2009

Modification of rice starch properties by addition of amino acids at various pH levels

Rosaly Vallejo Manaois
Louisiana State University and Agricultural and Mechanical College, rhio7@yahoo.com

MODIFICATION OF RICE STARCH PROPERTIES BY ADDITION OF AMINO ACIDS AT VARIOUS pH LEVELS

A Thesis

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
Master of Science

in

The Department of Food Science

by
Rosaly V. Manaois
B.S., Central Luzon State University, 2001
August 2009

ACKNOWLEDGMENTS

My sincere gratitude go to the following, without whom my Masters study and this work will not come into fruition:

Dr. Joan M. King, my advisor, for her expertise, time, patience, motivation and continuous support throughout my study and research; her making sure that I understand the concepts well and her putting importance on the fact that I gained more knowledge during the whole research process I deeply appreciate;

My committee members Dr. John W. Finley and Dr. Zhimin Xu, for their constructive comments and suggestions, which stimulated me to think more critically and encouraged me to further better my work;

My professors: Dr. Witoon Prinyawiwatkul, for sharing his expertise in statistics for the analysis and interpretation of my data, Dr. Subramaniam Sathivel, for providing me opportunity to use a rheometer and learn more about rheology, Dr. Lucina Lampila, for imparting her knowledge on phosphates, and Dr. Paul Wilson and Dr. Jack Losso, for assisting me on lyophilization and for volunteering very helpful information;

Dr. Alfredo Prudente Jr. and Jonathan Futch, my colleagues in the laboratory, for all their help, support and friendship, Huaixia (Eva) Yin, for her kind assistance in doing the rheology test, and Phantipha Charoenthaikij, for her valuable insights on starch analysis;

The Ford Foundation-International Fellowships Program, for allowing me to realize my aspiration to study abroad through the generous support they provided;

My family: my parents Rogelio and Fely, and my sisters Fely Rose, Fely Reina, and Fely II, for their moral and spiritual guidance, support and love;

And my Creator, for everything.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Amino acids were previously found to modify starch functionalities and potentially increase starch utilization. The effect of amino acids at different pH levels on the pasting properties, thermal characteristics, and resistant starch (RS) formation of rice starch was investigated. Prior to the analyses, pretreatment of starch was done by adding amino acid (aspartic acid, leucine, lysine and tyrosine) at 6% dry weight basis and dispersing the mixture in distilled water, solutions adjusted with HCl and NaOH to pH 4, 7 and 10, and buffers of acetate, phosphate and carbonate at the same pH levels, respectively. Samples in HCl/NaOH solutions were mixed at room temperature and at 40 ± 2^{o}C. The slurries were stored at -80^{o}C and lyophilized.

Lysine and aspartic acid raised the breakdown (BD) and reduced the total setback (TSB) at all pHs using HCl/NaOH, with aspartic acid exhibiting the greater effect. Lysine shortened the pasting time (PTime) without affecting the peak temperature (PT) and increased the peak and conclusion temperatures with or without pH adjustment. Tyrosine in pH 10 solution reduced the PTime. In buffers, lowering of the peak viscosity, PTime and PT was observed, but was mainly attributed to the buffers. Heating at 40 ± 2^{o}C likewise decreased the paste viscosities and gelatinization temperatures, but raised the PTime and PT, with lysine having the most profound effect. Samples added with aspartic acid and leucine generally caused substantial increases in RS yields. No conclusive results on RS formation were obtained based on effect of charges. Therefore, charges in additives played an important role in altering pasting and thermal properties of rice starch, but not in controlling RS formation.

To determine effect of hydroxyl-containing amino acid, starch was added with tyrosine, gelatinized, and lyophilized. The sample in pH 10 solution (HCl/NaOH) had higher BD and TSB

than native starch. RS yields of gelatinized samples were negatively correlated to treatment in pH 10 solution. Compared to pretreated samples, gelatinized samples had higher paste viscosities and RS values.

In conclusion, amino acids in combination with pH treatments can be used to alter rice starch functionalities, and may consequently enhance formation of RS.

CHAPTER 1. INTRODUCTION

Starches have been used in the food industry for numerous applications. This is made possible by modification of native starch to improve its functional properties either by physical, such as heat or moisture treatments, or chemical means through etherification, esterification, cross-linking and grafting of starch (Wurzburg, 1986). Among the properties improved by these treatments are low shear resistance, thermal resistance, and high retrogradation potential (Hui et al., 2009). These properties, called functional characteristics, relate to the behavior of a starch product when subjected to various processing treatments, and determine the applications suitable for the starch.

Gelatinization refers to the process in which starch undergoes order-disorder transition with the application of heat in the presence of excess water. The gelatinization temperature of starch (GT), or the temperature at which 90% of the starch granules have swollen irreversibly in hot water (IRRI, 2006), is commonly determined using the Differential Scanning Calorimeter (DSC). DSC measures the gelatinization onset, peak, conclusion, and enthalpy. These thermal properties provide information on the energy required to disrupt molecular order and therefore are of particular importance to food processors who need to optimize heat input, cooking time and temperature, and reduce process cost (Bao and Bergman, 2004).

The process in which starch is further heated in water is called pasting, which is the formation of a viscous material comprised of leached amylose and disintegrated starch granules. Pasting properties reflect the cooking behavior of starch, such as water binding capacity, cooking stability, retrogradation potential, and pasting time and temperature. Generally, these properties are important indicators of final product quality (Newport Scientific, 1998).

Incorporation of amino acids to native starch was previously demonstrated to alter starch functional properties (Liang and King, 2002, Ito et al., 2004, Ito et al., 2006a, 2006b, Lockwood et al., 2008, An and King, 2009). Using starches of different botanical origins, these studies indicated that charged amino acids impact the pasting and gelatinization behavior of starches more than neutral ones. Lysine, when added to ozonated rice starch, reduced the water binding capacity and pasting time, and produced starch with better cooking stability and lower pasting viscosities. Lysine also lowered the enthalpy of amylose-lipid complexes (An, 2005), which also affect starch pasting properties (Zhou et al., 2002). In their study on sweet potato starch, Lockwood et al. (2008) reported that aspartic acid produced a starch with lower cooking stability and retrogradation potential, while lysine made a starch that is more resistant to shear during cooking. Lysine (Ito et al., 2004) and glutamic acid (Kinoshita et al., 2008) depressed the peak viscosity of potato starch. These studies all indicated that charged amino acids have an effect on gelatinization temperature of the starches (Liang and King, 2002, Ito et al., 2004, Ito et al., 2006a, 2006b, Kinoshita et al., 2008, Lockwood et al., 2008, An and King, 2009).

Alteration of functional properties of starch can decrease its digestibility due to the formation of resistant starch (RS). Processing and storage conditions that affect gelatinization and retrogradation were demonstrated to influence RS formation (Eerlingen et al., 1993, Eggum et al., 1993, Garcia-Alonso et al., 1999, Kim et al., 2006, Park et al., 2009). Chemical modifications, such as oxidation, dextrinization and cross-linking of starches were likewise shown to increase RS yields (Wolf et al., 1999). Growing interest in RS is due to its reported physiological benefits such as hypoglycemic and hypocholesterolemic effects and anticancer properties (Sajilata et al., 2006). Because it is indigestible by body enzymes, RS elicit no glycemic response. RS is fermented in the gut to form short chain fatty acids (SCFAs) such as

propionate which was shown to inhibit cholesterogenesis and lipogenesis in animals (Lopez et al., 2001). SCFAs are beneficial substrates for colonic epithelial cells, and thus, RS had been implicated in colorectal cancer mitigation (Niba and Niba, 2003). Hence, modification of starch may increase its utilization and at the same time lead to the production of novel food ingredients with health promoting properties. It is therefore worthwhile to investigate whether amino acid additives show an effect on starch functional properties and influence the RS formation of starch.

Effects of amino acids on functional properties and RS formation of rice starch was investigated by An in 2004. In her study, she observed a significant increase in RS yield in rice starches with added aspartic acid. Ito et al. (2004) noted that pH affects charges of amino acids, so they fixed the pH at 7 when they assessed the impact of amino acid net charge on the gelatinization of potato starch. Their study confirmed the findings of Liang and King (2002) regarding the strong effect of charged amino acids without pH treatments. Varying the pH levels would therefore provide more understanding of the contribution of amino acids on starch functionalities and potentially, RS formation.

This study primarily aimed to determine the effect of various amino acids in different pH systems on the pasting and thermal properties, and RS formation of rice starch. The study also investigated changes in these starch properties using a treatment procedure in which starch and amino acid mixtures were slurried in different hydrating mediums and dried prior to starch analysis. Lastly, it investigated for the first time the use of a hydroxyl group-containing amino acid (tyrosine), which was postulated to markedly change starch properties due to its hydrogen bonding capability.

CHAPTER 2. REVIEW OF RELATED LITERATURE

2.1 CARBOHYDRATE

2.1.1 Starch

Starch is the major dietary source of carbohydrates and is the most abundant storage polysaccharide in plants. It is present in high amounts in roots, tubers, cereal grains and legumes (Eerlingen and Delcour, 1995) and also occurs in fruit and vegetable tissues (McCleary et al., 2006). Starch is a polymer of glucose linked together by α-D-(1-4) and/or α-D-(1-6) glycosidic bonds. The starch granule mass comprises 70% amorphous regions, which consists of amylose and branching points of amylopectin molecules, and 30% crystalline, which is mainly composed of the outer chains of amylopectin (BeMiller, 2007, Eerlingen and Delcour, 1995, Perdon et al., 1999, Sajilata et al., 2006).

Amylose is the linear portion of the starch, with glucose residues linked by α-D-(1-4) bonds. Depending on the species, amylose constitutes typically 20 to 30% of starch (Bertoft, 2004), has a degree of polymerization (DP) of 500 to 6000 (Eerlingen and Delcour, 1995), and molecular mass ranging from10^7 to 10^9 g/mol (Hizukuri, 1996). The variable number of 1,6-branching points, as well as amount of glucose monomers, makes it difficult to determine amylose content in different starches (Haase, 1993). The long chains of amylose can form single or double-helical structures (Sajilata et al., 2006) with hydrophobic cavities that can complex with lipids and iodine (Englyst et al., 2000). Amylose does not dissolve easily in water and forms rigid gels (McCleary et al., 2006).

Amylose is the main component of starch which undergoes retrogradation, or the recrystallization of gelatinized starch (Hibi, 1998). In this process, the long chains of amylose form helices, either singly or doubly (with other amylose chains), which then align to form

insoluble crystallites resistant to enzymatic action (BeMiller and Whistler, 1996).

Amylopectin is a larger branched molecule with 4 to 5% of its glycosidic bonds as α-D-(1-6) linkages (Klaus et al., 2000, Eerlingen and Delcour, 1995). Amylopectin is one of the largest molecules in nature, with a degree of polymerization (DP) averaging 2 million and a molecular mass severalfold greater than amylose (Hizukuri, 1996). It easily dissolves in hot water and does not form a gel (McCleary et al., 2006). Starches that contain only amylopectin are termed waxy starches. Most amylopectin molecules have three branch chain fractions that differ in lengths. The outermost chains, or the A chains, comprise the smallest fraction, whereas the short and long B chains form the two other fractions. The longer B chains and shorter A chains determine the properties of starch and starch-based foods (BeMiller, 2007).

2.1.2 Gelatinization

Gelatinization is a process by which starch granules irreversibly lose their molecular order, called birefringence, as a result of a series of events when starch granules are heated in excess water. First, the granules swell as hydrogen bonds in the amorphous portions are disrupted. Next, water, which acts as plasticizer, is absorbed. More hydration and swelling occur in the amorphous regions as the temperature rises, causing the crystallites to break apart, and then undergo hydration and melting. Lastly, polymer molecules, particularly those of amylose, leach out of the granules and viscosity increases (Biliaderis, 1991, Eerlingen and Delcour, 1995, BeMiller 2007).

Gelatinization temperature (GT) and the temperature range of gelatinization depend on the type of starch, method of measurement, starch-water ratio, pH, absence or presence of swelling-inhibiting or swelling–promoting salt, salt concentration, and presence and concentration of a solute (eg. sucrose) (BeMiller, 2007). Sugars and other polyhydroxy

compounds increase GT, while simple salts have a lowering effect (Evans and Haisman, 1982).

The Differential Scanning Calorimetry (DSC) is the most common technique used to study the thermal properties of starches. It measures first-order (melting) and second-order (glass transition) transition temperatures and heat flow changes in polymeric materials and gives information on order-disorder phenomena of starch granules (Biliaderis et al., 1986).

Gelatinization is an endothermic process. In the DSC curve of starch at intermediate water levels, three endothermic transitions are usually observed. The first two endotherms correspond to the disorganization of starch crystallites (Biliaderis et al., 1986), or gelatinization, wherein glass transitions of water-plasticized amorphous portions and then non-equilibrium melting of the microcrystallites of the partially crystalline amylopectin occur (Slade et al., 1996). The third endotherm, which occurs at higher temperature, relates to the melting of complexes formed by amylose and native lipids (Biliaderis et al., 1986). Crystallite quality and the overall crystallinity of the starch are measured by the peak temperature (Tp) and the enthalpy of gelatinization (ΔH), respectively (Tester and Morrison, 1990). Onset temperature (To) and completion temperature (Tc) determine the boundaries of the different phases in a semicrystalline material like starch (Biliaderis et al., 1986).

2.1.3 Pasting

Continued heating of starch in excess water with stirring causes the granules to further swell, the amylose to leach more, and the granules to disintegrate, forming a viscous material called paste (BeMiller, 2007). Pasting occurs after or simultaneously with gelatinization. Pasting properties of starch are important indicators of how the starch will behave during processing and are commonly measured using the Rapid Visco Analyzer (RVA). Figure 2.1 shows a typical RVA pasting curve. In the RVA test, starch is mixed with water to allow for hydration and held

for a short time above ambient temperature. Heating proceeds, resulting in swelling of starch granules. As heating continues, an increase in viscosity can be observed, which reflects the process of pasting. The temperature at the onset of viscosity increase is termed pasting temperature. Viscosity increases with continued heating, until the rate of granule swelling equals the rate of granule collapse, which is referred to as the peak viscosity (PV). PV reflects the swelling extent or water-binding capacity of starch and often correlates with final product quality since the swollen and collapsed granules relate to texture of cooked starch. Once PV is achieved, a drop in viscosity, or breakdown, is observed as a result of disintegration of granules. Breakdown is a measure of the ease of disrupting swollen starch granules and suggests the degree of stability during cooking (Adebowale and Lawal, 2003). Minimum viscosity, also called hot paste viscosity, holding strength, or trough, marks the end of the holding stage at the maximum temperature of the RVA test. Cooling stage begins and viscosity again rises (setback) which is caused by retrogradation of starch, particularly amylose. Setback is an indicator of final product texture and is linked to syneresis or weeping during freeze-thaw cycles. Viscosity normally stabilizes at a final viscosity or cold paste viscosity, which is related to the capacity of starch to form viscous paste or gel after cooking and cooling (Batey, 2007, Newport Scientific, 1998).

Other components naturally present in the starchy material or additives interact with starch and influence pasting behavior (Newport Scientific, 1998). The presence of proteins with disulfide linkages confers shear strength and gelatinized paste rigidity to rice starch (Hamaker and Griffin, 1993, Xie et al., 2008). Beta-glucans added to rice starch reportedly increase the paste viscosities (Banchathanakij and Suphantharika, 2009). Lipids complexed with amylose tend to enhance retrogradation of rice starch. Beta-cyclodextrin and amino acids also altered pasting behavior of rice starch (Liang and King, 2003). With amino acids, pasting profiles were

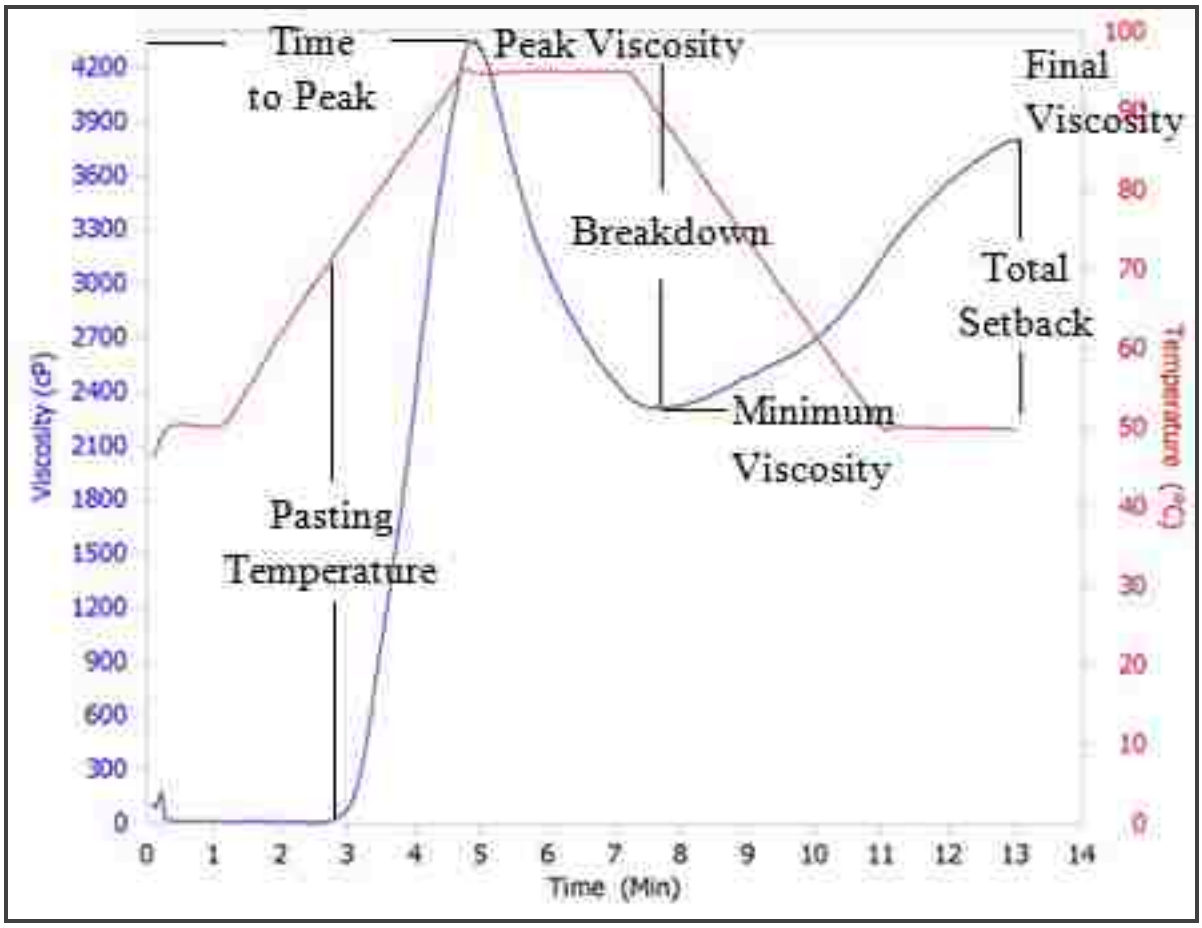


Figure 2.1 Typical Pasting Curve of Starch as Measured by RVA.

more affected by charged amino acids than neutral ones in rice (An and King, 2009, Liang and King, 2003), sweet potato (Lockwood et al., 2008), and potato starches (Ito et al. 2004, 2006a).

2.1.4 Retrogradation

Retrogradation refers to the processes that cause starch gels to become less soluble during cooling due to recrystallization of starch molecules (BeMiller and Whistler, 1996). Retrogradation occurs when the amylose leached from starch granules during gelatinization interacts with amylopectin chains of swollen starch granules, forming a rigid structure (Kurakake et al., 2009). This is the reason for the increased firmness of cooked food after cooling or storage. Both amylose and amylopectin fractions are important in the retrogradation process. Amylose undergoes rapid crystallization as soon as cooling begins and retrogradation depends on the amylose content in the sample, the amount that is free and uncomplexed with lipids, and its molecular weight distribution. Amylopectin, on the other hand, recrystallizes slowly and the degree of retrogradation depends on the chain length distribution of amylopectin (Philpot et al., 2006). Retrogradation due to amylose is favored at lower starch concentration (Orford et al., 1987) and results in a material very resistant to enzymatic hydrolysis (Ring et al., 1988). Recrystallization and retrogradation of amylopectin is dominant at a higher concentration of solids (Orford et al., 1987) and the polymer formed is more loosely bound than retrograded amylose (Englyst et al., 1992) and hence, highly susceptible to amylolysis (Ring et al., 1988).

2.2 RICE

2.2.1 Rice and Rice Starch

Rice (*Oryza sativa* L.) is a staple food of more than half of the world's population , particularly in Asian countries (Juliano, 1985). It has been cultivated on almost all continents and has been consumed by humans for at least 5,000 years (Bao and Bergman, 2004). China,

India and Indonesia were believed to be where rice was first cultivated, and thus the origin of the three races of rice – japonica, indica, and javanica (Juliano, 1993). Japonica and indica types are considered the two sub-species of rice, and each sub-species is comprised of genotypes with varying cooking and processing properties (Hizukuri et al., 1989, Bao and Bergman, 2004). The short and wide japonica rices typically cook soft, moist and sticky (Bao and Bergman, 2004) and retrograde slowly (Hizukuri et al., 1989), whereas the long and thin indica rices usually cook firm, dry and fluffy (Bao and Bergman, 2004) and retrograde rapidly (Hizukuri et al., 1989). Javanica rice belongs to the japonica race (IRRI, 2007a). The characteristics of the different rices are controlled by their starch composition.

Mostly consumed in its cooked milled form, rice is also made into flour or starch for use in pharmaceutical, food, and animal feed products. Rice starch has found many applications because of its many excellent characteristics. It has neutral taste and hence does not affect the final flavor of the product where it is incorporated in (Bao and Bergman, 2004). Rice starch has the smallest granules of the commercial starches (2-9 μm) (BeMiller, 2007), and it is known to form a soft gel, making it a desirable fat mimetic in a wide array of food products. Also, rice starch does not contain gluten and therefore do not invoke allergic responses in humans (Bao and Bergman, 2004).

2.2.2 Physicochemical Properties Related to Processing and Eating Quality

Milled rice contains about 90% starch. In rice starch, amylose has a greater effect on the processing properties and eating quality. Amylose is directly correlated to the hardness, whiteness and dullness of cooked rice and volume expansion and water absorption during cooking. Varieties with a low amylose level have a soft and sticky cooked texture while those with high amylose content have flaky and hard texture (Juliano, 1985). Rice varieties are usually

classified in terms of amylose content as waxy (1-2% amylose), very low (2-9%), low (10-20%), intermediate (20-25%), and high (25-30%) (IRRI, 2007b). Waxy rice occurs in both japonica and indica rice sub-species (Bao and Bergman, 2004).

While amylose content is the most important physicochemical property of rice related to its cooking and eating quality, GT also affects consumer preference and acceptance of a rice variety because GT is directly associated with cooking time (Juliano, 1993). Heat energy needed to completely gelatinize starch, on the other hand, is important for food processors, because this determines the heat input, cooking time, and temperature of processing (Bao et al., 2007). Classifications of starches according to GT as measured by the DSC are: low, 64 to 67^{o}C Tp; intermediate, 68 to 71^{o}C; and high, 75 to 79^{o}C (Tester and Morrison 1990).

2.2.2.1 Amylose Determination Methods

Complexation with iodine changes the color of amylose to blue-black and is the basis of the commonly used colorimetric method of determining the amylose content in a sample (Juliano et al., 1981). Mahmood et al. (2007) attributes the method's widespread use to its economical advantage and greater throughput per day over other methods available. The use of delicate reagents such as enzymes is also not required (Mahmood et al., 2007).

Yun and Matheson (1990), however, noted a major limitation of the colorimetric method relying on the color formation of the starch-iodine complex. The amylopectin portion of the starch also produces a reddish-purple compound when complexed with iodine (BeMiller and Whistler, 1996), which subjects the measurements to uncertainties. Amylose standards obtained from various sources that vary widely in terms of quality, the presence of lipids that could interfere with the assay, and the pH of the final solution are other possible sources of error (Bhattacharya, 2009). Therefore, results from this method could either be lower or higher than

the actual value (Singh et al., 2003), such that the value obtained is termed “apparent amylose” or “amylose equivalent” (Bhattacharya, 2009).

Gibson et al. (1997) developed a method that estimated the amount of the polysaccharide after precipitation with concanavalin-A (Con A), a lectin that can selectively precipitate amylopectin from starch through the formation of a complex under defined conditions of pH, temperature and ionic strength. Yun and Matheson (1990) refined this method by including an ethanol pretreatment of the starch sample to extract the lipids, which can also complex with amylose and interfere with colorimetric determinations. The amylose is then either reacted using phenol-sulfuric acid reagent or hydrolyzed enzymatically. The use of phenol-sulfuric acid reagent, however, could yield a higher amylose value, which may be due to the presence of non-starchy polysaccharide (Yun and Matheson, 1990). Megazyme International Ireland Ltd. (Co. Wicklow, Ireland) developed an amylose/amylopectin assay which is based primarily on the method of Yun and Matheson (1990), but utilized only the enzymatic hydrolysis.

2.3 MODIFIED STARCH

2.3.1 Starch Modification

Native starches have been used for a variety of food applications. However, they lack important functional characteristics. These characteristics include viscosity, texture and emulsifying properties, clarity of formed pastes, and binding properties (Keeling, 1997). In addition, some starch-based products are not usually made or consumed after gelatinization, but stored at low temperatures, which causes gels of native starches to shrink, undergo syneresis, and toughen in the process called retrogradation (Lillford and Morrison, 1997).

Modification either by chemical or physical means is done to overcome the shortcomings of native starches and to increase the usefulness of starch. Physically altered starches include

pregelatinized, redried, extruded, sonicated and irradiated starches (Wurzburg, 1986, Bao and Bergman, 2004). The types of chemical modifications commonly used are crosslinking of polymer chains, derivatization, depolymerization, pregelatinization, and combinations of these. With starch modification, the following properties can be achieved: reduced energy needed for cooking (improved gelatinization and pasting), altered cooking properties, enhanced solubility, increased or decreased paste viscosity, reduced or enhanced gel formation, improved gel strength, reduced gel syneresis, improved interaction with other substances, better stabilizing properties, enhanced film formation, improved water resistance of films, decreased paste cohesiveness, and improved stability to acid, heat, and shear (BeMiller, 2007).

Chemical modification of starch depends on the hydroxyl groups of the amylose and amylopectin, and very few of these hydroxyl groups are reacted, with degree of substitution (usually with ester or ether groups) values of <0.1. Chemical modifications currently allowed for use in foods in the United States include esterification with acetic anhydride, succinic anhydride, mixed anhydride of acetic and adipic acids, 1-octenylsuccinic anhydride, phosphoryl chloride, sodium trimetaphosphate, sodium tripolyphosphate, and monosodium orthophosphate; etherification with propylene oxide; reaction with hydrochloric and sulfuric acids; bleaching with hydrogen peroxide, peracetic acid, potassium permanganate, and sodium hypochlorite; oxidation with sodium hypochlorite; and treatment using various combinations of these chemical reactions (BeMiller, 2007).

2.4 RESISTANT STARCH

2.4.1 Forms

Starch can be subdivided into three types based on *in vitro* digestion: rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS) (Englyst et al., 1992).

RDS and SDS represent the starch fractions that are completely digested while RS is the portion which resists digestion in the small intestines of healthy individuals and is available for fermentation in the large bowel (Englyst et al., 2000). RS physiologically functions like dietary fiber (McCleary et al., 2006), notably by the reduction of plasma glucose and insulin levels and the increase in faecal bulk (Cairns et al., 1995). RS has been believed to account for 30% of the total fiber fraction in the diet (Englyst, 1989), the only difference being fiber not of starch origin (i.e. plant cell wall polysaccharides) (Englyst et al., 1987, Haralampu, 2000). RS is implicated in the prevention of gastrointestinal diseases like colon cancer, since its fermentation in the gut leads to the formation of short-chain fatty acids, such as acetate, propionate and butyrate, which have health-promoting properties (Hung et al., 2005, Zhang et al., 2007). RS acts as substrate for the growth of probiotic microorganisms (Birkett et al., 2000), reduces the formation of gall stones, decreases cholesterol levels, inhibits fat accumulation (Lopez et al., 2001), and improves the bioavailability of calcium (Younes et al., 2001), magnesium, zinc, iron and copper (Lopez et al., 2001, Sajilata et al., 2006).

RS is categorized into physically inaccessible starch (RS_1), starch made indigestible by inhibitory action of enzymes (RS_2), retrograded starch, particularly the amylose portion (RS_3), and chemically modified starch (RS_4) (Eerlingen and Delcour, 1995, Goñi et al., 1996, Sajilata et al., 2006). RS_1 represents starch present in foods with very dense structure such as whole grains and partially milled seeds and in some processed starchy foods and is heat stable in most normal cooking operations (Sajilata et al., 2006). Foods such as boiled rice, pasta, whole-grain bread, maize and legumes are also found to contain RS_1 (Englyst et al., 2000). RS_2 is the form which is tightly packed, has a high density and is partially crystalline, preventing enzymatic action. It can be found in foods with uncooked starch such as raw potato, bananas (Eerlingen and Delcour,

1995), raw cereal flours, dry-baked biscuits and legumes (Englyst et al., 2000). RS_3 is the fraction which forms when there is heat-moisture treatment involved, that is, during cooling of gelatinized starch (Eerlingen and Delcour, 1995, Sajilata et al., 2006). Cooling and ageing of the gel cause the reformation of a crystalline structure among the polymers, the phenomenon termed as retrogradation (Englyst et al., 1992). RS_4, on the other hand, is developed after some chemical or thermal treatments to the starch (Sajilata et al., 2006), with the indigestibility usually accounted to substituents or new glycosidic bonds formed by dry heat (Hung et al., 2005). Among these four types, RS_3 is the most common form in the diet. Furthermore, RS_3 is considered the most important because it is generated due to food processing (García-Alonso et al., 1998) and has a huge potential for use in a wide array of applications in the food industry due to its thermal stability (Haramlampu, 2000).

Studies have indicated a positive correlation between amylose content and amount of resistant starch (Berry, 1986, Sagum and Arcot, 2000, Rosin et al., 2002, Zhang et al., 2007). As described in Section 2.1.1, the amylose molecule has an extended shape that winds to form singular or double helical structures. On the outside surface of the single helical amylose are the hydrogen-bonding O2 and O6 atoms which can bond with aligned chains, causing retrogradation and syneresis, or the liberation of some of the bound water in the gel (BeMiller and Whistler, 1996). The aligned chains, which possess extensive inter- and intra-strand hydrogen bonding, may then form double stranded crystallites that are fairly hydrophobic, very slightly soluble, and resistant to amylases. The formed product is RS_3 (Chaplin, 2008).

Aside from the amylose content, many other factors influence the RS levels formed in a food product. These include the botanical source, starch interactions with other components, structure of starch granules, the presence of other components or antinutrients (eg. phytic acid),

processing, and storage conditions (Cairns et al., 1995, Escarpa et al., 1997, Rosin et al., 2002, Kumari et al., 2007). According to Zhang et al. (2007), milled rice samples with similar amylose contents can have different RS levels and the protein content was directly correlated to the amount of RS in foods. The physical form of starchy foods (eg. coarse ground cereals) (Birkett et al., 2000) and the degree of chewing (Muir and O'Dea, 1992) likewise affect RS levels. The presence of ions (potassium and calcium) and catechins greatly induces RS formation, while nutrients (albumin, olive oil and sucrose), pectins, gums and phytic acid affect it to a lesser extent (Escarpa et al., 1997). In regards to processing, factors contributing to RS formation are water content, heating temperature (Berry, 1986, Sagum and Arcot, 2000), pH, time, number of heating and cooling cycles, freezing, drying (Englyst et al., 1987), and storage time and temperature (Eerlingen et al., 1993). In a study by Sagum and Arcot (2000), the high amylose rice variety Doongara had significantly higher RS when pressure-cooked than when boiled. Modification of starch either by physical or chemical means were also shown to reduce starch digestibility (Saura-Calixto and Abia, 1991).

2.4.2 RS Assays

Because of the many beneficial physiological effects of RS, accurate estimation of RS levels in the diet is necessary. *In vitro* techniques that have been developed to measure RS in foods are either enzymatic-gravimetric or enzymatic-chemical (Englyst et al., 1987, Kim et al., 2003). Enzymatic-gravimetric approaches are based on the premise that resistant starch is the portion of starch that remains undigested by enzymes (Eerlingen et al., 1993). In this method, starch is hydrolyzed in phosphate buffer using three enzymes: heat stable α-amylase, protease, and amyloglucosidase (AOAC, 1995). After enzymatic digestion, precipitation with ethanol is carried out. The mixture is filtered, washed with ethanol and acetone, and dried. The resultant

residue is the RS. Eerlingen et al. (1993) used an enzymatic-gravimetric method to quantify RS in autoclaved starch and obtained comparable results with those in published reports. Meanwhile, Kim et al. (2003) suggested a simplified technique by using only the heat stable α-amylase after they found out that their results had correlated well with those assayed using three enzymes as in the AOAC method. RS obtained using enzymatic-gravimetric methods, however, does not necessarily represent RS obtained under *in vivo* conditions because of different pH and temperature conditions and the enzymes used (Eerlingen et al., 1993, Monro, 2004).

Enzymatic-chemical assays of measuring RS are either direct or indirect. Direct methods measure the RS after removal of digestible starch while indirect methods determine RS as the difference between total and digestible starch (Walter et al., 2005). Goñi et al. (1996) proposed a direct method of determining RS in food and food products, citing that a fraction of resistant starch often remains in analytically determined dietary fiber. This method involves addition of pepsin solution to the sample to remove proteins to enhance amylase accessibility, avoid starch-protein interactions, and simulate physiological conditions. Then, the enzyme α-amylase is added to remove digestible starch and then RS is solubilized and hydrolyzed using amyloglucosidase (AMG). The glucose concentration is determined using glucose oxidase-peroxidase reagent and then read against a glucose water standard curve. The quantification of RS is expressed as mg of glucose x 0.9 (Goñi et al., 1996). In this method, however, Zhang et al. (2007) noted that serious fermentation occurred in the incubation medium and that RS might or might not be affected by microbial growth in the supernatant. They, thus, investigated the impact of antimicrobial agents – antibiotics and sodium benzoate – on the RS levels. They found out that a significant decrease in RS levels had resulted from antibiotics addition, suggesting that without the antimicrobial agents, there was overestimation of RS since microbial growth

inhibited the action of α-amylase.

The method currently accepted by AOAC International and AACC International for measuring RS is that developed by McCleary and Monaghan (2002). This method involves incubation of starch with α-amylase with AMG to solubilize and hydrolyze the non-resistant fraction. The reaction is stopped by the addition of alcohol and the pellet is separated by centrifugation, washed with ethanol, and centrifuged again. The collected RS is dispersed in potassium hydroxide with stirring in an ice-water bath and then neutralized with acetate buffer. A high concentration of AMG is added to hydrolyze RS to glucose, which in turn is measured with glucose oxidase-peroxidase reagent (GOPOD) colorimetrically (McCleary and Monaghan, 2002). Data obtained using this method in an interlaboratory analysis were comparable with *in vivo* measurements (McCleary et al., 2002). The method, however, is best suited for finely milled samples (Monro, 2004) and samples containing more than 2% RS (Megazyme, 2002). The absence of protease in the assay could also overestimate the RS levels because starch-protein interactions or starch encapsulated in a protein matrix might be detected as RS. An earlier AOAC standard assay (AOAC Method 996.11, 1998), which also does not utilize protease, gave higher RS values than the method of Siljeström and Asp (1985), which involves hydrolysis of the sample with protease after α-amylase in the first step and solubilization with alkali (Walter et al., 2005).

Researchers led by Englyst (Englyst et al., 1992, Englyst et al., 2000) developed a procedure that quantifies RS indirectly, as well as other starch fractions from foods. The main procedure involves enzymatic hydrolysis of starch and then measurement of glucose released. Starch is first treated with protease and then incubated with amylolytic enzymes (pancreatic amylase, amyloglucosidase and invertase) under specified temperature, pH, viscosity and

mechanical mixing. RDS and SDS are measured after 20 min and 120 min incubation, respectively. RS is the fraction that remains undigested after 120 min and determined from the difference of total starch and digestible starch fractions. This method was validated *in vivo* using healthy ileostomy subjects as model for digestion in the small intestine (Englyst et al., 1996). The downside of the Englyst method, however, is the inaccurate measurements for foods with low RS levels due to accumulation of errors of two experimental determinations (Goñi et al., 1996).

2.5 AMINO ACIDS

2.5.1. Amino Acids and Their Properties

An amino acid is the building block of proteins. It consists of a carbon atom covalently bound to a hydrogen atom, an amino group, a carboxyl group, and a side-chain R group. The side chain R group determines the physicochemical properties of the amino acid, which include the net charge, solubility, chemical reactivity, and hydrogen bonding potential. Aliphatic (alanine, isoleucine, leucine, methionine, proline, and valine) and aromatic (phenylalanine, tryptophan, and tyrosine) side chains render hydrophobicity to the amino acids. The guanidino, amino and imidazole groups in arginine, lysine and histidine, respectively, have a basic character and hence, the net charge of the amino acids is positive at neutral pH. Carboxyl groups in aspartic and glutamic acids, on the other hand, make the net charge negative at neutral pH (Damodaran, 1996). The structures of representative amino acids are shown in Table 2.1.

Amino acids behave both as acids and bases and can exist in different ionized forms depending on the pH of the medium. When both of the acidic and amino groups of an amino acid are ionized (i.e. its net charge is zero), the amino acid becomes a zwitterion. This occurs at the isoelectric point (pI), which is specific to the amino acid. In a more acidic medium where

pH<pI, the amino acid becomes the weaker acid and therefore accepts proton, turning the amino acid positively charged. Conversely, in a more basic solution where pH>pI, the amino acid acts as the stronger acid and donates proton, causing its net charge to become negative. Several amino acids have side chains containing ionizable groups. The pH at which the concentrations of the protonated and deprotonated ionizable groups is called pK (Damodaran, 1996). The pK's and pI values of representative amino acids are presented in Table 2.2.

Table 2.1 Representative Amino Acids and Their Structures.

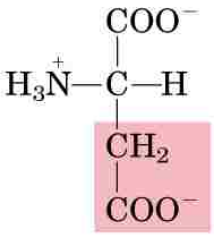
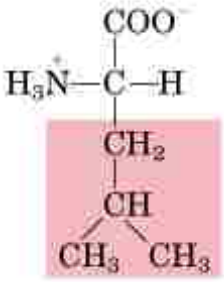
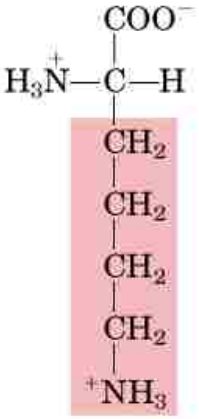
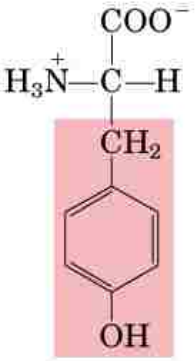
Name	Symbol	Structure at neutral pH
Aspartic acid	ASP	
Leucine	LEU	
Lysine	LYS	
Tyrosine	TYR	

Table 2.2 pK and pI Values of Ionizable Groups of Amino Acids Used in This Study.[1,2]

Name	pK			pI
	α-COOH	α-NH_3^+	Side Chain (Free AA)	
Aspartic acid	1.88	9.60	3.65	2.77
Leucine	2.30	9.60	--	5.98
Lysine	2.18	8.95	10.53	9.74
Tyrosine	2.20	9.11	10.07	5.96

[1]Values given at 25°C.
[2]Damodaran, 1996

CHAPTER 3. MODIFICATION OF RICE STARCH PROPERTIES BY ADDITION OF AMINO ACIDS AT VARIOUS pH LEVELS

3.1. INTRODUCTION

Rice starch possesses unique qualities, such as hypoallergenicity and bland taste, which make it a desirable food ingredient. Native starches, however, are unstable under various temperature, shear and pH conditions so that their application in the food industry is limited. Hence, to achieve more desirable functional characteristics and increase their utilization, native starches are oftentimes modified either through moisture-heat treatments, by reaction using various chemicals, through enzymatic means, or by genetic manipulation (Bao and Begman, 2004). Meanwhile, other components that co-exist with the starchy material can interact with starch and consequently affect its functional behavior (Newport Scientific, 1998). Not widely practiced as a starch modification technique, utilization of additives to alter rice starch properties is worth exploring.

The use of amino acids as additives to native starch holds promise as an alternative starch modification technique. Different amino acids have been demonstrated to alter functional characteristics in various native starches (Liang and King, 2003, Ito et al., 2004, Ito et al., 2006a, Lockwood et al., 2008, An and King, 2009). In rice starch, aspartic acid was shown to lower viscosity and setback values, resulting in a starch with increased retrogradation stability. Arginine, on the other hand, increased the tendency for retrogradation (Liang and King, 2003). Lysine lowers the swelling power and pasting time. The starch produced had better cooking stability and lower pasting viscosities (An and King, 2009). Lowering of peak viscosity by lysine (Ito et al., 2004) and glutamic acid was also observed in potato starch (Kinoshita et al., 2008). In general, charged amino acids had greater effect on controlling the pasting properties of starch

(Liang and King, 2003, Ito et al., 2004, An and King, 2009).

Significant alterations on the pasting behavior of starch are not the sole effects of amino acid additives. Charged amino acids, whether positive or negative, elevate the GT and reduce the gelatinization enthalpy of amylose-lipid complexes in rice starch (Liang, 2001, An, 2005). The enhancing effect on starch gelatinization of the charged amino acids is not specific to the starch source, as what was proven by Ito et al. (2004) and Lockwood et al. (2008) in their studies on potato starch and sweet potato starches, respectively. Moreover, larger increments in GT of potato starch were observed in amino acids with positive or negative net charge than in neutral ones when the amount of incorporated amino acids was increased (Ito et al., 2006a, Kinoshita et al., 2008).

Gelatinization is an essential step leading to the formation of enzyme-resistant starch. Resistant starch (RS) is the sum of all starch and its components that are not digested in the small intestine and become available for fermentation in the gut of healthy individuals (Englyst et al., 2000). RS is of current interest because of its numerous reported health effects. It is now well-known that RS physiologically behaves like dietary fiber and helps in the prevention of chronic diseases like colon cancer. Altering the functional properties of starch through the use of amino acid additives could also result in RS formation, and pave the way for the development of novel functional food ingredients. Lysine, when conjugated to starch via the Maillard reaction, was shown to lower the swelling and solubility of the starch, and thus believed to also reduce starch digestibility (Yang et al., 1998). Liang and King (2003) who observed an increase in relative crystallinity of rice starch after addition of amino acids also believed that this could enhance the formation of RS. In 2009, An and King confirmed this finding in their study on oxidized rice starch. Aspartic acid and leucine enhanced the RS yield of a commercial rice starch oxidized

with pure oxygen and ozone, respectively (An and King, 2009).

Based on published reports, amino acids contribute to changes in starch properties because of their properties, notably their charges. In this study, the effect of amino acids in combination with different pH conditions was tested. Also, an amino acid with a hydroxyl group capable of forming hydrogen bonds with starch has never been tested in altering the pasting and gelatinization properties, and was thus investigated using tyrosine. Unlike treatments adapted by other authors wherein amino acids were added to starch during functional properties measurements, treatments made in this study involved incorporation of amino acids to starch in the presence of a dispersing agent and then subsequently dried before analysis of starch properties. This study hypothesized that this treatment would allow interactions between amino acid and starch (eg. possible complex formation) to occur and consequently be more effective in altering starch functionalities. In addition, the effect of the different modifications on formation of RS was determined.

3.2. MATERIALS AND METHODS

3.2.1. Chemicals

Commercial rice starch (S7260) and amino acids, namely DL-aspartic acid (negatively charged), DL-leucine (neutral), DL-lysine (positive) and DL-tyrosine (hydroxyl group), were obtained from Sigma-Aldrich Co., Inc. (St. Louis, MO). Total dietary fiber assay kit (TDF-100A) was purchased from Sigma-Aldrich Co., Inc. (St. Louis, MO). Enzymes for amylose/amylopectin Con A method and enzymatic-chemical method for RS quantification were obtained from Megazyme International Ireland Ltd. (Bray, Co. Wicklow, Ireland). All chemicals used for the pH solutions and other reagents for the different tests were of analytical grade.

3.2.2. Sample Treatment and Preparation

The following treatments were used in this study:

A. Dispersal medium

1. distilled water
2. distilled water with pH adjusted to 4, 7, and 10 using 0.10 N solutions of HCl and NaOH
3. buffers of pH 4 (acetate, 0.10 M), 7 (phosphate, 0.10 M) and 10 (carbonate-bicarbonate, 0.05 M)

B. Temperature of reaction: (a) room temperature (1 min) and (b) $40 \pm 2^{o}C$ (15 min).

Distilled water was used to determine changes in behavior of starches due to amino acids only. pH levels were selected such that amino acids will not be at the pIs. Buffers were tested to ensure stability of the pHs during reactions. Heating of the starch suspension was done to check effects on the reaction of the starch-amino acid and dispersant at a temperature below the gelatinization point of starch.

Rice starch (15-20 g) was weighed into a beaker and amino acids were added at 6% starch dry weight basis. The mixture was dispersed in the liquid medium (1:4 wt/vol starch-to-liquid ratio) with continuous mixing under a magnetic stirrer. For the A.2. samples, mixing was carried out at different temperatures (B). Starch suspensions were transferred into weighing boats, covered with paper, stored at $-80^{o}C$ overnight, and lyophilized. Dry samples were ground using a Udy Cyclone Sample Mill (Udy Corp., Port Collins, CO) and stored at room temperature until analyzed. Two replicates were prepared per treatment. Figure 3.1 shows the schematic of the sample preparation.

A separate set of experiments were done to further test the effect of tyrosine on rice

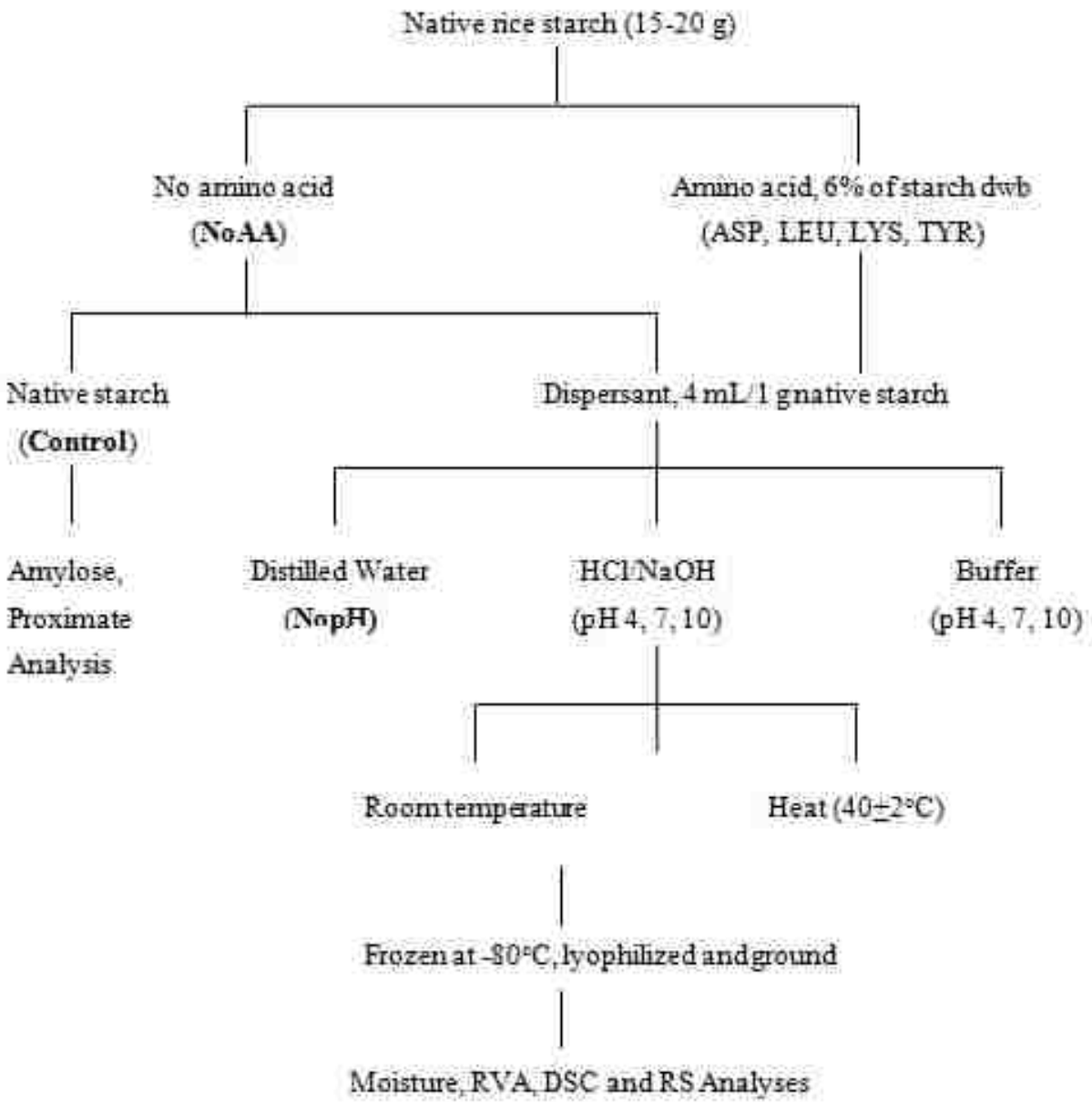


Figure 3.1 Flowchart of Sample Preparation and Treatment.

starch functionalities. Native rice starch was weighed into an RVA canister according to the procedure in Section 3.2.4 and then tyrosine (6% dwb) was added into it. The dispersant was added into the canister and the mixture subjected to RVA analysis. Gels obtained after the test were transferred into weighing boats, stored and lyophilized as the other samples for further analysis.

3.2.3. Properties of Native Rice Starch

3.2.3.1. Proximate Analysis

Native rice starch control was analyzed for crude protein (N x 5.95) (Method 954.01), crude fiber (Method 962.09), ash (Method 942.05) and lipid (Method 920.39) contents (AOAC, 2005). Moisture contents of the native rice starch control and lyophilized treated starch were determined using AOAC Method 925.10 (2005).

3.2.3.2. Amylose Content Determination

Analysis of the amylose content was performed using the Megazyme Amylose/ Amylopectin Assay kit (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). Briefly, 20-25 mg of starch sample was weighed into a screw capped tube and 1 mL of DMSO was added to the tube with gentle stirring at low speed on a vortex mixer. The tube was capped and heated in a boiling water bath until the sample was completely dispersed. The contents of the sealed tube was vigorously mixed at high speed on a vortex mixer, and then the tube was placed in a boiling water bath and heated for 15 min, with intermittent high-speed stirring on a vortex mixer. The tube was allowed to cool to room temperature for about 5 min and 2 mL of 95 % (v/v) ethanol was added with continuous stirring on a vortex mixer. A further 4 mL of ethanol was added, and the tube was capped and inverted to mix. The tube was allowed to stand for 15 min. After this, it was centrifuged at 2,000 x *g* for 5 min. The supernatant was discarded and the tube

was drained on tissue paper for 10 min, ensuring all of the ethanol drained. The pellet was mixed with 2 mL of DMSO with gentle vortex mixing. The tube was then placed in a boiling water bath for 15 min and mixed occasionally. Upon removing the tubes from the boiling water bath, 4 mL of Con A (Concanavalin A, a lectin protein) solvent was immediately added. The Con A solvent was prepared by diluting to 30% the concentrated Con A solvent. The concentrated Con A solvent was a solution of sodium acetate buffer containing the salts sodium chloride, $CaCl_2.2H_2O$, $MgCl_2.6H_2O$, and $MnCl_2.4H_2O$ and adjusted to pH 6.4.

The contents of the tubes with the Con A solvent were mixed thoroughly and then quantitatively transferred by repeated washing with Con A solvent to a 25-mL volumetric flask. The mixture was diluted to volume with Con A solvent (Solution A).

To a 2.0-mL Eppendorf® microfuge tube, 1.0 mL of Solution A was transferred. Then, 0.50 mL of Con A was added. The tube was capped and gently mixed by repeated inversion. The tube was allowed to stand for 1 h at room temperature, and then centrifuged at 14,000 x *g* for 10 min in a microfuge at room temperature. One milliliter of the supernatant was transferred into a 15-mL centrifuge tube and 3 mL of 100 mM sodium acetate buffer, pH 4.5 were added. The contents were mixed and the tube was lightly stoppered and heated in a boiling water bath for 5 min to denature the Con A. Then, the tube was placed in a water bath at 40°C. After equilibration for 5 min, 0.1 mL of amyloglucosidase (3300 U/mL)/α-amylase (500 U/mL) enzyme mixture was added and the tube was incubated at 40°C. After 30 min incubation, the tube was centrifuged at 2,000 x *g* for 5 min. An aliquot of 1.0 mL was taken from the supernatant and combined with 4 mL of glucose oxidase (>12,000 U)/peroxidase (>650 U) (GOPOD) reagent. The tube was incubated at 40°C for 20 min, along with the reagent blank and the D-glucose controls. For the reagent blank**,** 1.0 mL of 100 mM sodium acetate buffer was

used, while for the D-glucose controls 0.1 mL of D-glucose standard solution (1 mg/mL) and 0.9 mL of sodium acetate buffer was pipetted into the tube. The absorbance of each sample and the D-glucose controls was read at 510 nm against the reagent blank.

To determine the total starch, 0.5 mL of Solution A was mixed with 4 mL of 100 mM sodium acetate buffer, pH 4.5, in a screw capped tube. Then, 0.1 mL of amyloglucosidase/ α-amylase solution was added and the mixture was incubated at 40°C for 10 min. An aliquot (1.0 mL) of this solution was transferred into a glass test tube, combined with 4 mL of GOPOD reagent, and mixed well. It was incubated at 40°C for 20 min. This incubation was performed concurrently with the samples and standards.

Percentage amylose was calculated as:

$$\text{Amylose, \% (w/w)} = \frac{\text{Absorbance (Con A Supernatant)}}{\text{Absorbance (Total Starch Aliquot)}} \times \frac{6.15}{9.2} \times \frac{100}{1}$$

$$= \frac{\text{Absorbance (Con A Supernatant)}}{\text{Absorbance (Total Starch Aliquot)}} \times 66.8$$

where 6.15 and 9.2 are dilution factors for the Con A and Total Starch extracts, respectively.

3.2.3.3. Rheological Properties

Dynamic (oscillatory) rheological temperature sweep test of native rice starch was carried out using a rheometer (AR 2000ex, TA Instruments-Waters LLC, New Castle, DE). Starch was dispersed in distilled water at 9% wt/vol and about 1 ml of the suspension was loaded on the rheometer plate. A parallel plate geometry (40-mm diameter) was used and the gap was set at 0.2 mm. The frequency was maintained at 1 Hz and the strain was fixed at 3%. The temperature was equilibrated to 50^{o}C and maintained for 1 min. It was then raised from 50^{o}C to 95^{o}C at a rate of 12^{o}C/min. The storage (elastic) modulus (G') and loss (viscous) modulus (G") were determined using the software Rheology Advantage Data Analysis Program (TA Instruments-

Waters LLC, New Castle, DE). Duplicate samples were measured.

3.2.4. Pasting Characteristics Determination Using the Rapid Visco Analyzer (RVA)

Pasting characteristics of the rice starch samples were evaluated with a RVA-4 machine (Newport Scientific Pty. Ltd., Warriewood NSW, Australia) using the AACC Method 61-02 (Newport Scientific, 1998). Prior to analysis, the volume of water and weight of starch sample were determined based on the following formula:

$$S = 88 \times 3.00 / (100 - M)$$

$$W = 28.0 - S$$

where S is the corrected sample mass (g), M is the actual moisture content of the sample (% as is) determined based on AOAC Method 925.10, and W is the corrected water volume (mL). Briefly, distilled water (~25.4 g) was measured into an RVA canister. Then, an appropriate weight (~2.60 g) of starch sample was weighed into a pan and transferred into the canister with water. The paddle was placed into the canister and the sample was thoroughly dispersed into the liquid by vigorously jogging the blade up and down at least 10 times through the sample. The canister, with the paddle, was inserted into the instrument and the measurement cycle was started by carefully pressing the motor tower. Each sample was first held at 50^{o}C at a spindle speed of 960 rpm. After 10 sec, the rotating speed was reduced to 160 rpm. Next, the temperature was increased at 12^{o}C /min to 95^{o}C and held at the temperature for 2.5 min. It was finally cooled to 50^{o}C. The speed was kept at 160 rpm until the end of the test. The pasting temperature (PT), peak viscosity (PV), minimum viscosity (MV), final viscosity (FV), and peak time (PTime) were measured by the RVA with the ThermoCline for Windows v. 3 (TCW3) software. Total setback (TSB) and Breakdown (BD) were calculated as the difference between FV and MV, and PV and MV, respectively. Analysis was done in duplicate.

3.2.5. Thermal Properties Analysis Using a Differential Scanning Calorimeter (DSC)

Starch thermal properties were determined using a differential scanning calorimeter (DSC) (TA Q100, TA Instruments, Newcastle, DE). Starch (10 mg) was weighed into a steel DSC pan and 20 μL of distilled water was added. The pan was sealed with a lid and o-ring and equilibrated at room temperature for at least 1 hr. Heating was carried out from 35^{o}C to 140^{o}C at a rate of 5°C/min. A pan containing 20 μL of distilled water was used as reference. Onset (T_o), peak (T_p), and conclusion (T_c) gelatinization temperatures were measured and gelatinization enthalpy (ΔH) was calculated from the area of the peak endotherm using the Universal Analysis 2000 Software (version 4.5A, TA Instruments-Waters LLC, New Castle, DE). DSC runs were done in duplicate.

3.2.6. Resistant Starch Assay

3.2.6.1. Enzymatic-Gravimetric Technique

Resistant starch yield of native and treated rice starches, and starch-tyrosine dried gels were determined by the enzymatic-gravimetric method, as described in Sigma Technical Bulletin No. 74 TDFAB-3 with several modifications (Kim et al., 2003). Starch sample was weighed to 0.20g into a 125-mL Erlenmeyer flask and dispersed in 0.08 M phosphate buffer (20mL, pH 6.0). Next, 0.05 mL of heat stable α-amylase (68,300 U/mL) was added. The flask was covered with aluminum foil and placed in a water bath at 95°C for 15 minutes, agitating the flask gently at 5-min intervals. After cooling to room temperature, the solution was adjusted to pH 7.5$\pm$0.2 by addition of 0.275N aqueous NaOH solution and protease (P3910) (0.02mL, 50mg/mL solution of protease in phosphate buffer). The mixture was placed in a shaking incubator at 60°C for 30 min. The mixture was cooled to room temperature and then adjusted to pH 4.3$\pm$0.2 by adding 0.325 N aqueous HCl solution. Then, 0.02mL of amyloglucosidase (10,863 U/mL; A9913) was added.

The mixture was placed again in a shaking incubator at 60°C for 30 min. Four volumes of 95% ethanol (10 mL each) were added and the mixture was allowed to stand overnight at room temperature for complete precipitation. The insoluble residue was collected using a Whatman #2 filter paper. It was washed twice with 15mL of absolute ethanol and once with 10 mL acetone. The residue was dried in an oven at 40°C overnight.

The yield of resistant starch was determined as:

$$\text{Resistant starch (\%)} = \frac{\text{residue weight (g)}}{\text{sample weight (g)}} \times 100\% \text{ (dry weight basis)}$$

Crystalean, a commercial high amylose maize starch was used as check sample. Measurements were done in duplicate.

3.2.6.2. Enzymatic-Chemical Approach

The enzymatic-chemical method was performed according to the AOAC Method 2002.02 and AACC Method 32-40 using the Megazyme kit (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). Native starch, treated samples and RS control (52.5% dwb resistant starch) provided in the kit were weighed at 100±5 mg into screw cap tubes, which were gently tapped to ensure no sample adhered to the sides of the tube. Then, 4.0 mL of pancreatic α-amylase (3 Ceralpha Units/mg, 10 mg/mL) containing AMG (3 U/mL) was added to each tube. The tube was tightly capped, dispersed thoroughly on a vortex mixer, and attached horizontally in a shaking water bath, aligned in the direction of motion. The tube was incubated at 37^{o}C with continuous shaking (200 strokes/min). After shaking for exactly 16 hr, the tube was taken out of the water bath, uncapped, and the contents were treated with 4.0 mL of ethanol (99%) with vigorous mixing on a vortex mixer. After this, the tube was centrifuged at 1,500 x *g* (approx. 3,000 rpm) for 10 min (non-capped). The supernatant was carefully decanted and the pellet re-suspended in 2 mL of 50% ethanol and agitated using a vortex mixer. A further 6 mL of 50%

ethanol was added, the tube was mixed and centrifuged again at 1,500 x *g* for 10 min. Again, the supernatant was decanted and the suspension and centrifugation steps were repeated once more. Finally, the supernatant was decanted and the tube inverted on absorbent paper to drain excess liquid.

A magnetic stirrer bar (5 x 15 mm) was added to each tube, followed by 2 mL of 2 M KOH. The pellet was re-suspended (and the RS dissolved) by stirring for about 20 min in an ice/water bath over a magnetic stirrer. Then, 8 mL of 1.2 M sodium acetate buffer (pH 3.8) was added to each tube with stirring on a magnetic stirrer. Immediately, 0.1 mL of AMG (3300 U/mL) was added, the contents were mixed well under a magnetic stirrer, and the tube was placed in a water bath at 50^{o}C. The tube was incubated for 30 min with intermittent mixing on a vortex mixer.

The tube was directly centrifuged at 1,500 x *g* for 10 min. The final volume in the tube was approximately 10.3 mL ($\pm$0.05 mL). For the RS control, the contents of the tube was transferred into a 100-mL volumetric flask and then diluted to volume with distilled water. From this, an aliquot was taken and transferred into a screw cap tube. This was centrifuged together with the samples.

From each tube, 0.1 mL aliquot (in duplicate) of the supernatant was transferred into glass tubes, added with 3.0 mL of GOPOD reagent, and mixed well using a vortex mixer. A 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. Glucose standards were prepared (in quadruplicate) by mixing 0.1 mL glucose (1 mg/ mL) and 3.0 mL l GOPOD reagent. The samples, blank and standards were incubated for 20 min at 50^{o}C, cooled, and the spectrophotometer was set to 0 using the reagent blank. The absorbance was measured at 510 nm against the reagent blank.

The percentage of RS was calculated on "as is" basis using the following formula:

i. For samples

RS (g/100 g sample) = ΔA x F x (10.3/0.1) x (1/1000) x (100/W) x (162/180)

= ΔA x F/W x 9.27

ii. For Resistant Starch Control (>10% RS)

RS (g/100 g sample) = ΔA x F x (100/0.1) x (1/1000) x (100/W) x (162/180)

= ΔA x F/W x 90

where ΔA= average absorbance (reaction) read against the reagent blank; F = conversion factor from absorbance to micrograms (the absorbance obtained for 100 μg glucose in the GOPOD reaction is determined and F = 100 (micrograms 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 = "as is" weight of test portion 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 $\pm$ 0.05 mL.

3.2.7. Statistical Analysis

Analysis of variances (ANOVA) of the pasting parameters (PV, MV, BD, TSB, FV, PT, PTime), gelatinization temperatures (T_o, T_p, T_c), enthalpy of gelatinization, and resistant starch yield were determined using SAS (Statistical Analysis System) version 9.1 software package (SAS Institute, Cary, NC). Post hoc multiple comparisons were carried out using Tukey's studentized range test. When ANOVA was not suitable, comparison of treatments was performed using Student's *t* test. For the RS values, Grubb's test was performed to eliminate outliers. The

level of significance used in all tests was $p \leq 0.05$.

3.3. RESULTS AND DISCUSSION

3.3.1. Properties of Native Starch

3.3.1.1 Proximate Composition

The crude protein, lipid, crude fiber, and ash of the native starch are presented in Table 3.1. The native rice starch contained 0.64% crude protein, which may be residual endosperm storage protein or protein entrapped within the granules (BeMiller, 2007). A minimal amount of lipid (0.03%) was present while no detectable level of crude fiber was determined from the native rice starch. Lipids present could be free fatty acids and/or lysophospholipids, which may occur as free lipid or complexed with amylose (BeMiller, 2007).

3.3.1.2 Amylose Content

The native rice starch contained 26.26±0.65% amylose. Rice varieties containing this level of amylose belong to the high amylose category (≥25%) (IRRI, 2007b). This can be verified from its pasting profile, wherein a high cooling viscosity was observed (Figure 3.2). The increase in viscosity is due to the high degree of entanglements of amylose during retrogradation (Batey, 2007).

3.3.1.3 Rheological Properties

The results of the temperature sweep test of the native starch suspension are illustrated in Figures 3.3A-3.3B. At lower temperature (50-53.5^{o}C), the storage modulus (G") is greater than the loss modulus (G'), which indicates that the energy applied on the suspension was dissipated viscously and the behavior of the sample is liquid-like (sol) (Rao, 2007). This is due to the increasing dissolution of amylose molecules with temperature (Bao and Bergman, 2004). However, the value of G' became much higher at a temperature between 73.0^{o}C and 76.9^{o}C

Table 3.1 Proximate Composition of Native Rice Starch (Control).

Component	Level (g/100g dry basis)
Protein (*N* x 5.95)	0.64
Crude Fat	0.03
Crude Fiber, maximum	0.00
Ash	0.13

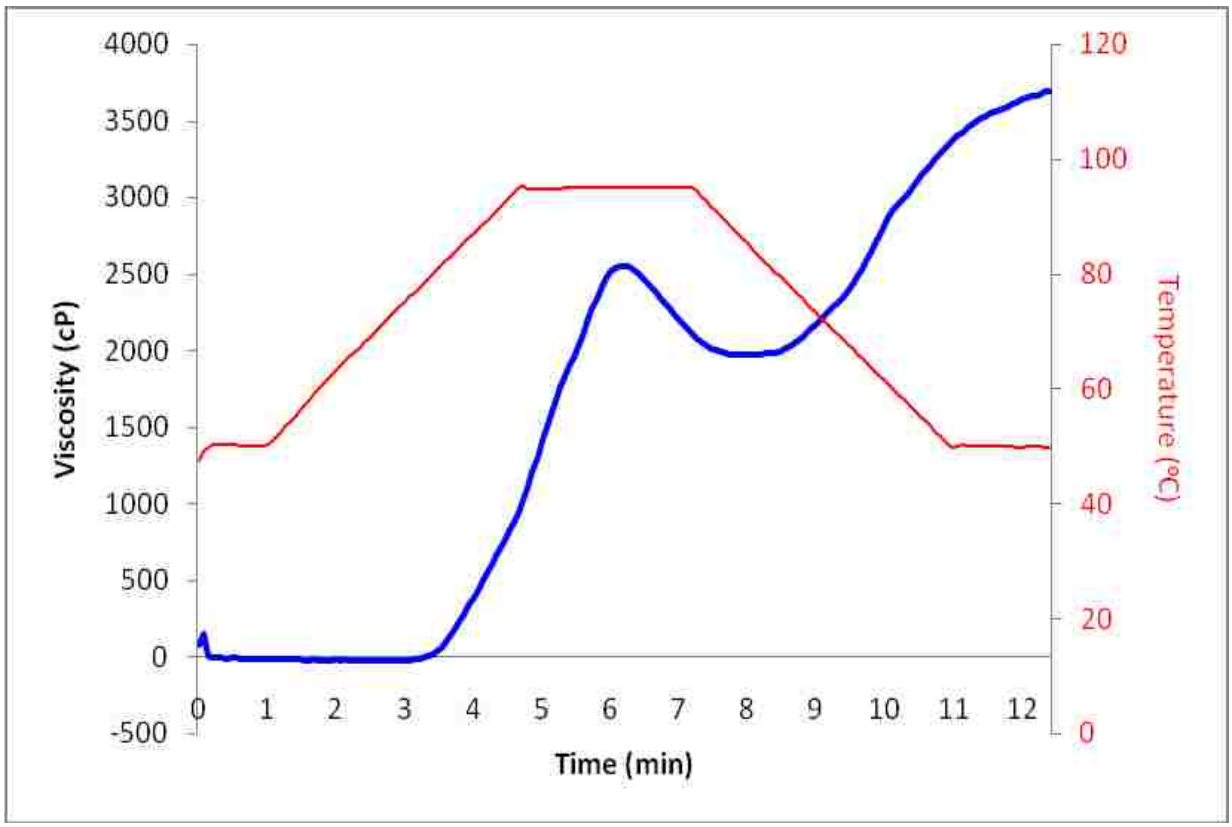


Figure 3.2 RVA Pasting Curve of Native Rice Starch (Control).

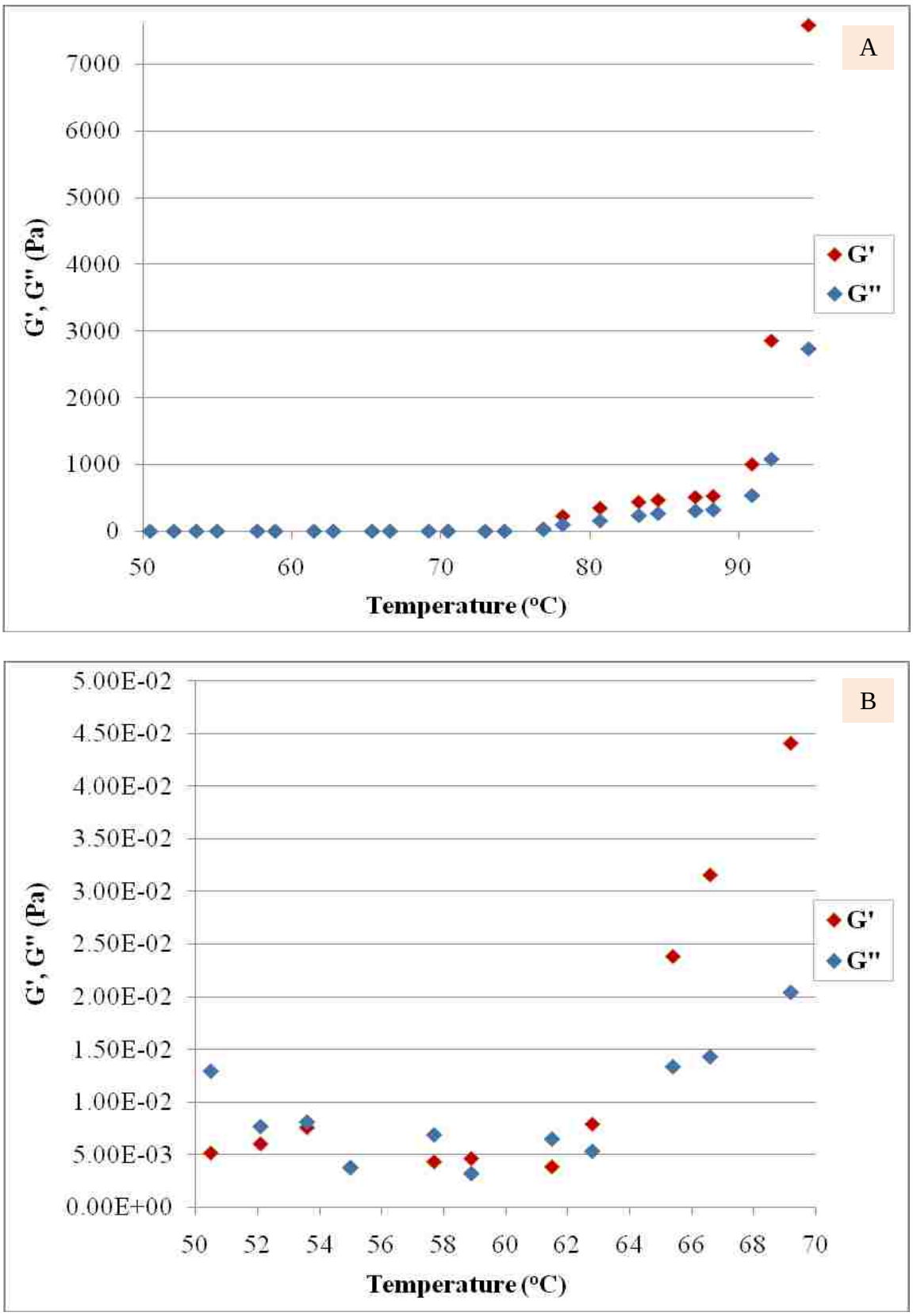


Figure 3.3 Storage Modulus (G') and Loss Modulus (G") of Native Rice Starch during the Temperature Sweep Test Showing (a) the Full Profile at 50-95^{o}C and (b) at Lower Temperatures.

Table 3.2 Dynamic Moduli during Rheological Temperature Sweep Test of Native Rice Starch.

Temperature (^{o}C)	G' (Pa)	G" (Pa)
50.5	0.005 ± 0.02	0.013 ± 0.02
52.1	0.006 ± 0.00	0.008 ± 0.01
53.6	0.008 ± 0.00	0.008 ± 0.00
55.0	0.004 ± 0.00	0.004 ± 0.00
57.7	0.004 ± 0.00	0.007 ± 0.00
58.9	0.005 ± 0.00	0.003 ± 0.00
61.5	0.004 ± 0.01	0.006 ± 0.00
62.8	0.008 ± 0.01	0.005 ± 0.01
65.4	0.024 ± 0.03	0.013 ± 0.01
66.6	0.032 ± 0.05	0.014 ± 0.01
69.2	0.044 ± 0.06	0.020 ± 0.02
70.5	0.053 ± 0.07	0.031 ± 0.03
73.0	0.124 ± 0.10	0.088 ± 0.04
74.3	0.324 ± 0.11	0.243 ± 0.06
76.9	36.933 ± 16.78	22.240 ± 3.49
78.2	226.467 ± 131.66	96.035 ± 25.12
80.7	347.133 ± 194.11	155.400 ± 25.03
83.3	436.600 ± 233.18	234.500 ± 57.56
84.6	466.033 ± 241.70	264.550 ± 69.37
87.1	509.533 ± 246.15	304.450 ± 79.27
88.3	526.200 ± 243.03	319.650 ± 86.20
90.9	1001.500 ± 462.02	534.750 ± 93.69
92.2	2853.667 ± 2171.40	1078.400 ± 446.33
94.7	7579.667 ± 4385.20	2732.500 ± 287.79

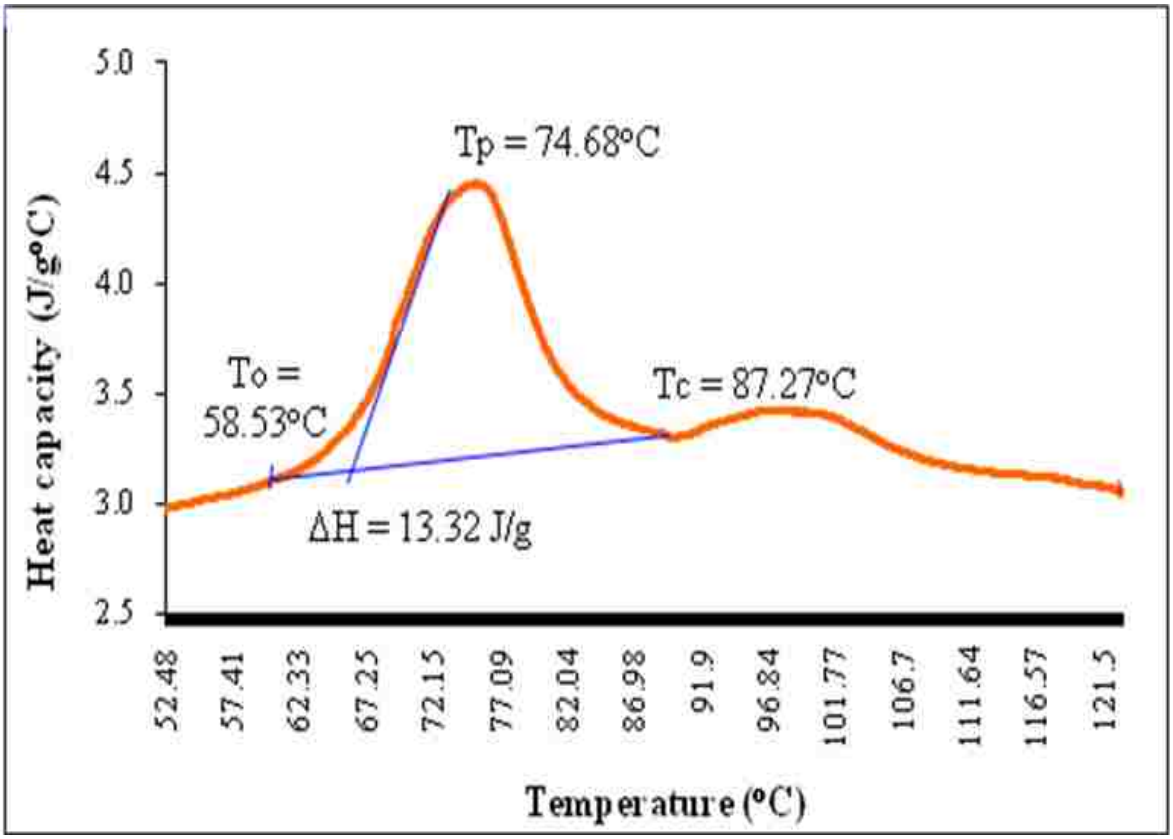


Figure 3.4 DSC Curve of Native Rice Starch.

(Table 3.2). The point where a cross-over (G'=G") was observed is called the gel point. At the gel point, the material behaves at the borderline between liquid- and gel-like. From this point on, the sample's gel- or solid-like character (G'>G") (Figure 3.3B) dominated the viscous behavior (Mezger, 2006). This change to the viscoelastic solid state is attributed to the interaction of leached amylopectin with the amylose matrix (Bao and Bergman, 2004).

The gel point determined by the rheometric test occurred near the gelatinization temperature (T_p = 74.68^{o}C) of native rice starch measured by the DSC (Figure 3.4). The storage modulus measured by rheometer gives information only on the disruption of intermolecular interactions that lead to the formation of paste, whereas gelatinization temperatures measured by DSC provide an insight of the melting of both intra- and intermolecular double helices of starch (Matalanis et al., 2009).

3.3.2 Pasting Properties

3.3.2.1 Amino Acids without pH Treatments

Pasting properties of rice starches with amino acids added differed significantly from those of the native rice starch control (Table 3.3). The treatments significantly lowered the paste viscosities of rice starch (Figure 3.5). Among treatments, LYS had the highest PV of all amino acids, 2066.0$\pm$11.6 cP. This treatment shortened the PTime without causing a considerable change in PT, which implies that addition of lysine to starch without pH adjustment would produce starch that cooks easily. ASP, likewise, reduced the PTime (Table 3.3). Addition of the neutral leucine and tyrosine had no effect. These findings indicate that charges in amino acids play a role in regulating the rate of swelling and collapse of starch granules. Ito et al. (2006b) attributed the changes in the pasting profile to the binding of the amino acids to starch chains. Lysine was believed to reduce swelling of starch granules by binding with starch chains and

Table 3.3 Effects of Additives on the Pasting Characteristics of Native Rice Starch without pH Treatment.[1,2,3,4,5]

Sample	Additive	PV	MV	BD	FV
Control	NoAA	2341.5±37.5a	1893.5±30.4a	448.0±7.1c	3433.0±46.7a
NopH	ASP	1882.8±31.8c	1294.3±34.5c	588.5±3.7a	2152.3±35.1c
	LEU	1951.0±78.9c	1603.0±46.2b	348.0±34.3d	3021.5±114.0ab
	LYS	2066.0±11.6b	1572.5±14.5b	493.5±11.5b	2895.0±25.7ab
	TYR	1903.8±16.3c	1594.5±19.8b	309.3±3.8d	2863.0±58.1bc
Sample	**Additive**	**SB**	**TSB**	**PTime**	**PT**
Control	NoAA	1091.5±9.2a	1539.5±16.3a	6.44±0.0a	81.6±0.0c
NopH	ASP	269.5±3.9d	858.0±4.8b	6.32±0.1b	91.7±0.4a
	LEU	1070.5±43.2a	1418.5±68.8ab	6.45±0.0a	87.9±1.7b
	LYS	829.0±15.9c	1322.5±21.4ab	5.60±0.0c	80.8±0.1c
	TYR	959.3±45.5b	1268.5±43.1ab	6.50±0.0a	90.3±0.0a

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BD=Breakdown; FV=Final Viscosity; SB=Setback; TSB=Total setback; PTime=Time to peak; PT=Pasting Temperature
[2]NopH=No pH Treatment
[3]NoAA=No Amino Acid; ASP=Aspartic Acid; LEU=Leucine; LYS=Lysine; TYR=Tyrosine
[4]Units: Viscosity (cP); Temperature (°C); Time (minute)
[5]Different letters within a column for each pasting property indicate means are significantly different at $p \leq 0.05$

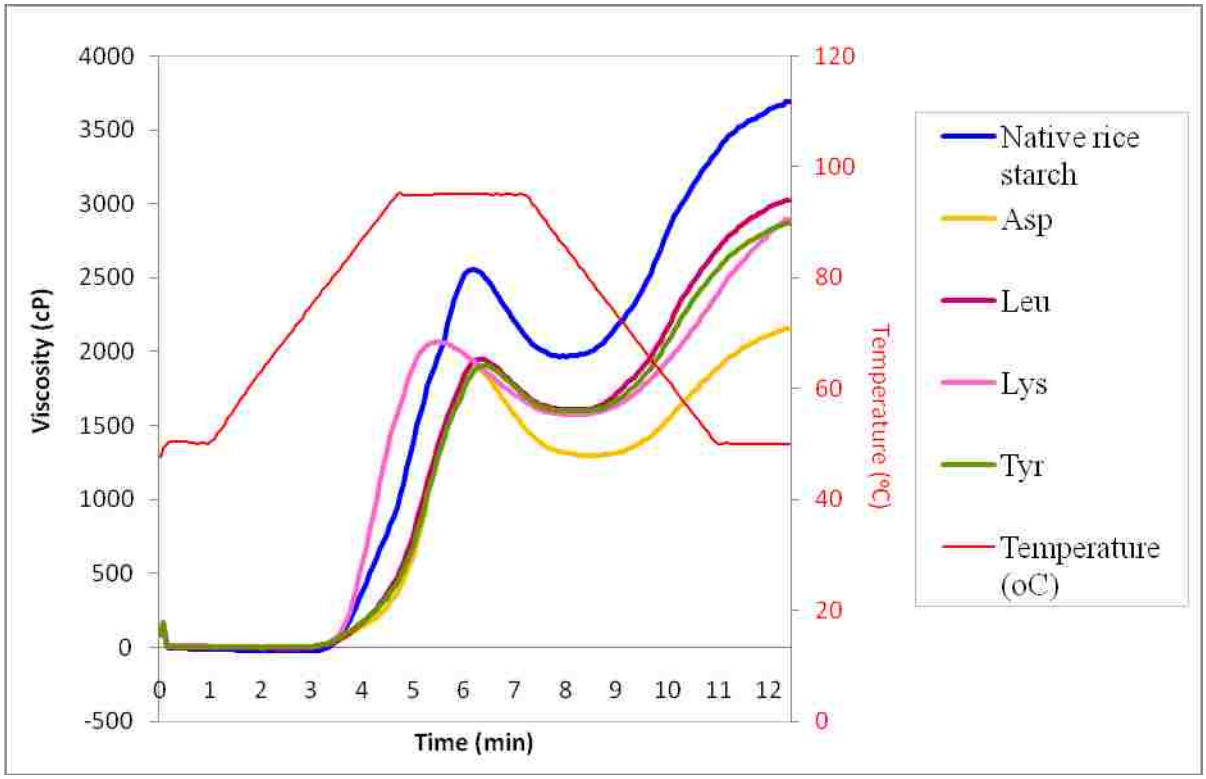


Figure 3.5 Pasting Curves of Rice Starches Added with Amino Acids without pH Treatment.

restricting starch-solvent interaction (Ito et al., 2006b). This binding could be electrostatic in nature (Ito et al., 2006a). In ozone-treated starch, lysine also exerted greater effect on altering the pasting behavior than aspartic acid, which may be due to formation of complex between the positively charged ammonium group of lysine and the anionic groups (carbonyl and carboxyl) of ozonated starch (An and King, 2009).

Leucine and tyrosine decreased the BD, suggesting that stability to shear during cooking was improved by both the neutral amino acids (Table 3.3). ASP was the only sample with significantly lower TSB than the native starch control (Figure 3.5). These results are in agreement with earlier reports (Liang and King, 2003, An and King, 2009).

3.3.2.2 Amino Acids with pH Treatment Using HCl/NaOH Solutions

Table 3.4-3.6 show the pasting profiles of the starches with amino acids at adjusted pH levels using solutions of HCl and NaOH. The use of the pH 4 solution did not result in significant hydrolysis of the starch, since PV was the only property affected (Table 3.4). With added amino acids, therefore, the changes in the pasting profile are due to the additives and not the pH medium. In this pH solution, the negative charged aspartic acid and positive charged lysine significantly increased the BD of rice starch. The neutral charged leucine and tyrosine had an opposite effect (Table 3.4), in which the viscosity of the warm paste dropped but the starches still had a strong tendency to retrograde as shown by their TSB values. However, all the amino acid additives had lower TSB values than the control, with aspartic acid having the lowest value at 882.3$\pm$17.2. This effect was attributed only to the amino acids, since no difference was observed without amino acids in pH 4 solution. Lysine was the only additive which changed the PTime (Figure 3.6) without affecting the PT. It reduced the PTime value by 14% (Table 3.4).

The pasting parameters of the starches treated in pH 7 solution using HCl and NaOH

Table 3.4 Effects of Additives on the Pasting Characteristics of Native Rice Starch Dispersed in pH 4 Solutions with HCl/NaOH.[1,2,3,4]

Sample	Additive	PV	MV	BD	FV
Control	NoAA	2341.5±37.5a	1893.5±30.4a	448.0±7.1c	3433.0±46.7a
pH 4	NoAA	2270.5±9.1b	1849.0±10.0a	421.5±11.1c	3398.8±15.6a
	ASP	1910.3±31.7d	1317.5±16.1d	592.8±16.4a	2199.8±31.0d
	LEU	1933.5±29.8cd	1570.3±31.6b	363.3±13.0d	2925.3±43.7b
	LYS	1974.3±18.3c	1433.3±20.8c	541.0±4.7b	2805.8±19.1c
	TYR	1903.8±14.8d	1522.5±10.7b	381.3±6.6d	2924.0±32.6b
Sample	**Additive**	**SB**	**TSB**	**PTime**	**PT**
Control	NoAA	1091.5±9.2a	1539.5±16.3a	6.44±0.0ab	81.6±0.0d
pH 4	NoAA	1128.3±17.1a	1549.8±25.0a	6.44±0.0ab	82.6±0.4d
	ASP	289.5±5.7d	882.3±17.2c	6.38±0.1b	92.4±0.8a
	LEU	991.8±20.6b	1355.0±30.5b	6.50±0.0a	89.5±1.0b
	LYS	831.5±18.3c	1372.5±21.6b	5.52±0.0c	81.4±0.4d
	TYR	1020.3±18.1b	1401.5±23.6b	6.44±0.0ab	87.5±1.0c

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BD=Breakdown; FV=Final Viscosity; SB=Setback; TSB=Total setback; PTime=Time to peak; PT=Pasting Temperature

[2]NoAA= No Amino Acid; ASP= Aspartic Acid; LEU= Leucine; LYS= Lysine; TYR=Tyrosine

[3]Units: Viscosity (cP); Temperature (°C); Time (minute)

[4]Different letters within a column for each pasting property indicate means are significantly different at $p \leq 0.05$

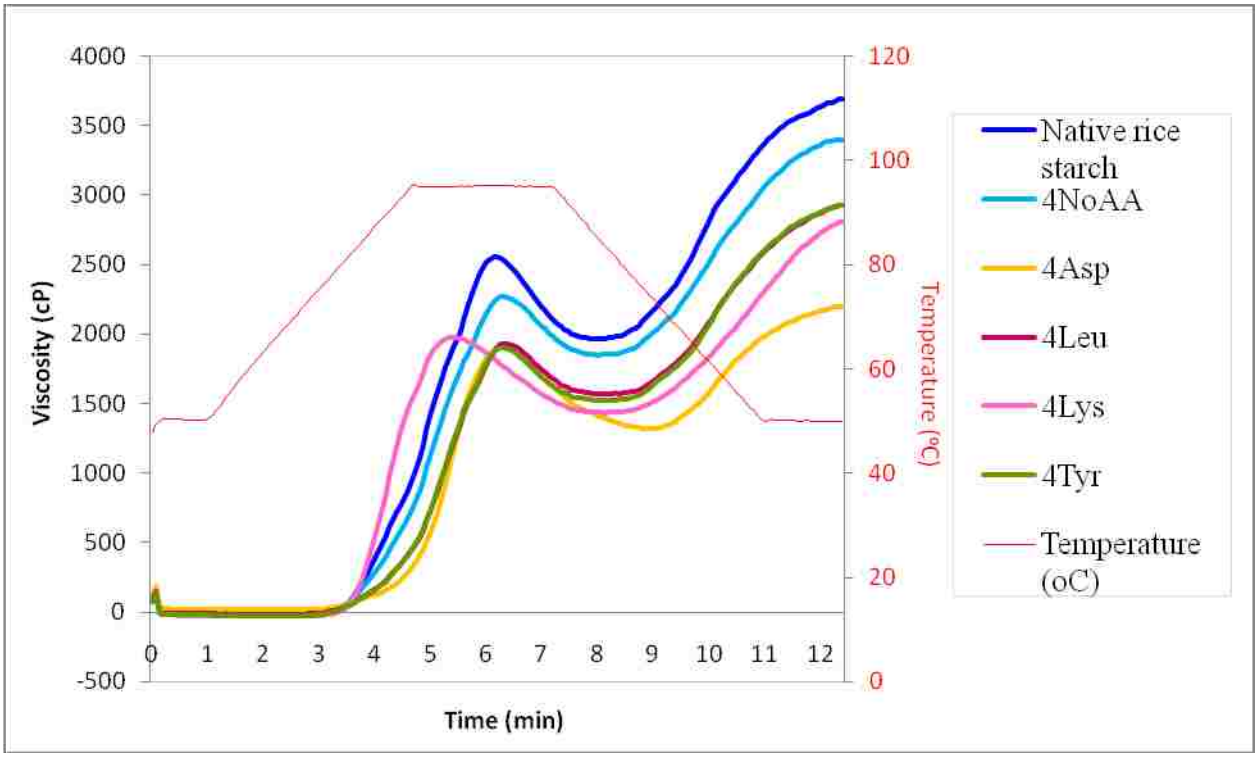


Figure 3.6 Pasting Curves of Rice Starches Added with Amino Acids in pH 4 Solutions.

solutions are presented in Table 3.5. All treatments, including pH 7 solution without amino acids, resulted in decreased PV, MV and FV (Figure 3.7). This denotes that the treatments stabilized the intermolecular cohesion within the starch granules resulting in more rigid swollen granules (Ito et al., 2004). Again, charges played an important role in regulating the pasting properties, as can be seen by the elevation of the BD by aspartic acid and lysine, which had negative and positive net charges at this pH, respectively. As in the previous treatments, lysine caused the PTime values to drop while the PT was unchanged. Leucine and tyrosine, which both had zero net charge in this pH solution, produced starches with very similar pasting profiles.

In pH 10 solution, the impacts of aspartic acid and lysine on BD and TSB were the same as in pH 7 (Tables 3.5-3.6). In terms of PTime and PT, lysine exhibited the same effects as in the previous treatments, indicating that it made the starch easier to cook at all pH levels tested in this study. This is in spite of its reduced amount of charge due to deprotonation of one ammonium group at this pH. These results support the findings of Ito et al. (2004) that charged amino acids impact the pasting properties, whether positive or negative.

Actual pH of the samples without amino acids in pH 4, 7 and 10 solutions were 6.14, 6.22 and 6.54, respectively. This indicated that the starch had a buffering effect. This is most likely why these were smaller, but significant effects on pasting properties, compared to samples with amino acids in the different solutions. The neutral amino acids followed the same pH pattern. The aspartic acid and lysine samples had pHs of around 3.11 and 9.65, respectively, in all pH solutions made with HCl and NaOH.

3.3.2.3 Amino Acids with pH Treatment Using Buffer Solutions

Table 3.7 shows the pasting profile of starches treated with amino acids in acetate buffer. The PV and MV of the starch were unaffected by the buffer treatment alone. Buffer effects were

Table 3.5 Effects of Additives on the Pasting Characteristics of Native Rice Starch Dispersed in pH 7 Solutions with HCl/NaOH.[1,2,3,4]

Sample	Additive	PV	MV	BD	FV
Control	NoAA	2341.5±37.5a	1893.5±30.4a	448.0±7.1c	3433.0±46.7a
pH 7	NoAA	2167.0±67.9b	1757.0±56.2b	410.0±12.8d	3323.5±52.9b
	ASP	1935.8±42.4c	1315.5±32.0e	620.3±13.7a	2241.0±46.7e
	LEU	2005.0±5.8c	1598.3±8.2c	406.8±11.0d	3054.5±16.6c
	LYS	1938.5±22.2c	1432.5±17.4d	506.0±14.3b	2752.5±12.2d
	TYR	1948.5±14.3c	1539.3±7.5c	409.3±21.0d	3003.5±24.6c
Sample	**Additive**	**SB**	**TSB**	**PTime**	**PT**
Control	NoAA	1091.5±9.2ab	1539.5±16.3ab	6.44±0.0a	81.6±0.0cd
pH 7	NoAA	1156.5±68.3a	1566.5±63.7a	6.45±0.0a	82.6±0.4c
	ASP	305.3±10.6d	925.5±14.8d	6.40±0.1a	91.8±0.1a
	LEU	1049.5±11.4b	1456.3±21.0b	6.47±0.1a	87.7±0.7b
	LYS	814.0±17.5c	1320.0±21.5c	5.58±0.1b	81.2±0.5d
	TYR	1055.0±12.1b	1464.3±30.1b	6.38±0.1a	87.3±0.7b

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BD=Breakdown; FV=Final Viscosity; SB=Setback; TSB=Total setback; PTime=Time to peak; PT=Pasting Temperature

[2]NoAA=No Amino Acid; ASP=Aspartic Acid; LEU=Leucine; LYS=Lysine; TYR=Tyrosine

[3]Units: Viscosity (cP); Temperature (°C); Time (minute)

[4]Different letters within a column for each pasting property indicate means are significantly different at $p \leq 0.05$

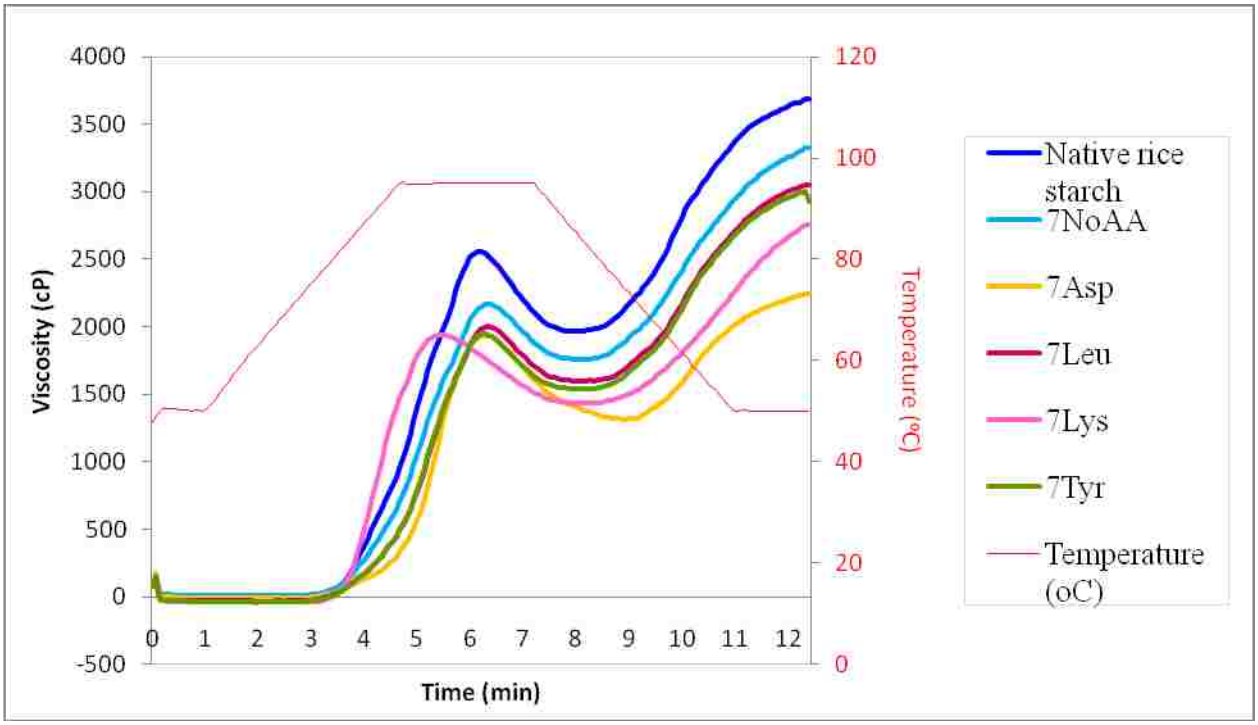


Figure 3.7 Pasting Curves of Rice Starches Added with Amino Acids in pH 7 Solutions.

Table 3.6 Effects of Additives on the Pasting Characteristics of Native Rice Starch Dispersed in pH 10 Solutions with HCl/NaOH.[1,2,3,4]

Sample	Additive	PV	MV	BD	FV
Control	NoAA	2341.5±37.5a	1893.5±30.4a	448.0±7.1cd	3433.0±46.7a
pH 10	NoAA	2215.0±47.1b	1777.3±36.4b	437.8±12.8cd	3371.8±51.7a
	ASP	1847.0±50.3d	1253.0±44.2d	594.0±28.1a	2155.0±57.5d
	LEU	1926.8±43.8cd	1539.3±24.9c	387.5±31.1d	2976.5±80.2b
	LYS	2023.8±38.9c	1492.0±11.2c	531.8±31.4b	2810.0±56.7c
	TYR	1965.0±36.5c	1515.8±21.2c	449.3±17.6c	3096.8±40.6b
Sample	**Additive**	**SB**	**TSB**	**PTime**	**PT**
Control	NoAA	1091.5±9.2bc	1539.5±16.3ab	6.44±0.0a	81.6±0.0c
pH 10	NoAA	1156.8±16.8a	1595.5±24.2a	6.42±0.0ab	82.4±0.1bc
	ASP	308.0±15.0e	902.0±21.8d	6.37±0.0ab	92.3±0.1a
	LEU	1049.8±43.1c	1437.3±73.0b	6.45±0.1ab	85.8±2.9b
	LYS	786.5±18.6d	1318.0±48.3c	5.60±0.1c	81.6±0.1c
	TYR	1131.8±6.1ab	1581.0±23.0a	6.32±0.0b	85.2±2.1bc

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BD=Breakdown; FV=Final Viscosity; SB=Setback; TSB=Total setback; PTime=Time to peak; PT=Pasting Temperature

[2]NoAA=No Amino Acid; ASP=Aspartic Acid; LEU=Leucine; LYS=Lysine; TYR=Tyrosine

[3]Units: Viscosity (cP); Temperature (°C); Time (minute)

[4]Different letters within a column for each pasting property indicate means are significantly different at $p \leq 0.05$

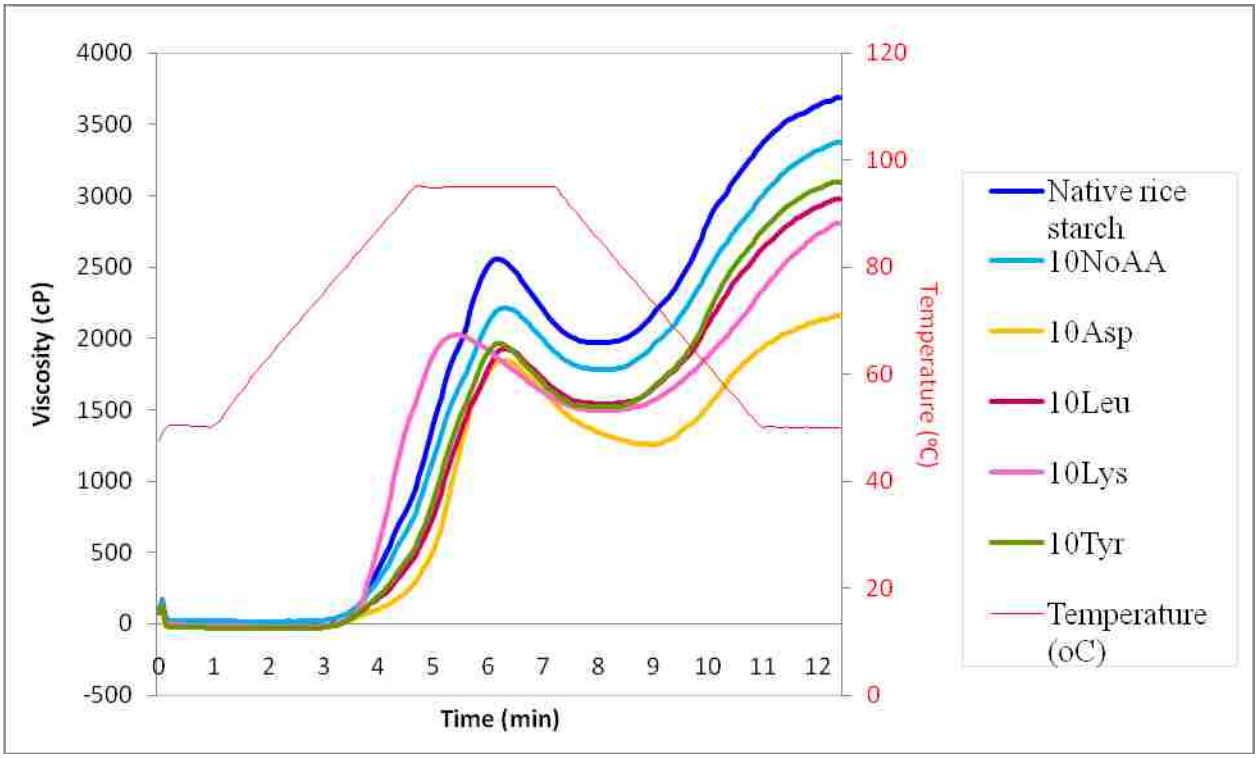


Figure 3.8 Pasting Curves of Rice Starches Added with Amino Acids in pH 10 Solutions.

Table 3.7 Effects of Additives on the Pasting Characteristics of Native Rice Starch Treated with Acetate Buffer, pH 4.[1,2,3,4]

Sample	Additive	PV	MV	BD	FV
Control	NoAA	2341.5±37.5a	1893.5±30.4a	448.0±7.1a	3433.0±46.7a
pH 4	NoAA	2083.0±163.6ab	1741.0±123.0a	342.0±45.9b	2556.5±214.8b
	ASP	1485.8±188.1c	981.5±141.2c	504.3±47.1a	1723.8±257.9c
	LEU	1726.8±138.3bc	1415.5±110.8b	311.3±30.5bc	2213.3±166.9b
	LYS	1887.5±53.7b	1651.3±27.5ab	236.3±32.0c	2494.5±111.8b
	TYR	1735.8±126.5bc	1403.5±101.3b	332.3±52.2bc	2238.3±155.7b
Sample	**Additive**	**SB**	**TSB**	**PTime**	**PT**
Control	NoAA	1091.5±9.2a	1539.5±16.3a	6.44±0.0c	81.6±0.0c
pH 4	NoAA	473.5±52.7c	815.5±98.3b	6.60±0.1b	90.7±0.4b
	ASP	238.0±69.9d	742.3±116.8b	6.27±0.0d	93.1±1.4ab
	LEU	486.5±37.1bc	797.8±66.3b	6.60±0.0b	93.5±1.5a
	LYS	607.0±62.9b	843.3±94.4b	6.82±0.1a	83.4±0.5c
	TYR	502.5±38.7bc	834.8±90.6b	6.50±0.1bc	93.0±1.0ab

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BD=Breakdown; FV=Final Viscosity; SB=Setback; TSB=Total setback; PTime=Time to peak; PT=Pasting Temperature

[2]NoAA=No Amino Acid; ASP=Aspartic Acid; LEU=Leucine; LYS=Lysine; TYR=Tyrosine

[3]Units: Viscosity (cP); Temperature (°C); Time (minute)

[4]Different letters within a column for each pasting property indicate means are significantly different at $p \leq 0.05$

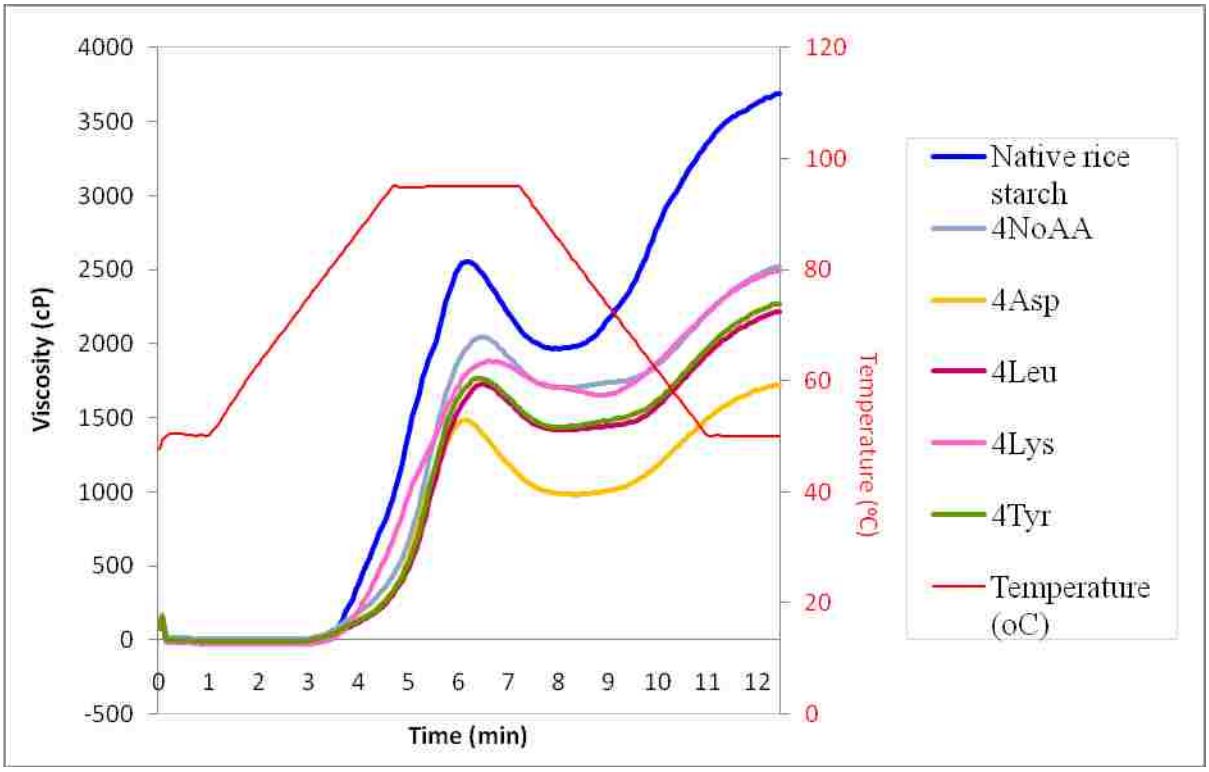


Figure 3.9 Pasting Curves of Rice Starches with Amino Acids Dispersed in Acetate Buffer, pH 4.

seen in the BD, TSB, FV, PTime and PT values. Acetate buffer decreased the BD, FV and TSB, while it increased the PTime and PT. Aspartic acid further lowered the PV and MV. It also produced a starch with the lowest cold paste viscosity and its pasting occurred the earliest. All TSB values, including that without amino acid, were significantly lower than the control, and thus the effect may be due to the buffer. Unlike the previous treatments, lysine added to starch in acetate buffer at pH 4 lengthened the PTime, although PT was still unchanged.

The results of the RVA test for starches treated at pH 7 with phosphate buffer are shown in Table 3.8. Except for MV and PT, the pasting properties of starch dispersed in phosphate buffer alone were significantly different from the parent native starch. With the additives, further decreases in the paste viscosities were observed (Figure 3.10). ASP had a higher BD than without amino acid, confirming the previous findings from the starches with aspartic acid added but with different pH systems. ASP also reduced the PTime and raised the PT. Lysine, on the other hand, did not affect the temperature of pasting.

Suspending rice starch in carbonate buffer at pH 10 resulted in a starch sample with increased BD and decreased SB and PTime, as shown in Table 3.9. Again, addition of amino acids contributed to further lowering of paste viscosities (Figure 3.11), but no differences in lowering the PV were observed between these additives. Aspartic acid significantly reduced the hot and cold paste viscosities, and retrogradation potential, though cooking stability was neither improved nor weakened. Moreover, it was the only additive which altered the PT of native rice starch from $81.6 \pm 0.0^{o}C$ to $92.9 \pm 0.7\ ^{o}C$.

3.3.2.4 Amino Acids with pH and Thermal Treatments

Application of heat to starch-amino acid mixture in the acidic medium below the gelatinization temperature caused significant changes in starch (Table 3.10). Aspartic acid and

Table 3.8 Effects of Additives on the Pasting Characteristics of Native Rice Starch Treated with Phosphate Buffer, pH 7.[1,2,3,4]

Sample	Additive	PV	MV	BD	FV
Control	NoAA	2341.5±37.5a	1893.5±30.4a	448.0±7.1a	3433.0±46.7a
pH 7	NoAA	2089.3±88.8b	1956.8±95.2a	132.5±8.2c	2346.0±133.4b
	ASP	1544.5±100.2d	1276.5±111.4d	268.0±26.4b	1785.8±160.8d
	LEU	1721.5±87.1cd	1587.3±84.4bc	134.3±8.3c	1955.0±165.6cd
	LYS	1831.0±27.2c	1685.5±18.2b	145.5±9.4c	2128.0±53.4bc
	TYR	1574.8±77.3d	1456.5±64.8cd	118.3±18.2c	1900.6±141.3cd

Sample	Additive	SB	TSB	PTime	PT
Control	NoAA	1091.5±9.2a	1539.5±16.3a	6.44±0.0c	81.6±0.0d
pH 7	NoAA	256.8±49.2b	389.3±46.9b	7.00±0.0a	84.4±0.5cd
	ASP	241.3±74.5b	509.3±81.2b	6.63±0.1b	92.9±0.8a
	LEU	233.5±79.4b	367.8±81.3b	6.97±0.1a	86.2±1.3bc
	LYS	297.0±30.9b	442.5±38.5b	6.87±0.1a	84.2±0.4cd
	TYR	325.9±64.9b	444.1±81.3b	6.98±0.0a	87.8±2.4b

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BD=Breakdown; FV=Final Viscosity; SB=Setback; TSB=Total setback; PTime=Time to peak; PT=Pasting Temperature
[2]NoAA=No Amino Acid; ASP=Aspartic Acid; LEU=Leucine; LYS=Lysine; TYR=Tyrosine
[3]Units: Viscosity (cP); Temperature (°C); Time (minute)
[4]Different letters within a column for each pasting property indicate means are significantly different at $p \leq 0.05$

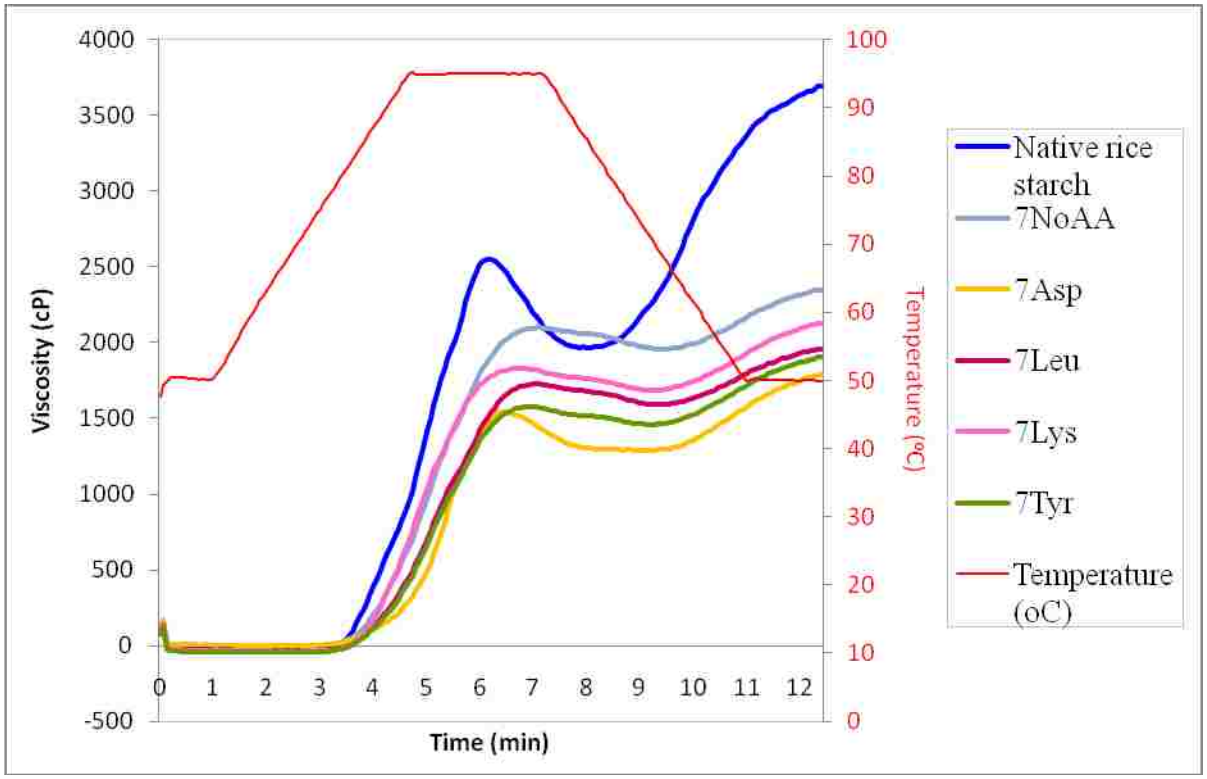


Figure 3.10 Pasting Curves of Rice Starches with Amino Acids Dispersed in Phosphate Buffer, pH 7.

Table 3.9 Effects of Additives on the Pasting Characteristics of Native Rice Starch Treated with Carbonate Buffer, pH 10.[1,2,3,4]

Sample	Additive	PV	MV	BD	FV
Control	NoAA	2341.5±37.5a	1893.5±30.4a	448.0±7.1b	3433.0±46.7a
pH 10	NoAA	2212.3±111.5a	1623.5±190.4abc	588.8±100.1a	3101.0±145.0a
	ASP	1705.8±170.1b	1366.3±136.3c	339.5±36.2bc	2061.5±222.8c
	LEU	1902.5±77.9b	1658.8±44.0ab	243.8±34.2c	2434.0±132.8b
	LYS	1831.5±108.0b	1578.5±90.4bc	253.0±34.9c	2554.3±146.9b
	TYR	1848.3±47.9b	1480.0±17.5bc	368.3±56.5bc	2575.3±96.0b
Sample	**Additive**	**SB**	**TSB**	**PTime**	**PT**
Control	NoAA	1091.5±9.2a	1539.5±16.3a	6.44±0.0a	81.6±0.0bc
pH 10	NoAA	889.3±36.4b	1478.0±80.1a	5.62±0.2d	83.2±0.9b
	ASP	355.8±55.2e	695.3±88.8d	6.55±0.0a	92.9±0.7a
	LEU	531.5±55.1d	775.3±89.3cd	6.28±0.1ab	82.0±0.5bc
	LYS	722.8±56.8c	975.8±77.6bc	6.03±0.1bc	83.2±1.1b
	TYR	727.0±48.8c	1095.3±105.2b	5.92±0.2cd	81.1±0.4c

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BD=Breakdown; FV=Final Viscosity; SB=Setback; TSB=Total setback; PTime=Time to peak; PT=Pasting Temperature

[2]NoAA=No Amino Acid; ASP=Aspartic Acid; LEU=Leucine; LYS=Lysine; TYR=Tyrosine

[3]Units: Viscosity (cP); Temperature (°C); Time (minute)

[4]Different letters within a column for each pasting property indicate means are significantly different at $p \leq 0.05$

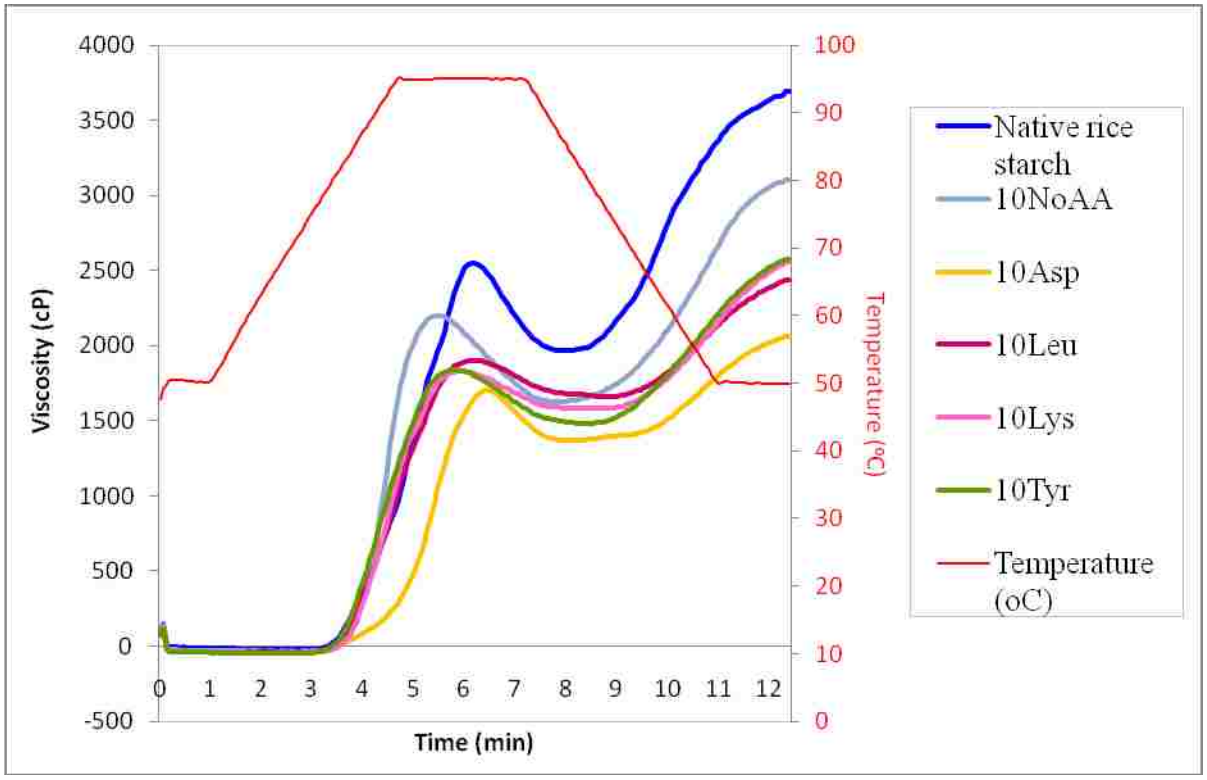


Figure 3.11 Pasting Curves of Rice Starches with Amino Acids Dispersed in Carbonate Buffer, pH 10.

Table 3.10 Effects of Additives on the Pasting Characteristics of Native Rice Starch Dispersed in pH 4 Solutions with HCl/NaOH and Heat-Treated.[1,2,3,4]

Sample	Additive	PV	MV	BD	FV
Control	NoAA	2341.5±37.5a	1893.5±30.4a	448.0±7.1a	3433.0±46.7a
pH 4	NoAA	2123.8±21.4b	1756.8±20.4b	367.0±40.3b	3087.0±62.4b
	ASP	1693.0±21.5e	1187.0±7.6f	506.0±23.8a	1978.5±28.5e
	LEU	1808.3±23.5cd	1518.5±3.8c	289.8±25.6c	2615.0±90.2cd
	LYS	1818.0±19.1c	1362.5±11.3e	455.5±10.3a	2585.3±21.9d
	TYR	1760.0±6.3d	1455.3±16.1d	304.8±15.1c	2736.5±35.4c

Sample	Additive	SB	TSB	PTime	PT
Control	NoAA	1091.5±9.2a	1539.5±16.3a	6.44±0.0b	81.6±0.0c
pH 4	NoAA	974.5±36.1a	1330.3±82.4b	6.65±0.1a	88.5±3.6b
	ASP	285.5±7.1c	791.5±30.2d	6.54±0.1ab	94.5±0.7a
	LEU	806.8±98.6b	1096.5±87.7c	6.67±0.1a	92.5±0.8ab
	LYS	767.3±16.3b	1222.8±18.9bc	5.67±0.1c	81.3±0.4c
	TYR	976.5±40.9a	1281.3±46.0b	6.50±0.1ab	87.5±0.7b

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BD=Breakdown; FV=Final Viscosity; SB=Setback; TSB=Total setback; PTime=Time to peak; PT=Pasting Temperature

[2]NoAA=No Amino Acid; ASP=Aspartic Acid; LEU=Leucine; LYS=Lysine; TYR=Tyrosine

[3]Units: Viscosity (cP); Temperature (°C); Time (minute)

[4]Different letters within a column for each pasting property indicate means are significantly different at $p \leq 0.05$

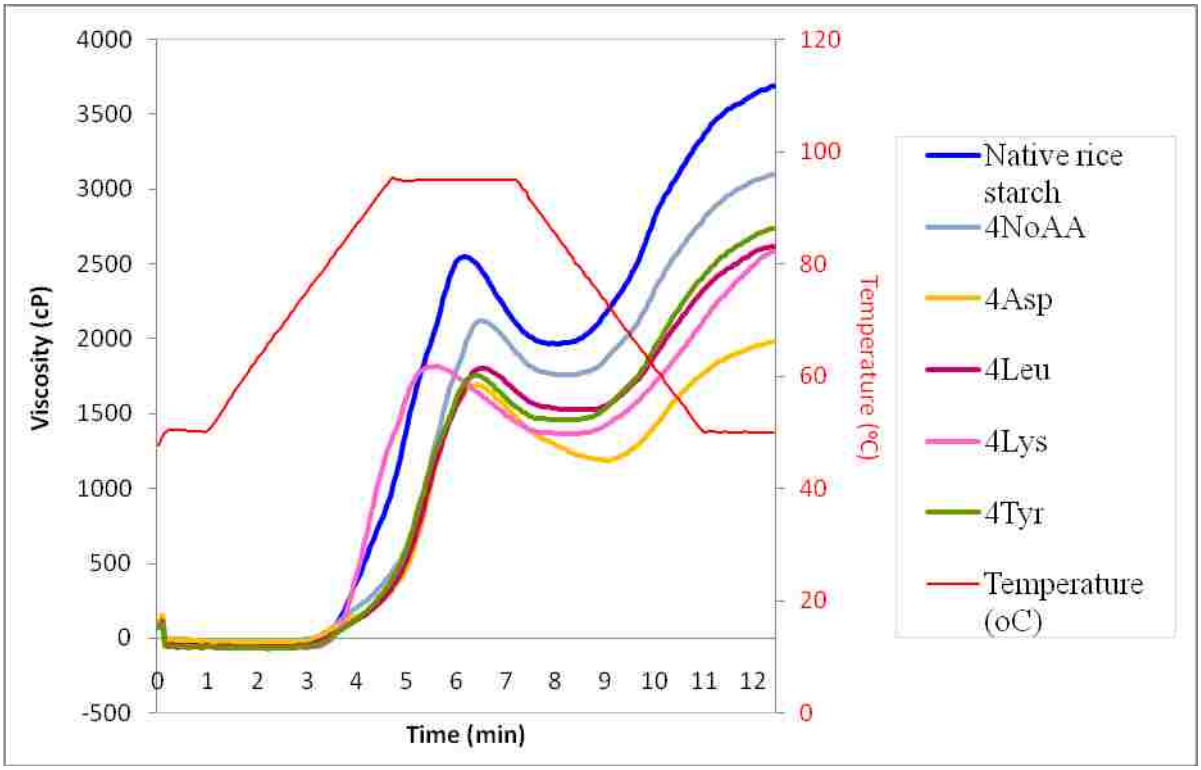


Figure 3.12 Pasting Curves of Rice Starches with Amino Acids in pH 4 Solutions with Heat Treatment.

lysine, however, showed a similar behavior in regards to BD. They both increased the BD of starch relative to the sample in pH 4 solution without amino acids, so that their values were comparable with that of the parent native starch. Lysine again decreased the PTime without changing the PT (Figure 3.12).

The pasting properties of starches treated in pH 7 solution with heating are presented in Table 3.11. Heating alone lowered the PV, BD, FV, SB, and TSB (Figure 3.13), and delayed the PTime. Similar trends with respect to elevation of BD by aspartic acid and reduction of PTime without change in PT by lysine were observed for this treatment. The neutral amino acids leucine and tyrosine, on the other hand, had lower BD than the control, but were not different from pH 7 solution alone.

Heating alone similarly caused the lowering of pasting viscosities, notably the PV, BD, and FV, and delayed the PTime in pH 10 solution (Table 3.12). Amino acids further lowered the FV, resulting in reduced retrogradation potential, except for LEU. ASP and LYS had the lowest TSB at 816.8$\pm$12.9 cP and 1129.5$\pm$22.6 cP, respectively. Their BD values were comparable with that of the native starch, whereas LEU and TYR had significantly lower BD levels, but were not different from pH 10 solution only samples.

3.3.2.5 Comparison of Treatments

The use of different liquids as dispersion agents yielded significantly different pasting properties when analyzed using Student's *t* test at $p \leq 0.05$. When the pH of the medium was adjusted using HCl and NaOH solutions, pasting viscosities were altered among all amino acids relative to those of water-dispersed starches. For aspartic acid-added starches, the SB and TSB were higher in the samples with pH solution added. For lysine-added starch, the TSB was higher and the PTime was lower in pH 4 solution than when distilled water was used. In pH 10 solution,

Table 3.11 Effects of Additives on the Pasting Characteristics of Native Rice Starch Dispersed in pH 7 Solutions with HCl/NaOH and Heat-Treated.[1,2,3,4]

Sample	Additive	PV	MV	BD	FV
Control	NoAA	2341.5±37.5a	1893.5±30.4a	448.0±7.1b	3433.0±46.7a
pH 7	NoAA	2161.0±16.7b	1786.8±48.7a	374.3±42.6cd	3138.5±46.6b
	ASP	1748.0±4.7c	1204.3±16.2d	543.8±14.4a	2024.8±18.8e
	LEU	1816.3±18.1c	1490.3±3.6b	326.0±21.3d	2767.0±45.0c
	LYS	1762.8±121.8c	1325.5±86.7c	437.3±36.5bc	2473.0±150.9d
	TYR	1699.3±23.9c	1359.8±24.9c	339.5±7.4d	2762.5±20.7c

Sample	Additive	SB	TSB	PTime	PT
Control	NoAA	1091.5±9.2a	1539.5±16.3a	6.44±0.0b	81.6±0.0c
pH 7	NoAA	977.5±37.9b	1351.8±75.5b	6.67±0.1a	85.7±6.2bc
	ASP	276.8±19.1d	820.5±30.2d	6.47±0.0b	94.1±0.7a
	LEU	950.8±27.6b	1276.8±48.3b	6.55±0.0ab	90.3±1.4ab
	LYS	710.3±51.4c	1147.5±70.3c	5.64±0.1c	80.8±0.0c
	TYR	1063.3±30.8a	1402.8±31.9b	6.42±0.0b	87.9±4.2abc

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BD=Breakdown; FV=Final Viscosity; SB=Setback; TSB=Total setback; PTime=Time to peak; PT=Pasting Temperature

[2]NoAA=No Amino Acid; ASP=Aspartic Acid; LEU=Leucine; LYS=Lysine; TYR=Tyrosine

[3]Units: Viscosity (cP); Temperature (°C); Time (minute)

[4]Different letters within a column for each pasting property indicate means are significantly different at $p \leq 0.05$

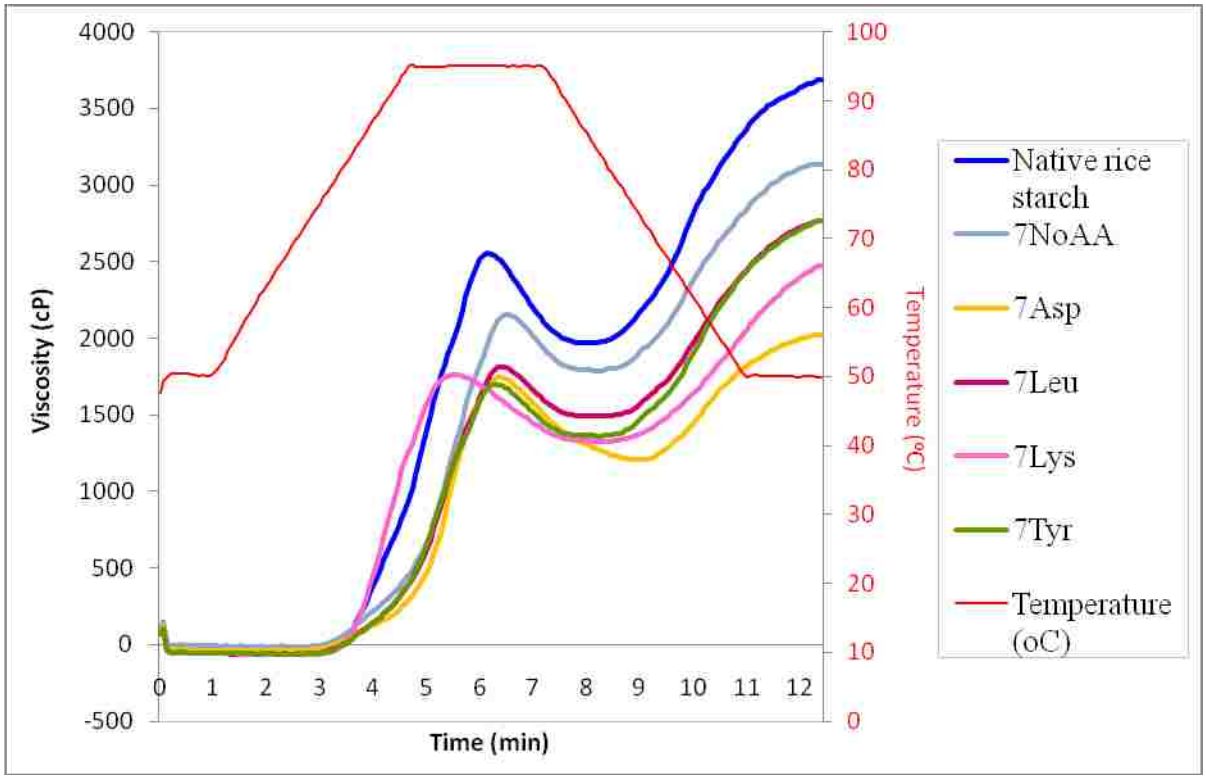


Figure 3.13 Pasting Curves of Rice Starches with Amino Acids in pH 7 Solutions with Heat Treatment.

Table 3.12 Effects of Additives on the Pasting Characteristics of Native Rice Starch Dispersed in pH 10 Solutions with HCl/NaOH and Heat-Treated.[1,2,3,4]

Sample	Additive	PV	MV	BD	FV
Control	NoAA	2341.5±37.5a	1893.5±30.4a	448.0±7.1ab	3433.0±46.7a
pH 10	NoAA	2187.7±28.9b	1815.0±38.4a	372.8±46.0c	3200.5±35.4b
	ASP	1653.5±45.2c	1157.0±29.2d	496.5±23.7a	1973.8±37.6e
	LEU	1649.0±52.6c	1314.3±38.1c	334.8±17.9c	2696.3±76.7c
	LYS	1742.0±6.5c	1305.8±4.8c	436.3±3.9b	2435.3±27.1d
	TYR	1773.5±97.0c	1457.0±105.0b	316.5±11.2c	2726.8±27.3c
Sample	**Additive**	**SB**	**TSB**	**PTime**	**PT**
Control	NoAA	1091.5±9.2a	1539.5±16.3a	6.44±0.0b	81.6±0.0b
pH 10	NoAA	1012.8±17.6ab	1385.5±62.1ab	6.65±0.1a	85.4±3.6b
	ASP	320.3±12.1d	816.8±12.9d	6.50±0.0ab	94.5±0.7a
	LEU	1047.3±28.7ab	1382.0±46.2ab	6.42±0.0b	87.1±2.8ab
	LYS	693.3±23.0c	1129.5±22.6c	5.67±0.1c	80.2±0.4b
	TYR	953.3±120.0b	1269.8±126.8cd	6.52±0.1ab	87.9±5.8ab

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BD=Breakdown; FV=Final Viscosity; SB=Setback; TSB=Total setback; PTime=Time to peak; PT=Pasting Temperature

[2]NoAA=No Amino Acid; ASP=Aspartic Acid; LEU=Leucine; LYS=Lysine; TYR=Tyrosine

[3]Units: Viscosity (cP); Temperature (°C); Time (minute)

[4]Different letters within a column for each pasting property indicate means are significantly different at $p \leq 0.05$

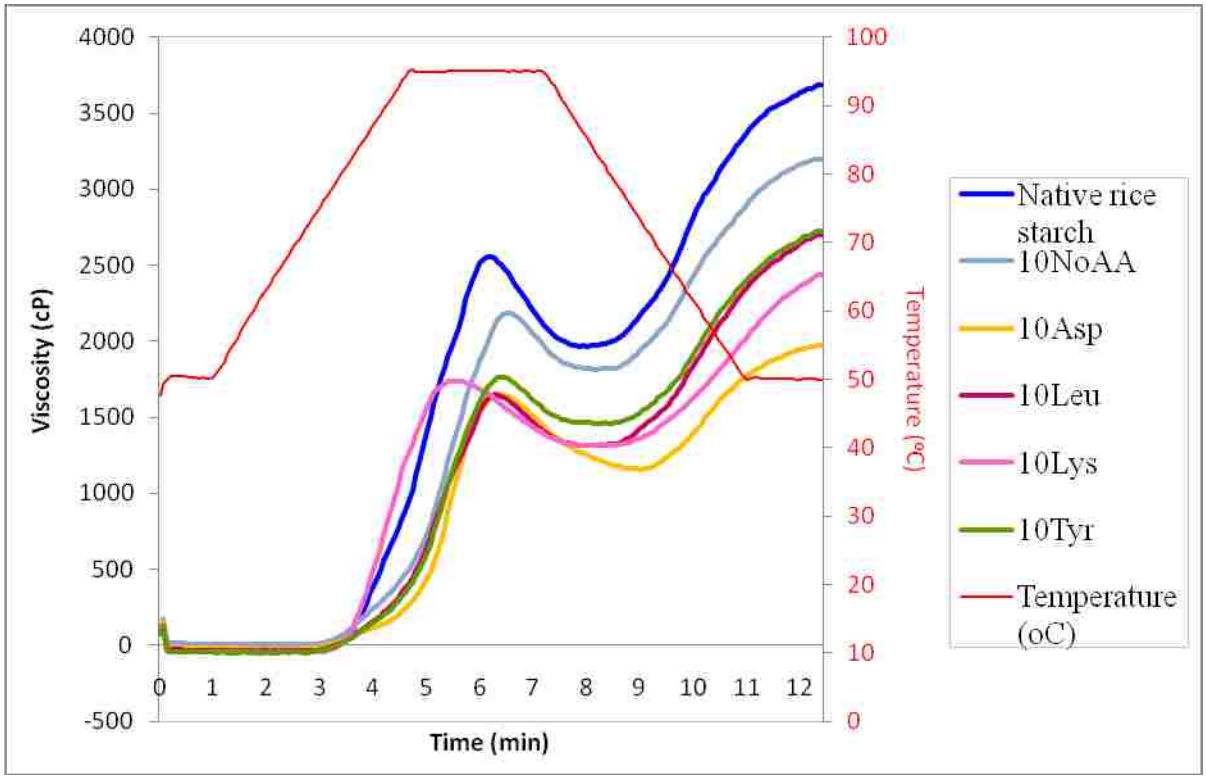


Figure 3.14 Pasting Curves of Rice Starches with Amino Acids in pH 10 Solutions with Heat Treatment.

starch with tyrosine had higher BD and shorter PTime than when just distilled water was added. Meanwhile, minor change in pasting properties of leucine-added starch was observed.

Likewise, buffers significantly affected the pasting profiles, especially for samples treated with leucine, lysine and tyrosine. Heating mostly lowered the viscosities of the samples, lengthened the pasting time and raised the PT. These results showed that addition of amino acids in combination with adjustment of pH with or without heating will yield starches of different functionalities, and can therefore be used for certain products depending on the intended application.

3.3.2.6 Starches with Tyrosine at Different pH Treatments Prepared Using the RVA

The different pH treatments using solutions of HCl/NaOH with tyrosine did not change most of the pasting parameters of native starch (Figure 3.15). Without any pH treatment, tyrosine increased the BD by about 18% (Table 3.13). pH 10 reduced the MV and therefore increased the BD. TSB also increased with alkalinity. These effects might be due to changes in hydrogen bonding that may be occurring.

Samples with tyrosine dispersed in the buffer systems displayed different pasting behaviors than native rice starch (Table 3.14). The PV and MV tended to increase with pH treatments, whereas FV, SB and TSB were all reduced. Incorporation of tyrosine in starch using pH 7 phosphate buffer as a dispersant produced an end-product with markedly different pasting profile than the untreated starch control and starch without pH treatment (Figure 3.16). It had higher PV and MV, lower BD, FV and TSB, and longer PTime. At alkaline pH, the cooking time was the shortest. The PT, however, was unchanged by the treatments.

Compared with the pretreatment step of dispersing starch-tyrosine mixture in pH adjusted mediums and lyophilizing the suspension, these results were statistically different according to

Table 3.13 Effects of Additives on the Pasting Characteristics of Native Rice Starch with Tyrosine Dispersed in Solutions of HCl/NaOH and Gelatinized Using the RVA.[1,2,3,4]

Sample	pH	PV	MV	BD	FV
Control	None	2341.5±37.5a	1893.5±30.4a	448.0±7.1c	3433.0±46.7a
	NopH	2369.5±13.4a	1842.5±2.1ab	527.0±11.3b	3457.5±20.5a
HCl/ NaOH	4	2381.0±1.4a	1882.5±10.6ab	498.5±9.2b	3388.0±9.9a
	7	2359.5±7.8a	1827.5±7.8b	532.0±15.6b	3462.0±0.0a
	10	2330.5±7.8a	1738.5±3.5c	592.0±11.3a	3488.5±44.5a

Sample	pH	SB	TSB	PTime	PT
Control	None	1091.5±9.2a	1539.5±16.3bc	6.44±0.0a	81.6±0.0a
	NopH	1088.0±7.1a	1615.0±18.4b	6.40±0.0a	80.8±0.1a
HCl/ NaOH	4	1007.0±8.5b	1505.5±0.7c	6.44±0.0a	81.6±0.1a
	7	1102.5±7.8a	1634.5±7.8b	6.33±0.0a	81.2±0.6a
	10	1158.0±36.8a	1750.0±48.1a	6.30±0.0a	80.8±0.0a

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BD=Breakdown; FV=Final Viscosity; SB=Setback; TSB=Total setback; PTime=Time to peak; PT=Pasting Temperature

[2]NopH=No pH Treatment (distilled water)

[3]Units: Viscosity (cP); Temperature (°C); Time (minute)

[4]Different letters within a column for each pasting property indicate means are significantly different at $p \leq 0.05$

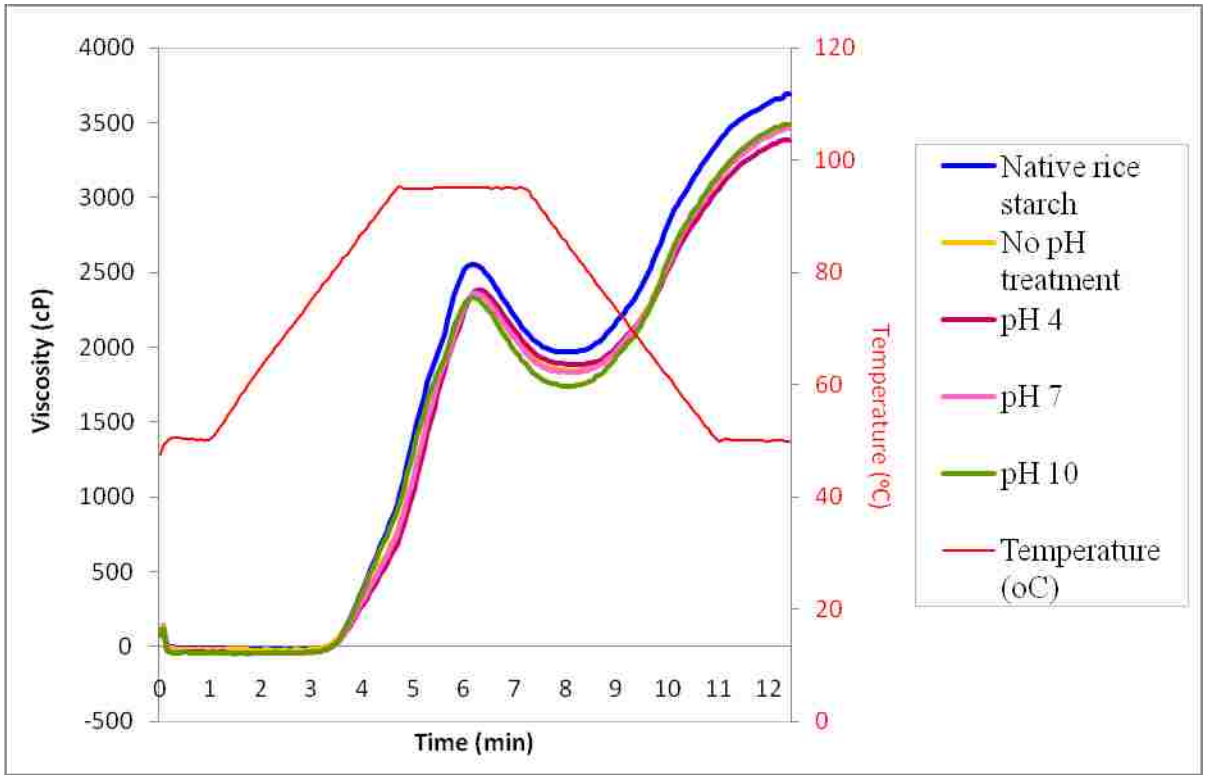


Figure 3.15 Pasting Curves of RVA Gelatinized Rice Starches with Tyrosine Dispersed in Solutions Adjusted to Different pHs Using HCl/NaOH.

Table 3.14 Effects of Additives on the Pasting Characteristics of Native Rice Starch with Tyrosine Dispersed in Buffer Solutions and Gelatinized Using the RVA.[1,2,3,4,5]

Sample	pH	PV	MV	BD	FV
Control	None	2341.5±37.5c	1893.5±30.4c	448.0±7.1c	3433.0±46.7a
	NopH	2369.5±13.4bc	1842.5±2.1c	527.0±11.3b	3457.5±20.5a
Buffer	4	2660.0±0.0a	1903.5±24.7c	756.5±24.7a	3128.5±0.7b
	7	2667.0±14.1a	2464.0±5.7a	203.0±8.5d	2860.0±26.9d
	10	2436.5±12.0b	2051.0±15.6b	385.5±27.6c	2977.5±7.8c
Sample	**pH**	**SB**	**TSB**	**PTime**	**PT**
Control	None	1091.5±9.2a	1539.5±16.3b	6.44±0.0b	81.6±0.0a
	NopH	1088.0±7.1a	1615.0±18.4a	6.40±0.0b	80.8±0.1a
Buffer	4	468.5±0.7c	1225.5±25.5c	6.60±0.1b	84.1±0.0a
	7	193.0±12.7d	396.0±21.2e	6.97±0.0a	84.0±0.0a
	10	541.0±19.8b	926.5±7.8d	6.10±0.0c	72.8±14.7a

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BD=Breakdown; FV=Final Viscosity; SB=Setback; TSB=Total setback; PTime=Time to peak; PT=Pasting Temperature
[2]NopH=No pH Treatment (distilled water)
[3]Units: Viscosity (cP); Temperature (°C); Time (minute)
[4]Buffers used were acetate (pH 4), phosphate (pH 7) and carbonate (pH 10)
[5]Different letters within a column for each pasting property indicate means are significantly different at $p \leq 0.05$

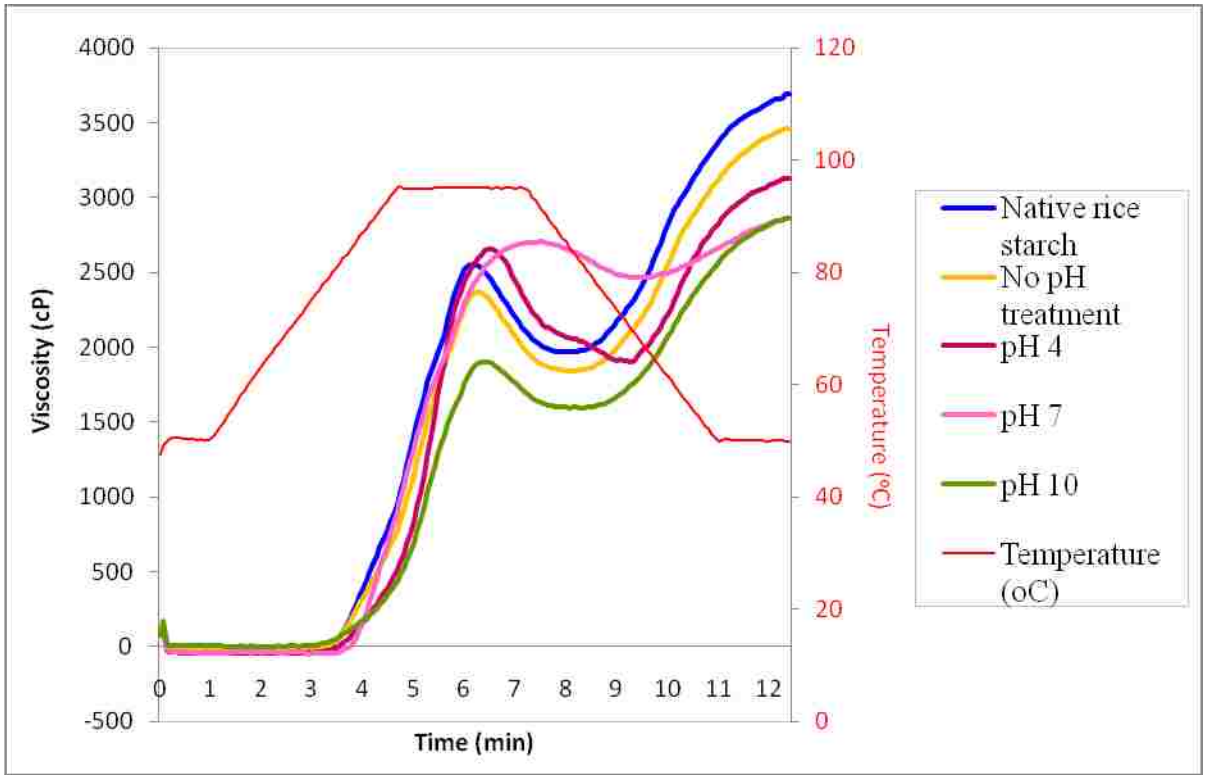


Figure 3.16 Pasting Curves of RVA Gelatinized Rice Starches with Tyrosine Dispersed in Solutions Adjusted to Different pHs using Buffers.

Student's *t* test ($p \leq 0.05$). All pasting viscosities were lower in the pretreated starch in all treatments, while pasting times were generally unchanged. The pretreatment step might have allowed more interactions between tyrosine and starch granules so that more pronounced changes in the effects on swelling and granular collapse were observed. These interactions might also be similar to effects of solutes which can compete with starch for hydration, since tyrosine contains a hydroxyl group capable of H bonding (Donald, 2004).

3.3.3 Thermal Characteristics by DSC

3.3.3.1 Amino Acids without pH Treatments

Without pH treatment, aspartic acid and lysine enhanced the ability of the starch to resist swelling, as shown by the higher T_p and T_c of LYS (Table 3.15). Starches which are more resistant to gelatinization require more energy to disorganize their structure (Biliaderis et al., 1986) and substances with net charges were shown to provide this resistance. This is attributable to the binding ability of these substances to starch (Ito et al., 2004. 2006b). Lysine- and aspartic acid-added starches also reduced the formation of amylose-lipid complex in the rice starch, as seen from the loss of the characteristic second transition endotherm occurring around 100^{o}C in their DSC curves (Figure 3.17). The neutral charged leucine and tyrosine had no effect on the thermal properties, similar to what was observed by Ito et al. (2006b).

3.3.3.2 Amino Acids with pH Treatments Using HCl/NaOH Solutions

Treatment with pH 4 solution did not change the thermal properties of rice starch (Table 3.16). This indicates that no cleavage of glycosidic linkages was effected by the mild acid treatment (Puchongkavarin et al., 2003). Even with addition of amino acids, no significant change was observed, except for lysine. The positive charged lysine increased the T_p and T_c of rice starch (Figure 3.18) without changing the enthalpy of melting, which signifies that swelling

Table 3.15 DSC Parameters of Rice Starch with Amino Acid Additives and No pH Treatment.[1,2,3,4]

Sample	Additive	Gelatinization Endotherm			
		T_o	T_p	T_c	ΔH
Control	NoAA	58.53±1.26a	74.68±0.47b	87.27±1.34b	13.32±1.94a
NopH	ASP	58.74±3.65a	76.33±0.21a	91.45±1.28a	12.52±1.14a
	LEU	57.97±0.51a	74.20±0.32b	86.88±0.50b	14.04±0.56a
	LYS	58.75±4.76a	76.46±0.73a	94.24±1.95a	15.05±1.50a
	TYR	59.85±2.45a	74.44±0.39b	86.48±1.10b	12.23±1.19a

[1]T_o=onset temperature; T_p=peak temperature; T_c=conclusion temperature; ΔH (Enthalpy)
[2]NoAA= No Amino Acid; ASP= Aspartic Acid; LEU= Leucine; LYS= Lysine; TYR=Tyrosine
[3]Units: Temperature (°C), Enthalpy (J/g, dry matter)
[4]Different letters within column indicate means are significantly different at $p \leq 0.05$

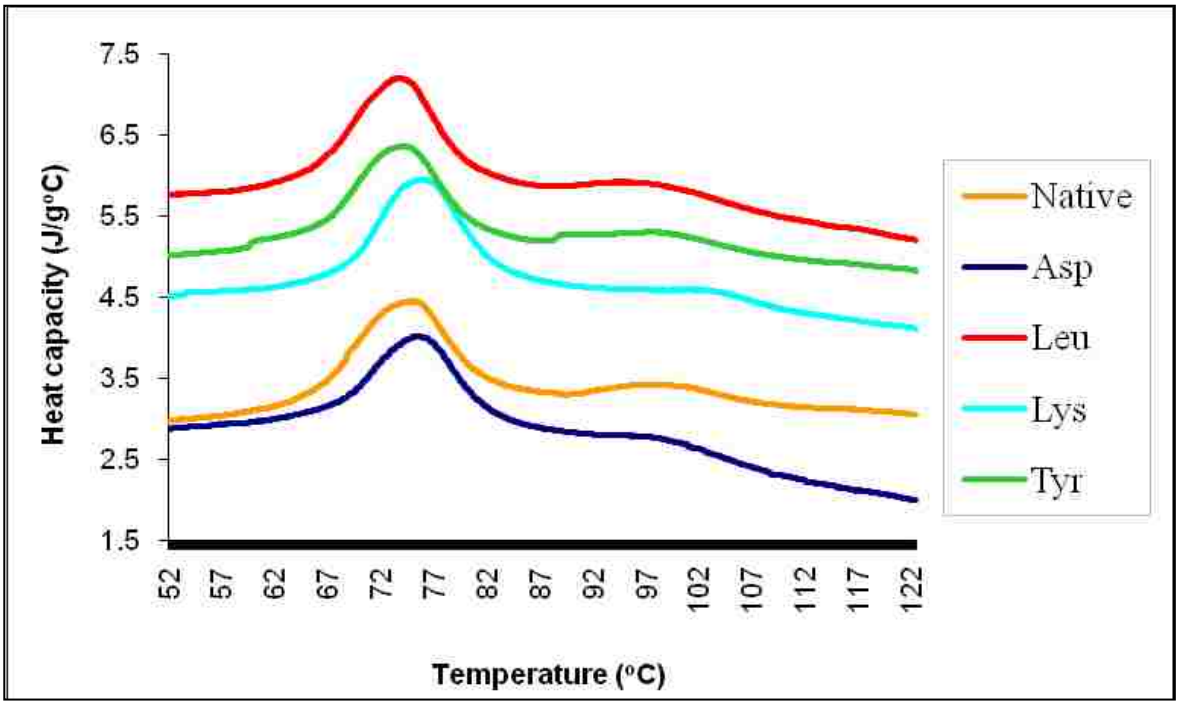


Figure 3.17 Thermal Curves of Rice Starches with Amino Acids without pH Treatment.

Table 3.16 DSC Parameters of Rice Starch with Amino Acid Additives and pH Treatments (HCl/NaOH).[1,2,3,4]

Sample	Additive	Gelatinization Endotherm			
		T_o	T_p	T_c	ΔH
Control	NoAA	58.53±1.26bcd	74.68±0.47cdef	87.27±1.34d	13.32±1.94abc
pH 4	NoAA	60.17±1.67abcd	75.28±0.86bcd	89.42±1.56abcd	12.59±1.02bc
	ASP	60.17±1.94abcd	74.77±0.36cde	87.82±1.07cd	12.92±0.95bc
	LEU	55.83±2.32d	74.07±0.20ef	87.56±1.16cd	15.24±0.98ab
	LYS	61.35±1.38abc	76.51±0.62a	92.60±0.79ab	15.36±0.67ab
	TYR	57.53±2.44bcd	73.87±0.18abc	86.54±0.62d	13.45±0.55abc
pH 7	NoAA	57.40±1.62cd	74.46±0.20def	88.91±1.19bcd	13.94±0.78abc
	ASP	63.63±2.55a	75.41±0.45abcd	87.68±0.46cd	11.15±2.04c
	LEU	59.18±1.86abcd	73.75±0.25ef	87.19±0.46d	13.78±1.28abc
	LYS	62.17±0.58ab	76.13±0.39ab	91.24±0.65abc	15.04±0.57ab
	TYR	57.50±0.31bcd	73.78±0.39ef	86.97±0.83d	14.24±0.59abc
pH 10	NoAA	60.31±2.04abcd	74.65±0.81cdef	88.06±2.07cd	12.39±1.44bc
	ASP	58.45±0.85bcd	74.41±0.35def	87.62±2.15cd	13.74±0.55abc
	LEU	58.62±1.86bcd	73.55±0.12f	87.74±0.82cd	14.61±1.39ab
	LYS	58.92±1.83abcd	75.65±0.20abc	93.25±2.39a	16.46±0.98a
	TYR	58.47±2.19bcd	73.60±0.31ef	86.02±2.76d	13.24±2.11bc

[1]T_o=onset temperature; T_p=peak temperature; T_c=conclusion temperature; ΔH (Enthalpy)
[2]NoAA= No Amino Acid; ASP= Aspartic Acid; LEU= Leucine; LYS= Lysine; TYR=Tyrosine
[3]Units: Temperature (°C), Enthalpy (J/g, dry matter)
[4]Different letters within column indicate means are significantly different at $p \leq 0.05$

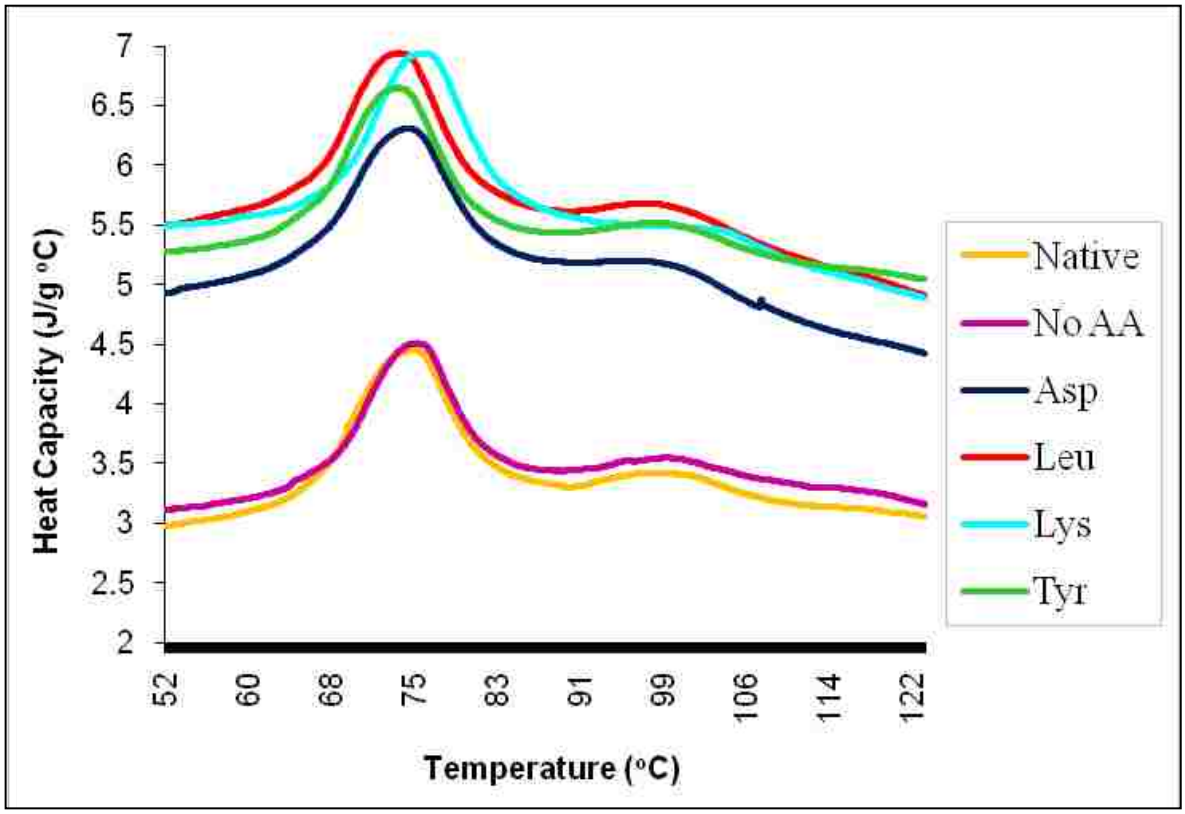


Figure 3.18 Thermal Curves of Rice Starches with Amino Acids in pH 4 Solution (HCl/NaOH).

of the granules was restricted but the overall crystallinity was unchanged. These properties were similar to those imparted by crosslinking reactions (Chatakanonda et al., 2000). Meanwhile, the negative charged aspartic acid did not cause any change in the thermal properties, contrary to what was observed by Liang (2001).

In pH 7 solution, a different gelatinization behavior was displayed by the samples. Shifting of gelatinization endotherm to higher T_p was again observed for LYS, while ASP delayed the onset of gelatinization (Figure 3.19).

In pH 10 solution, LYS was the only sample which displayed a different gelatinization temperature (Figure 3.20). Its T_c was higher at 93.25^{o}C than the control and the other samples at the same pH. However, the T_p was unchanged, unlike the treatments at lower pHs, which could be due to the reduced number of positive charges. According to Ito et al. (2006a), greater number of charges contributed more to increasing the gelatinization temperature.

3.3.3.3 Amino Acids with pH Treatments Using Buffer Solutions

Starch treated with acetate buffer at pH 4 without amino acid showed no observable difference compared to untreated rice starch (Table 3.17). At pH 7, the phosphate buffer elevated the T_p of all treated starch samples, as shown by their endotherms which were shifted to higher temperatures (Figure 3.22). The phosphate buffer, and not the amino acids, was primarily responsible for the shifting effect since NoAA displayed higher T_p and T_c than the untreated control starch. In sweet potato starches, the amount of phosphate was found to be directly correlated to the gelatinization temperature (Kitahara et al., 2005).

All additives caused the starch to resist swelling as shown by their elevated T_p's at pH 10 (Figure 3.23), but the buffer itself had the greatest effect on increasing the gelatinization temperature of rice starch, as displayed by NoAA. No change in the ΔH suggests that the

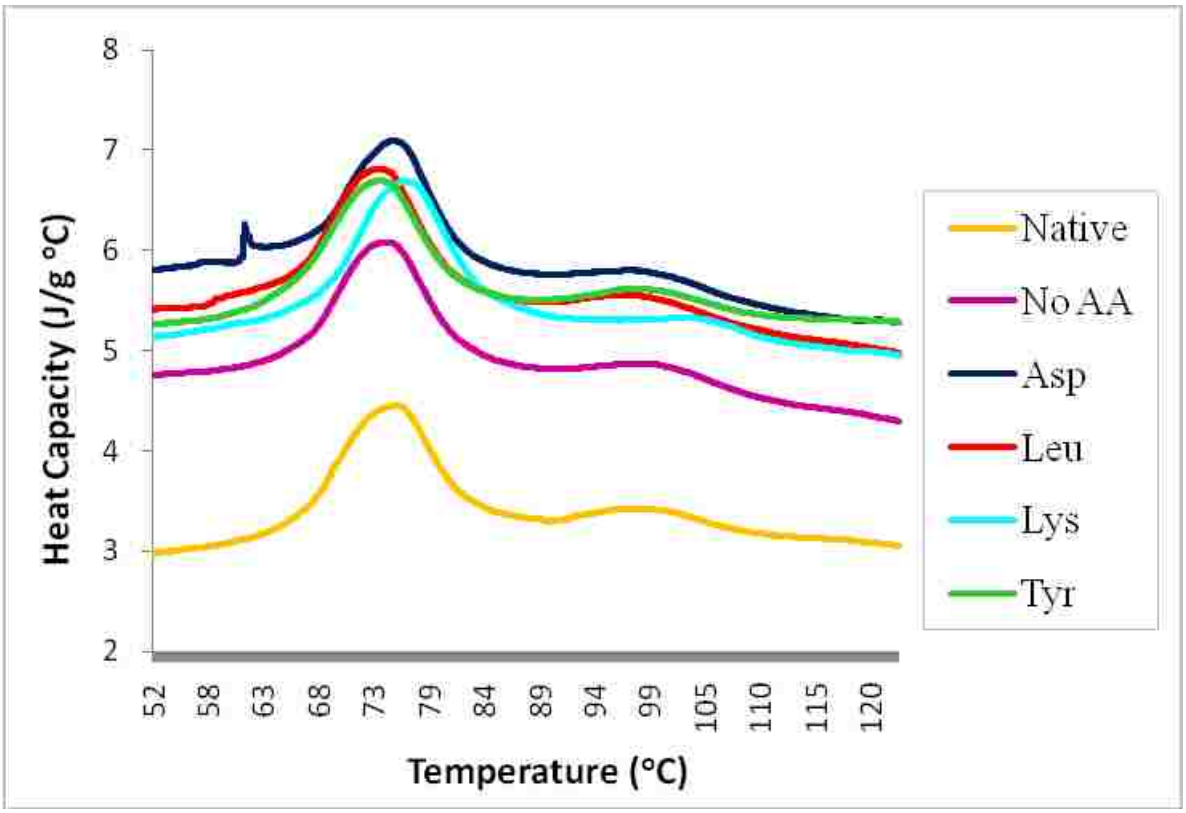


Figure 3.19 Thermal Curves of Rice Starches with Amino Acids in pH 7 Solution (HCl/NaOH).

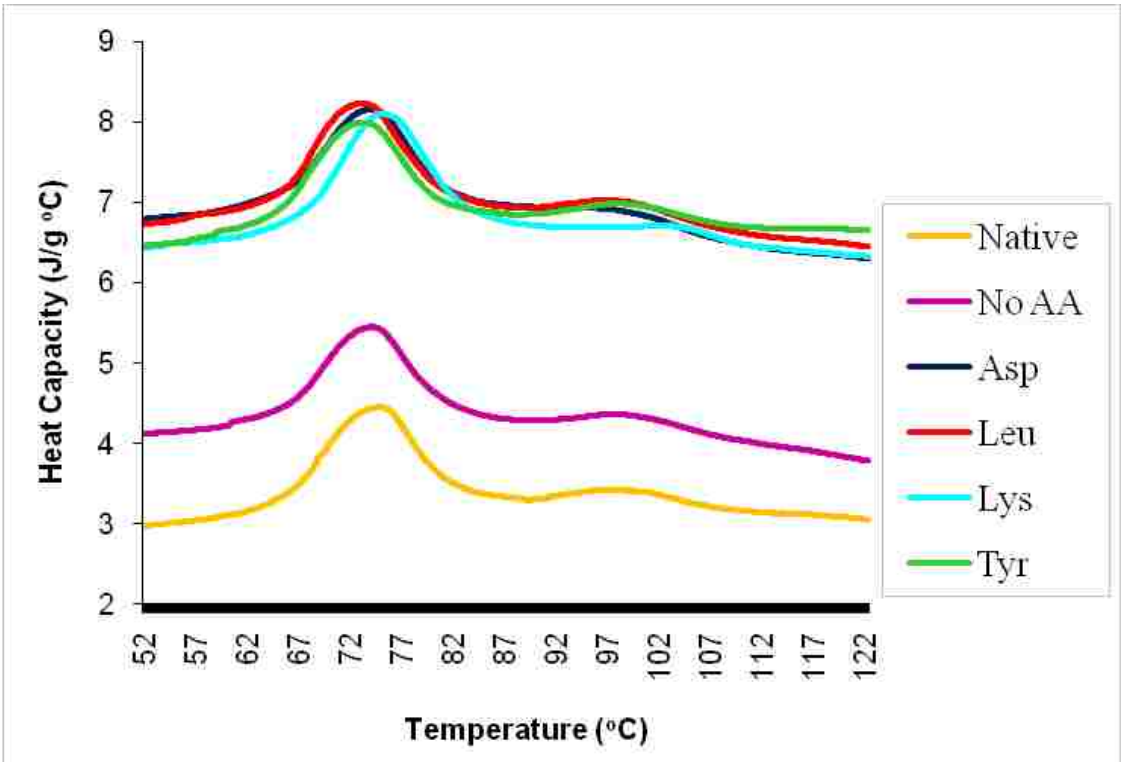


Figure 3.20 Thermal Curves of Rice Starches with Amino Acids in pH 10 Solution (HCl/NaOH).

Table 3.17 DSC Parameters of Rice Starch with Amino Acid Additives and pH Treatment (Buffers).[1,2,3,4,5]

Sample	Additive	Gelatinization Endotherm			
		T_o	T_p	T_c	ΔH
Control	NoAA	58.53±1.26bc	74.68±0.47d	87.27±1.34d	13.32±1.94a
pH 4	NoAA	61.50±1.81abc	76.19±0.71cd	89.96±3.42bcd	12.04±1.67a
	ASP	56.72±2.24ab	75.97±0.69cd	89.58±1.90bcd	13.60±0.57a
	LEU	60.77±2.68abc	76.33±0.72cd	89.35±2.27bcd	12.30±0.74a
	LYS	61.26±3.76abc	78.30±0.65ab	93.81±1.12abc	13.95±0.84a
	TYR	58.58±4.44bc	75.83±0.87cd	87.96±2.26cd	12.07±0.93a
pH 7	NoAA	63.96±1.56ab	79.87±0.58a	94.41±1.98ab	13.07±0.09a
	ASP	65.14±2.64a	80.24±1.18a	92.66±2.46abcd	11.97±1.30a
	LEU	66.13±2.64a	79.68±0.70a	95.25±2.59ab	12.11±2.25a
	LYS	64.88±1.74ab	80.02±0.86a	96.43±3.92a	13.15±0.64a
	TYR	64.80±2.51ab	78.86±0.90a	93.47±1.30abc	12.37±0.94a
pH 10	NoAA	64.51±2.09ab	79.26±0.14a	93.73±1.81abc	14.08±1.69a
	ASP	62.40±2.61abc	78.70±1.22ab	92.90±3.00abcd	13.46±0.67a
	LEU	65.30±1.75a	76.75±0.35bc	89.78±1.20bcd	12.01±2.06a
	LYS	62.15±1.02abc	78.30±0.18ab	91.77±1.60abcd	14.60±1.10a
	TYR	61.28±0.54abc	76.80±0.49bc	90.60±1.37abcd	14.21±0.90a

[1]T_o=onset temperature; T_p=peak temperature; T_c=conclusion temperature; ΔH (Enthalpy)
[2]NoAA=No Amino Acid; ASP=Aspartic Acid; LEU=Leucine; LYS=Lysine; TYR=Tyrosine
[3]Units: Temperature (°C), Enthalpy (J/g, dry matter)
[4]Buffers used were acetate (pH 4), phosphate (pH 7) and carbonate (pH 10)
[5]Different letters within column indicate means are significantly different at $p \leq 0.05$

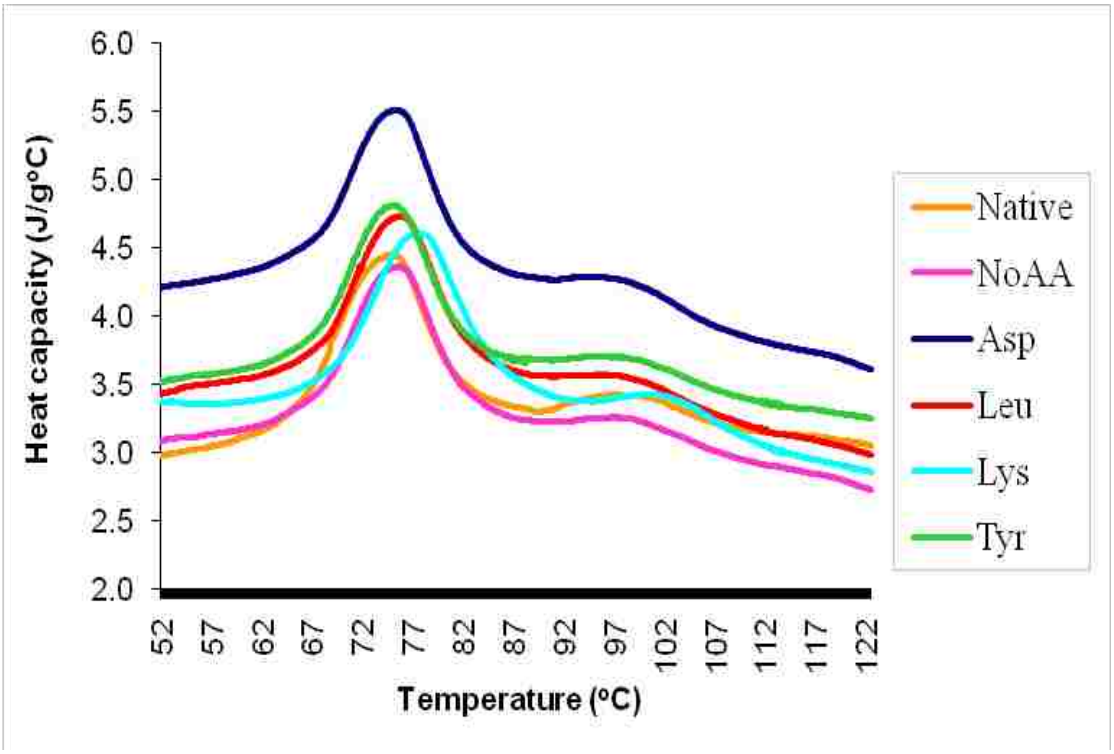


Figure 3.21 Thermal Curves of Rice Starches with Amino Acids at pH 4 (Buffer).

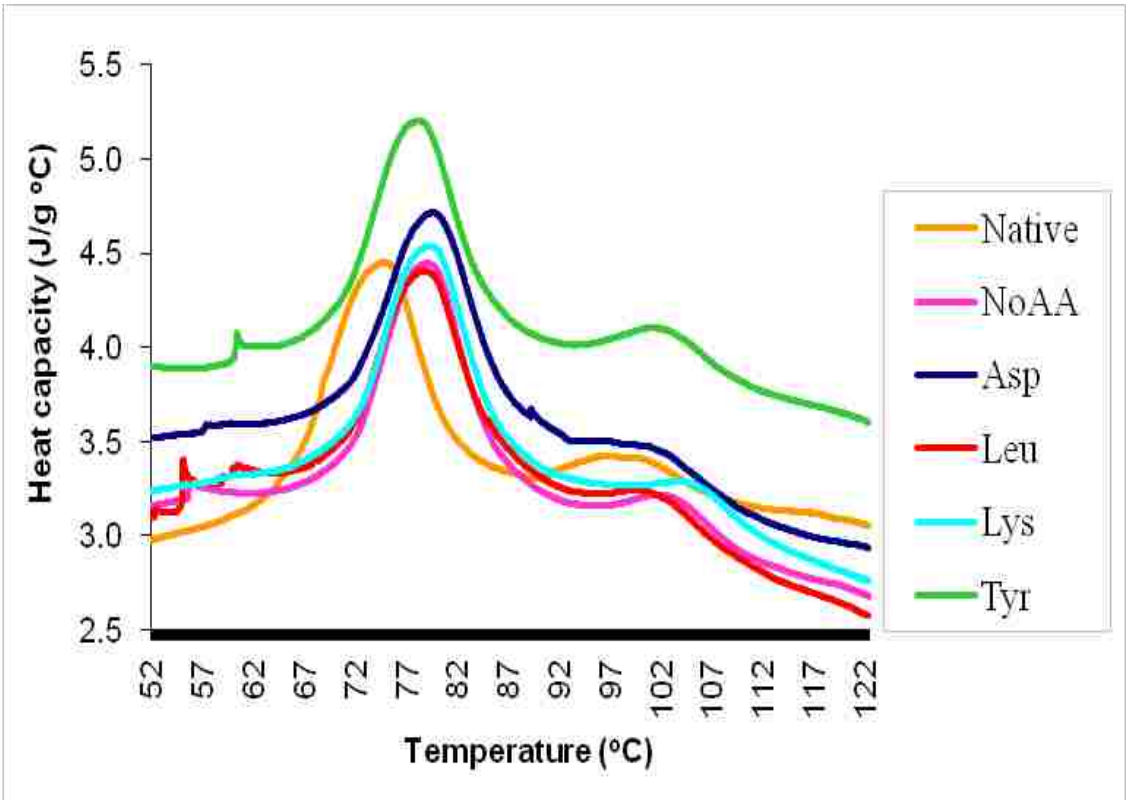


Figure 3.22 Thermal Curves of Rice Starches with Amino Acids at pH 7 (Buffer).

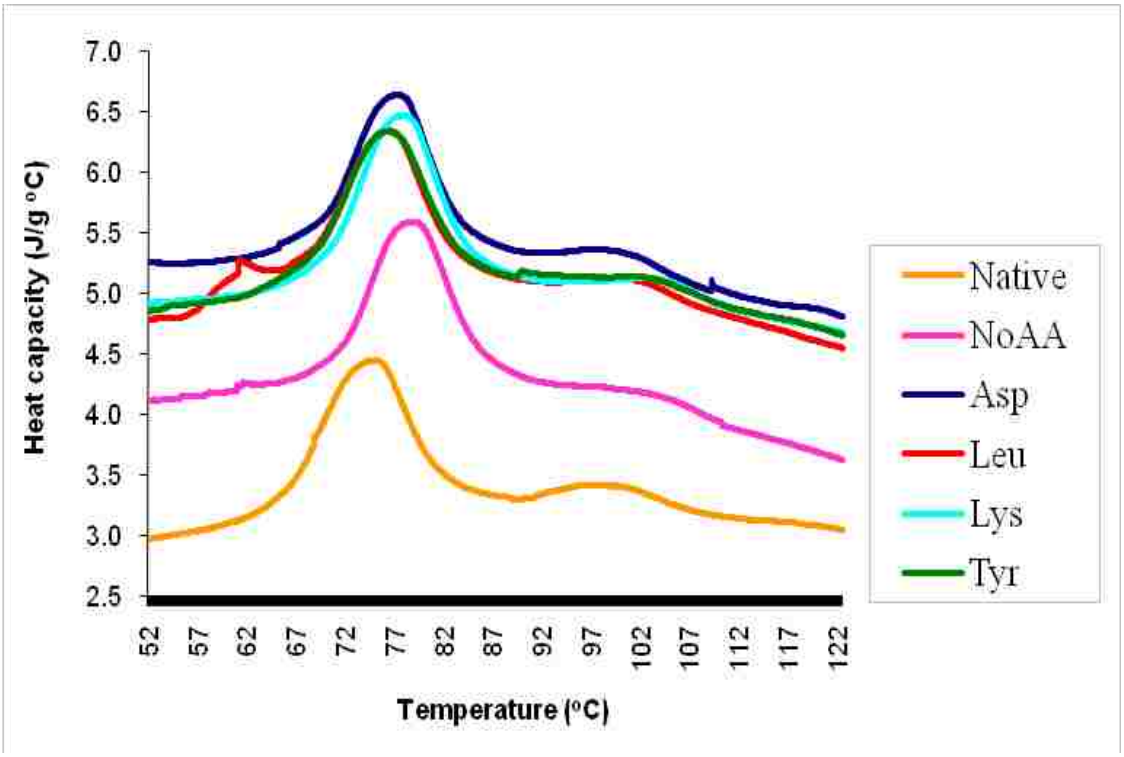


Figure 3.23 Thermal Curves of Rice Starches with Amino Acids at pH 10 (Buffer).

treatments did not cause a considerable change in the granular structure of the starch (Biliaderis, 1991).

3.3.3.4 Amino Acids with pH and Thermal Treatments

Heat treatment of starch without amino acid and dispersed in pH 4 solution increased the ΔH, and narrowed the range of granule melting ($T_c - T_o$) by about 1^oC (Table 3.18), which suggests that reorganization and increased crystallinity occurred (Biliaderis, 1991). However, the pH 4-heat treatment decreased the T_p, which could indicate that no significant hydrolysis occurred. During acid hydrolysis, the amorphous portions of the starch granule are attacked. The amorphous regions destabilize the crystallites, and attack by acids frees the crystallites. As a consequence, the crystallites melt at higher temperatures (Hoover, 2000). LYS was the only treatment which altered the gelatinization temperature of rice starch. It increased the T_p and T_c. Lysine broadened the gelatinization range which could mean that the starch produced had higher crystal heterogeneity (Vandeputte et al., 2003) or irregularly shaped granules (Singh et al., 2003). Heating might have increased the LYS-starch interactions, which in turn stabilized the amorphous regions. This restricted the hydration and ultimately, delayed the swelling and gelatinization of LYS and raised its temperature for gelatinization (Donald, 2004, Vandeputte et al., 2003).

At pH 7, heating caused a lowering of the T_p of native rice starch. LEU and TYR had comparable T_p's with NoAA, suggesting that even without the addition of leucine and tyrosine, restriction of gelatinization can be made by the combination of heat and pH treatment. Meanwhile, the positive charged LYS again caused an elevation of the T_p. These findings verified that neutral substances have little or no effect on gelatinization temperature, while those with unbalanced charges have greater contribution (Ito et al., 2006a).

Table 3.18 DSC Parameters of Rice Starch with Amino Acid Additives and pH (HCl/NaOH) and Heat Treatments.[1,2,3,4]

Sample	Additive	Gelatinization Endotherm			
		T_o	T_p	T_c	ΔH
Control	NoAA	58.53±1.26ab	74.68±0.47b	87.27±1.34cde	13.32±1.94bcde
pH 4	NoAA	60.34±1.31ab	73.71±0.45cd	88.01±1.44cde	16.09±1.05a
	ASP	64.08±3.69a	74.67±0.60b	89.58±2.60bcd	12.49±0.55de
	LEU	60.18±3.14ab	73.73±0.36cd	87.05±0.31cde	13.63±1.34abcde
	LYS	61.47±0.97ab	75.80±0.28a	91.62±0.73ab	16.02±0.20a
	TYR	60.37±1.95ab	73.31±0.15d	85.37±0.71e	13.89±0.29abcde
pH 7	NoAA	62.94±2.27ab	73.63±0.12cd	87.53±0.15cde	14.52±1.21abcde
	ASP	61.53±1.94ab	74.72±0.32b	86.35±0.39de	12.36±1.02e
	LEU	58.86±3.24ab	73.60±0.28cd	86.79±0.42cde	14.24±1.06abcde
	LYS	61.97±1.45ab	75.82±0.64a	89.99±0.59bc	15.27±0.46abc
	TYR	60.25±1.07ab	73.10±0.09d	86.14±1.22e	14.12±2.35abcde
pH 10	NoAA	60.67±1.06ab	73.75±0.12cd	87.18±0.37cde	15.42±0.63ab
	ASP	57.59±1.30b	74.46±0.09bc	87.44±1.48cde	12.66±0.56cde
	LEU	58.51±2.50ab	73.42±0.28d	87.30±1.22cde	15.15±1.05abcd
	LYS	62.12±3.18ab	75.99±0.35a	93.52±2.34a	15.74±0.41ab
	TYR	59.98±1.13ab	73.64±0.06cd	87.30±1.35cde	14.68±0.35abcde

[1]T_o=onset temperature; T_p=peak temperature; T_c=conclusion temperature; ΔH (Enthalpy)
[2]NoAA=No Amino Acid; ASP=Aspartic Acid; LEU=Leucine; LYS=Lysine; TYR=Tyrosine
[3]Units: Temperature (°C), Enthalpy (J/g, dry matter)
[4]Different letters within column indicate means are significantly different at the level of $p \leq 0.05$

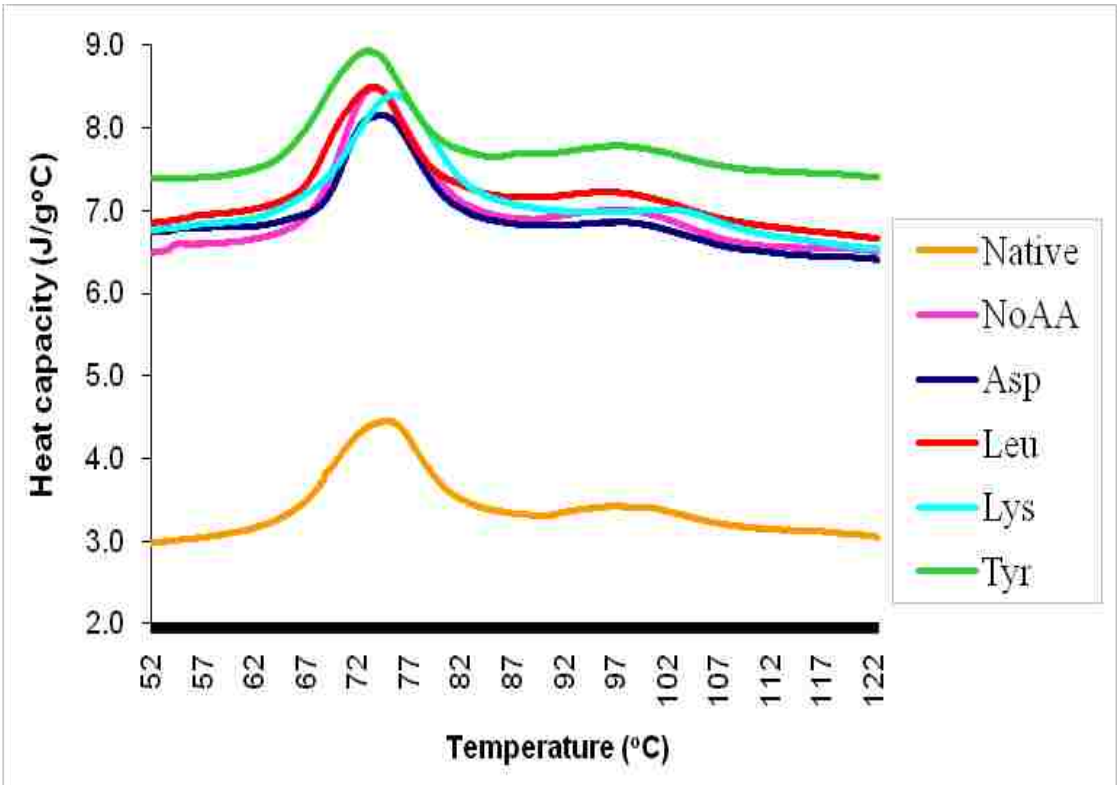


Figure 3.24 Thermal Curves of Rice Starches with Amino Acids in pH 4 Solution (Heat Treated).

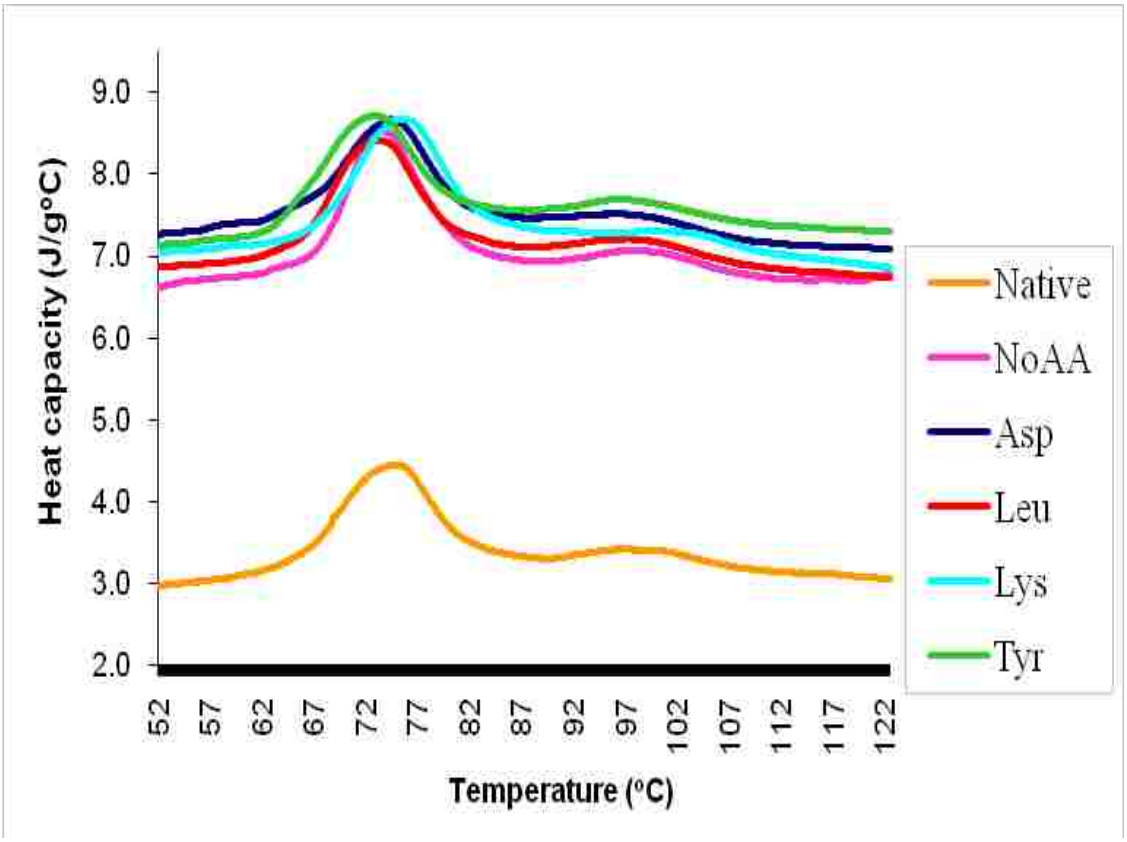


Figure 3.25 Thermal Curves of Rice Starches with Amino Acids in pH 7 Solution (Heat Treated).

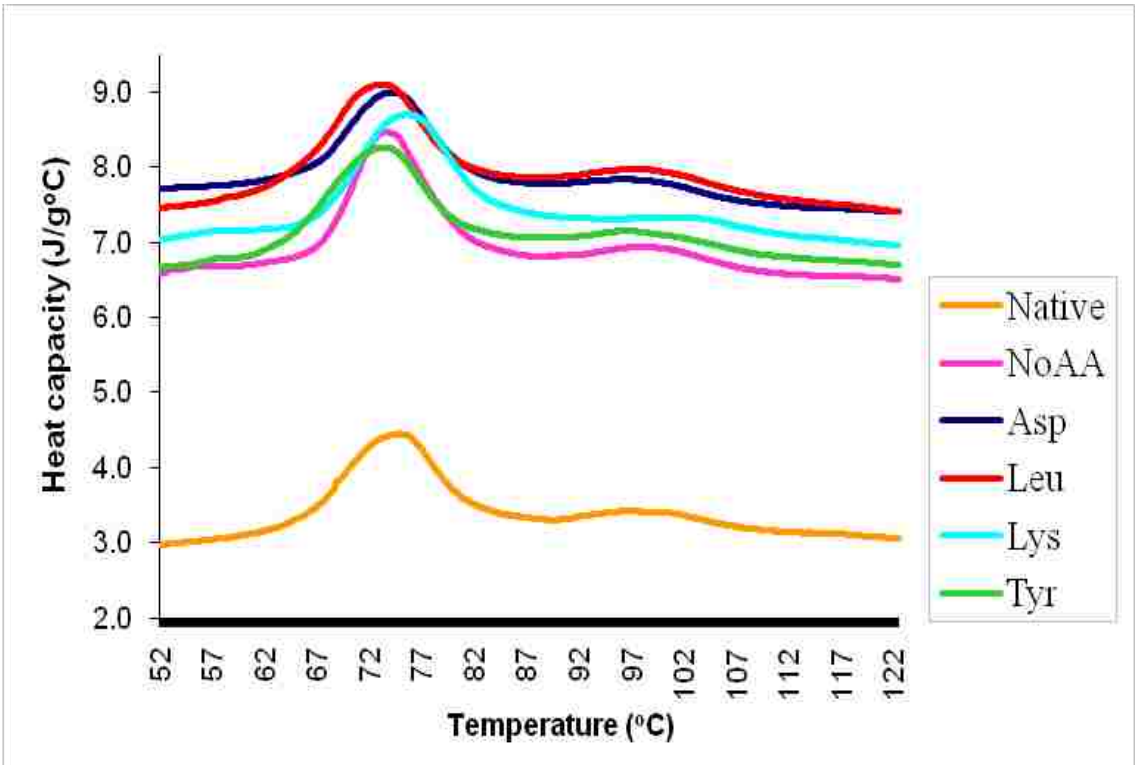


Figure 3.26 Thermal Curves of Rice Starches with Amino Acids in pH 10 Solution (Heat Treated).

The effects of amino acid treatment on the gelatinization temperature of rice starch samples in a pH 10 medium were similar to those of starches treated in pH 7 solution.

3.3.4 Resistant Starch

3.3.4.1 Non-thermally Treated Starches

Crystalean® (Opta Food Ingredients, Cambridge, MA), a commercial high amylose maize starch, was run along with the samples as check. It had 43.79±1.28% RS, which was close to the value (41%) reported by McCleary and Monaghan (2002) using the enzymatic-chemical assay described in this study.

Resistant starch levels of starches with amino acid additives dispersed in water and solutions containing HCl or NaOH and assayed by enzymatic-gravimetric approach are shown in Table 3.19. Addition of amino acids without adjusting the pH of the solution did not improve the RS formation of rice starch.

The starches with amino acid additives placed in the different pH solutions were able to form RS. The charged ASP and LYS were very effective in elevating the RS content of rice starch treated with pH 4 solution. LEU and TYR, on the other hand, had RS yields which were not significantly different from that of the control. For pH 7 treatment, a great increase in RS (196.49%) was observed for NoAA. LEU and TYR which both had zero net charge in pH 7 solution had higher RS yields than the control, but the values were lower than the starch without additives. pH 10 treatment, on the other hand, enhanced the RS formation of native rice starch. All treatments had RS levels which were at least twice as high as that of the untreated control. The higher RS content NoAA had, however, signifies that elevation of RS formation was mainly due to the pH 10 solution. Unlike pasting and thermal properties, therefore, amino acid charges

Table 3.19 Resistant Starch Yield (%) of Starches with Amino Acid Additives Without and With pH Treatments Using HCl/NaOH.[1,2,3]

Sample	Additive	Resistant Starch Yield (%)	
Control	NoAA	5.70±0.73h	
NopH	ASP	5.87±2.52h	
	LEU	5.41±0.71h	
	LYS	6.84±2.27gh	
	TYR	6.27±1.22h	
pH 4	NoAA	8.24±0.37fgh	
	ASP	16.14±2.31bc	183.37
	LEU	7.94±0.68fgh	
	LYS	23.34±1.72a	309.64
	TYR	6.04±0.98h	
pH 7	NoAA	16.89±2.35bc	196.49
	ASP	9.50±1.67efgh	
	LEU	10.76±1.27defg	88.87
	LYS	8.72±0.66efgh	
	TYR	11.41±1.68def	100.20
pH 10	NoAA	18.08±2.16b	217.33
	ASP	13.86±0.84bcd	143.37
	LEU	11.77±1.75def	106.71
	LYS	13.01±1.25cde	128.41
	TYR	11.50±0.59def	101.79

[1]NoAA=No Amino Acid; ASP=Aspartic Acid; LEU=Leucine; LYS=Lysine; TYR=Tyrosine
[2]Increase in RS Yield is relative to native rice starch (control)
[3]Different letters within column indicate means are significantly different at $p \leq 0.05$

were not the controlling factor in the formation of RS in rice starch, except for pH 4 solutions.

The results of RS determination in starch and amino acid mixtures suspended in buffer systems are presented in Table 3.20. Without amino acids, acetate and phosphate buffers increased the RS yield of starch, while carbonate buffer did not show an effect. At pH 4, all treated starches had significantly higher RS yields than the untreated starch control but not different from pH 4 alone. At pH 7 and 10 addition of ASP and LEU further enhanced RS formation conferred by the buffer; these amino acids doubled the RS content of native starch with phosphate buffer. At the same concentration in starch (6%), these amino acids were previously shown to enhance RS formation in unoxidized and oxidized rice starch: ASP on untreated laboratory-prepared rice starch and on commercial rice starch treated with pure oxygen, and LEU on commercial starch oxidized using ozone (An, 2005).

3.3.4.2 Thermally Treated Starches

Heat treatments were reported to contribute to RS formation in starches (Saura-Calixto and Abia, 1991). The RS yields of rice starches treated with different amino acids at various pH levels with the application of heat are presented in Table 3.21. In pH 4 solution, no increase was observed. This is in contrast to the findings when the samples were not heated, where ASP and LYS caused substantial RS increases. In pH 7 solution, gain in RS levels was observed for LEU and ASP added starches, with levels increasing by 72.93% and 78.6%, respectively (Table 3.21), compared to when no heating was employed, 88.87% (Table 3.19). Increasing the temperature was beneficial for starch with ASP, which had higher RS with thermal treatment than unheated samples. The amino acid additives ASP and TYR also enhanced the formation of RS in pH 10 solution with heating. However, lower amounts of RS were collected compared with those of the unheated samples (Table 3.19), except for TYR.

Table 3.20 Resistant Starch Yield (%) of Starches with Amino Acid Additives with pH Treatments (Buffers).[1,2,3,4,5]

Sample	Additive	Resistant Starch Yield (%)	%Increase in RS
Control	NoAA	5.70±0.73d	--
pH 4	NoAA	11.33±0.88bc	98.96
	ASP	10.24±2.23c	79.77
	LEU	9.61±2.23c	68.75
	LYS	10.70±1.41c	87.75
	TYR	10.47±2.29c	83.76
pH 7	NoAA	12.48±0.52bc	119.10
	ASP	16.62±0.91a	191.69
	LEU	14.64±1.51ab	157.04
	LYS	4.58±0.53d	NSD
	TYR	2.48±0.28d	NSD
pH 10	NoAA	4.42±2.20d	NSD
	ASP	14.88±0.84ab	161.21
	LEU	17.06±1.15a	199.52
	LYS	3.48±1.09d	NSD
	TYR	2.89±0.96d	NSD

[1]NoAA=No Amino Acid; ASP=Aspartic Acid; LEU=Leucine; LYS=Lysine; TYR=Tyrosine
[2]Increase in RS Yield is relative to native rice starch (control)
[3]Buffers used were acetate (pH 4), phosphate (pH 7) and carbonate (pH 10)
[4]NSD=Not significantly different from control
[5]Different letters within column indicate means are significantly different at $p \leq 0.05$

Table 3.21 Resistant Starch Yield (%) of Thermally Treated Starches with Amino Acid Additives in Different pH Levels (HCl/NaOH).[1,2,3,4]

Sample	Additive	Resistant Starch Yield (%)	%Increase in RS
Control	NoAA	5.70±0.73d	--
pH 4	NoAA	6.89±1.41cd	NSD
	ASP	8.54±0.96cd	NSD
	LEU	7.11±0.95cd	NSD
	LYS	8.06±1.54cd	NSD
	TYR	8.72±1.10cd	NSD
pH 7	NoAA	5.36±2.16d	NSD
	ASP	10.17±1.56abc	78.60
	LEU	9.85±1.55bc	72.93
	LYS	7.14±1.32cd	NSD
	TYR	8.19±1.01cd	NSD
pH 10	NoAA	8.00±0.42cd	NSD
	ASP	13.34±1.24ab	134.14
	LEU	9.70±1.66bc	70.31
	LYS	9.42±1.97c	65.40
	TYR	13.66±1.19a	139.81

[1]NoAA=No Amino Acid; ASP=Aspartic Acid; LEU=Leucine; LYS=Lysine; TYR=Tyrosine
[2]Increase in RS Yield is relative to native rice starch (control)
[3]NSD=Not significantly different from control
[4]Different letters within column indicate means are significantly different at $p \leq 0.05$

3.3.4.3 Starches with Tyrosine at Different pH Treatments Prepared Using the RVA

The RS levels of starches containing tyrosine under different pH conditions are presented in Table 3.22. The use of distilled water was effective in increasing the RS levels of rice starch with tyrosine added and after gelatinization in RVA. Using HCl and NaOH solutions in adjusting the pH, comparable values were obtained at pH 4 and 7, while RS level was not improved at pH 10. With buffers, on the other hand, only pH 4 increased the yield but it was not higher than that obtained with distilled water. These results verified the findings of García-Alonso et al. (1999) in which better RS yields could be obtained generally at lower pHs (3.5 and 5.5). A vital implication of the finding that the use of water as suspending medium for starch and tyrosine results in high RS yields translates to savings in terms of ingredients or processing aids (i.e. chemicals for pH adjustment) and facilities for processors (Garcia-Alonso et al., 1999).

3.3.4.4 Enzymatic-Chemical Technique (Megazyme)

Starches dispersed in water and buffers were the only samples analyzed for RS yield using the enzymatic-chemical method. The treatments apparently did not cause the formation of RS (Table 3.23). High pH, on the other hand, decreased the RS yield. This buffer also generally showed no enhancing effect in samples assayed using the enzymatic-gravimetric method.

There was no observed agreement between the RS values obtained using chemical and gravimetric methods. The latter consistently yielded higher RS values. In the Megazyme assay, residues remained after enzymatic hydrolysis of the RS fraction even with a high concentration of amyloglucosidase, which could indicate that the reaction was not completed under the specified experimental conditions. Thus, lower values were obtained using this procedure. Incomplete hydrolysis, as well as small polymer size of their modified starch, was also proposed by Wolf et al. (1999) as an explanation for the low yields obtained by enzymatic RS methods.

Table 3.22 Resistant Starch Yield (%) of RVA Gelatinized Starches with Tyrosine in Different pH Systems.[1,2,3,4,5,6]

Sample	Additive	Resistant Starch Yield (%)	%Increase in RS
Control	NoAA	5.70±0.73d	--
dH_2O	NopH	11.68±1.97ab	105.03
HCl/NaOH	4	14.69±2.21a	157.90
	7	12.22±1.95a	114.49
	10	5.00±0.15d	NSD
Buffer	4	10.92±1.21abc	91.64
	7	7.87±0.50bcd	NSD
	10	6.99±1.65cd	NSD

[1]NoAA=No Amino Acid
[2]NopH=No pH Treatment
[3]Increase in RS Yield is relative to native rice starch (control)
[4]Buffers used were acetate (pH 4), phosphate (pH 7) and carbonate (pH 10)
[5]NSD=Not significantly different from control
[6]Different letters within column indicate means are significantly different at $p \leq 0.05$

Table 3.23 Resistant Starch (%) of Rice Starches with Amino Acids at with and without pH Treatment (Buffers) Assayed by Enzymatic-Chemical Method (Megazyme).[1,2,3,4]

Sample	Additive	Resistant Starch Yield (%)
Control	NoAA	2.29±0.28abcde
NopH	ASP	2.16±1.23def
	LEU	1.61±0.54cdef
	LYS	1.70±0.37cdef
	TYR	1.17±0.10ef
pH 4	NoAA	2.44±0.07abcde
	ASP	2.22±0.12bcde
	LEU	2.60±1.05abcd
	LYS	1.97±0.19bcdef
	TYR	2.24±0.07bcde
pH 7	NoAA	3.57±0.88a
	ASP	3.07±0.66ab
	LEU	2.88±0.13abc
	LYS	2.60±0.16abcd
	TYR	2.70±0.79abcd
pH 10	NoAA	0.78±0.39f
	ASP	0.81±0.28f
	LEU	0.90±0.23f
	LYS	0.78±0.05f
	TYR	1.22±0.77ef

[1]NoAA=No Amino Acid
[2]NopH=No pH Treatment
[3]Increase in RS Yield is relative to native rice starch (control)
[4]Different letters within column indicate means are significantly different at $p \leq 0.05$

CHAPTER 4. SUMMARY AND CONCLUSIONS

Modification is an important process used to change functional properties of native starch, therefore increasing its utilization. Several physical or chemical modification techniques were shown to enhance RS formation. Amino acids have been demonstrated to alter starch functionalities. This study was conducted to determine the effect of amino acids in combination with different pH treatments on the pasting and thermal characteristics, and RS formation of rice starch.

Adding pH adjusted solutions using HCl and NaOH caused alterations in the pasting properties of rice starch. Without amino acids, the pasting properties were unchanged in pH 10 solution, whereas only the PV was affected in pH 4 solution. With amino acid additives, starches with ASP and LYS, consistently had significantly lower TSB and FV than the control and the other treatments. Samples with added ASP regardless of pH solution had the highest BD, while starches with LYS shortened the cooking time of the starch in all pH solutions. In general, ASP and LYS consistently increased the BD and decreased the TSB, which suggests that these amino acids had an effect on the starch structure, particularly amylose, since amylose correlates positively with setback and negatively with breakdown (Bhattacharya, 2009). The loss or reduction of the amylose-lipid complex peak but unchanged gelatinization enthalpy as shown by the DSC curves of the starches with these amino acids further support this assumption. It is evident that the charges that both of these amino acids contain at the tested pH levels influenced the said changes possibly though complex formation with starch, the nature of which needs further investigation. However, only lysine was consistent in elevating the gelatinization temperatures in all pH solutions, with or without heating. Aspartic acid, which had negative net charge at all the pH levels tested, did not show any effect on the first transition endotherm. The

positive charged lysine might have become attached to the starch molecules electrostatically, preventing their interaction with water, as seen from the increased gelatinization temperatures of LYS-starches in water or pH-adjusted solutions using HCl/NaOH.

Different trends were observed using buffers. BD, TSB, FV, PTime and PT values were altered by acetate buffer at pH 4 without amino acids. ASP reduced the PV, FV and PTime and increased the MV. LYS lengthened the PTime. However, no change in gelatinization temperatures of all treatments was observed. At pH 7 with phosphate buffer, ASP increased the BD and PT and decreased the PTime. No change in PT was observed for LYS. All treated starches, including the sample without amino acid, had elevated T_p's at this pH treatment. At alkaline pH using carbonate buffer, ASP had lower MV, FV, and TSB and higher PT. Other amino acids had properties comparable with either the control or the starch with buffer alone. Similar to the effect of phosphate buffer, carbonate buffer likewise increased the gelatinization temperature of rice starch.

The combination of pH treatment and amino acids proved to have an effect on RS formation. In pH 4 solution using HCl/NaOH, ASP and LYS yield high RS levels, while LEU and TYR had the same effect in pH 7 solution. ASP and LEU enhanced the RS formation of starch when treated with phosphate buffer or heated with HCl/NaOH at pH 7. Unlike pasting and thermal properties, therefore, RS formation is not controlled by charges of additives.

The effect of a hydroxyl containing amino acid on pasting, thermal and RS formation was tested using tyrosine. With the exception of BD, all RVA parameters of native rice starch were unchanged with tyrosine added in HCl/NaOH adjusted solutions at pH 4 and 7 and gelatinized in RVA. In pH 10 solution, MV was lower, while BD and TSB were higher than native starch. These results mean that pH 10 solution in combination with tyrosine made the swollen granules

of the treated starch easier to disrupt and increased the probability of the formation of three-dimensional network during cooling (Newport Scientific, 1998). pH solution was also negatively correlated with the formation of RS in starch with TYR. Compared with pretreated starch (i.e. lyophilized), lower paste viscosities were displayed by gelatinized starches with TYR.

In conclusion, amino acids alone or in combination with pH treatments would yield rice starches with varied functionalities. Significant alterations in the starch structure, particularly amylose, might have been exerted by ASP and LYS, and this may need further research. On the practical applications of this study, lysine can be incorporated into rice starch even without pH adjustment or heat treatments, which in turn would mean savings for the processor. The amino acids used in this study may find potential applications in the production of functional food ingredients. Further refinement of RS assays and establishment of the safe intakes of RS are thus recommended.

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APPENDIX 1
RVA RAW DATA OF RICE STARCHES WITHOUT pH TREATMENT

Treat	AA	Rep	PV	MV	BD	FV	SB	TSB	PTime	PT
1	None	1	2368	1915	453	3466	1098	1551	6.40	81.60
1	None	2	2315	1872	443	3400	1085	1528	6.47	81.55
2	Asp	1	1926	1341	585	2200	274	859	6.33	91.85
2	Asp	2	1885	1295	590	2156	271	861	6.40	91.85
2	Asp	3	1868	1282	586	2133	265	851	6.33	91.10
2	Asp	4	1852	1259	593	2120	268	861	6.22	91.80
3	Leu	1	2028	1641	387	3095	1067	1454	6.47	85.50
3	Leu	2	2010	1644	366	3142	1132	1498	6.40	88.70
3	Leu	3	1886	1572	314	2935	1049	1363	6.47	89.45
3	Leu	4	1880	1555	325	2914	1034	1359	6.47	87.95
4	Lys	1	2074	1568	506	2902	828	1334	5.60	80.85
4	Lys	2	2065	1565	500	2908	843	1343	5.60	80.85
4	Lys	3	2075	1594	481	2913	838	1319	5.60	80.75
4	Lys	4	2050	1563	487	2857	807	1294	5.60	80.70
5	Tyr	1	1890	1580	310	2837	947	1257	6.53	90.30
5	Tyr	2	1924	1619	305	2950	1026	1331	6.47	90.30
5	Tyr	3	1910	1602	308	2834	924	1232	6.53	90.20
5	Tyr	4	1891	1577	314	2831	940	1254	6.47	90.25

APPENDIX 2
RVA DATA OF RICE STARCHES (HCl/NaOH)

Treat	pH	AA	Rep	PV	MV	BD	FV	SB	TSB	PTime	PT
6	4	NA	1	2257	1843	414	3398	1141	1555	6.40	82.30
6	4	NA	2	2276	1838	438	3421	1145	1583	6.40	82.35
6	4	NA	3	2276	1858	418	3389	1113	1531	6.47	82.45
6	4	NA	4	2273	1857	416	3387	1114	1530	6.47	83.25
7	7	NA	1	2110	1713	397	3252	1142	1539	6.47	82.30
7	7	NA	2	2107	1704	403	3362	1255	1658	6.47	82.30
7	7	NA	3	2217	1803	414	3315	1098	1512	6.47	82.45
7	7	NA	4	2234	1808	426	3365	1131	1557	6.40	83.20
8	10	NA	1	2254	1804	450	3433	1179	1629	6.40	82.25
8	10	NA	2	2257	1811	446	3396	1139	1585	6.40	82.35
8	10	NA	3	2168	1735	433	3326	1158	1591	6.40	82.35
8	10	NA	4	2181	1759	422	3332	1151	1573	6.47	82.45
9	4	Asp	1	1932	1332	600	2218	286	886	6.40	92.60
9	4	Asp	2	1902	1309	593	2200	298	891	6.40	91.75
9	4	Asp	3	1938	1330	608	2225	287	895	6.33	91.85
9	4	Asp	4	1869	1299	570	2156	287	857	6.40	93.55
10	7	Asp	1	1971	1341	630	2277	306	936	6.40	91.75
10	7	Asp	2	1915	1311	604	2233	318	922	6.47	91.90
10	7	Asp	3	1971	1338	633	2276	305	938	6.40	91.80
10	7	Asp	4	1886	1272	614	2178	292	906	6.33	91.85
11	10	Asp	1	1806	1254	552	2127	321	873	6.40	93.50
11	10	Asp	2	1920	1315	605	2241	321	926	6.40	91.80
11	10	Asp	3	1837	1226	611	2131	294	905	6.33	92.70
11	10	Asp	4	1825	1217	608	2121	296	904	6.33	91.15
12	4	Leu	1	1915	1570	345	2884	969	1314	6.53	88.65
12	4	Leu	2	1978	1615	363	2984	1006	1369	6.53	88.60
12	4	Leu	3	1922	1550	372	2902	980	1352	6.47	90.30
12	4	Leu	4	1919	1546	373	2931	1012	1385	6.47	90.30
13	7	Leu	1	1997	1596	401	3034	1037	1438	6.47	88.65
13	7	Leu	2	2006	1607	399	3049	1043	1442	6.53	87.90
13	7	Leu	3	2011	1588	423	3072	1061	1484	6.40	87.10
13	7	Leu	4	2006	1602	404	3063	1057	1461	6.47	87.10
14	10	Leu	1	1990	1572	418	3074	1084	1502	6.40	86.70
14	10	Leu	2	1920	1512	408	3010	1090	1498	6.40	82.40
14	10	Leu	3	1906	1533	373	2918	1012	1385	6.53	89.35
14	10	Leu	4	1891	1540	351	2904	1013	1364	6.47	84.75
15	4	Lys	1	1964	1416	548	2811	847	1395	5.47	81.70
15	4	Lys	2	1955	1416	539	2781	826	1365	5.53	81.55

Treat	pH	AA	Rep	PV	MV	BD	FV	SB	TSB	PTime	PT
15	4	Lys	3	1996	1458	538	2804	808	1346	5.53	80.80
15	4	Lys	4	1982	1443	539	2827	845	1384	5.53	81.70
16	7	Lys	1	1930	1428	502	2751	821	1323	5.60	80.75
16	7	Lys	2	1911	1416	495	2742	831	1326	5.53	81.50
16	7	Lys	3	1956	1429	527	2770	814	1341	5.60	81.65
16	7	Lys	4	1957	1457	500	2747	790	1290	5.60	80.80
17	10	Lys	1	1982	1490	492	2751	770	1261	5.67	81.55
17	10	Lys	2	2000	1477	523	2772	772	1295	5.53	81.70
17	10	Lys	3	2063	1499	564	2859	796	1360	5.60	81.60
17	10	Lys	4	2050	1502	548	2858	808	1356	5.60	81.65
18	4	Tyr	1	1919	1537	382	2957	1038	1420	6.47	86.30
18	4	Tyr	2	1913	1524	389	2942	1029	1418	6.40	88.65
18	4	Tyr	3	1887	1514	373	2883	996	1369	6.47	87.90
18	4	Tyr	4	1896	1515	381	2914	1018	1399	6.40	87.10
19	7	Tyr	1	1951	1540	411	3018	1067	1478	6.40	87.20
19	7	Tyr	2	1961	1537	424	3024	1063	1487	6.33	87.85
19	7	Tyr	3	1954	1531	423	3003	1049	1472	6.33	86.40
19	7	Tyr	4	1928	1549	379	2969	1041	1420	6.47	87.85
20	10	Tyr	1	2000	1537	463	3132	1132	1595	6.33	83.25
20	10	Tyr	2	1990	1531	459	3126	1136	1595	6.27	83.90
20	10	Tyr	3	1948	1497	451	3084	1136	1587	6.33	85.55
20	10	Tyr	4	1922	1498	424	3045	1123	1547	6.33	88.00

APPENDIX 3
RVA DATA OF RICE STARCHES (BUFFER)

Treat	pH	AA	Rep	PV	MV	BD	FV	SB	TSB	PTime	PT
21	4	NA	1	2212	1843	369	2723	511	880	6.60	90.35
21	4	NA	2	1954	1639	315	2390	436	751	6.60	91.05
21	4	NA	3	2155	1757	398	2680	525	923	6.53	91.10
21	4	NA	4	1866	1566	300	2286	420	720	6.67	91.10
22	7	NA	1	2172	2039	133	2495	323	456	7.00	84.90
22	7	NA	2	2143	2020	123	2391	248	371	7.00	84.85
22	7	NA	3	2069	1938	131	2321	252	383	7.00	84.05
22	7	NA	4	1973	1830	143	2177	204	347	7.00	83.95
23	10	NA	1	2302	1806	496	3238	936	1432	5.73	83.95
23	10	NA	2	2107	1543	564	2964	857	1421	5.60	82.40
23	10	NA	3	2315	1751	564	3215	900	1464	5.73	84.05
23	10	NA	4	2125	1394	731	2989	864	1595	5.40	82.35
24	4	Asp	1	1666	1112	554	1968	302	856	6.27	91.85
24	4	Asp	2	1332	867	465	1509	177	642	6.27	94.35
24	4	Asp	3	1630	1095	535	1925	295	830	6.27	91.75
24	4	Asp	4	1315	852	463	1493	178	641	6.27	94.25
25	7	Asp	1	1606	1351	255	1936	330	585	6.60	93.50
25	7	Asp	2	1444	1140	304	1668	224	528	6.53	92.70
25	7	Asp	3	1652	1382	270	1912	260	530	6.67	91.90
25	7	Asp	4	1476	1233	243	1627	151	394	6.73	93.45
26	10	Asp	1	1818	1459	359	2233	415	774	6.53	91.95
26	10	Asp	2	1572	1245	327	1887	315	642	6.53	93.50
26	10	Asp	3	1884	1507	377	2274	390	767	6.53	92.75
26	10	Asp	4	1549	1254	295	1852	303	598	6.60	93.45
27	4	Leu	1	1866	1532	334	2370	504	838	6.60	91.85
27	4	Leu	2	1631	1353	278	2067	436	714	6.60	94.25
27	4	Leu	3	1823	1483	340	2345	522	862	6.60	92.75
27	4	Leu	4	1587	1294	293	2071	484	777	6.60	95.10
28	7	Leu	1	1741	1612	129	2010	269	398	7.00	86.30
28	7	Leu	2	1625	1499	126	1777	152	278	7.00	85.60
28	7	Leu	3	1831	1693	138	2158	327	465	6.87	84.85
28	7	Leu	4	1689	1545	144	1875	186	330	7.00	87.90
29	10	Leu	1	1975	1696	279	2565	590	869	6.20	82.30
29	10	Leu	2	1808	1606	202	2275	467	669	6.33	81.60
29	10	Leu	3	1957	1694	263	2519	562	825	6.33	82.45
29	10	Leu	4	1870	1639	231	2377	507	738	6.27	81.60

Treat	pH	AA	Rep	PV	MV	BD	FV	SB	TSB	PTime	PT
30	4	Lys	1	1872	1627	245	2514	642	887	6.73	83.15
30	4	Lys	2	1828	1629	199	2354	526	725	6.93	84.10
30	4	Lys	3	1957	1682	275	2626	669	944	6.73	83.10
30	4	Lys	4	1893	1667	226	2484	591	817	6.87	83.25
31	7	Lys	1	1832	1687	145	2155	323	468	6.73	84.00
31	7	Lys	2	1868	1709	159	2190	322	481	6.87	84.00
31	7	Lys	3	1804	1665	139	2087	283	422	6.93	84.80
31	7	Lys	4	1820	1681	139	2080	260	399	6.93	84.00
32	10	Lys	1	1904	1651	253	2587	683	936	6.13	84.00
32	10	Lys	2	1720	1462	258	2396	676	934	5.87	81.60
32	10	Lys	3	1942	1649	293	2741	799	1092	6.00	83.95
32	10	Lys	4	1760	1552	208	2493	733	941	6.13	83.15
33	4	Tyr	1	1757	1412	345	2277	520	865	6.40	92.70
33	4	Tyr	2	1869	1539	330	2371	502	832	6.53	92.75
33	4	Tyr	3	1753	1363	390	2292	539	929	6.47	91.95
33	4	Tyr	4	1564	1300	264	2013	449	713	6.60	94.40
34	7	Tyr	1	1635	1506	129	2018	383	512	7.00	87.80
34	7	Tyr	2	1504	1384	120	1788	284	404	7.00	91.10
34	7	Tyr	3	1648	1516	132	2028	380	512	6.90	86.70
34	7	Tyr	4	1512	1420	92	1769	257	349	7.00	85.65
35	10	Tyr	1	1796	1486	310	2469	673	983	6.13	81.55
35	10	Tyr	2	1896	1491	405	2658	762	1167	5.80	80.75
35	10	Tyr	3	1820	1489	331	2519	699	1030	6.00	81.45
35	10	Tyr	4	1881	1454	427	2655	774	1201	5.73	80.70

APPENDIX 4
RVA DATA OF RICE STARCHES (THERMAL)

Treat	pH	AA	Rep	PV	MV	BD	FV	SB	TSB	PTime	PT
36	4	NA	1	2120	1754	366	3077	957	1323	6.67	87.15
36	4	NA	2	2100	1771	329	3044	944	1273	6.73	84.10
36	4	NA	3	2123	1773	350	3049	971	1276	6.67	91.85
36	4	NA	4	2152	1729	423	3178	1026	1449	6.53	91.00
37	7	NA	1	2154	1725	429	3154	1000	1429	6.60	79.25
37	7	NA	2	2184	1813	371	3195	1011	1382	6.67	91.05
37	7	NA	3	2161	1836	325	3087	926	1251	6.80	81.55
37	7	NA	4	2145	1773	372	3118	973	1345	6.60	91.05
38	10	NA	1	2219	1830	389	3230	1011	1400	6.67	83.20
38	10	NA	2	2203	1825	378	3211	1008	1386	6.60	90.30
38	10	NA	3	2154	1846	308	3149	995	1303	6.73	85.60
38	10	NA	4	2175	1759	416	3212	1037	1453	6.60	82.40
39	4	Asp	1	1686	1197	489	1971	285	774	6.60	95.05
39	4	Asp	2	1666	1184	482	1942	276	758	6.60	95.05
39	4	Asp	3	1706	1179	527	1994	288	815	6.47	93.55
39	4	Asp	4	1714	1188	526	2007	293	819	6.47	94.25
40	7	Asp	1	1750	1207	543	2010	260	803	6.47	95.00
40	7	Asp	2	1751	1194	557	2015	264	821	6.47	93.55
40	7	Asp	3	1741	1190	551	2022	281	832	6.47	94.25
40	7	Asp	4	1750	1226	524	2052	302	826	6.47	93.45
41	10	Asp	1	1699	1196	503	2020	321	824	6.47	93.45
41	10	Asp	2	1685	1158	527	1988	303	830	6.47	94.30
41	10	Asp	3	1622	1148	474	1949	327	801	6.53	95.00
41	10	Asp	4	1608	1126	482	1938	330	812	6.53	95.05
42	4	Leu	1	1802	1516	286	2492	690	976	6.73	93.50
42	4	Leu	2	1843	1516	327	2603	760	1087	6.73	92.80
42	4	Leu	3	1795	1524	271	2684	889	1160	6.60	91.80
42	4	Leu	4	1793	1518	275	2681	888	1163	6.60	91.85
43	7	Leu	1	1791	1495	296	2701	910	1206	6.60	91.85
43	7	Leu	2	1817	1488	329	2783	966	1295	6.53	91.10
43	7	Leu	3	1833	1487	346	2802	969	1315	6.53	88.70
43	7	Leu	4	1824	1491	333	2782	958	1291	6.53	89.55
44	10	Leu	1	1692	1345	347	2749	1057	1404	6.40	88.65
44	10	Leu	2	1697	1347	350	2773	1076	1426	6.40	90.20
44	10	Leu	3	1601	1270	331	2649	1048	1379	6.40	84.05
44	10	Leu	4	1606	1295	311	2614	1008	1319	6.47	85.60

Treat	pH	AA	Rep	PV	MV	BD	FV	SB	TSB	PTime	PT
45	4	Lys	1	1803	1350	453	2566	763	1216	5.73	81.60
45	4	Lys	2	1801	1359	442	2572	771	1213	5.73	80.65
45	4	Lys	3	1828	1364	464	2615	787	1251	5.60	81.55
45	4	Lys	4	1840	1377	463	2588	748	1211	5.60	81.45
46	7	Lys	1	1651	1255	396	2360	709	1105	5.67	80.80
46	7	Lys	2	1664	1246	418	2333	669	1087	5.67	80.85
46	7	Lys	3	1861	1400	461	2644	783	1244	5.67	80.80
46	7	Lys	4	1875	1401	474	2555	680	1154	5.53	80.80
47	10	Lys	1	1751	1311	440	2466	715	1155	5.73	80.00
47	10	Lys	2	1736	1304	432	2437	701	1133	5.60	80.00
47	10	Lys	3	1739	1300	439	2400	661	1100	5.67	80.75
47	10	Lys	4	1742	1308	434	2438	696	1130	5.67	79.95
48	4	Tyr	1	1766	1478	288	2697	931	1219	6.60	87.95
48	4	Tyr	2	1752	1454	298	2760	1008	1306	6.53	88.75
48	4	Tyr	3	1758	1448	310	2772	1014	1324	6.40	82.35
48	4	Tyr	4	1764	1441	323	2717	953	1276	6.47	91.00
49	7	Tyr	1	1704	1365	339	2732	1028	1367	6.40	81.65
49	7	Tyr	2	1731	1390	341	2778	1047	1388	6.40	90.25
49	7	Tyr	3	1684	1354	330	2770	1086	1416	6.47	89.45
49	7	Tyr	4	1678	1330	348	2770	1092	1440	6.40	90.25
50	10	Tyr	1	1857	1545	312	2704	847	1159	6.60	90.95
50	10	Tyr	2	1858	1550	308	2711	853	1161	6.60	91.10
50	10	Tyr	3	1687	1354	333	2727	1040	1373	6.47	90.35
50	10	Tyr	4	1692	1379	313	2765	1073	1386	6.40	79.20

APPENDIX 5
RVA DATA OF RVA GELATINIZED RICE STARCHES WITH TYROSINE

Treat	Type	pH	Rep	PV	MV	BD	FV	SB	TSB	PTime	PT
51	NopH	NA	1	2379	1844	535	3472	1093	1628	6.40	80.80
51	NopH	NA	2	2360	1841	519	3443	1083	1602	6.40	80.70
52	HCl/NaOH	4	1	2380	1875	505	3381	1001	1506	6.40	81.65
52	HCl/NaOH	4	2	2382	1890	492	3395	1013	1505	6.47	81.50
53	HCl/NaOH	7	1	2365	1822	543	3462	1097	1640	6.33	81.60
53	HCl/NaOH	7	2	2354	1833	521	3462	1108	1629	6.33	80.80
54	HCl/NaOH	10	1	2325	1741	584	3457	1132	1716	6.33	80.80
54	HCl/NaOH	10	2	2336	1736	600	3520	1184	1784	6.27	80.85
55	Buffer	4	1	2660	1921	739	3128	468	1207	6.67	84.05
55	Buffer	4	2	2660	1886	774	3129	469	1243	6.53	84.10
56	Buffer	7	1	2657	2460	197	2841	184	381	7.00	84.00
56	Buffer	7	2	2677	2468	209	2879	202	411	6.93	84.00
57	Buffer	10	1	2445	2040	405	2972	527	932	6.13	62.40
57	Buffer	10	2	2428	2062	366	2983	555	921	6.07	83.15

APPENDIX 6
DSC DATA OF RICE STARCHES WITHOUT pH TREATMENT

Treat	AA	Rep	Gelatinization Endotherm			
			T_o	T_p	T_c	ΔH
1	NA	1	59.42	74.35	86.32	11.94
1	NA	2	57.64	75.01	88.21	14.69
2	Asp	1	59.42	76.63	92.03	12.29
2	Asp	2	53.55	76.13	92.79	14.15
2	Asp	3	62.08	76.28	89.81	11.48
2	Asp	4	59.90	76.29	91.18	12.16
3	Leu	1	57.53	74.34	86.91	14.24
3	Leu	2	57.88	73.87	87.38	14.64
3	Leu	3	58.71	74.00	87.03	13.94
3	Leu	4	57.76	74.58	86.20	13.32
4	Lys	1	61.72	76.67	95.15	13.98
4	Lys	2	51.72	75.49	91.41	17.15
4	Lys	3	61.67	77.24	94.61	13.96
4	Lys	4	59.90	76.42	95.80	15.12
5	Tyr	1	63.33	74.54	86.44	10.66
5	Tyr	2	57.64	74.63	86.44	12.28
5	Tyr	3	58.87	73.86	85.16	13.54
5	Tyr	4	59.54	74.72	87.86	12.43

APPENDIX 7
DSC DATA OF RICE STARCHES (HCl/NaOH)

Treat	pH	AA	Rep	Gelatinization Endotherm			
				T_o	T_p	T_c	ΔH
6	4	NA	1	61.32	75.99	91.29	12.45
6	4	NA	2	61.89	75.64	89.09	11.22
6	4	NA	3	58.67	75.46	89.76	13.13
6	4	NA	4	58.80	74.03	87.53	13.56
7	7	NA	1	55.53	74.50	90.51	14.27
7	7	NA	2	57.95	74.34	87.81	14.08
7	7	NA	3	59.33	74.72	89.09	12.82
7	7	NA	4	56.79	74.28	88.23	14.60
8	10	NA	1	59.09	74.29	88.09	13.92
8	10	NA	2	63.07	74.58	88.09	10.60
8	10	NA	3	58.48	73.94	85.49	13.09
8	10	NA	4	60.61	75.80	90.55	11.95
9	4	Asp	1	60.96	75.04	87.38	12.77
9	4	Asp	2	61.55	74.96	89.40	14.22
9	4	Asp	3	60.86	74.25	87.00	11.94
9	4	Asp	4	57.29	74.83	87.50	12.76
10	7	Asp	1	61.67	74.78	87.50	12.69
10	7	Asp	2	67.24	75.86	87.15	8.14
10	7	Asp	3	62.03	75.49	87.86	11.80
10	7	Asp	4	63.57	75.49	88.21	11.96
11	10	Asp	1	58.83	74.75	89.75	14.53
11	10	Asp	2	58.83	74.28	84.78	13.30
11	10	Asp	3	58.95	74.62	87.27	13.45
11	10	Asp	4	57.17	73.98	88.69	13.69
12	4	Leu	1	55.16	73.84	87.98	15.87
12	4	Leu	2	55.27	74.05	88.81	15.94
12	4	Leu	3	53.73	74.07	87.38	15.32
12	4	Leu	4	59.14	74.33	86.06	13.82
13	7	Leu	1	57.88	73.66	86.55	14.29
13	7	Leu	2	57.41	73.74	87.50	15.33
13	7	Leu	3	60.06	74.10	87.19	12.95
13	7	Leu	4	61.36	73.51	87.53	12.53
14	10	Leu	1	58.24	73.41	87.62	14.61
14	10	Leu	2	61.08	73.70	87.03	12.81
14	10	Leu	3	56.58	73.51	87.38	14.81
14	10	Leu	4	58.59	73.57	88.92	16.20

Treat	pH	AA	Rep	Gelatinization Endotherm			
				T_o	T_p	T_c	ΔH
15	4	Lys	1	62.15	76.33	92.24	14.79
15	4	Lys	2	59.30	75.75	92.24	16.26
15	4	Lys	3	61.79	76.75	92.12	15.48
15	4	Lys	4	62.15	77.21	93.78	14.91
16	7	Lys	1	62.74	76.50	91.29	14.45
16	7	Lys	2	62.50	75.86	92.12	14.79
16	7	Lys	3	62.00	75.74	90.62	15.78
16	7	Lys	4	61.44	76.43	90.94	15.15
17	10	Lys	1	58.12	75.70	93.55	17.62
17	10	Lys	2	59.78	75.85	96.51	15.51
17	10	Lys	3	60.96	75.68	91.77	15.80
17	10	Lys	4	56.81	75.38	91.18	16.89
18	4	Tyr	1	57.09	74.05	87.38	13.41
18	4	Tyr	2	54.56	73.65	86.2	13.99
18	4	Tyr	3	60.46	73.81	86.63	13.69
18	4	Tyr	4	58.00	73.97	85.96	12.70
19	7	Tyr	1	57.76	73.49	87.86	14.72
19	7	Tyr	2	57.17	74.34	86.67	13.54
19	7	Tyr	3	57.29	73.75	87.38	14.74
19	7	Tyr	4	57.76	73.54	85.96	13.97
20	10	Tyr	1	57.76	73.45	86.79	15.52
20	10	Tyr	2	57.76	73.79	87.86	14.37
20	10	Tyr	3	56.70	73.24	81.93	10.79
20	10	Tyr	4	61.67	73.91	87.5	12.28

APPENDIX 8
DSC DATA OF RICE STARCHES (BUFFER)

Treat	pH	AA	Rep	Gelatinization Endotherm			
				T_o	T_p	T_c	ΔH
21	4	NA	1	60.94	75.37	88.81	13.38
21	4	NA	2	60.23	76.38	89.52	11.95
21	4	NA	3	64.18	75.96	86.74	9.705
21	4	NA	4	60.65	77.06	94.78	13.11
22	7	NA	1	62.15	79.16	92.12	13.19
22	7	NA	2	65.94	80.32	95.68	12.96
22	7	NA	3	64.04	79.63	93.43	13.08
22	7	NA	4	63.69	80.37	96.39	13.05
23	10	NA	1	66.96	79.41	92.13	11.89
23	10	NA	2	64.16	79.15	94.02	15.16
23	10	NA	3	61.91	79.14	92.60	13.64
23	10	NA	4	64.99	79.34	96.15	15.64
24	4	Asp	1	57.37	75.98	87.48	13.32
24	4	Asp	2	56.93	75.76	90.11	13.99
24	4	Asp	3	53.62	75.25	88.81	14.14
24	4	Asp	4	58.95	76.90	91.93	12.94
25	7	Asp	1	62.50	79.41	92.60	13.33
25	7	Asp	2	64.28	80.32	95.09	12.83
25	7	Asp	3	65.01	79.36	89.31	10.72
25	7	Asp	4	68.76	81.88	93.64	11.01
26	10	Asp	1	60.49	77.51	89.64	12.98
26	10	Asp	2	61.67	79.44	95.68	14.13
26	10	Asp	3	61.20	77.82	91.06	13.93
26	10	Asp	4	66.25	80.02	95.20	12.79
27	4	Leu	1	58.95	75.68	88.14	13.20
27	4	Leu	2	62.79	76.67	92.22	11.74
27	4	Leu	3	58.00	75.80	87.04	12.61
27	4	Leu	4	63.33	77.18	89.99	11.64
28	7	Leu	1	68.76	78.78	93.99	8.80
28	7	Leu	2	67.00	80.41	98.88	13.03
28	7	Leu	3	62.50	79.50	92.93	13.82
28	7	Leu	4	66.25	80.02	95.20	12.79
29	10	Leu	1	66.59	76.55	88.06	9.84
29	10	Leu	2	66.94	77.26	89.84	10.97
29	10	Leu	3	64.35	76.48	90.51	12.69
29	10	Leu	4	63.33	76.71	90.70	14.55

Treat	pH	AA	Rep	Gelatinization Endotherm			
				T_o	T_p	T_c	ΔH
30	4	Lys	1	58.59	78.01	93.55	14.78
30	4	Lys	2	64.63	78.68	94.97	12.99
30	4	Lys	3	57.46	77.53	92.38	14.51
30	4	Lys	4	64.35	78.98	94.35	13.50
31	7	Lys	1	62.74	79.15	93.16	12.94
31	7	Lys	2	67.00	80.41	98.88	13.03
31	7	Lys	3	64.92	79.50	93.04	12.55
31	7	Lys	4	64.87	81.03	100.65	14.06
32	10	Lys	1	63.45	78.09	90.11	12.99
32	10	Lys	2	61.67	78.28	91.18	14.83
32	10	Lys	3	61.08	78.30	91.89	15.07
32	10	Lys	4	62.38	78.52	93.90	15.49
33	4	Tyr	1	60.34	74.90	86.72	12.29
33	4	Tyr	2	60.96	76.95	91.29	11.64
33	4	Tyr	3	51.94	75.50	87.39	13.26
33	4	Tyr	4	61.08	75.97	86.44	11.09
34	7	Tyr	1	64.07	78.02	93.48	13.53
34	7	Tyr	2	67.07	80.13	94.50	11.43
34	7	Tyr	3	61.57	78.78	91.63	12.70
34	7	Tyr	4	66.48	78.50	94.26	11.83
35	10	Tyr	1	61.70	76.24	89.33	14.60
35	10	Tyr	2	60.49	77.10	89.52	14.03
35	10	Tyr	3	61.39	76.55	91.54	15.15
35	10	Tyr	4	61.55	77.31	92.01	13.04

APPENDIX 9
DSC DATA OF RICE STARCHES (THERMAL)

Treat	pH	AA	Rep	Gelatinization Endotherm			
				T_o	T_p	T_c	ΔH
36	4	NA	1	58.83	74.05	89.28	17.60
36	4	NA	2	60.37	74.03	89.04	15.76
36	4	NA	3	62.03	73.09	87.50	15.85
36	4	NA	4	60.13	73.65	86.20	15.15
37	7	NA	1	65.20	73.66	87.38	12.88
37	7	NA	2	62.38	73.46	87.50	14.45
37	7	NA	3	60.01	73.74	87.50	15.72
37	7	NA	4	64.16	73.64	87.74	15.02
38	10	NA	1	59.90	73.80	87.38	16.19
38	10	NA	2	60.72	73.66	87.50	15.38
38	10	NA	3	59.90	73.64	86.67	15.46
38	10	NA	4	62.15	73.89	87.15	14.66
39	4	Asp	1	66.89	74.20	90.70	12.56
39	4	Asp	2	67.60	74.10	92.72	11.71
39	4	Asp	3	60.49	75.20	87.27	12.71
39	4	Asp	4	61.32	75.18	87.62	12.97
40	7	Asp	1	59.07	74.42	85.84	13.41
40	7	Asp	2	61.67	75.15	86.32	11.17
40	7	Asp	3	63.81	74.75	86.79	11.87
40	7	Asp	4	61.55	74.57	86.44	12.98
41	10	Asp	1	59.18	74.36	86.08	12.14
41	10	Asp	2	58.12	74.41	89.52	12.41
41	10	Asp	3	56.46	74.51	86.79	12.65
41	10	Asp	4	56.58	74.57	87.38	13.45
42	4	Leu	1	57.64	74.05	86.79	14.73
42	4	Leu	2	57.29	74.02	86.79	14.73
42	4	Leu	3	62.86	73.45	87.38	13.03
42	4	Leu	4	62.93	73.39	87.24	12.02
43	7	Leu	1	62.74	73.49	86.20	12.95
43	7	Leu	2	60.13	73.28	87.15	13.89
43	7	Leu	3	57.17	73.93	86.79	14.73
43	7	Leu	4	55.39	73.68	87.03	15.40
44	10	Leu	1	59.07	73.52	87.62	15.97
44	10	Leu	2	61.32	73.77	88.81	13.63
44	10	Leu	3	55.27	73.25	86.79	15.31
44	10	Leu	4	58.36	73.14	85.96	15.70

Treat	pH	AA	Rep	Gelatinization Endotherm			
				T_o	T_p	T_c	ΔH
45	4	Lys	1	60.13	76.15	91.77	15.98
45	4	Lys	2	62.38	75.79	90.70	15.97
45	4	Lys	3	61.91	75.78	92.48	16.31
45	4	Lys	4	61.44	75.46	91.53	15.83
46	7	Lys	1	62.50	74.89	90.23	15.65
46	7	Lys	2	63.45	75.96	90.70	15.49
46	7	Lys	3	60.01	76.31	89.64	15.32
46	7	Lys	4	61.91	76.13	89.40	14.61
47	10	Lys	1	64.52	75.68	90.70	15.13
47	10	Lys	2	64.99	76.48	93.78	15.90
47	10	Lys	3	58.36	75.79	96.39	15.94
47	10	Lys	4	60.61	76.01	93.19	15.98
48	4	Tyr	1	62.03	73.16	86.2	13.94
48	4	Tyr	2	62.03	73.32	85.13	13.49
48	4	Tyr	3	59.18	73.24	85.61	13.92
48	4	Tyr	4	58.24	73.50	84.54	14.20
49	7	Tyr	1	58.71	73.24	87.50	15.07
49	7	Tyr	2	60.49	73.09	84.54	10.61
49	7	Tyr	3	61.20	73.07	86.20	15.39
49	7	Tyr	4	60.61	73.02	86.32	15.42
50	10	Tyr	1	60.72	73.73	86.91	14.15
50	10	Tyr	2	60.96	73.61	86.32	14.79
50	10	Tyr	3	58.47	73.61	89.28	14.88
50	10	Tyr	4	59.78	73.60	86.67	14.89

APPENDIX 10
RS DATA OF RICE STARCHES (ENZYMATIC-GRAVIMETRIC)

Treat	Type	pH	AA	Rep	RS
1	dH2O	NopH	NA	1	5.18
1	dH2O	NopH	NA	2	6.21
2	dH2O	NopH	Asp	1	5.97
2	dH2O	NopH	Asp	2	3.94
2	dH2O	NopH	Asp	3	9.39
2	dH2O	NopH	Asp	4	4.17
3	dH2O	NopH	Leu	1	5.81
3	dH2O	NopH	Leu	2	4.41
3	dH2O	NopH	Leu	3	6.02
3	dH2O	NopH	Leu	4	5.40
4	dH2O	NopH	Lys	1	4.90
4	dH2O	NopH	Lys	2	5.18
4	dH2O	NopH	Lys	3	9.75
4	dH2O	NopH	Lys	4	7.54
5	dH2O	NopH	Tyr	1	5.89
5	dH2O	NopH	Tyr	2	5.43
5	dH2O	NopH	Tyr	3	8.08
5	dH2O	NopH	Tyr	4	5.66
6	H/N	4	NA	1	8.08
6	H/N	4	NA	2	7.98
6	H/N	4	NA	3	8.66
7	H/N	7	NA	1	16.67
7	H/N	7	NA	2	16.15
7	H/N	7	NA	3	14.58
7	H/N	7	NA	4	20.16
8	H/N	10	NA	1	16.98
8	H/N	10	NA	2	15.67
8	H/N	10	NA	3	20.48
8	H/N	10	NA	4	19.18
9	H/N	4	Asp	1	14.48
9	H/N	4	Asp	2	17.68
9	H/N	4	Asp	3	13.87
9	H/N	4	Asp	4	18.53
10	H/N	4	Leu	1	7.29
10	H/N	4	Leu	2	7.88
10	H/N	4	Leu	3	7.70
10	H/N	4	Leu	4	8.90
11	H/N	4	Lys	1	21.56
11	H/N	4	Lys	2	23.43
11	H/N	4	Lys	3	22.70
11	H/N	4	Lys	4	25.65
12	H/N	4	Tyr	1	7.29
12	H/N	4	Tyr	2	5.83
12	H/N	4	Tyr	3	4.91
12	H/N	4	Tyr	4	6.12
13	H/N	7	Asp	1	8.60
13	H/N	7	Asp	2	11.88
13	H/N	7	Asp	3	9.40
13	H/N	7	Asp	4	8.12
14	H/N	7	Leu	1	12.05

Treat	Type	pH	AA	Rep	RS
14	H/N	7	Leu	2	11.41
14	H/N	7	Leu	3	10.45
14	H/N	7	Leu	4	9.13
15	H/N	7	Lys	1	7.78
15	H/N	7	Lys	2	8.79
15	H/N	7	Lys	3	9.25
15	H/N	7	Lys	4	9.07
16	H/N	7	Tyr	1	10.60
16	H/N	7	Tyr	2	13.86
16	H/N	7	Tyr	3	10.09
16	H/N	7	Tyr	4	11.07
17	H/N	10	Asp	1	13.68
17	H/N	10	Asp	2	15.10
17	H/N	10	Asp	3	13.42
17	H/N	10	Asp	4	13.25
18	H/N	10	Leu	1	11.54
18	H/N	10	Leu	2	11.37
18	H/N	10	Leu	3	14.18
18	H/N	10	Leu	4	10.00
19	H/N	10	Lys	1	12.14
19	H/N	10	Lys	2	14.86
19	H/N	10	Lys	3	12.35
19	H/N	10	Lys	4	12.70
20	H/N	10	Tyr	1	10.95
20	H/N	10	Tyr	3	12.33
20	H/N	10	Tyr	4	11.40
21	Bfr	4	NA	1	12.35
21	Bfr	4	NA	2	10.91
21	Bfr	4	NA	3	10.74
22	Bfr	7	NA	1	12.42
22	Bfr	7	NA	2	11.76
22	Bfr	7	NA	3	12.83
22	Bfr	7	NA	4	12.90
23	Bfr	10	NA	1	7.22
23	Bfr	10	NA	2	4.78
23	Bfr	10	NA	3	1.94
23	Bfr	10	NA	4	3.74
24	Bfr	4	Asp	1	12.78
24	Bfr	4	Asp	2	9.34
24	Bfr	4	Asp	3	7.65
24	Bfr	4	Asp	4	11.18
25	Bfr	4	Leu	1	11.23
25	Bfr	4	Leu	2	9.31
25	Bfr	4	Leu	3	11.33
25	Bfr	4	Leu	4	6.57
26	Bfr	4	Lys	1	12.65
26	Bfr	4	Lys	2	9.66
26	Bfr	4	Lys	3	10.79
26	Bfr	4	Lys	4	9.68
27	Bfr	4	Tyr	1	7.57
27	Bfr	4	Tyr	2	10.02
27	Bfr	4	Tyr	3	13.00
27	Bfr	4	Tyr	4	11.29
28	Bfr	7	Asp	1	17.58
28	Bfr	7	Asp	2	16.64
28	Bfr	7	Asp	3	16.84

Treat	Type	pH	AA	Rep	RS
28	Bfr	7	Asp	4	15.40
29	Bfr	7	Leu	1	14.68
29	Bfr	7	Leu	2	14.97
29	Bfr	7	Leu	3	12.63
29	Bfr	7	Leu	4	16.28
30	Bfr	7	Lys	1	4.31
30	Bfr	7	Lys	2	5.34
30	Bfr	7	Lys	3	4.14
30	Bfr	7	Lys	4	4.52
31	Bfr	7	Tyr	1	2.13
31	Bfr	7	Tyr	2	2.71
31	Bfr	7	Tyr	3	2.37
31	Bfr	7	Tyr	4	2.69
32	Bfr	10	Asp	1	15.47
32	Bfr	10	Asp	2	14.21
32	Bfr	10	Asp	3	15.72
32	Bfr	10	Asp	4	14.11
33	Bfr	10	Leu	1	18.52
33	Bfr	10	Leu	2	16.18
33	Bfr	10	Leu	3	16.09
33	Bfr	10	Leu	4	17.45
34	Bfr	10	Lys	1	4.44
34	Bfr	10	Lys	2	2.23
34	Bfr	10	Lys	3	4.34
34	Bfr	10	Lys	4	2.90
35	Bfr	10	Tyr	1	2.20
35	Bfr	10	Tyr	2	2.88
35	Bfr	10	Tyr	3	2.23
35	Bfr	10	Tyr	4	4.25
36	Tml	4	NoAA	1	8.04
36	Tml	4	NoAA	2	4.84
36	Tml	4	NoAA	3	7.33
36	Tml	4	NoAA	4	7.36
37	Tml	7	NoAA	1	6.74
37	Tml	7	NoAA	2	2.70
37	Tml	7	NoAA	3	4.54
37	Tml	7	NoAA	4	7.45
38	Tml	10	NoAA	1	7.85
38	Tml	10	NoAA	2	7.75
38	Tml	10	NoAA	3	8.63
38	Tml	10	NoAA	4	7.76
39	Tml	4	Asp	1	9.65
39	Tml	4	Asp	2	7.30
39	Tml	4	Asp	3	8.66
39	Tml	4	Asp	4	8.56
40	Tml	4	Leu	1	7.14
40	Tml	4	Leu	2	7.22
40	Tml	4	Leu	3	8.20
40	Tml	4	Leu	4	5.88
41	Tml	4	Lys	1	9.65
41	Tml	4	Lys	2	8.88
41	Tml	4	Lys	3	6.15
41	Tml	4	Lys	4	7.55
42	Tml	4	Tyr	1	10.10
42	Tml	4	Tyr	2	7.52
42	Tml	4	Tyr	3	8.97

Treat	Type	pH	AA	Rep	RS
42	Tml	4	Tyr	4	8.28
43	Tml	7	Asp	1	11.45
43	Tml	7	Asp	2	11.55
43	Tml	7	Asp	3	9.19
43	Tml	7	Asp	4	8.50
44	Tml	7	Leu	1	7.69
44	Tml	7	Leu	2	10.48
44	Tml	7	Leu	3	11.30
44	Tml	7	Leu	4	9.93
45	Tml	7	Lys	1	8.25
45	Tml	7	Lys	2	6.92
45	Tml	7	Lys	3	8.02
45	Tml	7	Lys	4	5.36
46	Tml	7	Tyr	1	8.90
46	Tml	7	Tyr	2	6.70
46	Tml	7	Tyr	3	8.48
46	Tml	7	Tyr	4	8.68

Treat	Type	pH	AA	Rep	RS
47	Tml	10	Asp	1	15.14
47	Tml	10	Asp	2	12.64
47	Tml	10	Asp	3	12.40
47	Tml	10	Asp	4	13.17
48	Tml	10	Leu	1	7.40
48	Tml	10	Leu	2	9.84
48	Tml	10	Leu	3	11.35
48	Tml	10	Leu	4	10.22
49	Tml	10	Lys	1	7.13
49	Tml	10	Lys	2	8.61
49	Tml	10	Lys	3	11.64
49	Tml	10	Lys	4	10.30
50	Tml	10	Tyr	1	11.92
50	Tml	10	Tyr	2	14.19
50	Tml	10	Tyr	3	14.60
50	Tml	10	Tyr	4	13.93

APPENDIX 11
RS DATA OF RVA GELATINIZED RICE STARCHES WITH TYROSINE (ENZYMATIC-GRAVIMETRIC)

Treat	Type	pH	Rep	RS
51	dH2O	NopH	1	10.03
51	dH2O	NopH	2	11.61
51	dH2O	NopH	3	10.60
51	dH2O	NopH	4	14.47
52	H/N	4	1	12.34
52	H/N	4	2	16.98
52	H/N	4	3	16.11
52	H/N	4	4	13.33
53	H/N	7	1	14.59
53	H/N	7	2	12.86
53	H/N	7	3	11.37
53	H/N	7	4	10.05
54	H/N	10	1	4.59
54	H/N	10	2	4.85
54	H/N	10	3	5.57
55	Bfr	4	1	9.22
55	Bfr	4	2	12.03
55	Bfr	4	3	11.02
55	Bfr	4	4	11.40
56	Bfr	7	1	7.71
56	Bfr	7	2	7.29
56	Bfr	7	3	8.48
56	Bfr	7	4	7.99
57	Bfr	10	1	6.94
57	Bfr	10	2	8.12
57	Bfr	10	3	8.23
57	Bfr	10	4	4.68

APPENDIX 12
RS DATA OF RICE STARCHES (ENZYMATIC-CHEMICAL)

Treat	Type	pH	AA	Rep	RS
1	dH2O	NopH	NoAA	1	2.61
1	dH2O	NopH	NoAA	2	2.17
1	dH2O	NopH	NoAA	3	1.97
1	dH2O	NopH	NoAA	4	2.40
2	dH2O	NopH	Asp	1	1.55
2	dH2O	NopH	Asp	2	1.51
2	dH2O	NopH	Asp	3	1.57
3	dH2O	NopH	Leu	1	1.19
3	dH2O	NopH	Leu	2	2.30
3	dH2O	NopH	Leu	3	1.77
3	dH2O	NopH	Leu	4	1.19
4	dH2O	NopH	Lys	1	1.26
4	dH2O	NopH	Lys	2	2.11
4	dH2O	NopH	Lys	3	1.90
4	dH2O	NopH	Lys	4	1.55
5	dH2O	NopH	Tyr	1	1.17
5	dH2O	NopH	Tyr	2	1.18
5	dH2O	NopH	Tyr	3	1.29
5	dH2O	NopH	Tyr	4	1.04
6	Bfr	4	NA	1	2.35
6	Bfr	4	NA	2	2.44
6	Bfr	4	NA	3	2.53
6	Bfr	4	NA	4	2.45
7	Bfr	7	NA	1	3.11
7	Bfr	7	NA	2	3.32
7	Bfr	7	NA	3	4.87
7	Bfr	7	NA	4	2.98
8	Bfr	10	NA	1	0.82
8	Bfr	10	NA	2	1.27
8	Bfr	10	NA	3	0.71
8	Bfr	10	NA	4	0.31
9	Bfr	4	Asp	1	2.31
9	Bfr	4	Asp	2	2.26
9	Bfr	4	Asp	3	2.08
10	Bfr	4	Leu	1	2.00
10	Bfr	4	Leu	2	4.17
10	Bfr	4	Leu	3	2.09
10	Bfr	4	Leu	4	2.14
11	Bfr	4	Lys	1	1.96
11	Bfr	4	Lys	2	2.01
11	Bfr	4	Lys	3	2.19
11	Bfr	4	Lys	4	1.73
12	Bfr	4	Tyr	1	2.16
12	Bfr	4	Tyr	2	2.29
12	Bfr	4	Tyr	3	2.27
13	Bfr	7	Asp	1	2.77
13	Bfr	7	Asp	2	3.83
13	Bfr	7	Asp	3	2.61
14	Bfr	7	Leu	1	2.71
14	Bfr	7	Leu	2	3.00

Treat	Type	pH	AA	Rep	RS
14	Bfr	7	Leu	3	2.97
14	Bfr	7	Leu	4	2.85
15	Bfr	7	Lys	1	2.54
15	Bfr	7	Lys	2	2.69
15	Bfr	7	Lys	3	2.76
15	Bfr	7	Lys	4	2.39
16	Bfr	7	Tyr	1	2.45
16	Bfr	7	Tyr	2	2.07
16	Bfr	7	Tyr	3	3.58
17	Bfr	10	Asp	1	0.67
17	Bfr	10	Asp	2	0.62
17	Bfr	10	Asp	3	1.13
18	Bfr	10	Leu	1	1.01
18	Bfr	10	Leu	2	1.14
18	Bfr	10	Leu	3	0.61
18	Bfr	10	Leu	4	0.83
19	Bfr	10	Lys	1	0.77
19	Bfr	10	Lys	2	0.74
19	Bfr	10	Lys	3	0.75
19	Bfr	10	Lys	4	0.86
20	Bfr	10	Tyr	1	2.10
20	Bfr	10	Tyr	2	0.75
20	Bfr	10	Tyr	3	0.80

APPENDIX 13
SAS CODE FOR THE ANOVA OF RVA DATA OF RICE STARCHES

```
dm 'log;clear;output;clear';
data one;
input treat $ pH $ AA $ rep PV MV BD FV SB TSB PTime PT;
datalines;
;
proc sort; by treat;
proc means mean std n maxdec=2; by treat;
var PV --- PT;
proc anova;
class treat;
model PV --- PT = treat;
means treat/tukey lines;
run;
quit;
```

APPENDIX 14
SAS CODE FOR THE ANOVA OF RVA DATA OF RVA GELATINIZED RICE STARCHES WITH TYROSINE

```
dm 'log;clear;output;clear';
data one;
input treat type $ pH $ rep PV MV BD FV SB TSB PTime PT;
datalines;
;
proc sort; by treat;
proc means mean std n maxdec=2; by treat;
var PV --- PT;
proc anova;
class treat;
model PV --- PT =treat;
means treat/tukey lines;
run;
quit;
```

APPENDIX 15
SAS CODE FOR THE T-TEST OF RVA DATA OF RICE STARCH TREATMENTS

```
dm 'log;clear;output;clear';
data one;
input treat type $ pH $ aa $ rep PV MV BD FV SB TSB PTime PT;
datalines;
;
proc ttest;
class type;
var PV -- PT;
run;
```

APPENDIX 16
SAS CODE FOR THE ANOVA OF DSC DATA OF RICE STARCHES

```
dm 'log;clear;output;clear';
data one;
input treat pH $ aa $ rep To Tp Tc dH;
;
proc sort; by treat;
proc means mean std n maxdec=2; by treat;
var To Tp Tc dH;
proc anova;
class treat;
model To Tp Tc dH= treat;
means treat/tukey lines;
run;
quit;
```

APPENDIX 17
SAS CODE FOR THE ANOVA OF RS DATA OF RICE STARCHES

```
dm 'log;clear;output;clear';
data one;
input treat type $ pH $ AA $ rep RS;
datalines;
;
proc sort; by treat;
proc means mean std n maxdec=2; by treat;
var RS;
proc anova;
class treat;
model RS=treat;
means treat/tukey lines;
run;
quit;
```

VITA

The author was born in Nueva Ecija, Philippines, in 1980. She graduated at Central Luzon State University in 2001 with a bachelor's degree in chemistry. A Ford-International Fellowships Program fellow, she started her graduate study at Louisiana State University in August 2007 and will receive a degree in Master of Science in food science in August 2009.