26b | n/a[c] | n/a[c] | 175±57.89d | 7.89±0.2a | n/a[c] | n/a[c] |

a. PV, peak viscosity; MV, minimum viscosity; BKD, breakdown; FV, final viscosity; PT, pasting temperature; Ptime, peak time; TSB, total setback
b. All levels are based on starch dry weight
c. n/a because the values disappeared in the RVA curve
d. Values followed by the same letter in the same column are not significantly different (P >0.05)

Net charge of amino acids has been reported for regulating the gelatinization and pasting characteristics of potato starch; the decrease in the peak viscosity of potato starch followed a linear regression equation with the absolute value of the amount of net charge of amino acids (Ito, Hattori et al. 2006). In the present study, because a three-component system is involved, a mathematical model for relationship between starch pasting and level/net charges of additives would be a complicated one. There may be a similar mechanism btweenn rice starch, fatty acid and amino acids and that of potato starch and amino acids. The pI for lysine, cysteine and glutamine are 9.74, 5.07 and 5.65, so lysine's water solution, which is of pH 10, has the lowest charge. The weakened decrease in peak viscosity for added lysine at pH10 may be explained by its loss of negative charge, compared to added cysteine or glutamine at pH10. But different from the effects of amino acids on potato starch, stearic acid was an integral part for this interaction in rice starch.

**5.3.4 Determination of nitrogen**

Nitrogen contents of selected sample were determined to check if any covalent bonds formed for starch samples, especially those that showed inhibited swelling and pasting viscosity (Table 5.4). If any covalent bonds were developed during RVA heating, for example, carboxyl-amine bonds, the starch would be cross-linked, contributing to inhibited starch granule swelling. As a result, lysine would be attached to starch molecular and the nitrogen contents of starch samples would increase significantly. However, from the nitrogen contents in Table 5.3, we didn't find any lysine-attached starch after water rinsing. On the country, some starch samples even showed reduced nitrogen content. For starch with added stearic acid 1.0% and lysine 6.0%, their nitrogen contents were about 61% and 68% of control starch (without additives). Therefore we can infer that covalent crosslinking was not formed during the process; the reason for inhibited starch swelling and pasting must be something else.

Table 5.4 Nitrogen content of selected starch samples

| Additives | Nitrogen content % |
|---|---|
| N/A (control starch) | 0.135 |
| Lysine 6.0% | 0.146 |
| Glycine 6.0% | 0.138 |
| Stearic acid 1.0% +lysine 2.0% | 0.123 |
| Stearic acid 1.0% +lysine 4.0% | 0.127 |
| Stearic acid 1.0% +lysine 6.0% | 0.083 |
| Stearic acid 1.0% + glycine6.0% (pH 10) | 0.092 |

In chapter 3, we found stearic acid and oleic acid as major functional molecules that contribute to the inhibited starch swelling and pasting, while lysine is an integral part of the effect. In this chapter, we found that pH or charge of amino acids was crucial to the interaction among stearic acid, amino acids and rice starch. In theory, at basic pH condition (pH 10), the hydroxyl groups of starch molecules tend to be ionized and develop into alkoxide ions; the hydrogen bonding between hydroxyl groups of starch chains is interfered and the starch molecules become more flexible (Gray and BeMiller 2005). Indeed, increased pH was often found in production of cross-linked starch (Yangsheng and Seib1990, van Warners et al. 1994, Han and BeMiller 2005) because of increased flexibility of starch chain in a basic environment. Amylose-lipid complex would be promoted because of the hydrophilic environment in the negatively charged amino acid solution and increased flexibility of starch chains. Starch inside is filled with hydrophobic and non-polar molecules and outside is hydrophilic and polar molecules. The driving force for fatty acid to complex with amylose in presence of amino acids would be a tendency to minimize its interaction with charged amino acids.

The influence of pH indicates that there should be certain electrostatic attraction among those compounds leading to inhibited starch swelling. Genyi Zhang et al. (2010) proposed that fatty acid bridged the self-assembly of a three-component complex consisting of amylose, protein and free fatty acids. Correspondingly, if fatty acid bridges a three-component complex consisting of amylose, amino acids and stearic acid, it is possible that the complex result in inhibited starch swelling and pasting. However, it is impossible for fatty acid binding amino acid in a basic environment because both would be negatively charged. Also, Ito et al (2006) studied that binding of amino acids to starch chains but did not illustrate how the electrostatic attraction was formed. Because starch won't be charged, another possibility is that the electrostatic attraction may be caused by negatively charged amino acids and some surface compounds of starch granule that is positively charged, such as protein. It is unclear how this is related to stearic acid, though. The surface compound may be the reason that causes different pasting performance between corn starch and rice starch when same additives were added.

Debet and Gidley (2007) mentioned three hypothesis to explain the starch ghost formation, including a surface film around granules rich in protein and lipid inhibiting starch granule expansion; cross-linking of proteins inside of the granule; and cross-linking of amylose/or long amylopectin branches. Although their designed experiments ruled out the first two mechanisms, they could not validate the presence cross-linking among starch molecules by existing analytical instruments. While the present study cannot answer the whole question, it provides a new perspective to

view the function of protein and lipids in influencing starch pasting and how starches with added protein, amino acid and lipid perform uniquely during starch cooking. Not only big protein molecule, its hydrolysate amino acids also play important roles in changing pasting characteristics of rice starch.

## 5.4 CONCLUSION

Based on characterization of starch samples, especially rice starch with added stearic acid 1.0% and lysine 6% added, in terms of its pasting, thermal, retrogradation, digestibility, morphology and XRD patterns, the interaction among rice starch, stearic acid and lysine developed by RVA heating (from 50°C to 90°C at 12°C/min) was confirmed. A small amount of complex can inhibit starch granule swelling and pasting significantly. The existence of lysine promoted the formation of amylose-lipid V complex during starch cooking, including palmitic acid, stearic acid and linoleic acids. Negatively charge amino acids, like glycine, cysteine and glutamine at pH 10, were found to inhibited rice starch pasting greatly when stearic acid was present. The interaction among rice starch, amino acids and fatty acids was unlikely to be caused by covalent bonding, but electrostatic attraction instead. The altering of starch functionality through the use of simple additives offers great marketing potential for clean label starch as a food ingredient.

## 5.5 REFERENCES


Bhatnagar, S. and M. A. Hanna (1994). "Amylose-lipid complex formation during single-screw extrusion of various corn starches." Cereal Chemistry **71**(6): 582-586.

Debet, M. R. and M. J. Gidley (2007). "Why do gelatinized starch granules not dissolve completely? Roles for amylose, protein, and lipid in granule “ghost” integrity." Journal of agricultural and food chemistry **55**(12): 4752-4760.

Godet, M., H. Bizot and A. Buléon (1995). "Crystallization of amylose-fatty acid complexes prepared with different amylose chain lengths." Carbohydrate Polymers **27**(1): 47-52.

Gray, J. A. and J. N. BeMiller (2005). "Influence of reaction conditions on the location of reactions in waxy maize starch granules reacted with a propylene oxide analog at low substitution levels." Carbohydrate polymers **60**(2): 147-162.

Han, J.-A. and J. N. BeMiller (2005). "Rate of Hydroxypropylation of Starches as a Function of Reaction Time." Starch - Stärke **57**(9): 395-404.

Hayakawa, K., K. Tanaka, T. Nakamura, S. Endo and T. Hoshino (1997). "Quality characteristics of waxy hexaploid wheat (Triticum aestivum L.): Properties of starch gelatinization and retrogradation." Cereal Chemistry **74**(5): 576-580.

Ito, A., M. Hattori, T. Yoshida and K. Takahashi (2006). "Contribution of the net charge to the regulatory effects of amino acids and poly (L-lysine) on the gelatinization behavior of potato starch granules." Bioscience, biotechnology, and biochemistry **70**(1): 76-85.

Krog, N. (1971). "Amylose complexing effect of food grade emulsifiers." Starch-Stärke **23**(6): 206-210.

Lu, T.-j., J.-I. Jane and P. L. Keeling (1997). "Temperature effect on retrogradation rate and crystalline structure of amylose." Carbohydrate polymers **33**(1): 19-26.

Obanni, M. and J. BeMiller (1995). "Identification of starch from various maize endosperm mutants via ghost structures." Cereal Chemistry **72**(5): 436-442.

Tang, M. C. and L. Copeland (2007). "Analysis of complexes between lipids and wheat starch." Carbohydrate polymers **67**(1): 80-85.

van Warners, A., E. J. Stamhuis and A. A. Beenackers (1994). "Kinetics of the hydroxyethylation of starch in alkaline salt-containing aqueous slurries." Industrial & engineering chemistry research **33**(4): 981-992.

Yang, W., M. Hattori, T. Kawaguchi and K. Takahashi (1998). "Properties of starches conjugated with lysine and poly (lysine) by the Maillard reaction." Journal of agricultural and food chemistry **46**(2): 442-445.

Yangsheng, W. and A. SEIB (1990). "Acetylated and hydroxypropylated and distarchphosphates from waxy barly: Paste Properties and Free-Thaw Stability." Cereal Chemistry **67**(2): 202-208.

Zhang, G., M. Maladen, O. H. Campanella and B. R. Hamaker (2010). "Free fatty acids electronically bridge the self-assembly of a three-component nanocomplex consisting of amylose, protein, and free fatty acids." Journal of Agricultural and Food Chemistry **58**(16): 9164-9170.

Zobel, H. (1988). "Molecules to granules: a comprehensive starch review." Starch-Stärke **40**(2): 44-50.

# CHAPTER 6
# GENERAL CONCLUSIONS AND RECOMMENDATIONS

Effects of both added fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid) and amino acids (leucine, aspartic acid, lysine, cysteine and tyrosine) added were studied in rice starches regarding their pasting, thermal, retrogradation, enzyme digestibility and crystalline structure. Substantial low peak viscosity and delayed pasting time of starch paste was found when rice starch was combined with both 1.0% stearic acid and 6.0% lysine; within 0.6%-1.0% of stearic acid and 6.0% of lysine addition, there was a linear regression relationship between peak viscosity and the level of stearic acid.

Using starch with both 1.0% stearic acid and 6.0% lysine added as a model, when stored 10 days under refrigeration, the above starch was found to have lower retrogradation than starch with added stearic acid only or starch without additives. This finding was validated by both DSC thermal analysis and the syneresis test. Complex index measurement and X-ray diffraction indicated that lysine increased the formation of amylose-lipid complex when fatty acids were present. By observing starch samples under differential interference contrast microscopy, starch combined with 1.0% stearic acid and 6.0% lysine was found to have inhibited swelling under heating, compared to control starch without additive or with 1.0% stearic acid only. Therefore a three-way interaction among starch, lysine and stearic acid was confirmed (100:6:1, w/w/w) to inhibit starch swelling.

Because no increased yield of resistant starch and increased nitrogen content was found, this inhibited starch swelling is unlikely dominated by covalent bonds by starches molecules. Later, amino acids with charge, like glycine, cysteine and glutamine at pH 10, were also found to inhibit rice starch pasting greatly when 1.0% stearic acid was present. This suggests that electrostatic interaction plays a important role in this three-way interaction.

By water rinsing starch gel containing 1.0% stearic acid and 6.0% glycine (pH10) or 1.0% stearic acid and 6.0% lysine (pH 10), a decreased amount of nitrogen was observed, compared to starch without additives and starch with added amino acids only. This implies the existence of a nitrogen-containing water-soluble complex being washed way. In further study, more studies can be done to confirm the existence of this complex.

Corn starch was also used to test its pasting properties when combined with both amino acids and fatty acids. However, unlike rice starch, combination of stearic acid and lysine did not cause inhibited starch swelling. Therefore, another perspective to investigate this three-component interaction is to test the effects of amino acids

and fatty acids using starch of different physiochemical characteristics, like amylose/amylopectin ratio, surface compound removal and other types of starch.

Another interesting thing to delve into is to see the effects of different types of lipids used in altering starch properties. While stearic acid as the basic molecule in lipids interacted with amino acids and starch, lipids with different headgroups can also be checked for further study.

In terms of practical application, the study provides great marketing potential for clean label starch as a food ingredient by altering starch properties with simple additives. With the above added stearic acid and amino acids, starch exhibited delayed pasting, inhibited starch swelling and retrogradation, which may be used for food that requires high shear, acid processing, prolonged heating and long shelf life under refrigeration or frozen storage. Also the fact that addition of lysine increased the amount of amylose-lipid complex offers a possbility of using starch with additives for controlled release of fatty acids (e.g. ω -3 fatty acids).

# APPENDIX 1 SAS CODE FOR THE ANOVA OF RVA DATA OF STARCHES WITH ADDITIVES

```
dm 'log;clear;output;clear';
data one;
input Rep$ Peak Trough BKD FV PTime PTemp Tsetback Trt;
datalines;
;
proc means mean std maxdec=2;
class Trt;
var Peak Trough BKD FV PTime PTemp Tsetback=Trt;
run;
proc glm;
class Trt;
model Peak Trough BKD FV SBK PTime PTemp Tsetback=Trt;
means Trt/tukey lines;
run;
```

## APPENDIX 2 SAS CODE FOR THE ANOVA TEST OF RS/SDS DATA

```
*For TDF method*

dm 'log;clear;output clear';
title 'TDF';
ods rtf file='E:\SAS\TDF.rtf';

data RS;
input Additive$ RS@@;
cards;
;
proc glm;
class additive;
model RS=additive;
means additive/tukey lines;
run;
ods rtf close;


*For Megazyme*

dm 'log;clear;output clear';
title 'Megazyme';
ods rtf file='E:\SAS\Megazyme.rtf';

data RS;
input Additive$ RS@@;
cards;
;
proc glm;
class additive;
model RS=additive;
means additive/tukey lines;
run;
ods rtf close;
```

```
*For SDS*

dm 'log;clear;output clear';
title 'SDSassay';
ods rtf file='E:\SAS\ SDSassay.rtf';

data SDS;
input Additive$ SDS@@;
cards;
;
proc glm;
class additive;
model SDS=additive;
means additive/tukey lines;
run;
ods rtf close;
```

# THE VITA

Yu Jiang was born in Oct. 1986 in Jiangsu Province in People's Republic of China. She entered China Agricultural University in Beijing and obtained her Bachelor degree of Science in food quality and safety in 2007. In Aug. 2009, she started her doctoral program in food science at Louisiana State University in Baton Rouge with Economic Development Assistantship. She is expected to receive her degree of Doctor of Philosophy in Dec. 2013.