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Effects of ozonation and addition of amino acids on properties of rice starches

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EFFECTS OF OZONATION AND ADDITION OF AMINO ACIDS ON PROPERTIES OF RICE STARCHES

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ABSTRACT

In this study, the effects of ozonation and the addition of amino acids on rice starches were determined in terms of pasting properties by using rapid visco-analyzer, thermal characteristics by using differential scanning calorimeter, crystallinity by using x-ray diffraction, and resistant starch yield.

Results from viscosity analysis showed that the addition of lysine (6%) to ozonated Sigma rice starch significantly reduced peak viscosity (PV), minimum viscosity (MV), final viscosity (FV), and setback (SBK) by 918, 1024, 1023, and 105 cP, respectively. Moreover, it decreased pasting time, resulting in the faster swelling upon heating and less rigid gel formation upon cooling. The presence of lysine in ozonated white starch isolate (WSI) also significantly reduced all pasting properties and time to cook, and produced starch gel with the best cooking stability.

The amylose content of Sigma rice starch was increased by ozone, and the enzymatic-gravimetric analysis indicated that the resistant starch yield was enhanced by more than 3% when rice starch was ozonated for 30 minutes. Moreover, the addition of leucine to ozonated starch showed the highest resistant starch formation (9.13%). Ozonation of Sigma rice starch and white starch isolate (WSI) reduced gelatinization temperature, amylose-lipid complex endothermic temperature and enthalpy, but increased 1^{st} transition enthalpy. However, the presence of lysine (6%) increased gelatinization endotherm transition temperature but reduced 2^{nd} transition enthalpy.

Ozone treatment of Sigma rice starch induced B+V type XRD pattern and increased the relative crystallinity (RC), and addition of lysine showed A+B type XRD pattern and enhanced the RC further. In addition, ozonation caused some physical damage showing some broken small

pieces of particles and distorted starch granules with both Sigma rice starch and white starch isolate.

CHAPTER 1. INTRODUCTION

Starch is used in large quantities in many food industries; however, natural starches do not often have the properties required for a particular end-use. Starches show unstable viscosity when their pastes are subjected to high shearing action, prolonged heating or freeze-thaw cycles (Kantouch and Tawfik, 1998). Chemical modifications of starch have been applied to improve the gelatinization, cooking characteristics, and resistance of starch to degradation and to prevent retrogradation and gelling tendencies by using suitable treatments such as acid hydrolysis, oxidation, etherification or esterification. This allows polysaccharides to be used as thickeners, stabilizers or extenders for high-sugar foods such as glazes and bakery fillings.

Oxidation of starch is known to form carboxyl and carbonyl groups on the amylose chains having an influence on the retrogradation and gelling properties. It is known that oxidized starches are effective thickeners, producing low or high rigid textures depending on concentration (Kasapis, 2002). Resistant starch (RS) is defined as the fraction of starch, which resists digestion in the small intestine, but which is later fermented in the large intestine. Resistant food starch can be produced by either processing, such as autoclaving and parboiling (Eggum et al., 1993) or chemical modification such as enzyme or acid hydrolysis (Filer, 1988). Wolf et al. (1999) found that the extent of starch digestion was significantly decreased by modifications such as dextrinization and oxidation, and also found that as the degree of modification increased, the extent of digestion reduced, resulting in an increase in the amount of resistant starch.

Thus, chemical modification of starch might produce a slowly digested starch and serve as a good source of resistant starch in human and animal diets.

Ozone is known as a more powerful oxidant than oxygen that reacts with most substances at ambient temperatures, but creates no waste disposal problem. Therefore, utilizing ozone to replace the solvent and destroy undesirable toxins will be useful. Ozone is more reactive due to the extra oxygen atom, which can share an oxygen atom with other substances to oxidize them. Thus, ozonation could alter rheological properties of wheat flour to make it more elastic resulting in improved functionality (Voraputhaporn, 1996).

Starch-protein interaction has been studied in many modified starches by adding or removing protein. Mangala et al. (1999a) studied the effect of removal of protein and lipids on the resistant starch content of rice and ragi starches, and found that the percent recovery of RS increased significantly and that enthalpy decreased by Differential Scanning Calorimeter (DSC) after defatting and autoclaving. They also found that the formation of RS involved long unbranched chains of amylopectin as well because crystallization of amylopectin is an extremely slow process. Unbranched amylopectin might hydrogen bond to a certain extent, which might be attributed to the formation of RS. They also found that retrograded samples hydrolyzed slower than freshly gelatinized samples, indicating more resistance to amylolysis due to a higher degree of entanglement, and therefore a higher crystallinity index.

In spite of uses of chemical oxidizing agents for starch modification, they are generally undesirable because of safety and nutrition issues since it had been reported that some agents produce undesirable residues which remain in wheat flour, for example, benzoyl peroxide and chlorine dioxide which react immediately to leave a residue of benzoic acid and chlorine, respectively (Voraputhaporn, 1996). However, ozone does not leave any residue when it is introduced to a food product since single oxygen from triatomic oxygen is reacting with other substances, but O_2 that we breathe remains in the air. Thus, ozone treatment would be a good alternative to chemical treatment as ozone does not leave any solvent residues when utilized on a food product. There has been a lot of research on modified starch using many different suitable treatments and on the effect of lipid or protein on thermal properties. However, less research has been done to study the effect of ozonation and addition of amino acids on rice starch characteristics.

In this study, two different kinds of rice starches were ozonated and their physicochemical properties were examined by studying pasting characteristics by Rapid Visco-Analyzer (RVA), thermal properties by Differential Scanning Calorimeter (DSC), resistant starch content, crystallinity by X-ray Diffraction (XRD), and physical change of rice starch granules by Scanning Electron Microscopy (SEM). The addition of amino acids on ozonated rice starches were also studied by RVA and resistant starch content. Among the amino acids, lysine was further analyzed by DSC, XRD, and SEM.

CHAPTER 2. LITERATURE REVIEW

2.1. CARBOHYDRATE

2.1.1. Starch

Starch is the predominant food reserve substance providing 80% of the calories consumed by humans worldwide. Starch consists of two polysaccharides, amylose, a linear polymer of D-glucose units and highly branched amylopectin (Fennema, 1996; Paterson et al., 1996). Starch varies in types: high amylose (35-50%), regular (25%), and waxy (mostly amylopectin). Starch is a mixture of linear amylose and branched amylopectin in discrete particles. Starch granules are dense and insoluble; however, they make flowable slurries and hydrate slightly in cold water. Common starches contain about 75% amylopectin and 25% amylose (Fennema, 1996). Many studies have investigated rice starch to study the effect of composition and physical properties of the granules thermo- mechanically since it is composed of a single-size granule distribution (Biliaderis and Juliano, 1993). The starch granule is built up of a series of concentric growth rings, in which there are crystalline and amorphous regions in the starch granules and the crystalline regions contain oriented helices of starch chains (Atwell et al., 1988). In native starches, amylose and amylopectin associated by hydrogen bonding form readily oriented micelles or crystalline areas of various degrees of order (Kantouch and Tawfik, 1998).

Starch plays an important role as a determinant of food product quality (Zeng et al., 1997), and the functional properties of starch are determined by the temperature dependent interactions of starch involving water. The processes involved in cooking starch are gelatinization, pasting, and retrogradation (Atwell et al., 1988).

- **Gelatinization**

When starch is cooked in excess water over a critical temperature, the starch granule integrity is weakened, which leads water to penetrate inside the granule, therefore, hydration takes place. Gelatinization of starch is the loss of crystallinity and irreversible granule swelling (disruption of molecular order within granule) that produces a viscous mass consisting of a continuous phase of solubilized amylose and amylopectin. It is also an uptake of heat as the conformation of the starch alters, the starch hydrates, and there is a lost of integrity of the starch granule (Waniska and Gomaz, 1992). Natural granules are insoluble in cold water: therefore, heating is required to gelatinize in the endothermic process. The important factors that affect the gelatinization process are granule size, shape, amylose/amylopectin ratio, and possibly, the non-starch constituents of the starch granule, for example, lipids and proteins (Tester and Morrison, 1990a).

The linear amylose fraction presents the ability to form inclusion complexes with a variety of ligands such as iodine, which enter the helical cavities of the amylose molecules and form molecular inclusion complexes. During the formation with ligands, amylose changes its shape from coil to helix, which results in aggregation of helices into partially crystalline V structure (Eliasson and Krog, 1985). Due to this characteristic of starch, it is believed to complex with lipids whether they are present naturally inside starch granule or they are added before cooking. Moreover, starch is known to interact with protein as well. Amylose –lipid complexes and starch- protein interaction may influence viscoelastic properties of grain and flour (Hamaker & Griffin, 1990). According to Elliason (1986), the formation of amylose-lipid complexes is an exothermic process; thereby decreasing endothermic gelatinization enthalpy. In addition, it was explained that the complexes hinder amylose leaching out of the starch granule during gelatinization; therefore, inhibits the swelling of starch granules (Elliason, 1985).

- **<u>Pasting</u>**

When starch is heated further in excess water past gelatinization, the viscosity of starch granule starts to increase, which is called pasting. Total disruption of granules occurs, but granule remnants still remain with further swelling with shearing (Fennema, 1996). Swelling and disruption of granules result in a viscous mass containing a continuous phase of solubilized amylose and/or amylopectin and a discontinuous phase of granule remnants (Fennema, 1996). Factors affecting the viscosity of starch paste are the volume fraction occupied by swollen granules, rigidity of swollen granules, viscoelasticity of the continuous phase, and adhesion force between the dispersed and continuous phase (Elliason and Bohlin, 1982).

Viscosity happens after most of the granule swelling stops and the increase in viscosity is shown to be mainly because of the exudates released from the starch granules imbibing more free water as they swell (Miller et al., 1973). They also mentioned that the formation of partial junction zones and a network resulted from the leached soluble starch, which increased the viscosity. The main factors that influence paste viscosity are the capability of swollen granule dispersion, exudates from the inside of granule, and swelling potential (Morris, 1990). Furthermore, it was stated that the volume of swollen granules is an important determinant for the viscosity and properties of starch paste (Fannon and Bemiller, 1992). Generally, high amylose content starch shows larger viscosity and higher swelling capacity. Reddy et al. (1994) explained that starch granules of high amylose were rigid and elastic, and maintained their granule integrity and gave a strong, elastic paste upon cooking. In contrast, low amylose starch granules were soft, inelastic, and more fragile, therefore, gave thin and weak paste with extensive granule disintegration. They also implied that this difference in starch-granule rigidity probably resulted in various differences in cooked rice texture.

Many investigators have studied the effect of defatting on pasting characteristics. It was observed that lipid extracted starches increased the peak viscosity, but reduced the pasting temperature (Goshima et al., 1985; Melvin, 1979). In addition, Biliderис and Tonogai (1991) reported an increase in viscosity when lipids were removed from rice and wheat starches. On the other hand, Vasanthan and Hoover (1992) found that defatting made the pasting peak disappear and lowered viscosities, especially breakdown (BKD, indicator of cooking stability measured by Rapid Visco-Analyzer), resulting in thermal stability. Furthermore, Hoover et al. (1993) presented higher pasting temperature and lower final viscosity from defatted pigeon pea starch. This study also reported the effect of heat-moisture treatment on pasting characteristics of pigeon pea and they found that heat-moisture treatment increased the pasting temperature but reduced the viscosities during the holding period since starch granule stability was enhanced from the heat treatment.

- **<u>Retrogradation</u>**

Retrogradation is the precipitation of starch when gels or pastes are cooled and stored, and the starch becomes less soluble. Factors that affect the rate of retrogradation are the molecular ratio of amylose to amylopectin, structure of the amylose and amylopectin molecules, starch concentration, cooking temperature, and concentration of other ingredients such surfactants and salts (Fennema, 1996).

Retrogradation of starch consists of two separate stages; the short-term change and the long-term change. During cooling, the short-term changes have been attributed to crystallization of the amylose fraction because most of the initial stage of starch gelation involves the solubilization of amylose (Miles et al., 1985; Sievert and Wursch, 1993). During storage of starch gels, the long-term changes have been attributed to the recrystallization of amylopectin fraction (Elliason, 1985). Factors that influence the behavior of retrograded amylopectin are the starch source, concentration (Orford et al. 1987), storage temperature, and amylopectin structure (Fredriksson et al., 1998).

2.1.2. Amylose

Amylose is predominantly a linear chain of α (1-4) linked D-glucosyl units (Paterson et al., 1996). Amylose has a right-hand helix linear structure, and the inside of the helix is lipophilic and consists of only hydrogen atoms. On the other hand, there are hydrophilic hydroxyl groups on the outside of amylose chains. The molecular weights of amylose are about 10^6 (Fennema, 1996). There is evidence that amylose is located within the amorphous region of the granule (Zobel, 1992). Amylose is known to have a more rigid gel and a stronger film than amylopectin after cooking (Moore et al., 1984; Wurzburg, 1972). Gelatinization could facilitate starch granule swelling and lead to leached amylose molecules in aqueous solution (Lii et al., 1995). Leaching of amylose is an order-disorder phase transition within the swollen granule and it occurs when starch is heated with water (Tester and Morrison, 1990a). If continued heating of starch granules in excess water occurs, it gives additional leaching of soluble amylose, and finally total disruption of granules occurs, in other words, pasting. It was found that there is a linear relationship between the amount of leached amylose and the total amylose content of the rice after cooking. According to Lii et al. (1995), the leached amylose concentration was affected by the starch concentration and the heating temperature. Olkku and Rha (1978) found that starch solubility, paste consistency and paste clarity are related to the swelling of starch granules as well. Jacobs et al. (1995) stated that the leaching of macromolecules is the main factor that contributes to viscosity development in starch pastes. Moreover, Li and Corke (1999) found that higher amylose content starch showed twofold higher final viscosity than waxy starches that had comparably lower amylose content. They also observed that higher amylose containing starch showed a lower value of breakdown (BKD) indicating great resistance to shear thinning.

Lipid effects on amylose leaching have been shown by many studies. The solubility of defatted starch has been shown to increase (Lorenz, 1983; Goshima et al., 1985). Furthermore, Vasanthan and Hoover (1992) found defatting decreased the extent of amylose leaching in potato and lentil starches due to increased granular stability, whereas increased amylose leaching was observed in wheat and corn starch, probably because of the removal of lipids bound to amylose. They explained that a decrease of granule swelling and an increase of granular stability resulting from defatting were related to interaction between amylopectin chain clusters. Numfor et al. (1996) observed a lower amylose leaching when emulsifiers were added to starch since the leaching of amylose was hindered by amylose-lipid complexes.

Jacobs et al. (1995) noticed amylose leaching for annealed wheat starch was changed and explained that the remnants of the granules rather than the amount of the amylose leached from the granules determined viscosity after gelatinization. They also mentioned annealing made the swollen granules more rigid, and thus produced a starch resistant to heat. Heat-moisture treatment is a process in which starch-water slurry is heated at 100˚C for 16hours in an air oven. It decreased the swelling of starch and amylose-leaching in starches because the heat treatment enhanced starch activity in the amorphous regions of the granule between amylose and the outer chain branches of amylopectin resulting in greater rigidity of the starch (Hoover et al., 1993: Hoover et al., 1994: Hoover & Vasanthan, 1994).

2.1.3. Amylopectin

Amylopectin is a highly branched polysaccharide consisting of α (1, 4) linked glucose with α (1, 6) linkages. Amylopectin is a polymer with a massive molecular weight, which has degrees of polymerization of 15-20 glucose units and results in entanglements between amylopectin molecules with very long life times (Paterson et al., 1996).

Starch granules are composed of concentric layers; dense layer, which consists of 16 alternate crystalline and amorphous lamellae and less dense layer, which is largely amorphous (French, 1984). In the crystalline regions of the starch growth ring, the short amylopectin chains are associated into double helices. Thus, amylopectin branches rather than amylose are mainly attributed to the crystallinity of the granule (Jenkins et al., 1993). Moreover, amylose content had little effect on granule crystallinity since starches with different amylose contents showed the same crystallinity. If these double helical regions are examined by X-ray Diffraction (XRD) and birefringence under microscope, they show a periodicity in the radial direction and granule crystallinity (Jenkins and Donald, 1995). According to Moore et al. (1984) and Wurzburg (1972), starches with high amylopectin content, waxy starch, tend to produce a crispy starch paste. The shape of amylopectin molecules is described as the “cluster” model, which is responsible for prevention of the formation of hydrogen bond intermolecular interactions. Therefore, amylopectin results in a softer gel compared with the amylose gel (Zobel, 1988a). Amylopectin forms a complex with iodine to a lesser extent than amylose, and complexes are unstable owing to the shorter length.

2.1.4. Effects of Protein on Starch

Ellis et al. (1998) implied that starch granules react with proteins and that the amounts of starch granules associated with protein vary depending on the source. Starch proteins can be separated as surface proteins and integral proteins. It was reported that surface proteins are removed by extraction at temperatures below the gelatinization temperature, whereas the integral proteins are extracted at temperatures near or above the gelatinization temperature.

Hamaker and Griffin (1993) investigated the effect of protein on the gelatinization behavior of starch granules and found that the lack of protein in starch improved the fragility of the granules making starch more accessible to water; thereby, causing an increased viscosity and gel strength because of larger swelling. They also found that proteins with disulfide bonds in rice flour hindered granule swelling and made the swollen granules easily breakable when shear was applied. Moreover, Radosavljevic et al. (1998) studied treated amaranth starch with alkaline-protease and concluded that deprotenized starch had a greater starch yield and recovery, and showed greater pasting by amylograph and lower gelatinization temperature by DSC (Differential Scanning Calorimetry) than native starch. It can be postulated that the thermal parameters of starch gelatinization are very dependant on the structural changes of starch. Marshall et al. (1990) also studied protein removal effects on starch gelatinization and found that starch granules without protein showed an increase in the starch gelatinization endotherm meaning that they might act as a barrier so that water could not penetrate easily upon cooking. The treated starch granule also had visible damage on the surface of the grains.

Liang (2001) studied the effects of various amino acids on pasting characteristics, gelatinization, and X-ray diffraction pattern. He observed that amino acids increased the rate of starch swelling but reduced the swelling extent, resulting in lower pasting viscosities and lower cooking stability. It was found that positive charged and negative charged amino acids showed stronger influence on starch pasting than neutral amino acids, which might be related to their amphipathic characteristics and influenced by the charges that those amino acids carried. He also found that amino acids inhibited amylose-lipid complex formation, resulting in the reduction of the second transition enthalpy and that the 4.4 Å peak, which represents V-pattern in XRD, might be related to the content of protein or amino acids residues in the starch.

According to Resurreccion et al. (1993), there are two hydrophobic protein bodies: PB I (Prolamin) and PB Π (Glutelin) in rice. Surface and internal proteins in starch granules and their hydrophobic tendency are the reasons why rice starch extraction is difficult. Besides that, tiny rice starch granules are slow to sediment in water, causing losses during separation and purification (Lumdubwing and Seib, 2000). Furthermore, the Maillard-type reactions with polysaccharides might result in limitation in food industrial application (Ellis et al., 1998). However, the protein fraction could be a by-product using appropriate separation with carbohydrate-hydrolyzing enzymes (Shih and Daigle, 1997). The most dominant amino acids in rice protein are glutamic acid, aspartic acid, arginine (Juliano, 1985), and lysine (Shih and Daigle, 1997).

2.2. MODIFIED STARCH

2.2.1. Starch Modification

Natural starches may have properties that limit their use for particular food applications. The undesirable properties include insolubility, instability, and unstable viscosity when starch pastes are subjected to processing conditions such as high shearing action, heated for prolonged periods or subjected to freeze-thaw cycles (Kantouch and Tawfik, 1998). However, modifications of starch products can solve these problems. Chemical modifications have been conducted to change the gelatinization and cooking characteristics of granular starch, to reduce the retrogradation and gelling tendencies of amylose containing starches, to enhance the water holding capacity of starch dispersion at low temperature, to increase hydrophilic character, to impart hydrophobic properties and/or to introduce ionic substituents for use as thickening, gelling, binding adhesive, and film-forming functionality (Hebeish et al., 1989). Modification methods involved acid or enzyme hydrolysis, oxidation, esterification or etherification (Rapaille and Vanhemelrijek, 1997). This allows polysaccharides to be used as thickeners, stabilizers or extenders for high-sugar foods such as glazes and bakery fillings. Cross-linking provides more structural integrity by bridging one starch molecule to another within the granule resulting in dramatic improvement of stability to processing conditions such as acid, heat, and shear forces. Stabilization reduces retrogradation by interrupting the linear structure of the amylose and/or segments of the amylopectin branches resulting in less retrogradation during storage of the finished product (Filer, 1988).

Processing methods are known to modify paste viscosities. Malted or popped starches presented low peak viscosity and set back due to starch granule damage and amylose leaching. Modifications in starch affect viscosities as well. It was found that crosslinking in waxy wheat starch (WWS) decreased paste viscosity, and starch paste consistency was resistant to stirring (Reddy and Seib, 2000). However, crosslinked WWS at low levels increased paste viscosity. In addition, crosslinked waxy corn starch had increased paste viscosities at all levels. The doubly modified waxy starch with hydroxylation and acetylation had increased 'thickening power' through enhanced swelling power (Reddy and Seib, 2000).

Morikawa and Nishinari (2000) studied the thermal properties of crosslinked starch treated with hydroxypropylated phosphate (HPS) and found a shift to lower temperature and a decreased enthalpy by DSC. They explained that there may have been some structural change during the chemical modification. They also found that the endothermic peak appeared at a temperature from 45 to 46˚C for HPS treated starch, whereas it appeared at 60˚C for native potato starch. Furthermore, by dynamic viscoelastic measurements they also found that the HPS granules did not rupture when heated in the temperature range from 50 to 100˚C for 30 minutes, while native starch granules ruptured gradually on heating above 70˚C. Reddy and Seib (2000) also studied the effect of modification and found that hydroxypropylated/crosslinked or acetylated/crosslinked waxy starches presented lower gelatinization temperature and enthalpies compared to native starch. They explained that the substituted starch granule in the amorphous regions (Biliaderis, 1982; Hood and Mercier, 1978) promotes swelling and disrupts the crystalline phase which melts at a lower temperature than in unmodified starch. They also reported a lower gelatinization temperature from extruded starch, which could be because of granule damage.

2.2.2. Oxidized Starch

During oxidation, starch undergoes oxidative degradation where it causes depolymerization and introduces carbonyl and carboxyl groups on hydroxyl groups; therefore, it weakens starch granules resulting in lower viscosity (Rutenberg and Solarek, 1984) and less recrystallization since structural changes make three dimensional networks impossible during gel formation. For the matter of structural change, it was found that the hydroxyl groups of C-2, C-3, and C-6 positions are the primary places that the carbonyl and carboxyl groups attach (Wurzburg, 1986).

Paterson et al. (1996) also suggested that oxidative degradation may influence the integrity of the starch granule resulting in an important factor in the functional behavior of oxidizing agents in baked products. On the other hand, Kuakpetoon and Wang (2001) found that slightly oxidized corn and rice starch showed an increase in peak viscosity and higher viscosity. This meant that oxidized starch granules swelled more since electrical repulsion of carboxyl groups reduced starch integrity; therefore, it allowed more water into the granules. In deep fat frying systems, high viscosity is beneficial for inhibiting batter runoff from the food during coating (Mukprasirt et al., 2001).

Sodium hypochlorite is the most frequently used oxidizing agent (Boruch, 1985). Other oxidants involve hydrogen peroxide (Autio et al., 1992), permanganate, periodate (Wing, 1994), chlorites, oxygen, and ozone (Parovuori et al., 1995). Schmorak et al. (1962) found that hydrogen peroxide oxidation introduced more carbonyl groups than hypochlorite treatment and that the molecular weight of amylopectin decreased considerably with the degree of oxidation. A side reaction other than oxidation is hydrolytic degradation of starch molecules, in which there is the increase in starch reducing value and a decrease of viscosity due to glycosic scission on starch molecules (Boruch, 1985; Hebeish et al., 1989). According to Farley and Hixon (1942), oxidizing agents have been claimed to penetrate deeply into the granule and to act mainly on the amorphous regions. It was found that a low amount of carbonyl groups was beneficial for the stability of starch dispersions (Prey and Siklossy, 1971), whereas a high level of oxidation produced stable viscosity in potato starch dispersion (Fischer and Piller, 1978). Moreover, during oxidation with hypochlorite, starch molecules alter their shape and spatial system, therefore, color complexes with iodine changes and resistance to the action of amylolytic enzymes becomes greater with greater flexible gels in the form of films (Boruch, 1985). On the other hand, Han and Ahn (2002) reported that corn starch oxidized with NaOCl did not change the size, shape, and amylose content compared to non treated samples. However, as the extent of oxidation increased, solubility, swelling power and the amount of soluble amylose increased.

Oxidized starches have been developed for the purpose of extending uses in many areas and are widely used in food, paper, and textile industries (Li and Vasanthan, 2003). Commercial oxidized starches have been applied in the food industry offering shorter cooking times, lower viscosities, higher stability (Kuakpetoon and Wang, 2001), reduced retrogradation and better clarity (Scallet and Sowell, 1967).

For nutritive perspectives, oxidized starch can be utilized as thickeners, edible film for packaging and gelling agents when making jelly, pudding, sauces or marmalade for better product stability (Boruch, 1985; Kokini, 1994). In addition, slightly oxidized starch has been used in batters and breadings for fried foods because of an excellent adhesiveness (Han, 2002). In high sugar foods, oxidized starch has been utilized as glazes and bakery filling (Kasapis, 2002). It also has been used in food industry for baby food formulas requiring small gel strength and transparency (Radly, 1982). In the paper and textile industries, oxidized starch has been applied as sizing agents (Hebeish et al., 1992b), film former (Kamel et al., 1971; EL-Thalouth et al., 1977), and stabilizer in internal sizing emulsions displaying better performance than untreated starch (Teleman et al., 1999).

Wade (1972) reported that oxidizing agents were extensively used as bread improvers and sulfite in particular was used at low levels to reduce the elastic properties of biscuit dough. It was also stated that the conventional understanding was that sulfite acted entirely on the protein component (Wade, 1972; Fitchett and Frazier, 1986). The integrity of starch granule may be influenced by the oxidative environment, so it seemed possible that the oxidizing agents could also have an effect on the starch component (Paterson et al., 1996).

High starch concentrations, higher temperature, and granular structure damage increases the rate of oxidation reaction (Wing, 1994); moreover, starch type influences the rate of oxidation and changes viscosity properties since different starches present differences in physical and molecular structure (Kuakpetoon and Wang, 2001). The degree of oxidation affected the starch gel with a high concentration of hypochlorite causing a much weaker gel than that of low concentrations (Forssell et al., 1995). The reaction rate was increased when gelatinized solution was used instead of starch granules (Schmorak et al., 1962). Wing (1994) reported that hypochlorite oxidation at pH 10-12 resulted in a high proportion of carboxyl groups, whereas lower pH increased the oxidation rate and yielded a greater percentage of crosslinkable carbonyl groups. It was also found that the size and crystallinity of starch granules affect the oxidation process and that characteristics of its product would differ according to the type of starch used for oxidation (Boruch, 1985).

Amylose content has been shown to be influenced by oxidation. It was found that the iodine binding capacities of starches decreased after oxidation due to the degradation of amylose and changes in the structure of the amylose molecules (Boruch, 1985). Forssell et al. (1995) agreed with previous result that total amylose content of barley and potato starch was decreased after hypochlorite oxidation. However, apparent amylose content of oxidized barley starch did not change and the second enthalpy, which indicates amylose-lipid complex in Differential Scanning Calorimetry (DSC), was reduced after oxidation. These facts indicated that mainly lipid-bound amylose was oxidized and it might be explained if amylose –lipid complexes are enriched near the granule surface (McDonald et al., 1991).

It was demonstrated that defatting enhanced oxidation since starch granule crystallinity was reduced due to partial gelatinization during defatting and the more open granular structure increased oxidation. However, no change in lipid content revealed that the oxidized amylose did not lose most of the lipids, but there was a decrease in complexion power of iodine with amylose from decreased total amylose content (Forssell et al., 1995). This result was in accordance with that of Boruch (1985), in which amylose content was decreased with the increase of starch oxidation degree because of reduction of natural amylose content of the spiral structure of the chain. In addition, it was also found that amylose content returns with storage, thus oxidation changed the form of starch molecules but did not cause degradation of amylose chains.

It was reported that low levels of catalysts such as sodium sulfite and sodium chloride markedly reduced the paste viscosity of potato starch as a result of a non-specific ionic effect (Mat Hashim et al., 1992). Moreover, low levels of sodium sulfite affected the degree of swelling of the starch granule pasted at high temperature because of decreases in swollen volume (Paterson et al., 1994). These facts suggested that sulfite acted as promoter that caused granule disintegration where a free radical attack was associated with the formation of oxygen and hydroxyl radicals followed by the breakage of bonds within the polymer chains, in other words, oxidative reductive depolymerization (Paterson et al., 1996).

2.2.3. Resistant Starch

Starch is divided into three different categories, RDS (Rapidly digestible starch), SDS (Slowly digestible starch), and RS (Resistant starch). RDS is starch that is rapidly and completely digested in the small intestine, whereas SDS is starch that is slowly but completely digested in the small intestine. RS, on the other hand, is the sum of starch and starch degradation products that is not digested in small intestine but reaches the human large intestine and may be fermented by microorganisms in it. RS is divided into 4 different categories. RS 1 is physically trapped starch in rigid cell walls that inhibits swelling and dispersion of starch, as in legumes or whole grains. RS 2 is ungelatinized granules or RS granules that are very densely packed, thus highly resistant to digestion by α-amylase until gelatinization in a food such as spaghetti or green banana. RS 3 is retrograded starch polymer, mainly amylose, produced when starch is cooled after gelatinization. RS consists of both amorphous and crystalline material of two distinct populations of α- glucans, semi crystalline material and retrograded amylose (Faisant et al., 1993; Eerlingen et al., 1993b). Furthermore, several studies revealed that the extent of retrogradation of amylose was found to be of primary importance in determining the RS content of starch (Cairns et al., 1996; Eerlingen et al., 1994a; Sievert and Pomeranz, 1989). Formation of RS 3 is also influenced by granular swelling and amylose leaching, and the molecular characteristics (chain length) of amylose. Amylose crystallization takes place through chain elongation by double helical formation, and the elongated amylose chains fold and facilitate helix-helix aggregation by formation of interhelical hydrogen bonds (Miles et al., 1985). As a result, the intimate packing of starch double helices form crystals (Wu and Sarko, 1978) and their binding to starch chains resist the diffusion of starch hydrolyzing enzymes into the region. In addition, the amylose fraction in hot concentrated starch dispersion forms a continuous matrix that penetrates swollen gelatinized granules composed essentially of amylopectin. Cooling leads to gelation due to phase separation of amylose as a network (Russell, 1989). Crystallization of amylopectin is recognized to take place in the swollen gelatinized granules (Eerlingen et al., 1993a). It was found that an increase in RS in breadcrumb after bread making could be retrograded amylopectin (Eerlingen et al., 1994a). The formation of RS involved long unbranched chains of amylopectin due to the fact that crystallization of amylopectin is an extremely slow process. Unbranched amylopectin might hydrogen bond to a certain extent, which might be attributed to the formation of RS. Furthermore, amylopectin crystal resists digestion by incorporation of a small proportion of amylopectin molecules into amylose crystals because of amylose-amylopectin interactions during gelation as well. Thermal transition at 40-60˚C by Differential Scanning Calorimeter was considered a staling endotherm of amylopectin (Russell, 1983; Eliasson, 1985). The amounts of RS 3 depends on the type of starch (Sievert and Pomerenz, 1989), amylose content (Leloup et al., 1992), moisture, heating temperature (Alejandra et al., 1998), additives (Eerlingen et al., 1994b; Sievert et al., 1991), amylose chain length (Eerlingen et al., 1993b; Kim et al., 1997; Zhang and Jackson, 1992), storage time and temperature.

RS 4 is resistant starch that could be developed by using chemical modification. Modified food starch can be produced by either processing, such as autoclaving and parboiling, which increases the resistant starch content of non-waxy starches (Eggum et al., 1993) or chemical modification such as enzyme or acid hydrolysis, cross-linking and stabilization (Filer, 1988). Enzyme hydrolysis with amylase debranches amylopectin chains and frees more linear amylose chains which participate in crystal formation by chain elongation and folding, therefore, increasing resistant starch content after heating (Vasanthan and Bhatty, 1998). For the production of resistant starch in the food industry, α –amylase, amyloglucosidase, and pullulanase are the most commonly used enzymes. Alpha-amylase is an endo-enzyme that cleaves α (1, 4) –D-glucosidic linkages in starch, while pullulanase debranches starch molecules by cleaving α (1, 6) –D-glucosidic linkages. Both amylose and amylopectin are digested by α-amylase treatment, and the end-products are glucose, maltose, maltotriose and branched α-limit dextrins (French et al., 1972). Furthermore, it was revealed that α-amylase isolated RS from amylose-lipid complexes at high temperatures (Holm et al., 1983). Incubation time during enzyme hydrolysis is an important factor since it helps remove degradable structures and thus isolate and concentrate RS (Sievert and Pomeranz, 1989). Eerlingen et al. (1993a) found that incubation time and temperature affected the yield of resistant starch. Acid treatment is similar to pullulanase hydrolysis, in which hydrolysis of amylopectin could yield starch entities such as linear chains, double helices, and crystallites (Vasanthan and Bhatty, 1998). Moreover, the hydrolysis of retrograded starch gels by pullulanase enzyme or acid was revealed to enhance the RS formation during annealing (Vasanthan et al., 1999).

Cooked rice generally contained more resistant starch than raw rice (Eggum et al., 1993). When gelatinization disrupts granule structure completely, crystallization of amylose polymers occurs readily, and the amylose fraction forms a continuous matrix, which penetrates swollen gelatinized granules composed of amylopectin. Crystallization of the amylose is more rapid than the crystallization of amylopectin (Russell et al., 1989). In addition, resistant starch is correlated positively with amylose content. Besides cooking, RS also could be produced by methods using relatively high moisture contents, for example, baking, parboiling, puffing, drying, extrusion (Mercier, 1980; Eggum et al., 1993), or autoclaving. Twin-extrusion treatment of starch decreased solubility and the susceptibility of the extrudates to α-amylase digestion (Galloway et al., 1989). Autoclaving with heating/cooling cycles are known as the most effective method (Brown, 2001). Russell et al. (1989) found that autoclaving enhances starch granule swelling during heating and allows amylose leaching that dominates crystallites. Moreover, annealed starch showed higher resistant starch content from starch isolations as the numbers of heating/cooling cycles increase (Bjorck et al., 1987; Berry, 1986; Sievert et al., 1990). According to Sievert and Pomeranz (1989), RS content is influenced by starch water ratio, autoclaving temp, and the number of autoclaving/cooling cycles in autoclaving process.

Digestion rate is another important factor to consider when forming resistant starch. Wolf et al. (1999) found that the extent of starch digestion was significantly decreased by modifications such as dextrinization (Flickinger et al., 1998), etherification, and oxidation. They also found that as the degree of modification increased, the extent of digestion decreased, resulting in an increase in the amount of resistant starch. Hence, chemical modification of starch may allow for the production of a slowly digested starch since substitution in modified starch interferes with the binding of α -amylase and/or amyloglucosidase, thus decreasing starch digestion.

Starch digestion is also slowed in the small intestine if the physical form of the food hinders access of pancreatic amylase causing reduced or delayed postprandial glucose and insulin responses (Englyst et al., 1992; Jenkins et al., 1987). The digestion rate is influenced by the physical and chemical characteristics of the food (Araya et al., 2002). Dietary carbohydrates are digested and absorbed at different rates and to different extents, depending on their botanical source, the physical form of the food, and the degree of food processing (Englyst et al., 1999). In case of legumes and minimally processed cereal grains, nutrients are encapsulated within the cell walls (dietary fiber), which retard the release and hence digestion and absorption of starch and sugars. It was found that high amylose starches are considered to have more hydrogen bonding resulting in more crystallinity in their structure that is not swelled or gelatinized as readily upon cooking, and hence are digested more slowly. However, the polymers of retrograded amylopectin are less firmly bound than that of amylose. A lot of studies have found that amylose-lipid complexes decreased starch digestibility in vitro (Larsson and Miezis, 1979; Mercier et al., 1980; Holm et al., 1983; Eliasson and Krog, 1985). The factors affecting starch digestion are degree of gelatinization, particle size, amylose/amylopectin ratio, starch-protein interaction, amylose-lipid complexes, and percentage of retrograded starch (Vasanthan et al., 1999).

Mangala et al. (1999a) studied the effect of removal of protein and lipids on the resistant starch content, and found that the percent recovery of RS increased significantly and that enthalpy decreased by DSC after defatting and autoclaving. Fewer lipid molecules were present for complexing with amylose resulting in more free and uncomplexed linear fraction. In contrast, deprotenization did not make any major difference. They also found that retrograded samples hydrolyzed slower than freshly gelatinized samples, indicating more resistance to amylolysis due to a higher degree of entanglement, and therefore a higher crystallinity index. Foods such as bread, breakfast, cereals and biscuits are known to contain appreciable quantities of resistant starch (RS), starch that is resistant to the action of amylolytic enzymes, either in vivo or vitro (Russell et al., 1989).

Amylose-lipid complexes are one of the most effective factors in RS formation. In defatted starch, more amylose is available to aggregate to each other since fewer lipids are present that form enzyme-digestible complexes with amylose during gelatinization, resulting in higher RS yields. On the other hand, added lipids interacted with amylose chains, and the formation of crystallized amylose was thereby reduced. Therefore, amylose-amylose association was prevented by the addition of lipids because of a competitive mechanism of amylose association and amylose-lipid complex formation (Szczodrak and Pomeranz, 1992). Furthermore, it was known that amylose changes its structure from linear to alpha helix conformation while it complexes with lipid, thus crystallization of amylose decreases, resulting in a reduction in resistant starch formation. However, in some studies, amylose-lipid complexes are considered to decrease the susceptibility of amylose to amylolysis (Eliasson and Krog, 1985; Holm et al., 1983). Moreover, amylose-lipid complexes are known as native enzyme inhibitors since defatted starch granules were more susceptible to enzyme degradation (Baker and Woo, 1992). In contrast to amylose, amylopectin binds to lipids to a lesser extent due to short branches (Guraya et al., 1997). Starch –protein interaction is another important factor. It was found that milled-rice protein reduced digestibility upon cooking, in which the denaturation of 'core proteins' of Prolamin (protein body 1) in rice endosperm takes place (Tanaka et al., 1978). RS content was high in egg noodle, which might be related to interaction between starch and proteins (Brighenti et al., 1998). In addition, Greenwell et al. (1985) revealed that surface proteins act as an obstacle to amylolytic enzymes. Escarpa et al. (1997) found that protein provides a rigid cover, in which starch is encapsulated. Moreover, protein bound to starch during starch retrogradation similar to the way amylose chains are formed by hydrogen bonds.

In Differential Scanning Calorimetry, the third peak represents the resistant starch peak. Sievert and Pomeranz (1989) investigated isolated resistant starch and found the RS peak at 120-165°C, which apparently contributed to the melting of amylose crystallites, and higher enthalpy meaning increased yield of RS. They also investigated the effect of heating/cooling cycles by autoclaving and found that 4 time cycles presented higher third peak enthalpy, which might be due to stabilization of starch. Thermal properties could be also changed by modifications and chemical composition of starch. Higher amylose is related positively to higher enthalpies by Differential Scanning Calorimetry (DSC). Sievert and Pomeranz (1990) explained that it could be either starch-protein interaction that required more energy or lipid in RS residues that attach to non-hydrolyzed starch. Sievert and Wursch (1993) also reported an increase in amylose –lipid complexing enthalpy and a decrease in resistant starch enthalpy after lipids were added. Moreover, resistant peak was shown at 155°C with lower enthalpy indicating lower resistant starch content when lipids were present (Czuchajowska et al., 1991).

There has been a considerable interest in RS owing to nutritional significance in the dietary fiber concept (Sievert and Pomeranz, 1990). Resistant starch is known to give origin to fermentation endpoints, mostly short chain fatty acids, and to affect lipid and N metabolism like soluble fiber. Resistant starch does not influence postprandial insulin and it moderately increases stool weight in the colon like insoluble fiber (Brighenti et al., 1998). When microbial degradation of RS takes place in the colon, an increase in short-chain fatty acids, especially butyrate, stabilizes colonic cell proliferation and modulates a wide range of cellular enzymes including those involved in glycol-conjugate metabolism. Diets having slowly digested starch may protect against chronic disease, whereas rapidly digested starch elevates blood glucose and insulin responses. In addition, clinical studies have found improved glucose metabolism and reduction in the risk of developing type 2 diabetes mellitus with food such as whole grains (Englyst et al., 1999; Hallfrisch and Behall, 2000). Physical properties and structure of grains are related to slow rate of starch hydrolysis in the gastrointestinal tract of humans and may have some of the physiological effects of dietary fiber (Englyst and MacFarlane, 1986).

2.3. OZONE

2.3.1. Definition of Ozone

Ozone (O_3) is a form of oxygen gas with three oxygen atoms in its molecule. Bablon et al. (1991) reported that ozone breaks down naturally, and can be considered as non-persistent chemical. Ozone is known as a more powerful oxidant than oxygen; it reacts with most substances at ambient temperature, but creates no disposal problem or leaves no harmful residues. Furthermore, ozone reacts faster and is more reactive than other oxidizing agents for example chlorine due to extra oxygen atoms, which can share an oxygen atom with other substances to oxidize them, leaving the remaining two ozone atoms to form regular oxygen found in air. Ozone can last up to 12 hours in air and about 20 minutes as dissolved gas solution. On the other hand, when ozone reacts with a food product it will be degraded quickly. Ozone gas is known to have a perceptibly blue color, which makes it possible for ozone concentration to be measured by spectrophotometer. Ozone effect is achieved by a direct kill attack and oxidation of the biological material. For bacteria, ozone will approach and bind with organic compounds in the cell wall of bacteria. For substances with double bonds, the free radicals of ozone will breakdown double bonds in the cell wall and destroy the cell permeability of the structure. In solution, ozone degradation occurs and produces hydroxyl group, which attack almost every substance. Therefore, utilizing ozone to replace the solvent and destroy undesirable toxins will be useful.

2.3.2. Application of Ozone

In Europe, application of ozone has been known mostly for disinfecting contaminated water (Rogers et al., 1992), deodorization purposes in industrial processes, removal of taste, odors, and colors, and cleaning various wastewaters (Bablon et al., 1991; Verostko et al., 1992; Hitchens et al., 1994). Due to the sterilizing action of ozone, it may be utilized to degrade harmful microbes and toxic compounds. Compared to chlorine, ozone has been proved a stronger and more rapid antimicrobial agent for the treatment of spore, fecal, and pathogenic microorganisms, and viruses (Da Silva et al., 1998). According to EPRI (1997), the ability of ozone as disinfectant was found in 1886 and its anti-microbial effectiveness was discovered in 1891. It was at Whiting, Indiana in 1940 that ozone was first used for water treatment. Furthermore, ozone has been used in food production since the 20^{th} century for the preservation of food and food ingredients such as milk, meat products, casein, and albumin (Graham, 1997; Kim et al., 1999). Most of the food research that involves ozone has focused primarily on microbiological control or sanitation. Ozone also has been applied for detoxification of oysters (Blogoslawski et al., 1979), for the treatment of spent chill bath water in poultry processing (Chang and Sheldon, 1989), for extending shelf-life of foods, for control of microbes in poultry products, for destruction of pesticide residues, for microbial destruction and sanitation on catfish, or for bleaching of grains.

Ozone has been utilized in grains or starch products. It was found that packaged

Japanese raw noodles showed an increase of 2-5 times in the shelf life, when treated with ozone at 0.5-50 ppm for 6 hours (Naito et al., 1989). It could possibly be used for improving wheat flour quality, which involves bleaching and changes of rheological properties to make it more elastic. Therefore, ozonation of wheat flour could result in rapid bleaching, improved functionality, and decreased microbial load. Moreover, Voraputhaporn (1996) investigated ozonated wheat starch and reported an increased peak viscosity (PV), a higher peak gelatinization temperature, but a lower enthalpy and lower temperatures for amylose-lipid complexes than unoxidized starch. However, he also reported that a higher enthalpy of the second endotherm from ozonated starch compared to the control might be related to the oxidation of starch lipid, which might affect the endothermic transition of amylose-lipid complexes.

2.3.3. Methods to Produce Ozone

There are three different methods to create ozone, corona discharge, UV radiation, and an electrochemical ozone production. In corona discharge, air is converted to ozone by high electrical discharge process. The electric current excites oxygen electrons, resulting in splitting of oxygen molecules and the separated atoms combine with other oxygen molecules to form ozone (Kim et al., 1999). However, this procedure has some disadvantages: high capital and operating costs, possible toxic contamination by the electrode material, and low ozone concentrations of 2.5 to 7.5 wt% (Nebil, 1981). Corona discharge is similar to how lightening produces ozone in nature and UV radiation is similar to how UV rays from the sun produce ozone in nature. An electrochemical ozone production is an alternative approach for the production of ozone which involves the use of water in an electrolytic cell, in which the oxygen in the water is converted to ozone by passing water through a positively charged surface and a negatively charged surface (Murphy et al., 1994; Hitchens et al., 1994). The advantages of this method are simple required equipment, safe reactants and products and high concentrations of ozone production, approximately more than 20 wt%. This method has been developed by Lynntech, Inc. (College Station, TX).

2.3.4. Safety of Ozone

Ozone is very toxic compound and all investigators must approach its use with respect and caution. The maximum allowable exposure for an eight hour period is 0.10ppm as required by Occupational Safety and Health Administration (OSHA). In 1982, the US Food and Drug Administration affirmed ozone as generally recognized as safe (GRAS), with specific limitation as a disinfectant in portable and bottled water. In July 1997, FDA affirmed ozone as a GRAS substance for broad food applications and for use as a disinfectant or a sanitizer in food processing (EPRI, 1997). Ozone in solution is unstable and it normally reverts back to oxygen in a short period of time. Twenty minutes treatment of ozone could be considered a reasonable estimate even though the half-life of ozone in solution varies.

CHAPTER 3

EFFECTS OF OZONATION AND ADDITION OF AMINO ACIDS ON PASTING CHARACTERISTICS OF RICE STARCHES BY USING RAPID VISCO ANALYSIS (RVA)

3.1. INTRODUCTION

Starch gelatinization is a disruption of molecular order within granules, which means the loss of crystallinity and irreversible granule swelling. It produces a viscous mass consisting of a continuous phase of solubilized amylose and amylopectin (Fennema, 1996). Pasting takes place when the starch granule swells further above gelatinization temperature, and then viscosity begins to increase. During starch gelatinization, the starch viscosity usually increases after maximum granule swell. This is the reason the gelatinization temperature is not recorded by Rapid Visco-Analyzer (RVA). The exudates from the soluble starch granule, usually amylose, are responsible for the increase in viscosity, because the leached soluble starch formed a network as it imbibed water upon swelling (Miller et al., 1973). However, Hermansson et al. (1996) reported that amylose maintains the integrity of swollen starch granules by inhibiting granule swelling and that amylopectin is mainly attributed to starch swelling. According to Tester and Morrison (1990b), waxy starch could swell rapidly but the swollen granules disintegrated at lower temperature indicating low paste viscosity stability. The factors that affect paste viscosity are exudates from the inside of the granule, the amount of solubilized amylose, starch granule swelling, the capability of swollen granule dispersion (Morris, 1990), the volume of swollen granules and their deformability (Fannon and Bemiller, 1992).

Amylose content and quantitative and qualitative differences in the various non-carbohydrate constituents, such as lipid and protein change pasting characteristics (Mangala et al., 1999a). Hamaker & Griffin (1993) studied the effect of protein on the pasting behavior of starch granules and found that deproteinization produced starch granules that were more fragile allowing more water into the granules, resulting in increased viscosity because of greater swelling. Radosavljevic et al. (1998) also concluded that the treated amaranth starch with alkaline-protease had a greater amount of starch yield and recovery, and showed greater pasting by amylograph. Ravi et al. (1999) observed that glycerol monosterate (GMS) decreased the peak viscosity by reducing the starch swelling because of the adsorption of lipid on the starch granule surface, and that protease treatment reduced the peak viscosity and final viscosity. Furthermore, Biliaderis and Tonogai (1991) concluded that defatted starches increased viscosity, explaining that granular lipids inhibit amylose leaching from the granules during gelatinization so that a softer starch paste was produced. Starch granule swelling was decreased when lipids are present (Tester and Morrison, 1990a; Tester et al., 1991). Moreover, added oleic acid in extrusion reduced paste viscosities (Schweizer et al., 1986). According to Shamekh et al. (1998), released fatty acids from hydrolyzed starch lipids hindered swelling and gelatinization of starch granules. Amylose – lipid complexes in nonwaxy starch decreased viscosities and showed pseudoplastic behavior, which was suitable for semisolid food applications (Guraya et al., 1997).

The main desired effects of oxidation are lower viscosity and improved stability of starch dispersions (Forssell et al., 1995; Hebeish et al.,1992a; Hebeish et al., 1992b). It was found that oxidation decreased paste viscosities and that decrease in diameter of starch granule might be attributed to loss of swelling capacity of oxidized field pea starches (Li and Vasanthan, 2003). Reduced capability of gel formation and increased viscosity stability were found with an increased degree of oxidiation (Han, 2002). Therefore, oxidation reduced peak viscosity and resulted in lower setback in cooled paste (Han and Ahn, 2002). Furthermore, Mukprasirt et al. (2001) reported that oxidized corn starch had decreased peak viscosity, breakdown, and setback.

The setback indicates the precipitation and reassociation of solubilized starch polymer and insoluble granular fragments during the cooling process. Oxidized starch was found to contribute to a lower viscosity but to exhibit an increased fluidity of starch paste due to molecular oxidative scission (Wurzburg, 1986), and damaged starch that was attributed to thickening of the continuous phase of the paste. In addition, Thiewes and Steeneken (1997) found that viscosity was reduced with increased degree of oxidation, and pasting temperature and the time to peak were also lower than untreated sample. In addition, lower setback with oxidized starch, in other words, less retrogradation potential was found due to structural change (Mukprasirt et al., 2001). Moreover, Wurzburg (1972) explained that carbonyl and carboxyl groups formed on the starch molecules in oxidized starch are bulkier than hydroxyl groups, resulting in a reduction in the tendency to associate.

In contrast, Kuakpetoon and Wang (2001) found that oxidation reduced pasting temperature and final viscosity but that the peak viscosity increased with low oxidation (0.8% w/w). They explained that the decrease in pasting temperature and increase in peak viscosity suggests that oxidized starch granules were easier to swell thus more water was allowed inside the granules. However, lower viscosity of hypochlorite oxidized starches with 2% NaOCl resulted from partial cleavage of the glucosidic linkages. Extensive oxidation caused a decrease in molecular weight (Morton and Solarek, 1984). Therefore, depolymerized starch molecules could not maintain the integrity of starch granules. Teleman et al. (1999) also had a similar result that hypochlorite-oxidized potato starch showed low viscosity with reduced tendency to retrogradation and gelling in solution. It suggests that oxidized starch does not form a strong network-like structure compared with non-oxidized starch because oxidized starch suspensions that were treated at low concentrations of NaOCl showed elastic and viscous characteristics (Han, 2002). Forssell et al. (1995) reported that the solubility of oxidized starch was increased and also found that a more rigid gel was formed with a lower degree of oxidation; thereby, the gelling ability of oxidized starches suggested that amylose dominated the structure.

Amylographs had been utilized to evaluate the pasting characteristics on starch products. However, Rapid Visco-Analysis (RVA) is often used instead of amylographs for research of many food products including starch pasting characteristics and product quality for extrusion of snacks and cereals (Almeida-Dominguez et al., 1997). The advantages of using RVA include small sample size, short experiment time, the capability of easy operation, great sensitivity giving optimized conditions of heat-cooling cycles, and variations: different heating and cooling rates, starting and ending temperature, and shear rate conditions (Batey et al., 1997; Almeida-Dominguez et al., 1997; Walker et al., 1988).

The objectives of this study were 1) to develop oxidized rice starches with ozone treatment and 2) to evaluate the effect of ozonation and amino acids on pasting properties of rice starches.

3.2 MATERIALS AND METHODS

3.2.1. Materials

Sigma rice starch was purchased from Sigma Chemical Co. (S7260) while white rice flour was obtained from Riviana Foods Inc. (Abbeville, LA). The three different amino acids utilized included positive charged (lysine), negative charged (aspartic acid), and neutral amino acid (leucine). Potato amylose (A0512), amylopectin (A8515), and protease (P5147) along with the amino acids were purchased from Sigma Chemical Co. (St. Louis, MO).

3.2.2. Preparation of White Starch Isolate from Rice Flour

3.2.2.1. Lipid Extraction from Rice Flour

A modified soxhlet extraction method (Yang and Chang, 1999) was applied to remove lipid from white rice flour. A 30g white rice flour sample was transferred into a 80mm–high extraction thimble, and then covered with cotton and put into a Soxhlet extraction tube. A 500ml flask with 100ml of petroleum ether was connected with an Allihn condenser and Soxhlet extraction tube. Then, the flask was placed on a water bath with a temperature setting of 45˚C. The condenser was then connected to a cooling system with coolant temperature setting at 3˚C. A vacuum pump was connected with a Soxhlet extraction tube to help solvent evaporate. The petroleum ether extraction was done for 12 hours, methanol extraction followed with 100ml methanol at 65˚C for another 12 hours extraction. After defatting the flour sample, it was air-dried under a vacuum hood. Eight batches were prepared.

3.2.2.2 Protein Removal from Defatted Rice Flour

A modified alkaline protease digestion method (Lumdubwing and Seib, 2000) was used to remove protein from the defatted rice flour. A 40g defatted flour sample was placed into a 500ml flask. Then 150ml of 0.001M NaOH solution and 0.2g protease powder was added to the flask. The mixture was then adjusted to pH 10 by adding 1M sodium hydroxide solution, and the flask was covered with parafilm and placed in a shaking water bath for 18 hours at 55˚C.

The slurry was then centrifuged at 3,000g for 20 minutes, and then the supernatant was discarded, whereas the sediment was washed twice with 150ml distilled water and centrifuged at 3,000g for 15 minutes. Then, the residue was suspended in 150ml-distilled water and adjusted to pH 7 by adding 1M hydrochloric acid. The pH-adjusted slurry was centrifuged at 10,000g for 20minutes. The supernatant was discarded, and the dark tailings layer atop the starch was carefully scraped away and discarded. The starch was finally washed three times with 100ml-distilled water until the tailing fraction became negligible after centrifuging. The isolated starch was dried in a convection oven at 40˚C for 48 hours, and then the dried starch was milled by using 0.5mm sieve (Brinkmann, Retsch). Four batches were prepared.

3.2.3. Pure Oxygen and Ozone Treatment

- **Ozone Concentration Measurement**

A Lynntech, Inc. ozone generator was equipped with a water trap, filter, and a 3-way valve. A spectrophotometer (Spectronic® Genesys™ 8, Spectronic Instruments) set at 254nm was turned on and was warmed up for 15 minutes. The tube from the 3-way valve, which was going into the sensor and spectrophotometer units, was detached and a reference gas was attached, either N_2 or O_2. After running the reference gas through the sensor units for 15 minutes, the spectrophotometer reading was adjusted to 0.00 abs. Then, the tubing to the 3-way valve was connected again and the knob was turned to the direction of the sensors. The first valve knob was turned to the "in –use" position, and the spectrophotometer reading was checked. The Ecosensor was used for detecting any gas leaks before reading. Finally, the reading was transferred to wt% by using the equation as follows:

Wt % ozone = A × 3000 / 24.313 + A (Lynntech, Inc.)

Where A is absorbance from the spectrophotometer

The concentration of ozone was about 20 wt%, and the flow rate was 170 mL/minute with 22 DCV and 10 DCAmp.

- **Sample Treatment**

Forty grams of each rice starch was mixed in 200mL of distilled water. The slurry was transferred into a 500mL flask and the sample flask was plugged with a silicone stopper with 2 holes in it. One hole was for the ozone line and the other was for tubing connected to the ozone destruct unit, which provides a closed system. The ozone knob was turned in the direction of sample. The starch –water solution was treated for 0, 15, or 30 minutes at room temperature, in which ozone was dissolved into the solution. The slurry was placed on a stir plate constantly during treatment. After treating with ozone, the vent from the ozone destruct unit was checked with the Ecosensor to ensure no ozone leaks. Pure oxygen treatment obtained from a pure oxygen tank was done the same way as the ozone treatment with the same flow rate. All samples were prepared in triplicate.

3.2.4. Proximate Analysis

Commercial rice starch and isolated white rice starch were examined for the lipid (method 945.16, AOAC 1995), protein (N × 5.95) (method 992.15, AOAC 1995), ash content (method 920.153, AOAC 1995), and moisture (method 985.14, AOAC 1995).

3.2.5. Amylose Content Measurement

A standard iodine colorimetry method (Juliano et al., 1981) was used for determination of amylose content. One hundred mg of potato amylose, amylopectin, or 90 mg samples of rice starch or isolated rice flour was transferred to 100 ml volumetric flasks in duplicate. One ml of 95% of ethanol was added for washing down any adhering starch to the flasks. Then 9 ml of 1N NaOH was added. The solutions were kept for 15-24 h at room temperature for starch dispersion. The flasks were brought to volume with distilled water and mixed.

The standard solutions were prepared by mixing the amylose and amylopectin solutions with 2ml 0.09N NaOH as shown in Table 3.1. A 5 ml aliquot of the starch dispersion was transferred into 100 ml volumetric flasks. In addition, 1 ml of 1 N acetic acid was added and mixed. Finally, 2 ml iodine solution (0.2% I_2 in 2.0% KI) was added and the solutions were made up to 100 ml with distilled water and mixed. The samples were left for 20 min for iodine color development before absorbance was read at 620 nm. For the blank, 5 ml 0.09 NaOH, 1 ml 1 N acetic acid, and 2 ml iodine solution with distilled water was used. A standard curve was

Table 3.1 Amylose and Amylopectin Content in Solution for Standard Curve

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

plotted using 5ml aliquots of a mixture of potato amylose and amylopectin, and the amylose content of rice starch samples were determined from the curve.

3.2.6. Rapid Visco-Analyzer (RVA) Analysis

Amino acids, aspartic acid (negative charged), leucine (neutral), or lysine (positive charged), were used as additives to test their effects at 6% of starch dry basis (Liang and King, 2003). A RVA (Newport Scientific, Foss Food Technology, Eden Prairie, MN) was used to evaluate pasting characteristics of the rice starch and isolated rice flour treated with pure oxygen or ozone. The Newport RVA rice method 10 (Version 3, June 1997) that is approved as a standard method of the AACC was used. Twenty five grams of distilled water was mixed with 2.65 gram of starch samples and 159mg of additive. Then, the paddle was placed and plunged up and down 10 times for mixing. Next, the canister with the paddle was inserted into RVA sample holder. Finally, the RVA tower was pushed down slowly and carefully until the motor clicked. Each sample was heated and gelatinized for 12.5 minutes with temperature increasing from 50˚C where the starch solution was stirred at 960 rpm for 10 sec. Spindle speed was then slowed to 160 rpm throughout the whole process. The RVA temperature was set at 50 ˚C for 1 minute, then increased at 12˚C/minute to 95˚C, and held for 2.5 minutes, decreased to 50˚C, and held for about 1 minute. The RVA measured pasting temperature (PT), peak viscosity (PV), minimum viscosity (MV), final viscosity (FV), and time to peak (Ptime). The total setback (TSB), breakdown (BD) and setback (SB) were calculated as the difference between FV and MV, PV and MV, and between FV and PV, respectively. All samples were tested in duplicate.

3.2.7. Statistical Analysis

SAS (Statistical Analysis System) software (version 8.0) was used for RVA data analysis. Analysis of Variance (ANOVA) with Tukey's studentized range (HSD) test was performed to

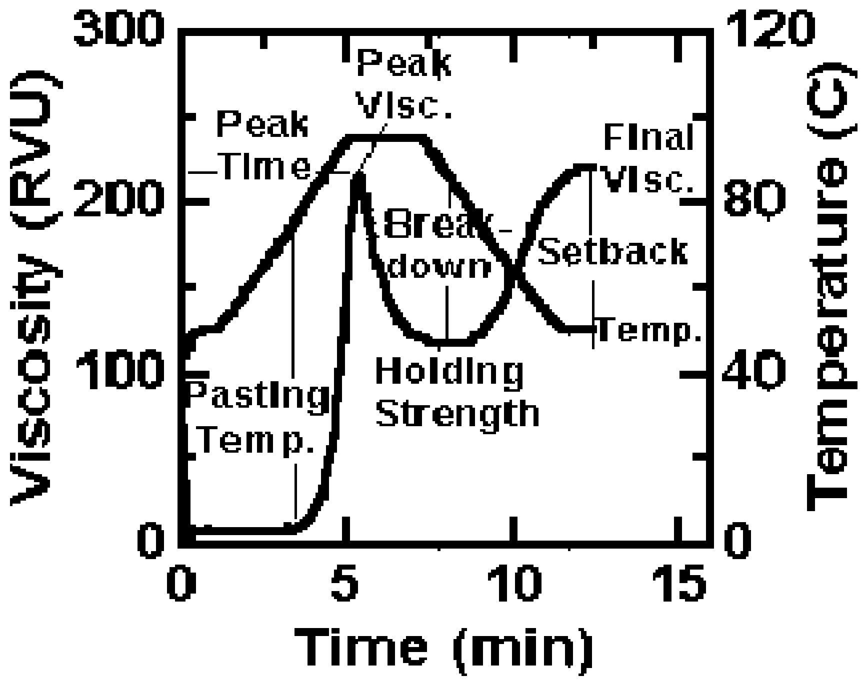

Figure 3.1 RVA Pasting Curve

examine the effects of ozonation and the additives (aspartic acid, leucine and lysine) on the pasting characteristics of rice starches. The treatments were pure oxygen and ozone. The durations of treatment were 0, 15, and 30 minutes. Abbreviations were PO for pure oxygen, OZ for ozone, NA for no additives, ASP for aspartic acid, LEU for leucine, LYS for lysine, and WSI for white starch isolate. The analysis used was a 2-tailed t-test ($P \leq 0.05$).

3.3. RESULTS AND DISCUSSION

Figure 3.1 shows the parameters of pasting characteristics of RVA. Pasting temperature (PT) is the temperature when starch granule swells further and viscosity increases. PT is above the gelatinization temperature, and associated with the minimum temperature that is required to fully cook starch and is an indication of the associated energy costs. Pasting results in granule rupture and reduction in the viscosity due to soluble amylose leaching following gelatinization, which weakens the granule. The peak viscosity (PV) relates to the water-binding capacity of the starch and occurs between an increase in viscosity from swelling and a decrease caused by granule rupture. Breakdown is the difference between peak viscosity and minimum viscosity (viscosity during the holding period at 95˚C) indicating the stability of the gel paste during cooking. Minimum viscosity usually indicates gelatinization behavior of the starch granule (Morris, 1990). The viscosity after the drop from peak to minimum viscosity rises to a final viscosity (FV) during the temperature change from 95˚C to 50˚C. This region determines total setback (TSB), which reflects the retrogradation tendency of the starch (Newport Scientific Pty Ltd). Jacobs et al. (1995) explained that the viscosity increase during the cooling period measures the retrogradation tendency of the starch since the amylose tends to aggregate to form a gel when it cools down.

Table 3.2 Chemical Composition of Rice Starches [1,2]

Sample	Moisture	Protein (N × 5.95)	Lipids	Ash	Amylose
Sigma Rice Starch	11.1	0.56	0.01	0.31	21.12
White Rice Flour	10.79	7.77	0.71	0.59	12.02
White Starch Isolate	10.4	2.62	0.26	0.52	15.91

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

3.3.1. Proximate Analysis

Proximate analysis of rice starches are shown in Table 3.2. Sigma rice starch consists of the lower protein, lipids, and ash, but shows the higher amylose content than white starch isolate. Petroleum ether and methanol extraction left a residue of lipids of 0.26% from white rice flour, and alkaline protease digestion had a residue of protein of 2.62% in white starch isolate. It has been known that lipids from rice were harder to be removed than other cereal starches such as wheat or potato since they have hydrophobic tendency in rice endosperm (Lumdubwong and Seib, 2000).

3.3.2. Amylose Content of Rice Starches Treated with Pure Oxygen or Ozone

Both pure oxygen and ozone treatments increased amylose content in Sigma rice starch with 30 minutes ozone exposure producing the highest amylose content (Table 3.3). This result was in contrast to many studies that showed a decrease in amylose content after oxidation. However, Han and Ahn (2002) found that sodium hypochlorite treated starch increased the amount of soluble amylose compared to non treated samples. For white starch isolate, pure oxygen treatments for 15 and 30 minutes increased amylose content; however, those of ozone treatments were lower than non-treated samples. This result agrees with studies of Boruch (1985), in which oxidation reduced the iodine binding capacities of starches due to the degradation of amylose and changes in the structure of the amylose molecules. In comparison with amylose content in Sigma rice starch, that of white starch isolate was lower probably because of residues of lipids and protein after defatting and deprotenization.

3.3.3. Effects of Pure Oxygen and Ozone on Sigma Rice Starch

Pure oxygen treatment for 15 and 30 minutes (PO15 and PO30) did not change paste viscosity compared to untreated sample. However, they decreased breakdown (BKD) by 101 and

Table 3.3 Amylose Measurement of Rice Starches

Sample	Treatment	Minutes	Amylose content (%)[1]
Sigma rice starch	None	0	21.11
	Pure oxygen	15	25.76
	Pure oxygen	30	26.02
	Ozone	15	26.78
	Ozone	30	27.03
White starch isolate	None	0	15.91
	Pure oxygen	15	16.44
	Pure oxygen	30	16.91
	Ozone	15	15.72
	Ozone	30	15.33

[1]Amylose measurement in percent calculated (Juliano et al., 1981)

96 centerpoise (cP), respectively and increased pasting temperature (PT) by 5˚C, resulting in greater cooking stability than untreated starch (Figure 3.2 and Table 3.4). In addition, PO15 and PO30 reduced final viscosity (FV) slightly and total setback (TSB) by 335 and 263 cP, respectively indicating a reduction in retrogradation potential tendency (Table 3.4).

Ozone treatment for 15 minutes (OZ15) increased peak viscosity (PV) by 284 cP, but decreased final viscosity (FV), setback (SBK), and total setback (TSB) by 458, 742, and 616 cP, respectively compared to non-oxidized starches. Similarly, ozonated rice starch for 30 minutes (OZ30) enhanced PV but reduced FV, SBK, and TSB by 689, 1057, and 757 cP, respectively (Figure 3.2 and Table 3.4). The result of higher peak viscosity might be similar to that of slightly oxidized starch treated with chemical oxidizing agents such as sodium hypochlorite (Kuakpetoon and Wang, 2001). It could be explained that more amylose was leached out during ozonation (Table 3.3) because of oxidation degradation and structural change, therefore, the integrity of starch granules were weakened, thus, viscosity increased. Moreover, Forssell et al. (1995) observed that a more rigid gel was formed with a lower degree of oxidation and that amylose dominated the gelling ability of oxidized barley starches. However, starch granule integrity was weakened and resulted in softer paste as starch was cooled to 50˚C. The same result was shown in many studies (Teleman et al., 1999; Mukprasirt et al,. 2001; Han and Ahn, 2002; Han, 2002).

Among treatments, OZ30 showed the highest peak viscosity and the lowest final viscosity, setback, and total setback. Therefore, OZ30 exhibited the greatest swelling extent, yet the weakest cooled paste at 50˚C and least retrogradation tendency. Moreover, pure oxygen treated Sigma rice starch had the best cooking stability.

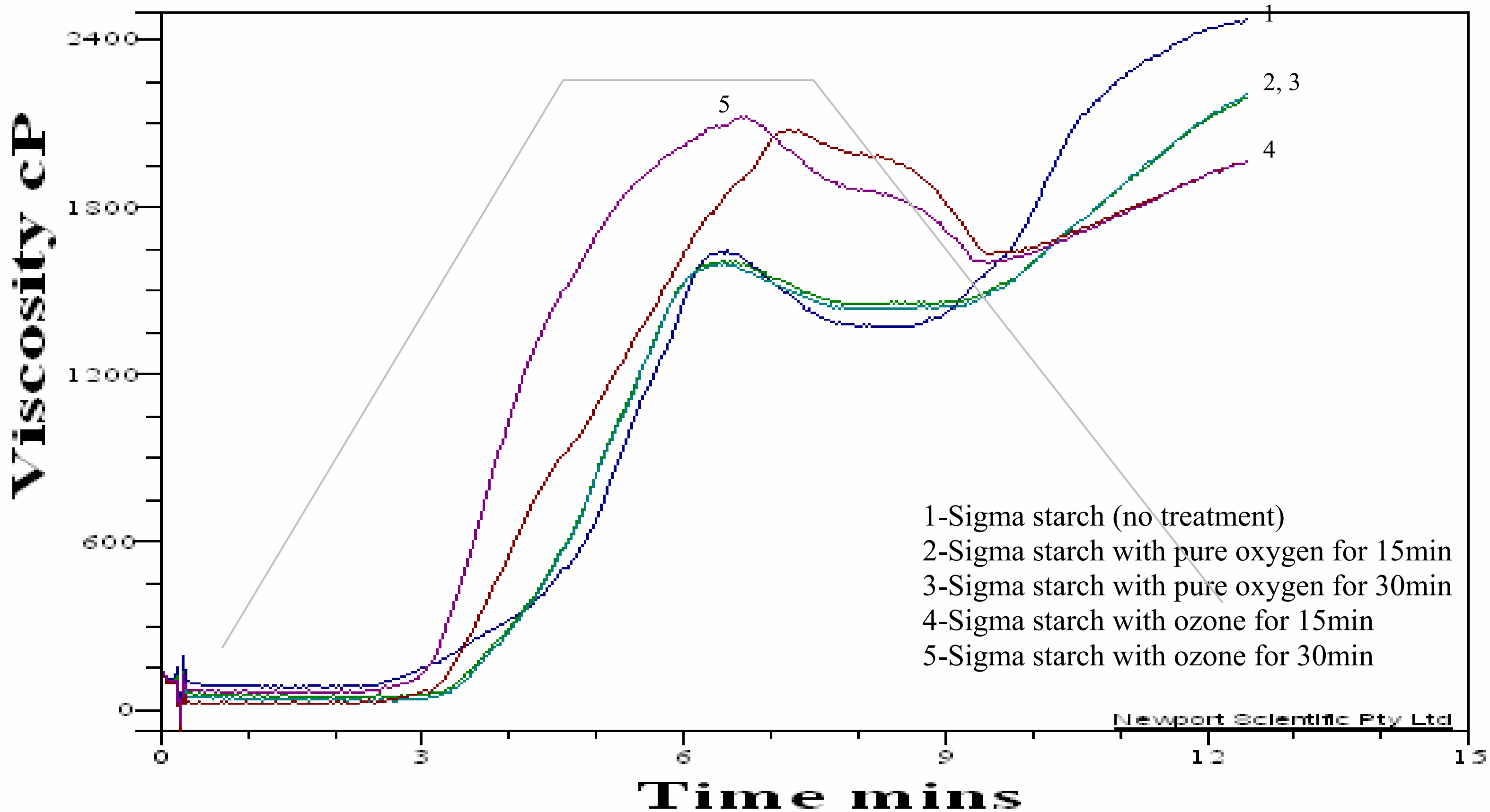

Figure 3.2 Effects of Pure Oxygen and Ozone Treatments for 0, 15, and 30 minutes on Sigma Rice Starch

Table 3.4 Effect of Pure Oxygen or Ozone Treatments on Sigma Rice Starch with No Additives[1, 2, 3]

Sample	Treatment	Time	PV	MV	BKD	FV
Sigma Rice Starch	None	0	1665.2±34.2b	1401±33.2b	264±15c	2475.8±47.8a
	Pure Oxygen	15	1606.8±40.0b	1444±22.1b	163±22d	2183.3±117b
		30	1604.5±30.5b	1436±26.5b	168±8cd	2247.3±36 b
	Ozone	15	1949.7±81.1a	1560±57.7a	390±29b	2017.6±60.6c
		30	2033.8±125a	1468±124ab	565±127a	1786.7±150d
	Treatment	**Time**	**SBK**	**TSB**	**Ptime**	**PT**
	None	0	810.7±30a	1075±38a	6.50±0.03ab	70.4±0.67c
	Pure Oxygen	15	576.5±97b	739.5±103b	6.48±0.1ab	75.5±0.96a
		30	642.8±30ab	811.3±32b	6.38±0.06ab	75.6±0.43a
	Ozone	15	68.00±135c	458.2±113c	6.88±0.09a	72.3±1.28b
		30	-247.2±147d	317.8±30d	6.23±0.77b	71.5±0.9bc

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BKD=Breakdown; FV=Final Viscosity; SBK=Setback; TSB=Totalsetback; Ptime=Time to peak; PT=Pasting Temperature
[2]Units: Viscosity (cP); Temperature (˚C); Time (minute)
[3]Different letters within column for each pasting property indicate means are significantly different at the level of $p \leq 0.05$

3.3.4. Effects of Amino Acids on Sigma Rice Starch

- **Amino Acids Effects on Non-treated Starch**

Compared to no additives, addition of 6% aspartic acid increased peak viscosity (PV) and breakdown (BKD) by 208 and 367 centerpoise (cP), but reduced minimum viscosity (MV), breakdown, setback (SBK), and total setback (TSB) by 160, 367, 441, and 282 cP, respectively (Table 3.5 and Figure 3.3). On the other hand, leucine showed no difference in paste viscosity, statistically. In contrast, the presence of 6% lysine reduced pasting characteristics of Sigma rice starch except breakdown, and shortened pasting time resulting in less swelling, faster cooking time, and a low cool paste viscosity and retrogradation tendency. However, none of the additives altered pasting temperature of starch pastes.

Among those amino acids, the highest PV, BKD, and pasting time (Ptime) were observed with 6% aspartic acid. Moreover, lysine showed the lowest PV, FV, TSB, and Ptime. These results agreed with Liang and King (2003), in which they found that charged amino acids (aspartic acid and lysine) had a greater effect on pasting characteristics than neutral amino acids (leucine). The reduction in paste viscosities for lysine added starch was also supported by the result from Hamaker and Griffin (1993) and Guraya and James (2002), in which they found that the removal of protein on starch enhanced paste viscosities. Moreover, protein bound to starch during starch retrogradation in a similar way amylose chains are formed by hydrogen bonds. The starch is associated with proteins, and the protein body is hydrophobic and resists swelling (Lumdubwong and Seib, 2000).

- **Amino Acid Effects on Rice Starches Treated with Pure Oxygen and Ozone for 15 minutes**

In comparison to PO15 with no additives (NA), OZ15 with NA showed higher PV, MV,

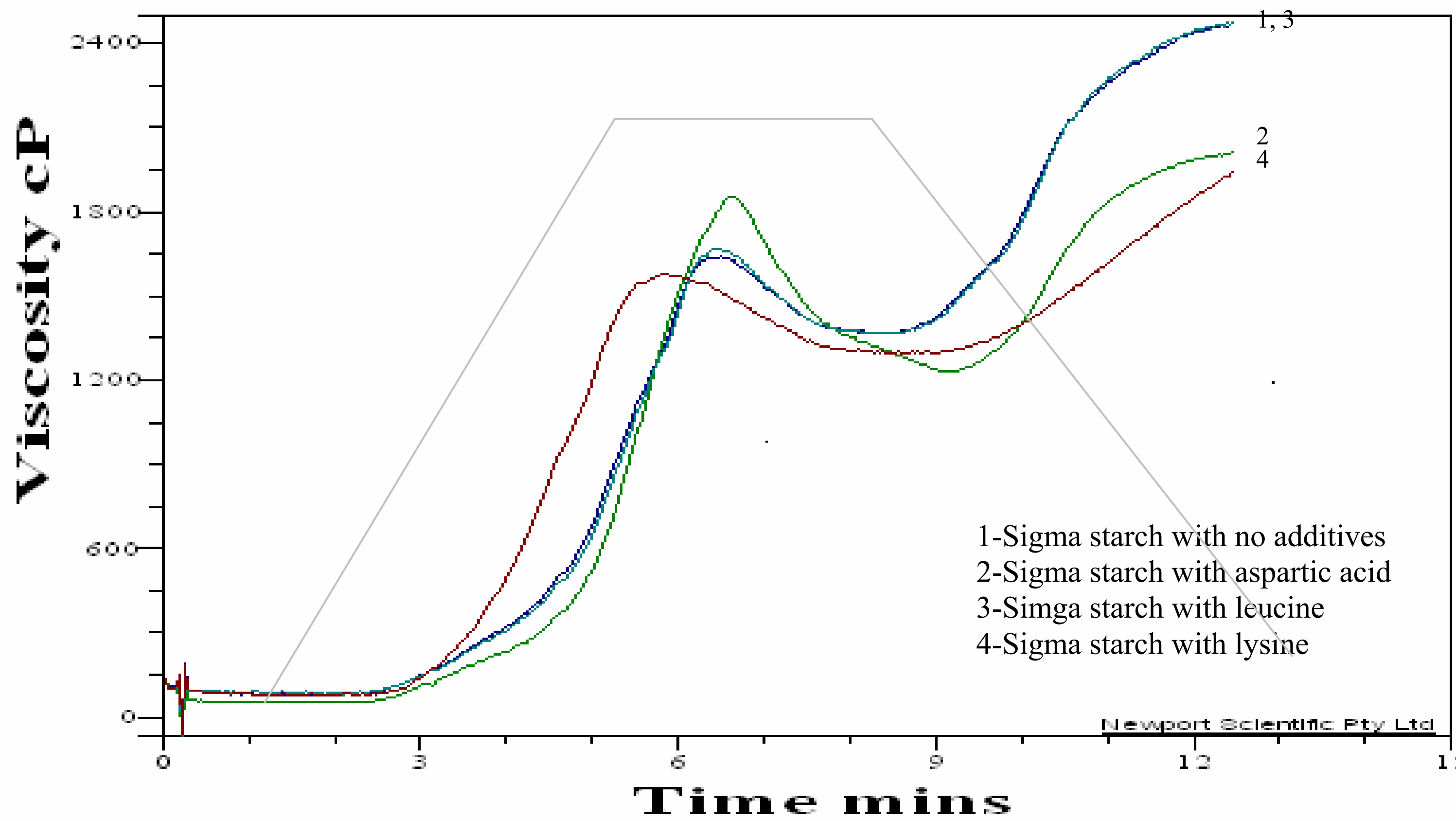

Figure 3.3 Pasting Characteristics of Untreated Sigma Rice Starch with Additives

Table 3.5 Effects of Additives on Pasting Characteristics of Untreated Sigma Rice Starch [1,2,3,4]

Sample	Additives	PV	MV	BKD	FV
Rice Starch (No Treatment)	NA	1665.2±34b	1401.0±33a	264.2±15c	2475.8±48a
	ASP	1873.2±54a	1241.8±35c	631.3±22a	2034.0±41b
	LEU	1674.7±23b	1380.5±20a	294.2±11b	2512.8±31a
	LYS	1580.7±28c	1313.2±26b	267.5±12c	1953.3±42c
	Additives	**SBK**	**TSB**	**Ptime**	**PT**
	NA	810.7±30a	1074.8±38b	6.50±0.03b	70.4±0.67a
	ASP	160.8±18c	792.17±9.1c	6.62±0.03a	71.0±0.46a
	LEU	847.2±23a	1141.3±23a	6.45±0.07b	70.0±1.01a
	LYS	372.7±17b	640.2±24d	5.87±0.08c	71.1±0.75a

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BKD=Breakdown; FV=Final Viscosity; SBK=Setback; TSB=Totalsetback; Ptime=Time to peak; PT=Pasting Temperature
[2]NA= No Additives; ASP= Aspartic Acid; LEU= Leucine; LYS= Lysine
[3]Units: Viscosity (cP); Temperature (˚C); Time (minute)
[4]Different letters within column for each pasting property indicate means are significantly different at the level of $p \leq 0.05$

BKD, and Ptime but lower FV, SBK, and TSB (Table 3.6). Moreover, pasting temperatures of ozonated samples were lower than those of PO treated starches by approximately 3˚C except for the lysine added sample. Therefore, ozone produces starch paste that shows greater and faster swelling but less retrogradation tendency than pure oxygen does. On the other hand, PO15 with leucine and PO15 without additives had lower breakdown by 145 and 101 cP, respectively compared to untreated samples resulting in greater cooking stability (Table 3.5 and Table 3.6).

Addition of 6% of aspartic acid to PO15 reduced MV, FV, SBK, and TSB, but increased BKD compared to PO15 with no additives resulting in less stable paste to cooking (Figure 3.4). Aspartic acid (6%) addition to OZ15 did not influenced PV, FV, and TSB but decreased MV and SBK and increased pasting temperature (PT) by 2˚C. Leucine (6%) addition to PO15 and OZ15 did not affect pasting properties as much as other amino acids showing almost the same paste behavior compared to their treated samples with no additives. On the other hand, addition of lysine (6%) to PO15 increased breakdown, final viscosity, and total setback by 283, 144, and 391 cP, respectively. In addition, lysine decreased pasting time but increased PT by 2˚C. Lysine showed greater effect on pasting characteristics of rice starch with ozone than pure oxygen for 15 minutes treatment. When lysine (6%) was added, the PV, MV, FV, and SBK were decreased significantly by 595, 815, 816, and 220, respectively, but increased BKD and PT by 219 cP and 5˚C (Table 3.6 and Figure 3.5). Moreover, OZ15 with lysine had reduced pasting time resulting in faster cooked paste compared to OZ15 with no additives and PO15 with lysine. This showed that ozone treatment is more powerful than pure oxygen in combination with lysine on paste viscosity. These results agreed with our hypothesis that ozone is more active than oxygen because of an extra single oxygen atom.

Among those amino acids, the present of lysine (6%) in OZ15 rice starch showed the

Table 3.6 Effects of Additives on Pasting Characteristics of Sigma Rice Starch Treated with Pure Oxygen and Ozone for 15 minutes [1,2,3,4]

	Additives	PV	MV	BKD	FV
Rice Starch with Pure Oxygen	NA	1606.8±40b	1443.8±22c	163.0±21e	2183.3±116b
	ASP	1687.2±54b	1329.5±37d	357.7±25d	1911.3±50e
	LEU	1673.8±68b	1524.5±63abc	149.3±13e	2114.7±97bc
	LYS	1643.3±68b	1196.5±43e	446.8±29c	2327.0±95a
Rice Starch with Ozone	NA	1949.7±81a	1559.5±57a	390.2±28cd	2017.7±60cde
	ASP	1974.5±51a	1452.7±73bc	521.8±31b	1955.0±35de
	LEU	1948.7±86a	1548.7±72ab	400.0±17cd	2065.5±69bcd
	LYS	1354.0±42c	744.7±25f	609.3±63a	1201.8±9.5f
	Additives	**SBK**	**TSB**	**Ptime**	**PT**
Rice Starch with Pure Oxygen	NA	576.5±96ab	739.5±103b	6.48±0.1c	75.6±0.96bc
	ASP	224.2±30c	581.8±10c	6.28±0.01d	77.0±0.8ab
	LEU	440.8±36b	590.2±37c	6.6±0.06bc	76.1±0.9ab
	LYS	683.7±45a	1130.5±63a	5.28±0.06e	77.2±0.1a
Rice Starch with Ozone	NA	68.00±134d	458.2±112c	6.88±0.09a	72.3±1.28d
	ASP	-19.50±62de	502.3±91c	6.64±0.11bc	74.1±0.58c
	LEU	116.8±121cd	516.8±107c	6.74±0.15ab	74.2±0.49c
	LYS	-152.2±44e	457.2±24c	4.96±0.06f	77.3±0.66a

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BKD=Breakdown; FV=Final Viscosity; SBK=Setback; TSB=Totalsetback; Ptime=Time to peak; PT=Pasting Temperature
[2]NA= No Additives; ASP= Aspartic Acid; LEU= Leucine; LYS= Lysine
[3]Units: Viscosity (cP); Temperature (˚C); Time (minute)
[4]Different letters within column for each pasting property indicate means are significantly different at the level of $p \leq 0.05$

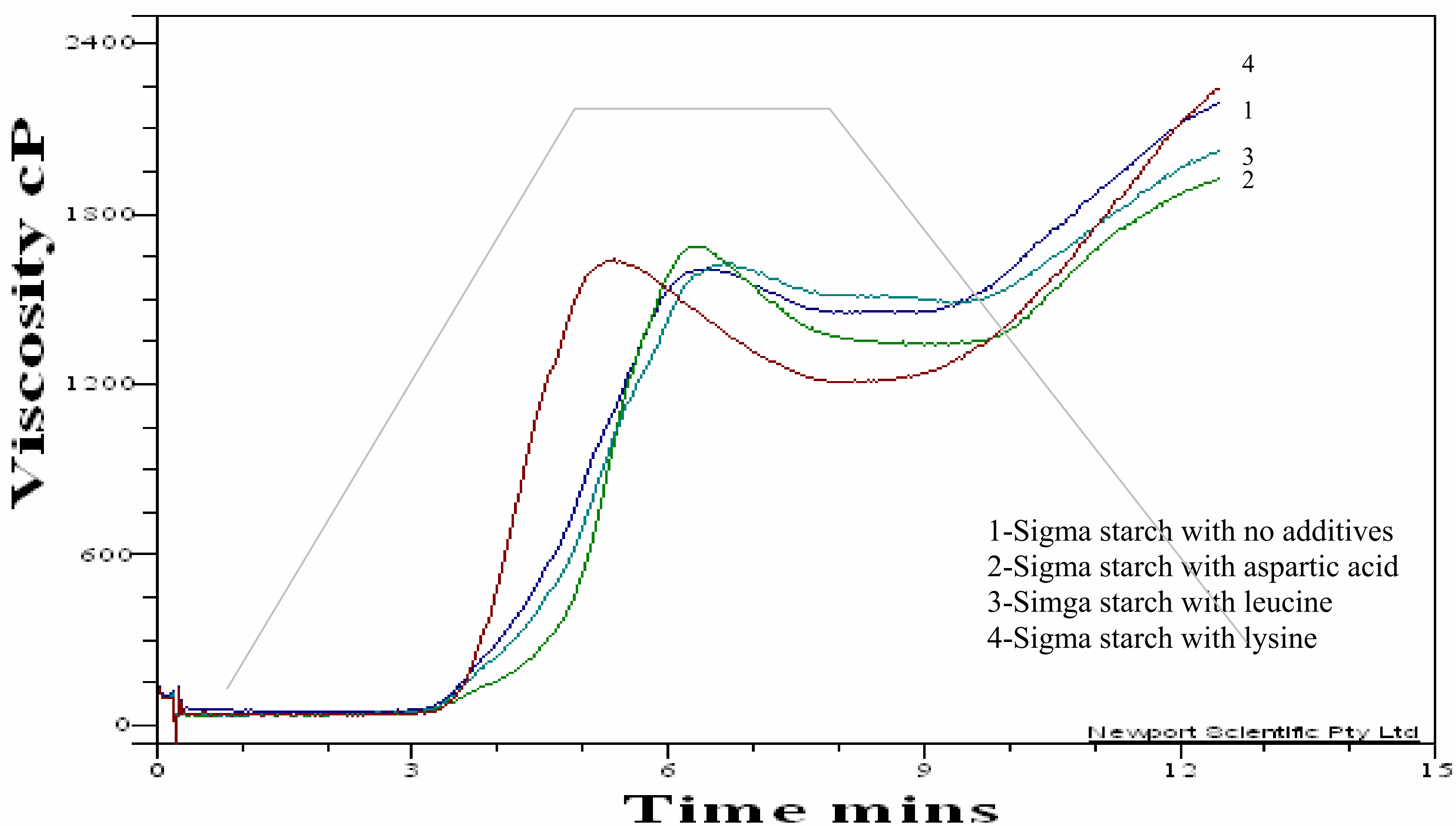

Figure 3.4 Pasting Properties of Sigma Rice Starch with Pure Oxygen for 15 minutes with Additives

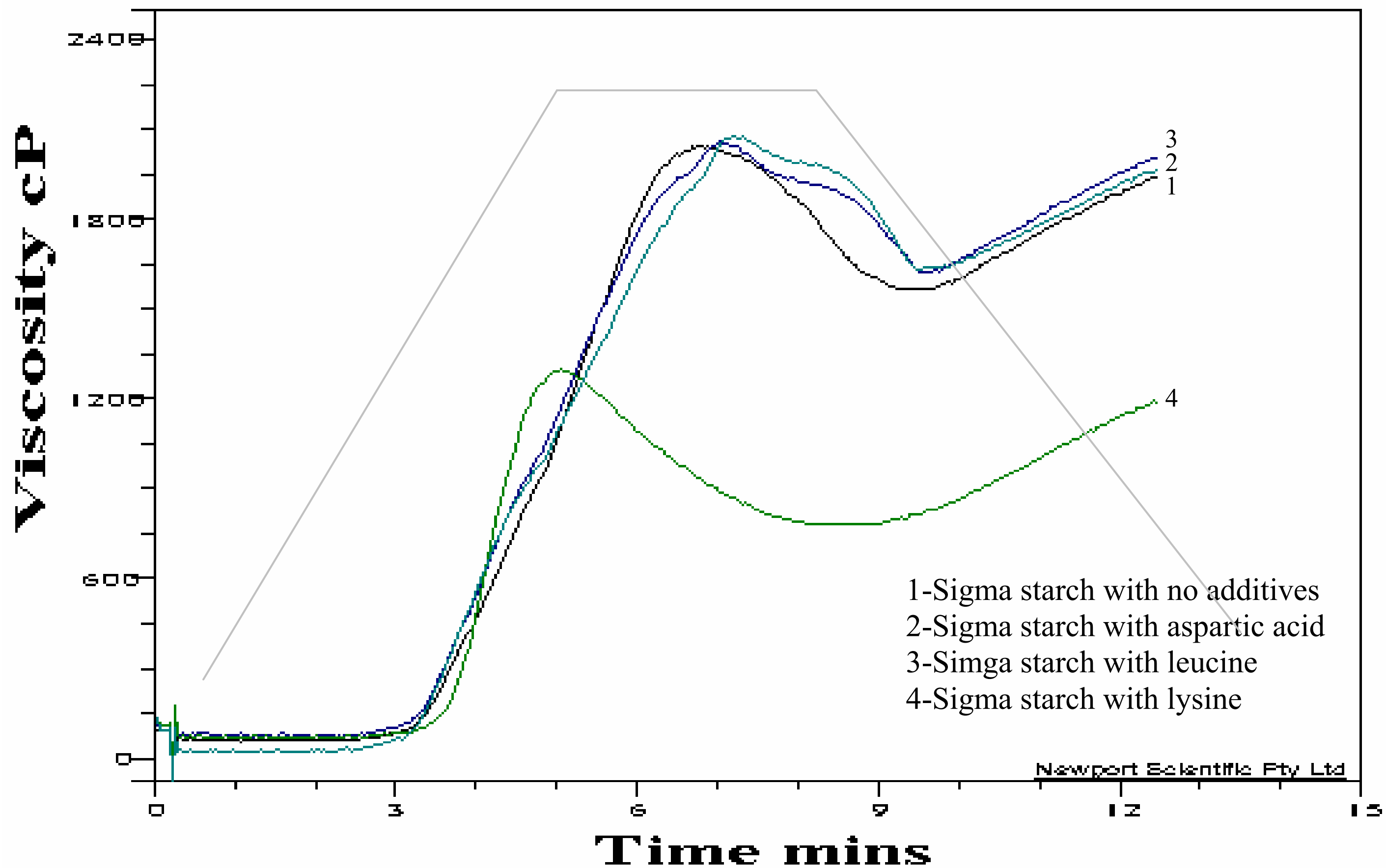

Figure 3.5 Pasting Characteristics of Sigma Rice Starch with Ozone for 15 minutes with Additives

lowest PV, MV, BKD, FV, SBK, TSB, and pasting time. Besides that, the pasting temperature was the highest when lysine was added to OZ15 starch sample. These results indicate that ozone treatment had a greater influence on pasting properties of starches than pure oxygen treatment in combination with lysine resulting in a less rigid paste and gel during heating and cooling.

- **<u>Amino Acid Effects on Rice Starches Treated with Pure Oxygen and Ozone for 30 minutes</u>**

Compared to PO30 with no additives (NA), ozone treatment for 30 minutes (OZ30) with NA increased peak viscosity (PV) and breakdown (BKD) by 429 and 397 centerpoise (cP), respectively. However, final viscosity (FV) and total setback (TSB) were reduced resulting in a low paste gel and low retrogradation tendency, and PO30 starches with leucine and without additives showed great cooking stability, which is a similar result to the 15 minute treated starch samples. Besides that, OZ30 lowered pasting temperature (PT) by 5˚C, which indicated a faster cooked paste gel, compared to PO30 except lysine added samples (Table 3.7). The presence of aspartic acid in PO30 starches increased BKD by 186 cP, but decreased FV, SBK, and TSB by 314, 409, and 224 centerpoise (cP), respectively compared to PO30 with no additives (Figure 3.6). However, aspartic acid (6%) did not affect pasting characteristics of OZ30 starches compared to OZ30 with no additives. Moreover, addition of leucine (6%) in PO30 and OZ30 starch samples did not influence paste viscosity much except slightly reducing SBK and TSB for PO30 samples but slightly increasing SBK for OZ30 starch samples. On the other hand, the MV was reduced and BKD and TSB were increased when lysine (6%) was added in PO30 samples. Pasting time (Ptime) was decreased, but PT was increased by 2˚C. In comparison to OZ30 with no additives, lysine (6%) decreased significantly PV, MV, FV by 918, 1024, and 1023 cP, respectively (Table 3.7 and Figure 3.7). In addition, Ptime was reduced, but PT was increased by

Table 3.7 Effects of Additives on Pasting Characteristics of Sigma Rice Starch Treated with Pure Oxygen and Ozone for 30 minutes [1,2,3,4]

	Additives	PV	MV	BKD	FV
Rice Starch with Pure Oxygen	NA	1604.5±30b	1436.0±26ab	168.5±7.5e	2247.3±36ab
	ASP	1700.3±13b	1345.8±11b	354.5±10d	1933.7±23c
	LEU	1688.5±29b	1543.3±25a	145.2±5.5e	2157.3±34b
	LYS	1654.7±36b	1211.0±27c	443.7±20cd	2322.7±60a
Rice Starch with Ozone	NA	2033.8±125a	1468.8±124ab	565.0±126b	1786.7±150cd
	ASP	1948.8±95a	1423.8±101ab	525.0±42bc	1774.7±118d
	LEU	1956.0±99a	1480.7±78a	475.3±32bc	1835.7±105cd
	LYS	1115.8±110c	444.17±44d	671.7±67a	763.0±69e
	Additives	**SBK**	**TSB**	**Ptime**	**PT**
Rice Starch with Pure Oxygen	NA	642.83±30a	811.33±32b	6.38±0.06a	75.6±0.43b
	ASP	233.33±27c	587.83±18c	6.31±0.06a	77.0±0.6ab
	LEU	468.83±20b	614.00±22c	6.57±0.05a	76.2±1.0b
	LYS	668.00±25a	1111.7±39a	5.34±0.05b	77.2±0.7ab
Rice Starch with Ozone	NA	-247.17±147ef	317.83±29d	6.23±0.77a	71.6±0.9c
	ASP	-174.17±30de	350.83±48d	6.16±0.31a	72.4±0.8c
	LEU	-120.33±31d	355.00±30d	6.67±0.09a	71.8±1.4c
	LYS	-352.83±42f	318.83±25d	4.70±0.02c	78.1±0.7a

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BKD=Breakdown; FV=Final Viscosity; SBK=Setback; TSB=Totalsetback; Ptime=Time to peak; PT=Pasting Temperature
[2]NA= No Additives; AA= Aspartic Acid; LEU= Leucine; LYN= Lysine
[3]Units: Viscosity (cP); Temperature (˚C); Time (minute)
[4]Different letters within column for each pasting property indicate means are significantly different at the level of $p \leq 0.05$

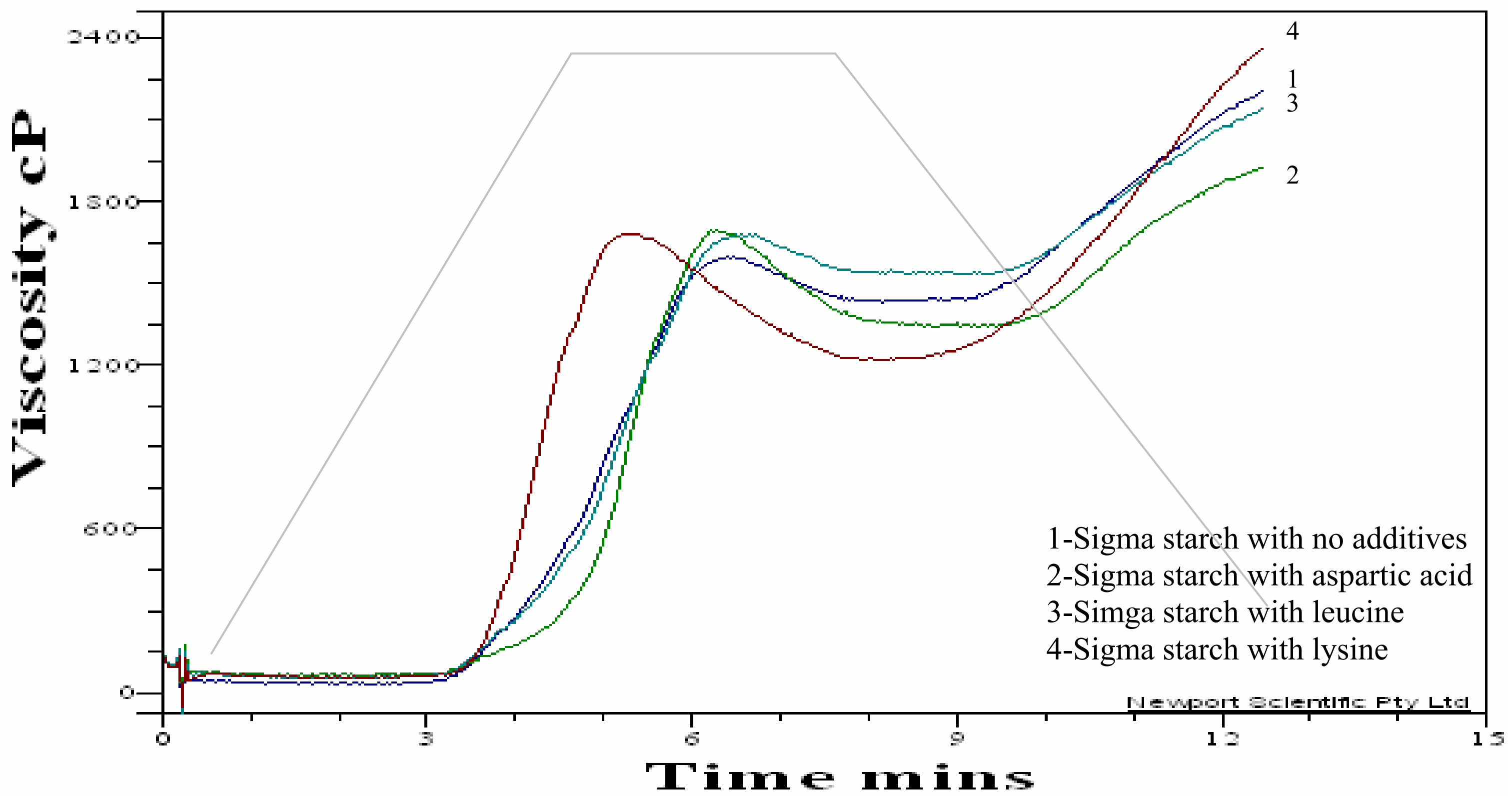

Figure 3.6.Pasting Properties of Sigma Rice Starch with Pure Oxygen for 30 minutes with Additives

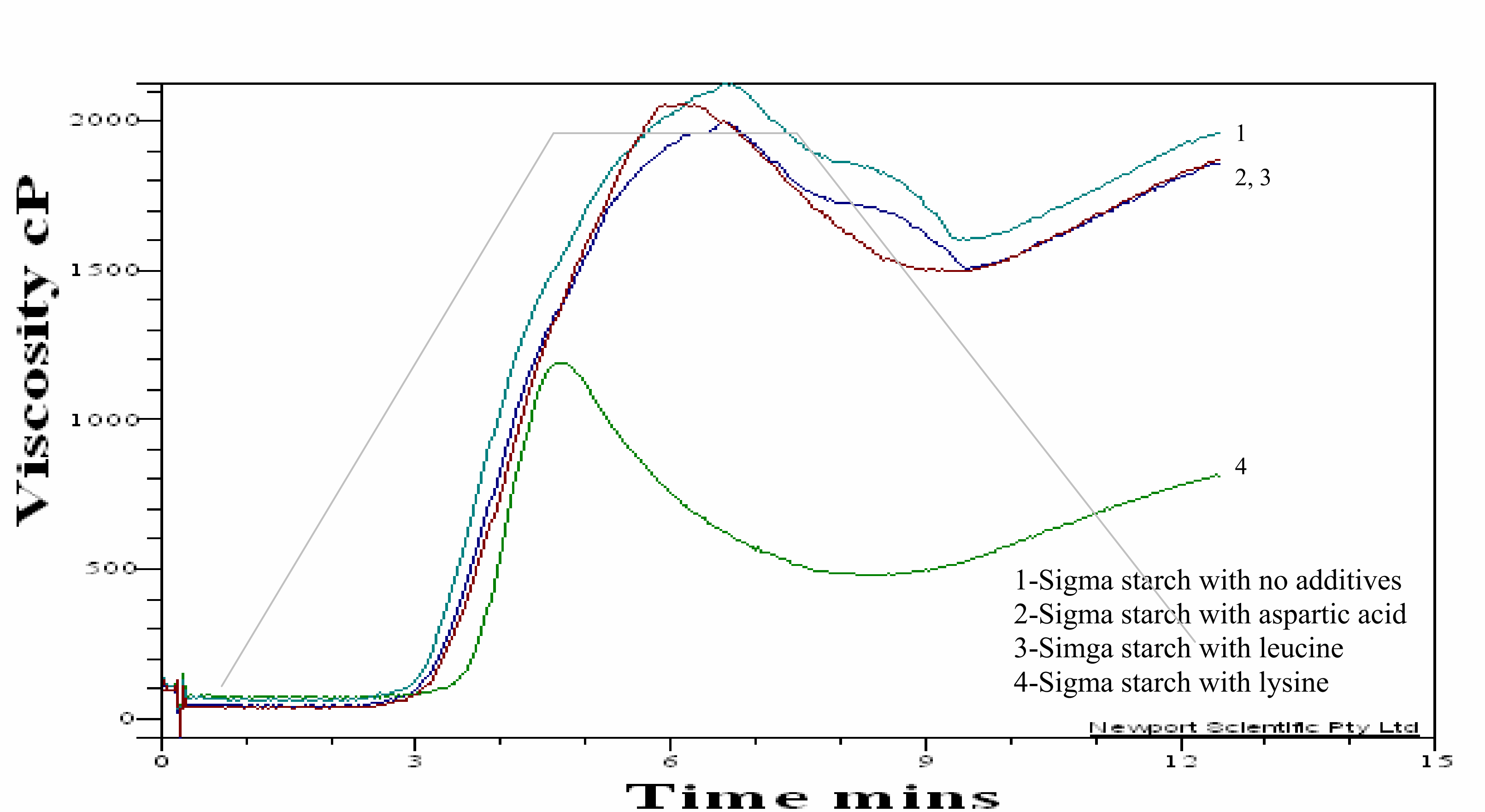

Figure 3.7 Pasting Characteristics of Sigma Rice Starch with Ozone for 30 minutes with Additives

7˚C. OZ30 decreased paste viscosity and pasting time even greater than PO30 when lysine was added, resulting in less rigid gel in faster time. This result may indicate that ozone is more effective on increasing pasting properties by weakening starch integrity, therefore, OZ30 samples swell faster, but less owing to the suppressing effect of lysine. Moreover, the paste viscosities of OZ30 with lysine were even lower than those of OZ15 with lysine so that longer duration of treatment showed greater modification effect on treated starches. The difference in time was an important factor in this experiment since there was only one concentration of ozone produced by electrochemical ozone generator. This result was similar with findings on the effect of concentrations of oxidizing agents that greater concentration caused lower paste viscosities (Abdel-Hafiz, 1997; Hebeish, et al., 1989; Li and Vasanthan, 2003; Han and Ahn, 2002).

Among those amino acids, the lowest BKD was shown in PO30 samples with leucine and without additives indicating the best cooking stability. Moreover, all of OZ30 starch samples with or without additives represented the lowest SBK and TSB. Besides that, OZ30 treatment reduced PT more than 5˚C except when lysine was added. The presence of lysine was the most influencing with the lowest paste viscosity and the shortest time to peak. Kweon et al. (2001) reported that oxidized corn starches showed anionic metal binding properties, which adsorbed cationic metal ions effectively.

3.3.5. Effect of Pure Oxygen and Ozone on White Starch Isolate (WSI)

In comparison to untreated white starch isolate, PO15 increased PV, MV, BKD, FV, SBK, and TSB by 582, 326, 257, 651, 68, and 326 cP, respectively, but had no influence on time to peak or pasting temperature (Table 3.8 and Figure 3.8). On the other hand, PO30 did not affect pasting characteristics of white starch isolate compared to non-treated samples statistically. OZ15 enhanced PV, MV, BKD, FV, SBK, and TSB, which was similar to PO15 samples (Figure

3.8). As mentioned earlier for treated Sigma rice starch, this behavior might be similar to that of slightly oxidized starch with chemical oxidizing agent, in which oxidation gave water more access into the starch granule, hence swelled to a greater extent resulting higher viscosities (Kuakpetoon and Wang, 2001). However, OZ30 starch samples did not change paste viscosity, but increased Ptime and reduced PT compared to untreated WSI. Therefore, PO and OZ30 treatments were not effective as much as PO15 and OZ15. It might be explained that 15 minutes of ozone or pure oxygen treatments saturated starch; thus, more duration of treatments were not necessary. The different results from Sigma rice starch was probably because of different sources of rice with different constituents such as less amylose and more lipids and proteins residues on WSI than on Sigma rice starch and isolation process. Kuakpetoon and Wang (2001) reported that starch type affects the oxidation rate and changes viscosity properties due to differences in physical and molecular structure present in different starches.

3.3.6. Effects of Amino Acids on White Starch Isolate

- **Amino Acids Effects on Non-treated Starch**

The addition of aspartic acid (6%) increased PV and BKD by 152 and 208 cP, but depressed SBK by 192 cP (Table 3.9). However, leucine (6%) did not affect on pasting properties of non-treated white starch isolate except for increasing TSB. The FV, SBK, and TSB were reduced by 124, 207, and 66 cP, respectively when lysine was added, resulting in less retrogradation tendency. Moreover, time to peak was decreased, but pasting temperature was increased by 2˚C.

Among those amino acids, the presence of lysine showed the lowest MV, FV, SBK, and TSB, but the fastest time to peak indicating that lysine was the additive to produce the starch gel

Table 3.8 Effect of Pure Oxygen or Ozone Treatments on White Starch Isolate with No Additives[1, 2, 3]

Sample	Treatment	Time	PV	MV	BKD	FV
White Starch Isolate	None	0	1503.5±36.9b	1143.7±16.5b	359.8±42.7c	1561.5±24.6b
	Pure Oxygen	15	2085.5±283.8a	1469.2±138.8a	616.3±150.3a	2212.2±366a
		30	1675.5±83.2b	1250.3±59.8b	425.2±43.1bc	1682.2±64.2b
	Ozone	15	1991.7±37.3a	1489±65.2a	502.7±36.9ab	2123.2±35.7a
		30	1631.3±71b	1208.7±52.4b	422.7±60.9bc	1636.8±79.4b
	Treatment	**Time**	**SBK**	**TSB**	**Ptime**	**PT**
	None	0	58±16.4ab	417.8±30.8b	6.08±0.1b	70.6±0.3a
	Pure Oxygen	15	126.7±121.7a	743±246.5a	6.17±0.08b	70.1±0.49ab
		30	6.7±20.3b	431.8±28.2b	6.09±0.06b	70.0±0.65ab
	Ozone	15	131.5±12.2a	634.2±39a	6.45±0.01a	70.4±0.49a
		30	5.5±26.2b	428.2±73.5b	6.37±0.08a	69.5±0.37b

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BKD=Breakdown; FV=Final Viscosity; SBK=Setback; TSB=Totalsetback; Ptime=Time to peak; PT=Pasting Temperature
[2]Units: Viscosity (cP); Temperature (˚C); Time (minute)
[3]Different letters within column for each pasting property indicate means are significantly different at the level of $p \leq 0.05$

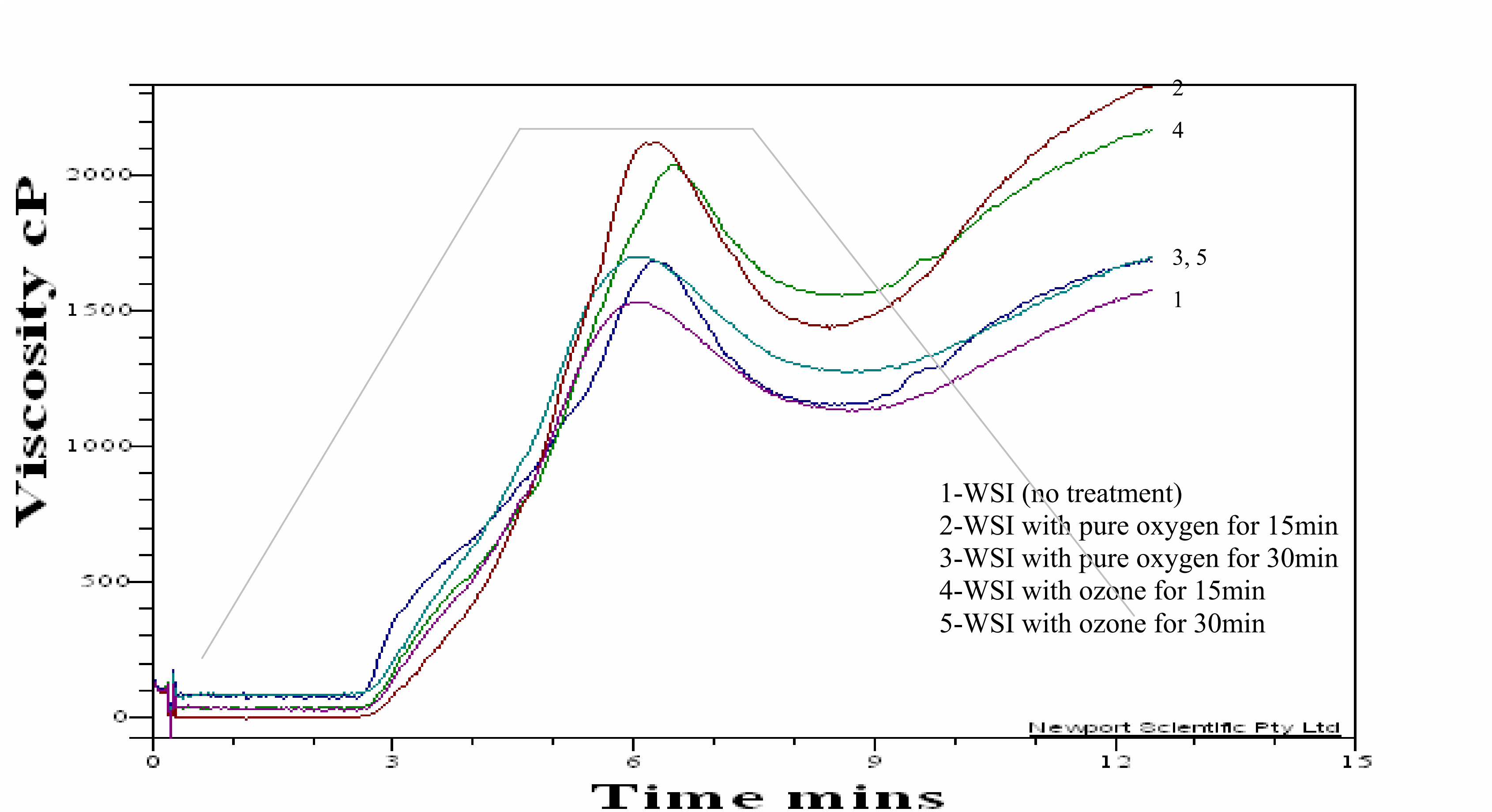

Figure 3.8 Pasting Characteristics of Untreated White Starch Isolate with no Additives

Table 3.9 Effects of Additives on Pasting Characteristics of Untreated White Starch Isolate (WSI) [1,2,3,4]

Sample	Additives	PV	MV	BKD	FV
WSI (No Treatment	NA	1503.5±36b	1143.7±16a	359.8±42c	1561.5±24a
	ASP	1655.3±39a	1087.7±53a	567.7±56a	1520.5±76ab
	LEU	1552.2±91ab	1138.8±73a	413.3±26c	1619.2±80a
	LYS	1586.8±73ab	1085.5±62a	501.3±18b	1437.0±72b
	Additives	**SBK**	**TSB**	**Ptime**	**PT**
	NA	58.00±16a	417.8±30b	6.08±0.1b	70.58±0.3b
	ASP	-134.83±53b	432.8±48ab	6.22±0.09a	70.84±0.21b
	LEU	67.00±11a	480.3±17a	6.04±0.07b	70.63±0.31b
	LYS	-149.83±24b	351.5±28c	5.79±0.05c	72.49±0.13a

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BKD=Breakdown; FV=Final Viscosity; SBK=Setback; TSB=Total setback; Ptime=Time to peak; PT=Pasting Temperature
[2]NA= No Additives; ASP= Aspartic Acid; LEU= Leucine; LYS= Lysine
[3]Units: Viscosity (cP); Temperature (˚C); Time (minute)
[4]Different letters within column for each pasting property indicate means are significantly different at the level of $p \leq 0.05$

with the lowest retrogradation tendency and the weakest cooled paste. These results were similar to those that occurred for non-treated Sigma rice starch with additives.

- **<u>Amino Acid Effects on White Starch Isolate Treated with Pure Oxygen or Ozone for 15 minutes</u>**

Compared to non-treated white starch isolate, both PO15 and OZ15 starch samples with no additives increased pasting characteristics, which was different from Sigma rice starch, in which only OZ15 enhanced paste viscosity. However, ozone treatment for 15 minutes on white rice isolate showed longer time to peak than pure oxygen treatment (Table 3.9 and 3.10).

The presence of aspartic acid in PO15 increased BKD by 286 cP, but reduced MV, FV, SBK, and TSB by 278, 480, 487, and 202 cP, respectively, therefore, produced a less cooking stable and less rigid starch gel compared to PO15 with no additives (Table 3.10).
Similarly, aspartic acid (6%) in OZ15 increased BKD 193 cP, but decreased MV, FV, and SBK by 256, 357, and 293 centerpoise (cP), respectively. On the other hand, the addition of leucine (6%) in PO15 and OZ15 did not affect on pasting properties of white rice isolate, which was similar to Sigma rice starch. In addition, lysine (6%) in PO15 did not influence paste viscosity except that it increased pasting temperature by 2˚C (Figure 3.9). However, the paste viscosities including PV, MV, BKD, FV, SBK, and TSB were significantly reduced by 647, 548, 99, 849, 200, and 301 cP, respectively when lysine (6%) was added in OZ15 white starch isolate. Besides that, pasting time (Ptime) was reduced, but pasting temperature (PT) was increased by 2˚C. These results indicate that the combination of ozone treatment and lysine produces a starch paste that has better cooking stability, less retrogradation tendency, and faster swelling with faster time to peak (Figure 3.10). Among those amino acids, the lowest PV, MV, BKD, FV, SBK, TSB, and Ptime were exhibited when lysine (6%) was added in OZ15 white starch isolate.

Table 3.10 Effects of Additives on Pasting Characteristics of White Starch Isolate (WSI) Treated with Pure Oxygen and Ozone for 15 minutes [1,2,3,4]

	Additives	PV	MV	BKD	FV
WSI with Pure Oxygen	NA	2085.5±283a	1469.2±138abc	616.3±150b	2212.2±366ab
	ASP	2094.7±171a	1191.8±92de	902.8±208a	1732.8±93c
	LEU	2113.2±252a	1449.7±110abc	663.5±144b	2208.3±313ab
	LYS	2244.7±318a	1673.3±315a	571.3±40bc	2269.2±486a
WSI with Ozone	NA	1991.7±37a	1489.0±65ab	502.7±37bc	2123.2±36abc
	ASP	1928.8±39a	1233.3±41cd	695.5±32b	1766.3±33bc
	LEU	1945.0±58a	1417.0±61bcd	528.0±23bc	2071±48abc
	LYS	1344.0±61b	941.0±33e	403.0±32c	1274.2±32d
	Additives	**SBK**	**TSB**	**Ptime**	**PT**
WSI with Pure Oxygen	NA	126.67±121a	743.00±240a	6.2±0.08cd	70.14±0.5c
	ASP	-361.83±109d	541.00±100ab	6.13±0.02de	71.02±0.51b
	LEU	95.17±71ab	758.67±209a	6.15±0.07de	70.60±0.43bc
	LYS	24.50±175ab	595.83±172a	6.02±0.17e	72.10±0.31a
WSI with Ozone	NA	131.50±12a	634.17±39a	6.45±0.02a	70.43±0.5bc
	ASP	-162.50±19c	533.00±38ab	6.30±0.05bc	70.54±0.34bc
	LEU	126.17±11a	654.17±26a	6.37±0.05ab	70.54±0.39bc
	LYS	-69.83±34bc	333.17±9b	5.66±0.03f	72.12±0.42a

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BKD=Breakdown; FV=Final Viscosity; SBK=Setback; TSB=Totalsetback; Ptime=Time to peak; PT=Pasting Temperature
[2]NA= No Additives; ASP= Aspartic Acid; LEU= Leucine; LYS= Lysine
[3]Units: Viscosity (cP); Temperature (˚C); Time (minute)
[4]Different letters within column for each pasting property indicate means are significantly different at the level of $p \leq 0.05$

- **<u>Amino Acid Effects on White Starch Isolate Treated with Pure Oxygen or Ozone for 30 minutes</u>**

In comparison to non-treated samples, PO or OZ30 did not influence on pasting behavior of white starch isolate with no additives (Table 3.9 and Table 3.11). The presence of aspartic acid in PO30 white starch isolate increased BKD by 203 cP, but reduced SBK by 216 cP. On the other hand, OZ30 with aspartic acid reduced MV, FV, and SBK by 183, 218, and 185 cP, respectively (Table 3.11). Leucine (6%), however, did not affect on paste viscosity of PO30 and OZ30 white starch isolate compared to their controls with no additives (Figure 3.11 and Figure 3.12). However, the addition of lysine in OZ30 significantly depressed all of pasting properties including PV, MV, FV, SBK, and TSB by 692, 626, 819, 127, and 194 cP, respectively. Moreover, Ptime was decreased, but PT was increased by 2˚C. This result was similar to Sigma rice starch at the same treatment where faster swelling occurred and less rigid gel was produced during heating and cooling. Lysine (6%) in PO30, on the other hand, did not influence pasting properties as much as in OZ30. The reduction in paste viscosities with addition of aspartic acid and lysine but no change with leucine was supported by Liang (2001).

Among those amino acids, lysine showed the lowest PV, MV, BKD, FV, TSB, and Ptime resulting in paste gel with the fastest swelling, the most cooking stability and the lowest retrogradation tendency. Besides that, lysine represented the shortest time to peak.

3.4. CONCLUSION

The results showed that ozone increased commercial starch granule swelling but decreased retrogradation tendency and pasting property during cooling, resulting in lower cooking stability and viscous hot paste but less rigid cooled paste compared to untreated starch, whereas pure oxygen treatment enhanced the cooking stability. Kuakpetoon and Wang (2001)

Table 3.11 Effects of Additives on Pasting Characteristics of White Starch Isolate (WSI) Treated with Pure Oxygen and Ozone for 30 minutes [1,2,3,4]

	Additives	PV	MV	BKD	FV
WSI with Pure Oxygen	NA	1675.5±83ab	1250.3±60a	425.2±43de	1682.2±64a
	ASP	1782.0±110a	1153.8±90a	628.2±30a	1571.7±88a
	LEU	1676.5±104ab	1244.2±64a	432.3±50cde	1708.7±86a
	LYS	1712.3±104ab	1196.2±57a	516.2±51bc	1567.7±69a
WSI with Ozone	NA	1631.3±71ab	1208.7±52a	422.7±60de	1636.8±80a
	ASP	1599.3±59b	1025.2±40b	574.2±51ab	1418.7±58b
	LEU	1626.7±75ab	1152.3±50a	474.3±66cd	1622.7±86a
	LYS	939.7±69c	582.5±57c	357.2±22e	817.3±78c
	Additives	**SBK**	**TSB**	**Ptime**	**PT**
WSI with Pure Oxygen	NA	6.67±20a	431.8±28ab	6.10±0.07d	70.1±0.65bc
	ASP	-210.33±27d	417.8±5ab	6.15±0.06cd	70.4±0.44b
	LEU	32.17±20a	464.5±33a	6.10±0.04d	70.0±0.33bc
	LYS	-144.67±36bc	371.5±18b	5.79±0.05e	72.1±0.21a
WSI with Ozone	NA	5.50±26a	428.2±74ab	6.38±0.08a	69.5±0.37c
	ASP	-180.67±18cd	393.5±55ab	6.22±0.1bc	69.9±0.33bc
	LEU	-4.00±15a	470.3±74a	6.30±0.06ab	69.9±0.36bc
	LYS	-122.33±25b	234.8±21c	5.38±0.07f	72.3±0.2a

[1]PV=Peak Viscosity; MV=Minimum Viscosity; BKD=Breakdown; FV=Final Viscosity; SBK=Setback; TSB=Totalsetback; Ptime=Time to peak; PT=Pasting Temperature
[2]NA= No Additives; ASP= Aspartic Acid; LEU= Leucine; LYS= Lysine
[3]Units: Viscosity (cP); Temperature (˚C); Time (minute)
[4]Different letters within column for each pasting property indicate means are significantly different at the level of $p \leq 0.05$

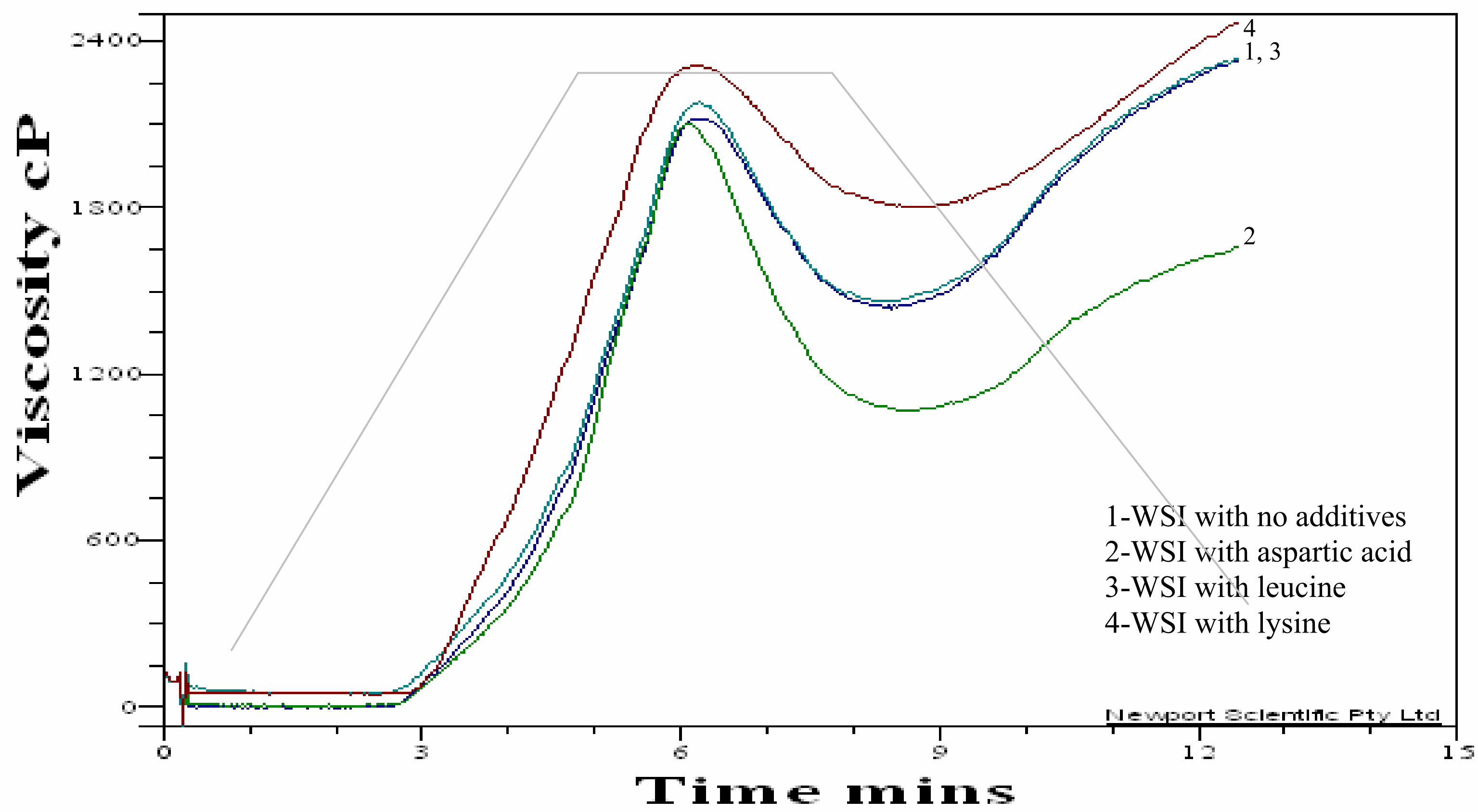

Figure 3.9 Pasting Characteristics of White Starch Isolate Treated with Pure Oxygen for 15 minutes with Additives

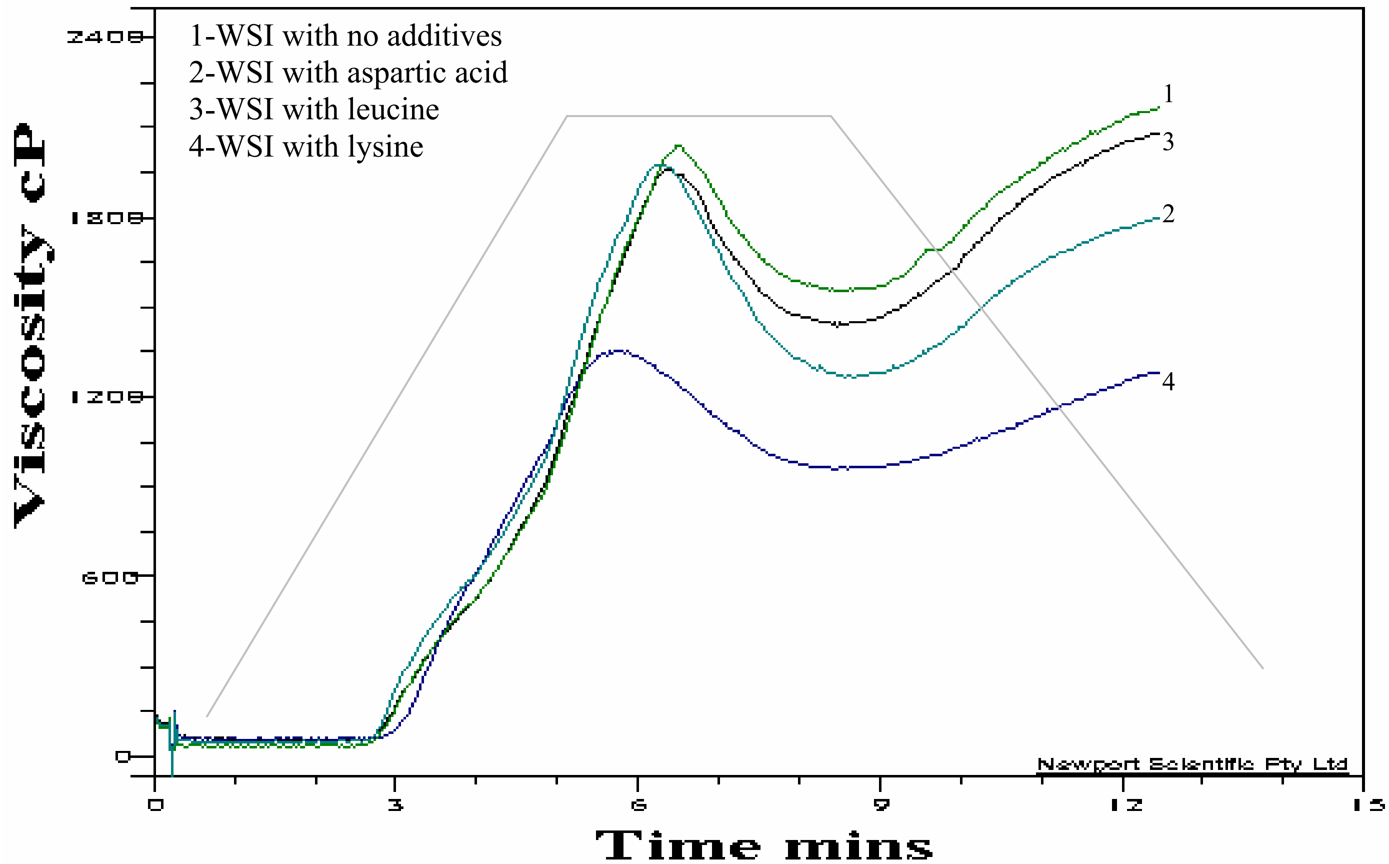

Figure 3.10 Pasting Characteristics of White Starch Isolate with Ozone for 15 minutes with Additives

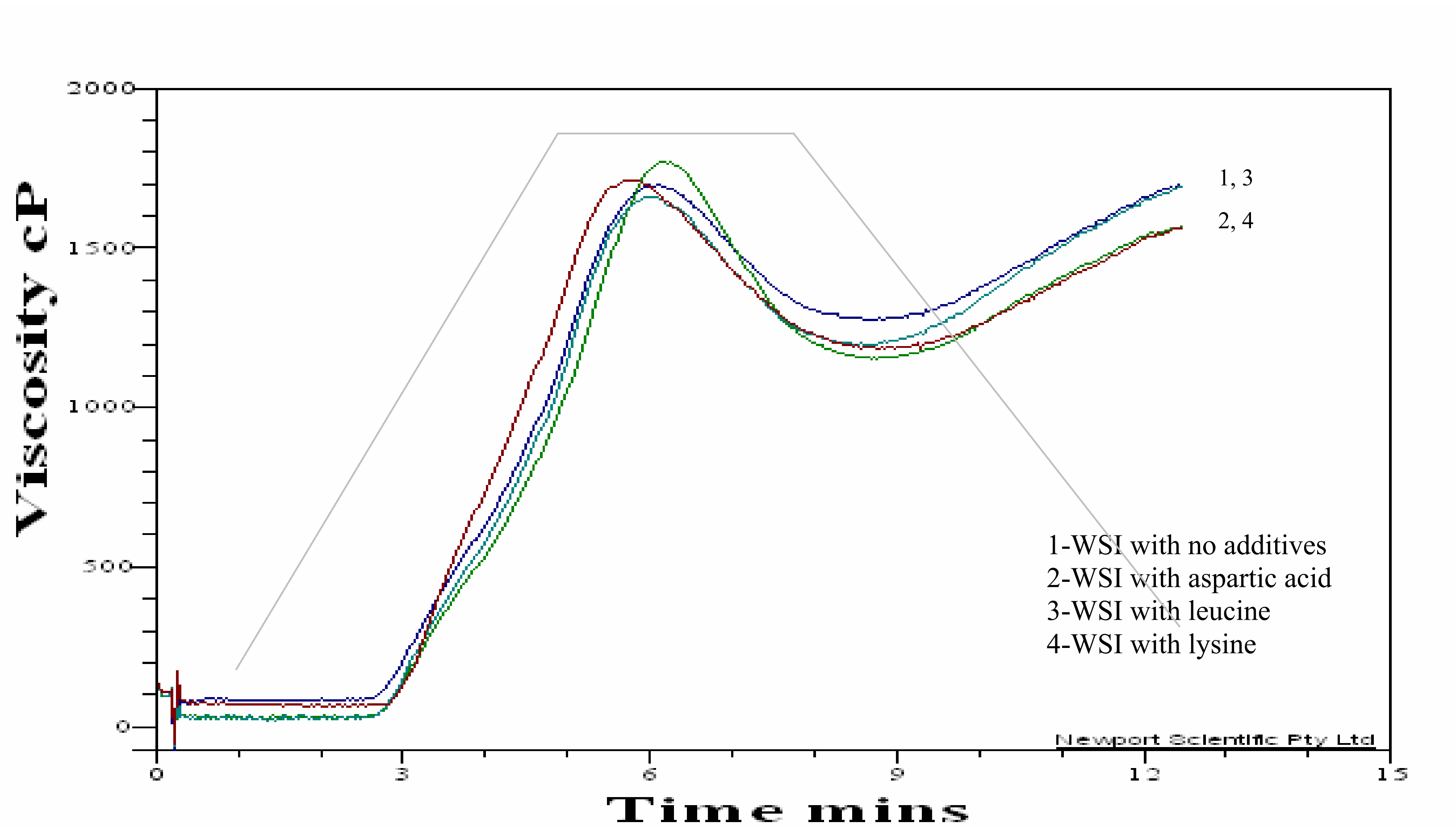

Figure 3.11 Pasting Characteristics of White Starch Isolate Treated with Pure Oxygen for 30 minutes with Additives

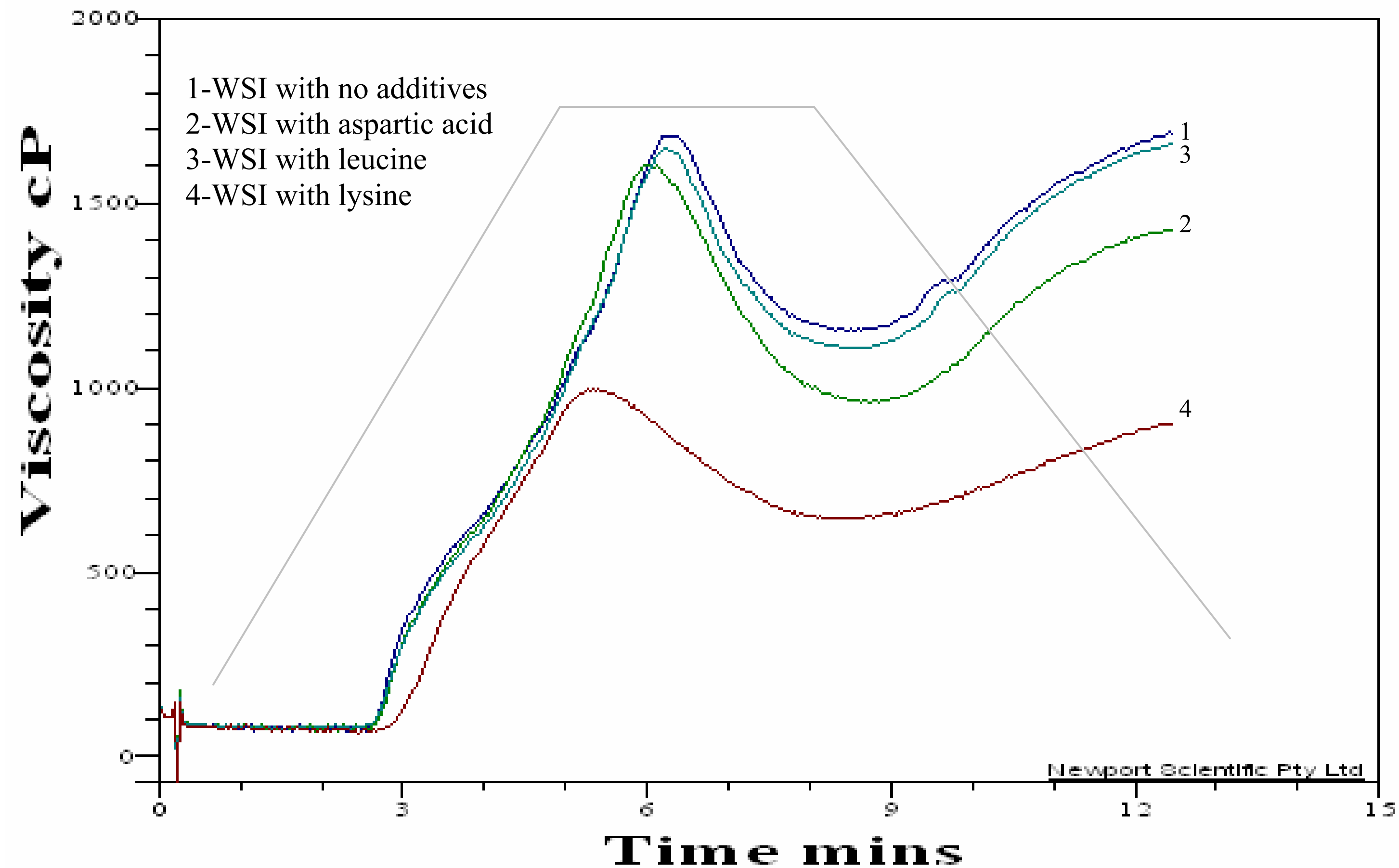

Figure 3.12 Pasting Characteristics of White Starch Isolate with Ozone for 30 minutes with Additives

reported that slightly oxidized starch showed more swelling due to loss of starch integrity, resulting in more water access to starch granule. Moreover, an increase in amylose content in ozonated starch might have been contributed to a greater extent of granule swelling. Rutenberg and Solarek (1984) reported that less retrogradation resulted from oxidative scission because structural changes make three dimensional networks impossible during gel formation. Therefore, ozonated starch exhibited similar pasting properties to those from oxidized starch treated with chemical oxidizing agents.

This study also showed that lysine in combination with ozone treatment for 30 minutes reduced commercial starch granule swelling extent and pasting time significantly, resulting in less rigid and viscous but faster cooking starch paste. Moreover, lysine on 30 minutes ozonated WSI presented the lowest paste viscosities and pasting time, resulting in starch paste with the fastest swelling, the lowest retrogradation tendency, and the greatest cooking stability. Liang and King (2003) reported that amino acids reduced the swelling extent and cooking stability and that the charged amino acids had a greater influence on pasting characteristics than the neutral ones probably because of the charges that they carried. In addition, catalysts such as sodium sulfite and sodium chloride have been used as promoters during oxidation since they caused granule disintegration due to the ionic effects (Mat Hashim et al., 1992). The fact that positive amino acid (lysine) exhibited greater effect on the pasting property of ozonated starch than negative amino acid (aspartic acid) might be related to the positive ions of lysine that ozonated starch easily combine. The ozonated starch could be used for viscous foods, whereas ozonated starch with lysine could be an alternative for commercial oxidized starch.

CHAPTER 4

EFFECTS OF OZONATION AND ADDITION OF AMINO ACIDS ON FORMATION OF RESISTANT STARCH

4.1. INTRODUCTION

There are three different categories in starch, RDS (Rapidly digestible starch), SDS (Slowly digestible starch), and Resistant starch. RS (resistant starch) is the starch that is not digested in small intestine but may be fermented by microorganisms in large intestine; thereby, has been recognized as a dietary fiber (Sievert and Pomeranz, 1990). There are 4 different categories in RS. RS1 is physically trapped starch in rigid cell walls, whereas RS2 is ungelatinized granules or RS granule. RS3 is retrograded starch polymer after gelatinization (Englyst et al., 1992). Formation of RS3 is affected by granular swelling, amylose leaching, and the amount of retrograded amylose. Finally, RS4 is the starch that treated with chemical modification methods, such as cross-linking, stabilization (Filer, 1988), etherification, and esterification (Wolf et al., 1999). Cooked rice generally contains higher resistant starch yield than raw rice due to the crystallization of amylose (Eggum et al., 1993). Enzyme hydrolysis or acid treatment debranches amylopectin chains; therefore more linear amylose chains reassociates after heating (Vasanthan and Bhatty, 1998).

Mangala et al. (1999b) reported a significant increase in resistant starch content after defatting because of lack of lipid molecules for complexing with amylose, resulting in more uncomplexed linear fraction. On the other hand, amylose-lipid complexes decrease the susceptibility of amylose to amylolysis in some studies (Eliasson and Krog, 1985; Holm et al., 1983). Starch –protein interaction also has an influence on RS content. It was found that protein bound to starch during starch retrogradation and the associated starch with protein is hydrophobic and resists swelling (Lumdubwong and Seib, 2000). Brighenti et al. (1998) reported a high yield of RS in egg noodle probably due to proteins in egg involved in association with starch. Moreover, Holm et al. (1985) found that the starch availability to α –amylase in raw and boiled wheat decreased without pepsin digestion, which indicated that a large fraction of the starch was encapsulated in a protein matrix.

When resistant starch is fermented by microorganisms in the colon, butyrate, which stabilizes colonic cell proliferation, is produced. Moreover, slowly digested starch may protect against chronic disease and reduce blood glucose and insulin responses, while rapidly digested starch elevates them. Therefore, improved glucose metabolism reduces the risk of diabetes mellitus (Englyst et al., 1999). Resistant starch has been related to the slow rate of starch hydrolysis in the gastrointestinal tract of humans; thereby, may have some of the physiological effects of dietary fiber (Englyst and MacFarlane, 1986). Foods, that are known to be resistant to the action of amylolytic enzymes and to have certain amount of resistant starch content, include bread, breakfast cereals and biscuits (Russell et al., 1989).

Wolf et al. (1999) reported that oxidized corn starch had lower extent of starch digestion compared to untreated starch, causing an increased resistant starch content and that substitution in modified starch interferes with digesting enzymes; thus, producing a slowly digested starch. Oxidation also has shown an influence on starch that reacts with enzyme since there are structural change as well as oxidative scission. Boruch (1985) demonstrated that oxidation changed a starch molecule’s shape and spatial system thus made the action of glycoamylase more difficult. It was also found that only slightly oxidized samples, which contained a small amount of reducing value and no carboxyl content slowed enzymatic saccharification significantly. Highly chemically oxidized waxy corn starch (100% amylopectin) was found to have slightly reduced digestibility, resulting in a slight increase in the amount of resistant starch by 1.3% (Wolf et al., 1999).

The objectives of this study were 1) to investigate the effect of ozonation on the formation of rice resistant starch 2) to study the effect of addition of amino acids on rice resistant starch yield.

4.2. MATERIAL AND METHODS

4.2.1. Materials

Sigma rice starch was purchased from Sigma Chemical Co. (S7260) while white rice flour was obtained from Riviana Foods Inc. (Abbeville, LA). Three different amino acids included positive charged (lysine), negative charged (aspartic acid), and neutral amino acid (leucine). Potato amyloses (A0512), amylopectin (A8515), protease (P5147, 4.0 units/mg), Dietary fiber kit (TDF-100A) along with the amino acids were purchased from Sigma Chemical Co. (St. Louis, MO).

4.2.2. Sample Preparation and Pure Oxygen and Ozone Treatments

For details of sample preparations, pure oxygen and ozone treatments, refer to Chapter 3.

4.2.3. Resistant Starch Analysis

Sigma rice starch and white starch isolate treated with either pure oxygen or ozone for 15 or 30 minutes were freeze- dried and ground into powder for further analysis. Three different types of amino acids, aspartic acid (negative charged), leucine (neutral), or lysine (positive charged) were used as additives to see the effects on pasting characteristics by using Rapid Visco- Analysis. Then, the Rapid-Visco Analyzed starch samples were stored at 4˚C, then freeze dried and ground for examining the resistant starch content.

The yield of resistant starch of treated rice starch and white starch isolate was determined by the enzymatic-gravimetric method, as described in Sigma Technical Bulletin No.

TDFAB-3 (Kim et al., 2003). Sigma rice starch or white starch isolate with or without additives (0.5g) was dispersed in 0.08 M phosphate buffer (25mL, pH 6.0) and 0.05 mL heat stable α - amylase (68,300 units/ml) was added. The beaker was covered with aluminum foil and placed in a water bath at 95°C for 15 minutes, agitating the beaker gently at 5 min intervals. After cooling to room temperature, the solution was adjusted to pH 7.5 by adding 0.275N aqueous NaOH solution and protease (P3910) was added (0.05mL, 50mg/mL solution of protease in phosphate buffer). The mixture was placed in a shaking incubator at 60°C for 30 min. After cooling to room temperature, the solution was adjusted to pH 4.3 by adding 0.325 N aqueous HCl solution and 0.05mL amyloglucosidase (10,863 units/ml; A9913) was added. The mixture was placed in a shaking incubator at 60°C for 30 min. Four volumes of 95% ethanol were added and the mixture was allowed to stand overnight at room temperature for complete precipitation. The insoluble residue was centrifuged at 1,500 rpm for 10 minutes twice with 15mL of absolute ethanol and washed once with 10 mL of acetone at a same rate. The residue was dried in an oven at 40°C overnight.

The yield of resistant starch was determined as:

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

4.2.4. Statistical Analysis

SAS (Statistical Analysis System) software (version 8.0) was used for resistant starch analysis. Analysis of Variance (ANOVA) with Tukey's studentized range (HSD) test was performed to determine the effects of the additives (aspartic acid, leucine and lysine) on the formation of resistant rice starches. The effects on duration of treatments were analyzed statistically ($P \leq 0.05$) using a two-tailed t-test. The treatments were pure oxygen and ozone. The durations of treatment were 0, 15, and 30 minutes. Abbreviations were PO for pure oxygen, OZ for ozone, NA for no additives, ASP for aspartic acid, LEU for leucine, LYS for lysine, and WSI for white starch isolate.

4.3. RESULTS AND DISCUSSION

4.3.1. Effects of Duration of Pure Oxygen and Ozone Treatments on Sigma Rice Starch with or without Additives

In comparison to untreated starch samples, pure oxygen for 15 and 30 minutes (PO15 and PO30) with no additives (NA) increased resistant starch yield (RSY) by 2.3 and 2.7%, respectively (Table 4.1). In addition, ozone treatment for 15 and 30 minutes (OZ15 and OZ30) with NA showed higher resistant starch content by 2.9 and 3.0 %, respectively. The addition of aspartic acid (ASP) and lysine (LYS) of PO15, PO30, OZ15, and OZ30 showed similar results with no additives, in which RSYs were enhanced by 3.4, 2.9, 3.1, and 2.7 %, respectively for AA and by 2.0, 2.3, 2.2, and 3.1 %, respectively, for LYS. The presence of leucine (6%) also increased RSY of PO15, PO30, OZ15, and OZ30 by 2.1, 2.6, 3.1, and 4.2 %, respectively. Statistically, only leucine added rice starch showed significant difference in RSY between PO and OZ treatments, having the highest content for OZ30 at 9.13% (Table 4.1).

Among duration time of treatments, OZ30 treatment presented the greatest resistant starch content with leucine (LEU) at 9.13%, lysine at 8.52% or NA at 8.42%. On the other hand, aspartic acid had the highest RSY with PO15. Overall, the longer treatment produced the higher formation of resistant starch at both pure oxygen and ozone treatments. In addition, ozone treatment was the most effective method compared to non-treated and pure oxygen treatment for resistant starch yield. These results might be supported by Wolf et al. (1999). They found that chemical modification including oxidation might allow for the production of a slowly digested

Table 4.1 Effects of Duration of Pure Oxygen and Ozone Treatment on Resistant Starch Yield of Sigma Rice Starch with or without Additives [1, 2]

	No Additives	
Sample	**Minutes**	**Resistant Starch Yield (%)**
No Treatment	0	5.3700 ± 0.48b
Pure Oxygen	15	7.7667 ± 0.94a
	30	8.1467 ± 0.89a
Ozone	15	8.3367 ± 0.78a
	30	8.4200 ± 0.84a
	Aspartic Acid	
Sample	**Minutes**	**Resistant Starch Yield (%)**
No Treatment	0	5.4633 ± 0.62b
Pure Oxygen	15	8.8250 ± 1.24a
	30	8.3983 ± 1.2a
Ozone	15	8.5967 ± 1.28a
	30	8.1933 ± 0.43a
	Leucine	
Sample	**Minutes**	**Resistant Starch Yield (%)**
No Treatment	0	4.9467 ± 0.69c
Pure Oxygen	15	7.0183 ± 0.75b
	30	7.5483 ± 0.99b
Ozone	15	8.0017 ± 0.97ab
	30	9.1333 ± 1.49a
	Lysine	
Sample	**Minutes**	**Resistant Starch Yield (%)**
No Treatment	0	5.4467 ± 0.57b
Pure Oxygen	15	7.4533 ± 1.00a
	30	7.7183 ± 0.99a
Ozone	15	7.6583 ± 0.85 a
	30	8.5233 ± 1.24a

[1]Resistant starch yield in percent calculated (Kim et al., 2003)
[2]Different letters within column for each additive indicate means are significantly different at the level of $p \leq 0.05$

starch; thereby, suggesting an increase in the amount of resistant starch as the extent of digestion decreased with increasing the degree of modification. These results also might be related to higher amylose content produced by both PO and OZ treatments (Table 3.1). The relationship between amylose content and resistant starch is known to be positively correlated. Besides that, the retrogradation of amylose was found to be of primary factor in the formation of RS content of starch (Sievert and Pomeranz, 1989; Eerlingen et al., 1994a; Cairns et al., 1996). Formation of resistant starch type 3 is also influenced by granular swelling and the molecular characteristics (chain length) of amylose. It could be postulated that more amylose leached out during heating that was obtained by RVA due to the structural change resulting from pure oxygen and ozone treatments; thus, the amount of retrograded amylose was increased as well.

4.3.2. Effects of Amino Acids on Sigma Rice Starch

- **Amino Acids Effects on Non-treated Starch**

Resistant starch yields (RSY) of non- treated commercial rice starch with additives are shown in Table 4.2. The RSY of untreated Sigma rice starch with no additives, and with aspartic acid, leucine, and lysine were 5.37, 5.46, 4.94, and 5.44%, respectively. Statistically, none of the amino acids significantly changed the resistant starch yield for Sigma rice starch.

- **Amino Acid Effects on Rice Starch Treated with 15 minutes Pure Oxygen or Ozone**

When 6% (dry base) of aspartic acid was added, resistant starch yield for Sigma rice starch with pure oxygen for 15 minutes (PO15) was 8.82%. Leucine (LEU) and lysine (LYS) nonsignificantly decreased resistant starch content compared to starch with pure oxygen treatment with no additives (7.76%). Ozone treatment showed similar results with PO treatment (Table 4.3).

Among all samples, ASP added Sigma rice starch treated with OZ15 showed the highest

Table 4.2.Effects of Additives on Resistant Starch Yield of Sigma Rice Starch [1, 2, 3]

Sample	Additives	Resistant Starch Yield (%)
Rice Starch with No Treatment	NA	5.3700 ± 0.48a
	ASP	5.4633 ± 0.62a
	LEU	4.9467 ± 0.69a
	LYS	5.4467 ± 0.57a

[1]NA= No Additives; ASP= Aspartic Acid; LEU= Leucine; LYS= Lysine
[2]Resistant starch yield in percent calculated (Kim et al., 2003)
[3]Different letters within column indicate means are significantly different at the level of $p \leq 0.05$

Table 4.3 Effects of Additives on Resistant Starch Yield of Sigma Rice Starch Treated with Pure Oxygen and Ozone for 15 minutes [1, 2, 3]

Sample	Additives	Resistant Starch Yield (%)
Rice Starch with Pure Oxygen	NA	7.7667 ± 0.94abc
	ASP	8.8250 ± 1.24a
	LEU	7.0183 ± 0.75c
	LYS	7.4533 ± 1.00bc
Rice Starch with Ozone	NA	8.3367 ± 0.78ab
	ASP	8.5967 ± 1.28ab
	LEU	8.0017 ± 0.97abc
	LYS	7.6583 ± 0.85abc

[1]NA= No Additives; ASP= Aspartic Acid; LEU= Leucine; LYS= Lysine
[2]Resistant starch yield in percent calculated (Kim et al., 2003)
[3]Different letters within column indicate means are significantly different at the level of $p \leq 0.05$

RSY. These results agreed with the fact that starch –protein interaction is one of the factors affecting starch digestion and formation of resistant starch (Vasanthan et al., 1998; Holm et al., 1983). According to Greenwell et al. (1985), surface proteins act as an obstacle to amylolytic enzymes. Moreover, RS content was high in egg noodle, which might be related to interaction between starch and proteins (Brighenti et al., 1998). It was also reported that starch availability was reduced when pepsin digestion was omitted, meaning that protein matrix encapsulates the starch fraction (Holm et al., 1985).

- **Amino Acid Effects on Rice Starch Treated with 30 minutes Pure Oxygen or Ozone**

Pure oxygen treated samples for 30 minutes with the addition of aspartic acid had RSY of 8.4%, and PO30 with no additives contained 8.2% of resistant starch. In addition, they were significantly higher than those of RSYs of PO30 in the presence of leucine and lysine, 7.0 and 7.45%, respectively (Table 4.4). On the other hand, OZ30 showed different results. Resistant starch yield was the highest for ozonated rice starch when leucine was added. Moreover, lysine also nonsignificantly increased the resistant starch content compared to that with no additives.

Among amino acids, leucine was the additive that showed the highest RSY for ozonated Sigma rice starch for 30 minutes. Among treatments, OZ30 had the most resistant starch content compared to pure oxygen or non-treated starch samples. Overall, ozone treatment is more effective in yielding resistant starch content than pure oxygen treatment with or without additives. The result was expected since ozone reacts a lot faster than pure oxygen due to the extra single oxygen atom.

4.3.3. Sigma Rice Starch versus White Starch Isolate (WSI) on Resistant Starch Yield

The resistant starch contents of WSI with no additives, aspartic acid, leucine, and lysine were higher than Sigma rice starch for non-treated starch samples by 3.5 %, 3.78%, 2.6%, and

Table 4.4 Effects of Additives on Resistant Starch Yield of Sigma Rice Starch Treated with Pure Oxygen and Ozone for 30 minutes [1, 2, 3]

Sample	Additives	Resistant Starch Yield (%)
Rice Starch with Pure Oxygen	NA	8.1467 ± 0.89ab
	ASP	8.3983 ± 1.2ab
	LEU	7.5483 ± 0.99b
	LYS	7.7183 ± 0.99b
Rice Starch with Ozone	NA	8.42 ± 0.84ab
	ASP	8.1933 ± 0.43ab
	LEU	9.1333 ± 1.49a
	LYS	8.5233 ± 1.24ab

[1]NA= No Additives; ASP= Aspartic Acid; LEU= Leucine; LYS= Lysine
[2]Resistant starch yield in percent calculated (Kim et al., 2003)
[3]Different letters within column indicate means are significantly different at the level of $p \leq 0.05$

3.4 %, respectively (Table 4.2 and Table 4.6). This is probably because of the residues of lipid and protein on white starch isolate since non-constituents of starch interfere with the binding of amylolytic enzymes such as α -amylase or amyloglucosidase; thus, reduce starch digestion. It was found that amylose-lipid complexes reduced the susceptibility of amylose to amylolysis (Eliasson and Krog, 1985). Moreover, lipid is native enzyme inhibitor since it was found that defatted starch was more susceptible to enzyme degradation (Baker and Woo, 1992). According to Escarpa et al. (1997), protein is encapsulated inside the starch granule providing a rigid cover. Moreover, protein is known to bind to starch in a same way that amylose aggregate to each other by hydrogen bonds during retrogradation.

4.3.4. Effects of Duration of Pure Oxygen and Ozone Treatments on White Starch Isolate (WSI) with or without Additives

In comparison to non-oxidized starch with no additives (NA), PO30 nonsignificantly increased resistant starch yield (RSY) by 0.5 % (Table 4.5). However, OZ15, and OZ30 with NA reduced RSYs. Moreover, RSYs of the treated starch samples decreased with PO and OZ when ASP (6%) was present. On the other hand, the presence of leucine (LEU) nonsignificantly increased RSY for PO30 by 1 %, and RSYs of OZ15 and OZ30 were not significantly different from that with non-treated starch samples. Similarly, lysine (LYS) with PO30 exhibited higher RSY than that with OZ30 treatment. However, RSY of PO with LYS was not statistically different from that of non-treated starch.

With no additives, resistant starch yield of WSI was nonsignificantly the highest (9.38%) for PO30 (Table 4.5). For ASP and LYS, non-treated starch showed the highest resistant starch content. In the presence of leucine, PO30 nonsignificantly exhibited the highest RSY. Overall, ozone treatment on WSI was not as strongly effective as it was on Sigma rice starch.

Table 4.5 Effects of Type and Duration of Pure Oxygen and Ozone Treatment on Resistant Starch Yield of White Starch Isolate with or without Additives [1, 2]

	No Additives	
Sample	**Minutes**	**Resistant Starch Yield (%)**
No Treatment	0	8.8883 ± 0.76ab
Pure Oxygen	15	8.3433 ± 0.78b
	30	9.38 ± 1.07a
Ozone	15	7.0733 ± 0.98c
	30	6.4383 ± 0.52c
	Aspartic Acid	
Sample	**Minutes**	**Resistant Starch Yield (%)**
No Treatment	0	9.2467 ± 0.73a
Pure Oxygen	15	7.575 ± 1.05bc
	30	8.7683 ± 1.12ab
Ozone	15	6.8800 ± 0.73c
	30	7.895 ± 1.50bc
	Leucine	
Sample	**Minutes**	**Resistant Starch Yield (%)**
No Treatment	0	7.5533 ± 1.15ab
Pure Oxygen	15	8.085 ± 1.10a
	30	8.530 ± 0.83a
Ozone	15	7.5917 ± 1.09ab
	30	6.6350 ± 1.43b
	Lysine	
Sample	**Minutes**	**Resistant Starch Yield (%)**
No Treatment	0	8.845 ± 1.84a
Pure Oxygen	15	7.92 ± 1.42a
	30	8.0633 ± 1.02a
Ozone	15	7.4117 ± 1.55ab
	30	6.3167 ± 0.85b

[1]Resistant starch yield in percent calculated (Kim et al., 2003)
[2]Different letters within column for each additive indicate means are significantly different at the level of $p \leq 0.05$

Table 4.6 Effects of Additives on Resistant Starch Yield of White Starch Isolate (WSI) [1, 2, 3]

Sample	Additives	Resistant Starch Yield (%)
WSI with No Treatment	NA	8.8883 ± 0.76a
	ASP	9.2467 ± 0.73a
	LEU	7.5533 ± 1.15b
	LYS	8.8450 ± 1.84ab

[1]NA= No Additives; ASP= Aspartic Acid; LEU= Leucine; LYS= Lysine
[2]Resistant starch yield in percent calculated (Kim et al., 2003)
[3]Different letters within column indicate means are significantly different at the level of $p \leq 0.05$

Compared to non-treated starch samples, it even reduced resistant starch yield. The results could be related to a lower starting concentration of amylose content in WSI (Table 3.2).

4.3.5. Effects of Amino Acids on White Starch Isolate (WSI)

- **Amino Acids Effects on Non-treated WSI**

Compared to no additives, aspartic acid (ASP) added white starch isolate showed higher RSY, but they were not different statistically (Table 4.6). In addition, there was no significant difference in resistant starch content when lysine was added to white starch isolate. However, leucine (6%) lowered resistant starch content of white starch isolate.

- **Amino Acid Effects on WSI Treated with 15 minutes Pure Oxygen or Ozone**

Both PO and OZ showed lower RSYs than non-treated white starch isolate with and without additives except leucine; RSY was slightly increased with PO and OZ when leucine (6%) was added, but was not significantly different statistically (Table 4.6 and Table 4.7). During the isolation process, white starch went through physical damage with reducing agents and washing steps, and pure oxygen and ozone treatments involve oxidation degradation resulting in another physical change. Therefore, treated starch isolate granules might be more easily breakable compared to untreated starches making the starch more digestible to enzymes.

Resistant starch contents of PO15 treatment with aspartic acid, leucine or lysine were not significantly different from that with no additives. OZ15 showed similar results with leucine and lysine. However, aspartic acid (6%) had lower RSY than no additives (Table 4.7). Overall, none of amino acids increased resistant starch yields for both PO15 and OZ15. PO15 exhibited the greatest RSY (8.34%) with no additives.

- **Amino Acid Effects on WSI Treated with 30 minutes Pure Oxygen or Ozone**

The effects of amino acids on white starch isolate treated with PO or OZ treatment for 30

Table 4.7.Effects of Additives on Resistant Starch Yield of White Starch Isolate (WSI) Treated with Pure Oxygen and Ozone for 15 minutes [1, 2, 3]

Sample	Additives	Resistant Starch Yield (%)
WSI with Pure Oxygen	NA	8.3433 ± 0.78a
	ASP	7.5750 ± 1.05ab
	LEU	8.0850 ± 1.10ab
	LYS	7.92 ± 1.42ab
WSI with Ozone	NA	7.0733 ± 0.98ab
	ASP	6.88 ± 0.73b
	LEU	7.5917 ± 1.09ab
	LYS	7.4117 ± 1.55ab

[1]NA= No Additives; ASP= Aspartic Acid; LEU= Leucine; LYS= Lysine
[2]Resistant starch yield in percent calculated (Kim et al., 2003)
[3]Different letters within column indicate means are significantly different at the level of $p \leq 0.05$

Table 4.8 Effects of Additives on Resistant Starch Yield of White Starch Isolate Treated with Pure Oxygen and Ozone for 30 minutes [1, 2, 3]

Sample	Additives	Resistant Starch Yield (%)
WSI with Pure Oxygen	NA	9.3867 ± 1.07a
	ASP	8.7683 ± 1.12ab
	LEU	8.53 ± 0.83ab
	LYS	8.0633 ± 1.02ab
WSI with Ozone	NA	6.4383 ± 0.52d
	ASP	7.895 ± 1.50bc
	LEU	6.635 ± 1.43cd
	LYS	6.3167 ± 0.85d

[1]NA= No Additives; ASP= Aspartic Acid; LEU= Leucine; LYS= Lysine
[2]Resistant starch yield in percent calculated (Kim et al., 2003)
[3]Different letters within column indicate means are significantly different at the level of $p \leq 0.05$

minutes are shown in Table 4.8. The RSY for PO30 with no additives (NA) were higher than any amino acids added PO30, but they were not significantly different statistically. Except for ASP, OZ30 samples with or without additives showed lower RSYs than those of OZ15 (Table 4.7 and 4.8). Among PO30 and OZ30 treated samples, none of the additives showed effectiveness on resistant starch yields. PO30 with no additives on WSI exhibited the greatest RSY (9.38%).

4.4 CONCLUSION

Ozonation increased resistant starch content of Sigma rice starch. In addition, the longer ozonation took place, the higher the resistant starch yield was. Ozonated Sigma rice starch for 30 minutes exhibited the highest resistant starch content. The addition of amino acids also enhanced formation of resistant starch. The addition of aspartic acid (6%) showed the highest resistant starch content with pure oxygen, while leucine (6%) did with ozone. These results addressed ozonated starch with amino acids could be health beneficial for comparably higher resistant starch content and for the additional essential amino acids compared to untreated starch.

In comparison to untreated Sigma rice starch, untreated white starch isolate exhibited greater resistant content than any of the treated samples. Moreover, the presence of aspartic acid (6%) presented the highest resistant starch yield in untreated white starch isolate. Among all of treated samples, pure oxygen for 15minutes with no additives showed the highest resistant starch content. However, ozone did not change resistant starch yield in white starch isolate.

CHAPTER 5

EFFECTS OF OZONATION AND ADDITION OF LYSINE ON THERMAL PROPERTIES OF RICE STARCHES BY DIFFERENTIAL SCANNING CALORIMETER (DSC)

5.1. INTRODUCTION

Gelatinization of starch takes place when starch is heated over a critical temperature in excess water and includes (1) the loss of crystallinity and disruption of molecular order within granule, that produces a viscous mass consisting of a continuous phase of solubilized amylose and amylopectin; (2) an uptake of heat as the conformation of the starch alters; and (3) hydration of the starch. Starch gelatinization determines the overall cooking behavior and product characteristics of foods, and it follows changes in properties, viscosity and heat uptake during heating. Amylose, the swollen granules, attributes to the mechanical properties and the structure of the gel or paste (Lii et al., 1995). The mixture of amylose and amylopectin is leached out from the granules. Amylose is known to be responsible for gel structure in the short term, whereas amylopectin is considered for gel structure in the long term (Ring, 1985). Starches with higher amylose content have shown greater amounts of resistant starch than the lower amylose content starches after extrusion (Biliaderis & Juliano, 1993).

The important factors that influence gelatinization are heating conditions such as temperature, heating period, and rate (Lii et al., 1995), granule size, shape, amylose content, starch varieties, degree of crystallinity and chain length of the amylopectin fractions, and the content of non-starch constituents such as lipid and protein (Juliano et al., 1987; Hamaker and Griffin, 1990; Tester and Morrison, 1990a). Starch lipid complexes with both amylose and amylopectin for interchain association, and they change the thermal and mechanical properties of starch gelatinization (Biliaderis & Juliano, 1993). Removing starch lipids has been investigated over several decades and has been found to increase granule swelling and to lower the gelatinization temperature of rice starch (Maningat and Juliano, 1980; Tester and Morrison, 1990a). Moreover, Marshall et al. (1990) reported a decrease in peak and final gelatinization temperature of starch with lipid and protein removed. On the other hand, amylose –lipid complexes increased gelatinization temperature (Eliasson et al., 1981), while defatted starch had the opposite effect (Morrison et al., 1993). In addition, Czuchajowska et al. (1991) reported that the 2nd peak in DSC appeared at 95-110C° with higher enthalpy when lipids were added, while untreated starch showed it at 100°C. Huang et al. (1994) found that rice flour with higher lipid and protein contents than rice starch had greater gelatinization temperature, but lower enthalpy since the non-starch constituents in flour prohibited starch gelatinization. Radosavljevic et al. (1998) agreed that isolated starch consisting of lower protein decreased gelatinization temperature, but enhanced enthalpy greater than those of untreated starch.

Several studies suggested that both endosperm matrix protein and granule associated protein influence the gelatinization properties of starch (Chandrashekar and Kirleis, 1988; Seguchi, 1986). Deprotenized flour decreased the gelatinization temperature, which indicates an inhibitory effect of rice protein on the swelling upon cooking (Marshall et al., 1990; Yang and Chang, 1999). It was reported that the starch is associated with two protein bodies; prolamin and glutelin, which are hydrophobic and resist swelling (Resurreccion et al., 1993). These findings indicate that rice starch extraction is more difficult than that of wheat starch or corn starch (Lumdubwong and Seib, 2000).

Modification of starch also has shown an effect on thermal transition properties. Jacobs et al. (1995) studied the effect of annealing starch on the thermal properties and reported that annealing made starch resistant; therefore showed a higher gelatinization temperature. There has been a controversial result with gelatinization temperature for oxidized starches. Muhrbeck et al.

(1990) found a decrease in transition temperature with increased degree of oxidation with bromine for potato starch, whereas a higher gelatinization temperature was observed from oxidized potato starch with hydrogen peroxide (Parovuori et al., 1995), where the explanation was the selective dissolution of the amorphous parts of the granule. The different effect of oxidizing agents might have caused different oxidation mechanisms depending on chemicals. It was also stated that difference in granular structure might have had an influence after oxidation when different types of starches were treated. Forssell et al. (1995) explained that an increase in gelatinization temperature after hypochlorite oxidation of barley starch might be due to the increase of the glass transition temperature of the amorphous regions, due to the reorientation of the crystal structure or to the denser crystal structure compared to potato starch, which showed unchanged gelatinization temperature (Forssell et al., 1995). Oxidation did not change the gelatinization enthalpy of potato and barley starches, however it decreased that of defatted barley starch.

Differential Scanning Calorimetry (DSC) has been utilized to study food thermal integrity both qualitatively and quantitatively (Biliaderis et al., 1986). Moreover, it has been recognized as the most appropriate method to examine gelatinization transition. It measures heat absorbed or given off by a sample during cooking at a specific heating rate. The advantages of this equipment are sensitivity and dependable results for starch gelatinization under a various experimental conditions (Biliaderis et al., 1986). A lot of studies have been conducted to investigate thermal properties of starches for different areas including the effect of lipid and protein on starch gelatinization (Marshall et al., 1990; Radosavljevic et al., 1998; Hoover et al., 1993), the effect of degree of milling on thermal properties (Marshall, 1992; Normand and Marshall, 1989), and the effect of annealing on starch properties by DSC (Jacobs et al., 1995).

The objectives of this study were 1) to determine the effect of ozonation on thermal properties of rice starches and 2) to investigate the effect of addition of lysine with ozonation on thermal characteristics of rice starches.

5.2. MATERIAL AND METHODS

5.2.1. Materials

Sigma rice starch was purchased from Sigma Chemical Co. (S7260) while white rice flour was obtained from Riviana Foods Inc. (Abbeville, LA). Positive charged amino acid (lysine), potato amylose (A0512), amylopectin (A8515), and protease (P5147) were purchased from Sigma Chemical Co. (St. Louis, MO).

5.2.2. Sample Preparation and Pure Oxygen and Ozone Treatments

For details of sample preparation and pure oxygen and ozone treatments, refer to Chapter 3.

5.2.3. Differential Scanning Calorimeter Analysis

For the preparation of additive solution, 300mg of lysine was weighed into a 10ml flask. Distilled water then was added to a total volume of 10ml. The solutions were mixed well and let stand for 10 minute to equilibrate before use.

A DSC (TA Instruments, USA) was utilized to measure the degree of starch gelatinization for each treated starch sample. Ten mg of sample and 20 mg of distilled water or solution with additives were transferred into DSC pans. The pans was hermetically sealed and inserted in the calorimeter. Thermal curves that included onset temperature (To), peak temperature (Tp), the endothermic peak area, and conclusion temperature (Tc) were achieved at a heating rate of 5°C/min from 35 to 140°C. Gelatinization energy (enthalpy, ΔH) is the area that is calculated by drawing a straight line between onset temperature and conclusion temperature and is determined in joules per gram (J/g) on a dry weight basis of rice starches.

5.2.4. Statistical Analysis

SAS (Statistical Analysis System) software (version 8.0) was used for DSC data analysis. Analysis of Variance (ANOVA) with Tukey's studentized range (HSD) test was performed to examine the effects of ozonation and addition of lysine on the thermal properties of rice starches. The treatments were pure oxygen and ozone. The durations of treatment were 0, 15, and 30 minutes. Abbreviations were PO for pure oxygen, OZ for ozone, NA for no additives, LYN for lysine, and WSI for white starch isolate. The analysis used was a 2-tailed t-test ($P \leq 0.05$).

5.3 RESULTS AND DISCUSSION

5.3.1 Effects of Pure Oxygen and Ozone on Thermal Properties of Sigma Rice Starch

In comparison to non-treated starches, both pure oxygen treatment for 15 and 30 minutes (PO15 and PO30) increased peak gelatinization temperature by 8 and 7°C, respectively, but did not change amylose-complex transition and either enthalpies statistically (Table 5.1 and Figure 5.1). PO15 and PO30 also increased onset gelatinization temperature (To) by 9 and 8˚C, respectively. On the other hand, ozone treatment for 15minutes (OZ15) and for 30minutes (OZ30) lowered To of gelatinization transition by 4 and 6°C, respectively. OZ15 and OZ30 also decreased 1st conclusion temperature (Tc) by 3.3 and 3.1˚C, respectively. However, OZ15 and OZ30 increased 1st peak temperature (Tp) by 3.7 and 3.4˚C, respectively. Moreover, OZ15 and OZ30 increased gelatinization enthalpy (J/g) and reduced conclusion temperature (Tc) for 2nd transition and amylose-complex enthalpy compared to untreated starches.

In the presence of lysine (6%), PO15 and PO30 also enhanced gelatinization transition including enthalpies by 3.7 and 2.7˚C for To, by 6.95 and 6.92˚C for Tp, by 3.8 and 3.6˚C for Tc, and by 1.5 and 2.2 (J/g) for enthalpies (Table 5.1 and Figure 5.2). However, they decreased second transition temperatures compared to non-treated starches. OZ15 and OZ30 decreased 1st onset and conclusion temperatures by 4 and 3.3 ˚C for To, and by 3.3 and 3.2 °C for Tc, respectively. However, they increased 1st peak temperature by 4.2 and 4.6˚C. For 2nd transition temperatures, PO15 and PO30 reduced onset temperature by 4.3 and 4.4°C. In addition, OZ15 and OZ30 with the addition of lysine also decreased second transition temperatures including enthalpy.

Whether lysine (6%) was added or not, PO increased thermal properties for gelatinization transition. On the other hand, OZ reduced onset and conclusion temperature of 1st endotherm and 2nd transition temperatures including enthalpy, but increased 1st Tp and enthalpy. The thermal characteristics of PO and OZ treated starches were supported by several studies, where a higher gelatinization temperature was demonstrated from oxidized starch (Parovuori et al., 1995) and from hypochlorite oxidized starch (Forssell et al., 1995). The results of lower 1st onset temperature for ozonated starches agreed with Muhrbeck et al. (1990), in which a decrease in transition temperature might be due to oxidative cleavage of starch granule causing water to get access inside the starch granule easily; therefore, more gelatinization occurs resulting in higher energy needed to cook. The different results on thermal properties of oxidized starch might be due to different oxidation mechanisms depending on different treatments. OZ30 showed the lowest second enthalpy among all treated samples. This result agreed with Forssell et al. (1995). They found a decrease in second enthalpy but no change in apparent amylose content, which meant that lipid-bound amylose was oxidized by using sodium hypochlorite and concluded that this phenomenon could be explained if amylose complexes are enriched near the granule surface. It matched with our results that there was not a decrease in amylose content (Table 3.1).

Table 5.1 Effects of Pure Oxygen and Ozone on Thermal Properties of Sigma Rice Starch [1, 2, 3]

		First Transition (Gelatinization Endotherm)				Second Transition (Amylose-lipid Complex)			
Treatment	**Minutes**	**To**	**Tp**	**Tc**	**ΔH**	**To**	**Tp**	**Tc**	**ΔH**
		NO ADDITIVES							
None	0	60.19ef	69.3e	84.0cd	5.87c	96.05bcd	102.5cde	109.7bc	0.91a
Pure oxygen	15	69.59a	77.88a	89.2ab	6.54bc	97.92bc	105.1abc	111.8ab	0.73ab
	30	68.34ab	76.3ab	89.36ab	6.74bc	97.12bcd	104.2bc	111.5ab	0.77ab
Ozone	15	56.04g	72.98cd	80.74d	7.99ab	94.23d	100.3de	105.8d	0.75ab
	30	55.38g	72.66d	80.88d	7.84ab	94.75cd	100.1e	105.2d	0.49bc
		LYSINE							
None	0	61.86de	70.95de	85.77bc	6.4bc	103.0a	107.5a	113.38a	0.57bc
Pure oxygen	15	65.57bc	77.87a	89.54a	7.92ab	98.66b	105.5ab	111.3ab	0.52abc
	30	64.12cd	77.90a	89.40ab	8.55a	98.60b	105.6ab	111.58ab	0.71ab
Ozone	15	57.88fg	75.12bc	82.48cd	8.66a	97.71bc	103.1bcd	107.3cd	0.38c
	30	58.58efg	75.52ab	82.59cd	8.97a	98.54b	103.8bc	107.3cd	0.27c

[1]To=onset temperature; Tp= peak temperature; Tc= conclusion temperature; ΔH (Enthalpy)
[2]Units: Temperature (°C), Enthalpy (J/g, dry matter)
[3]Different letters within column indicate means are significantly different at the level of $p \leq 0.05$

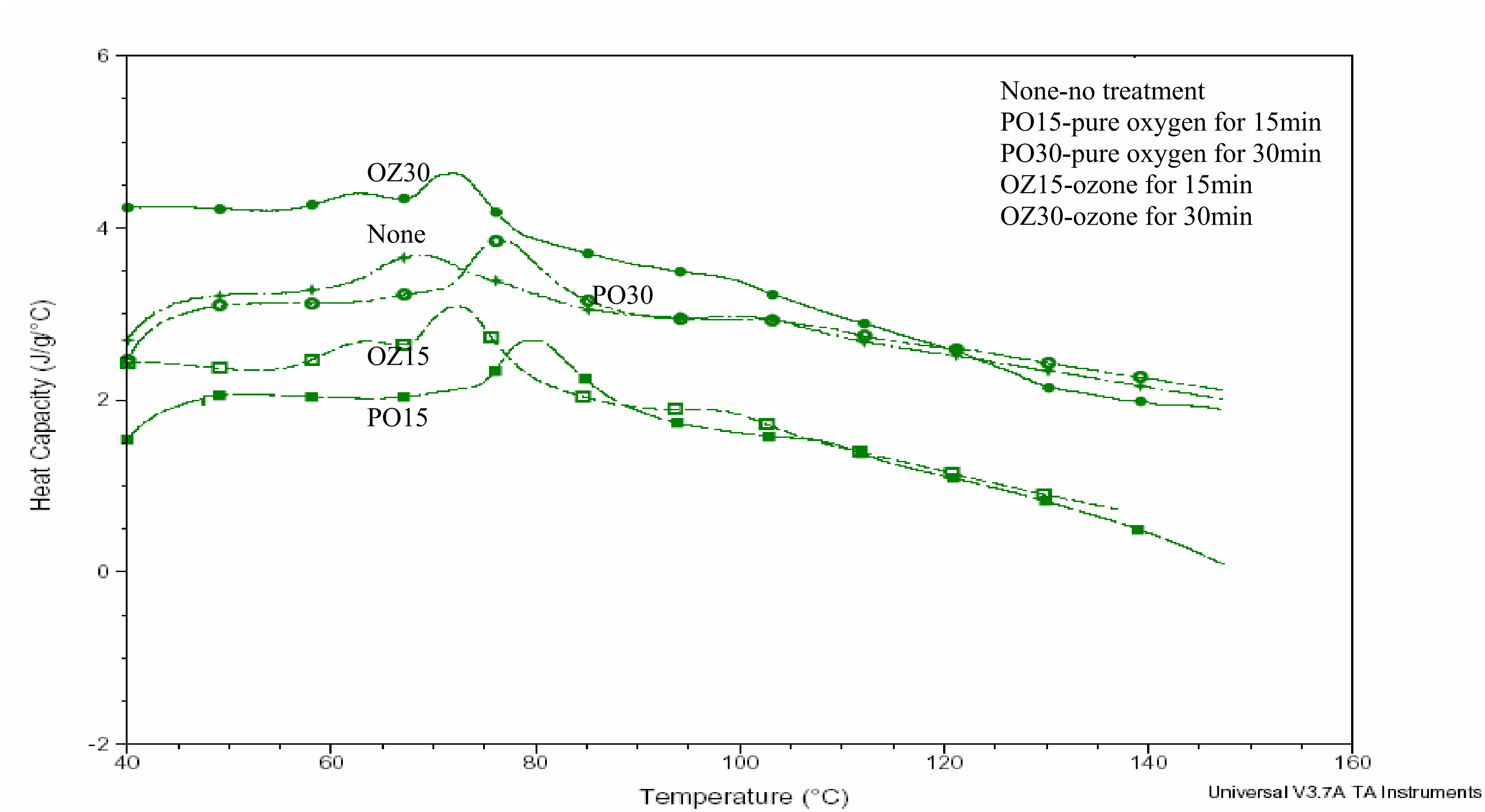

Figure 5.1 DSC Analysis of Sigma Rice Starch with no Additives

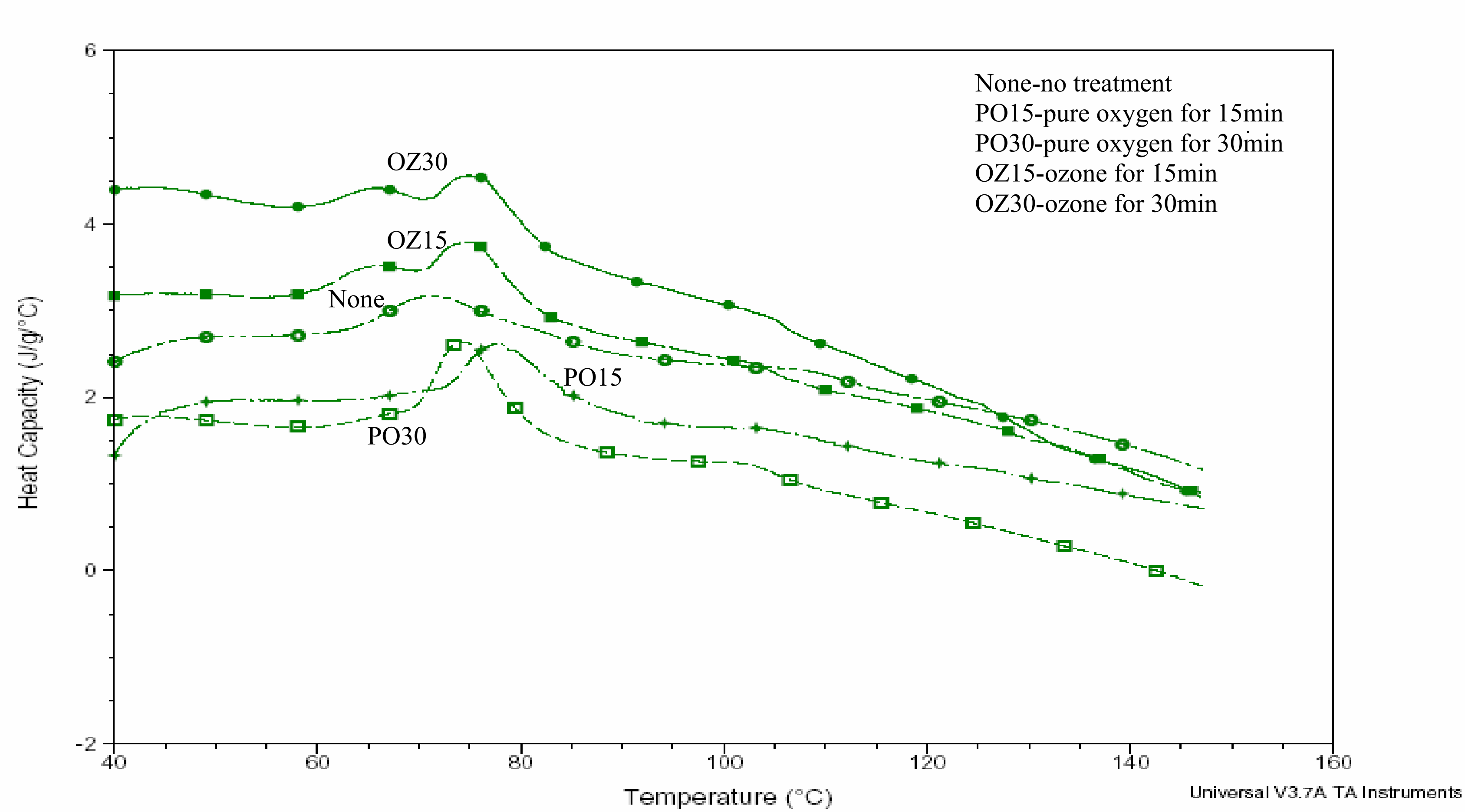

Figure 5.2 DSC Analysis of Sigma Rice Starch with Lysine

5.3.2 Effects of Lysine on Thermal Properties of Sigma Rice Starch

The presence of lysine (6%) increased the gelatinization endotherm slightly and amylose-complex transition temperatures, but lower enthalpy for untreated starches (Figure 5.3). Lysine on PO15 decreased 1st onset temperatures and 2nd enthalpy but increased 1st transition temperatures and 1st enthalpy compared to PO15 treated starch (Figure5.4). In addition, PO30 with lysine also reduced 1st To, but enhanced other transition temperatures and 1st enthalpy by 2 (J/g) compared to PO30 with no lysine (Figure 5.5). Furthermore, the addition of lysine (6%) on OZ15 increased gelatinization peak temperature (Tp) and second transition temperatures compare to without lysine, but reduced amylose-lipid complex enthalpy compared to OZ15 with no additives (Figure 5.6). In addition, the presence of lysine (6%) on OZ30 enhanced both gelatinization and amylose-lipid complex transition temperature, but decreased second enthalpy slightly compared to OZ30 with no additives (Figure 5.7). These results agreed with many studies, in which an increase in gelatinization transition temperature was observed. Liang (2001) reported that the presence of amino acids (aspartic acid, arginine, lysine, and glutamic acid) increased gelatinization temperature due to the protein effect on resistance in starch swelling. He also found a decrease in the enthalpy for amylose-lipid complex formation. In addition, Huang et al. (1994) found that rice flour with higher lipid and protein contents than rice starch had greater gelatinization temperature, but lower enthalpy since the non-starch constituents in flour prohibited starch gelatinization. Similarly, Marshall et al. (1990) reported a decrease in peak and final gelatinization temperature after lipid and protein removal from starch. Furthermore, Radosavljevic et al. (1998) agreed that isolated starch consisting of lower protein decreased gelatinization temperature, but enhanced enthalpy than those of untreated starch.

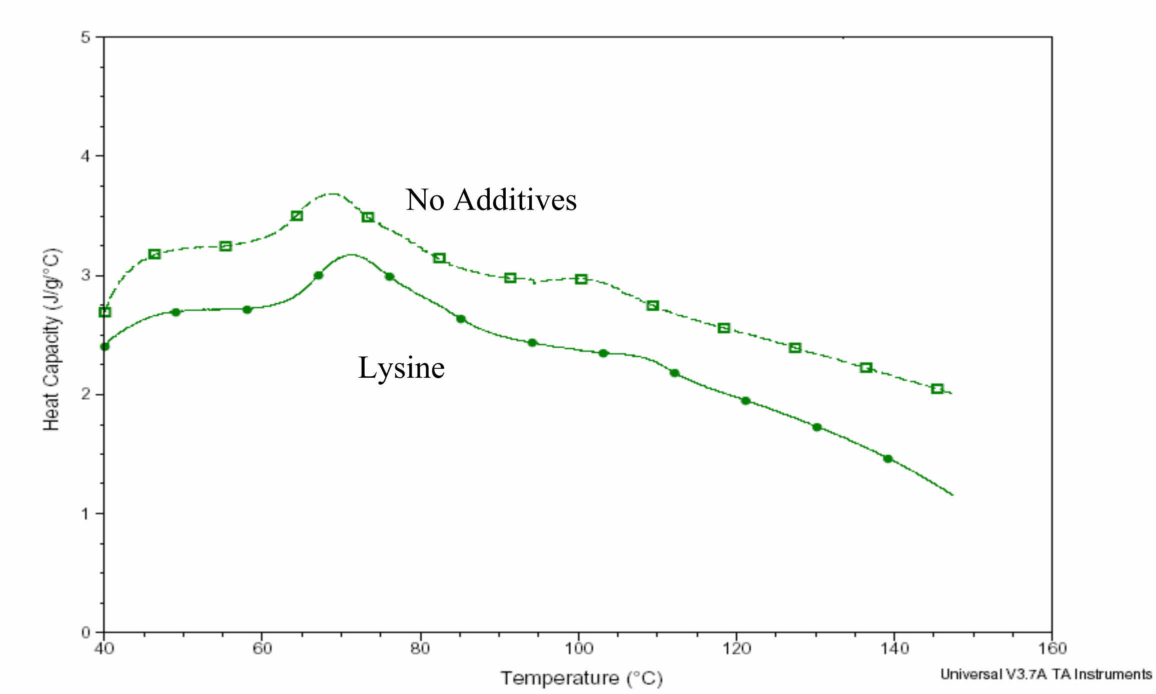

Figure 5.3 DSC Analysis of Sigma Rice Starch with no Treatment

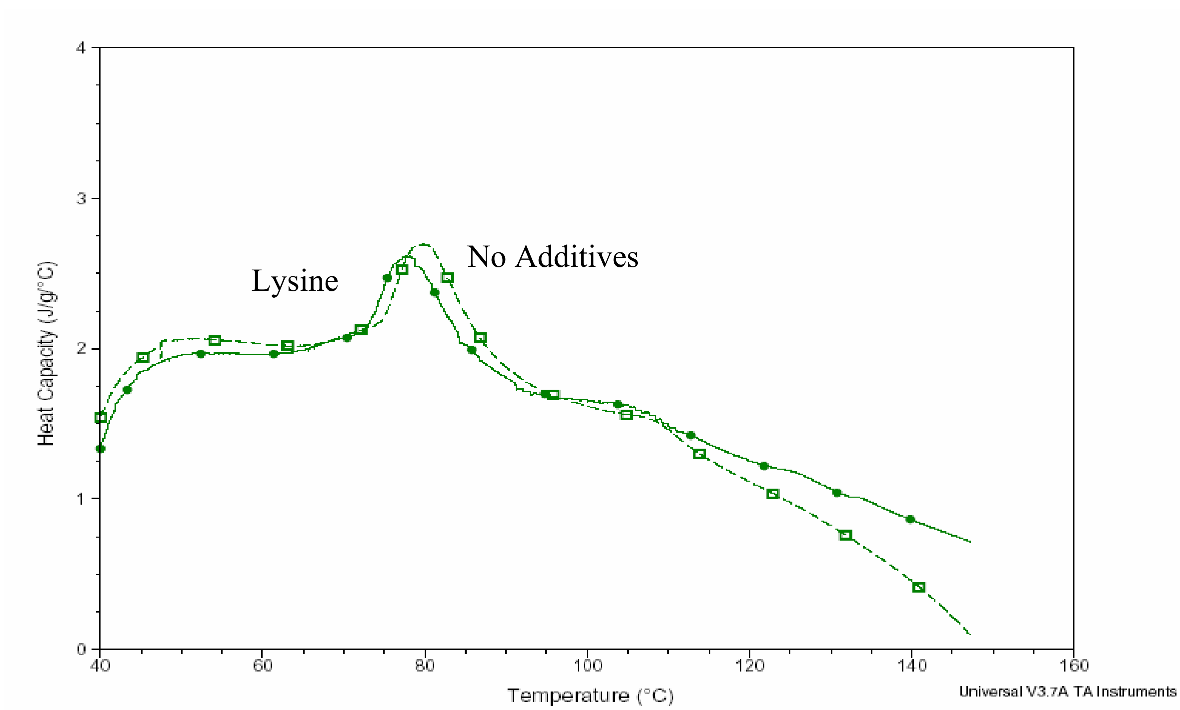

Figure 5.4 DSC Analysis of Sigma Rice Starch Treated with Pure Oxygen for 15 minutes

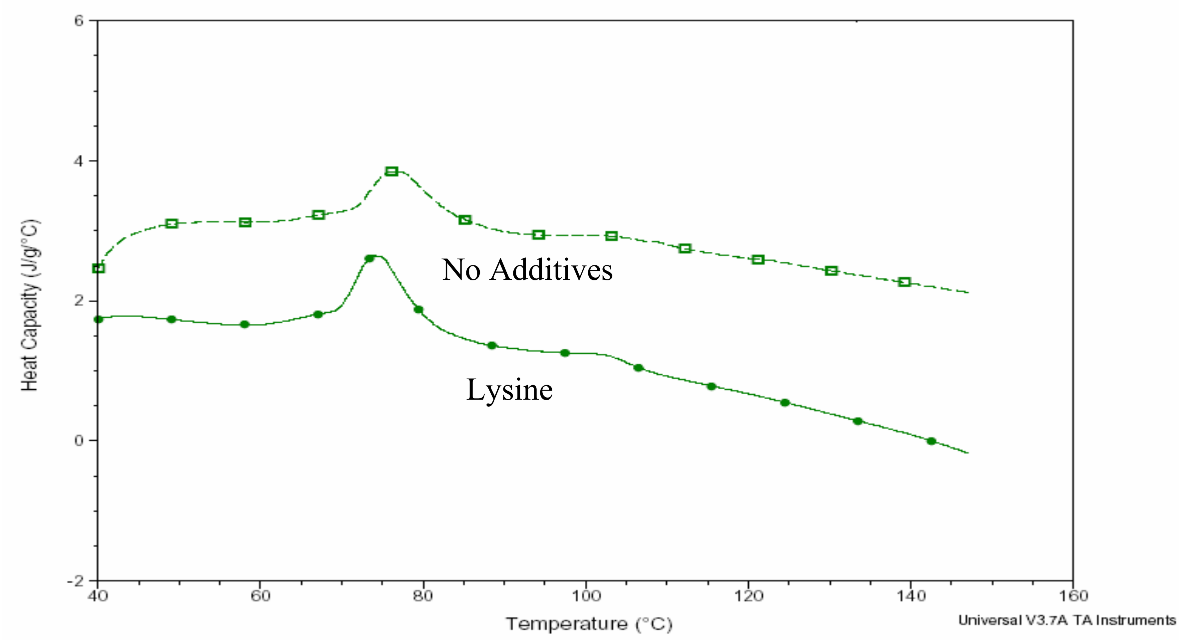

Figure 5.5 DSC Analysis of Sigma Rice Starch Treated with Pure Oxygen for 30 minutes

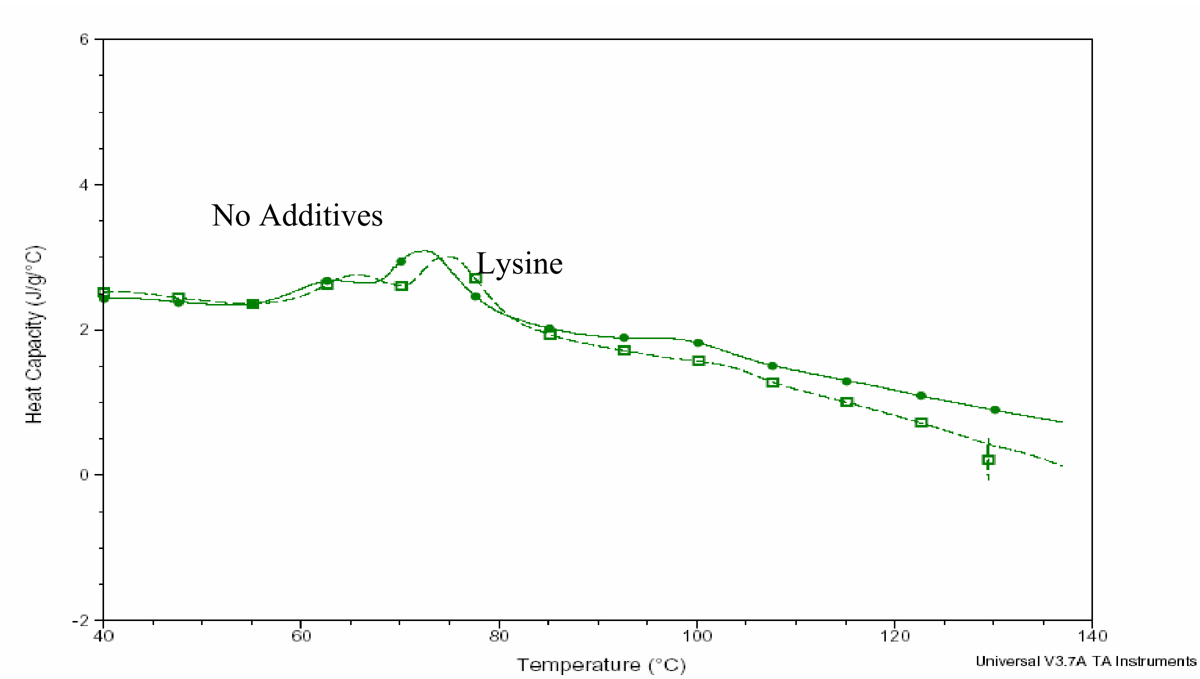

Figure 5.6 DSC Analysis of Sigma Rice Starch Treated with Ozone for 15 minutes

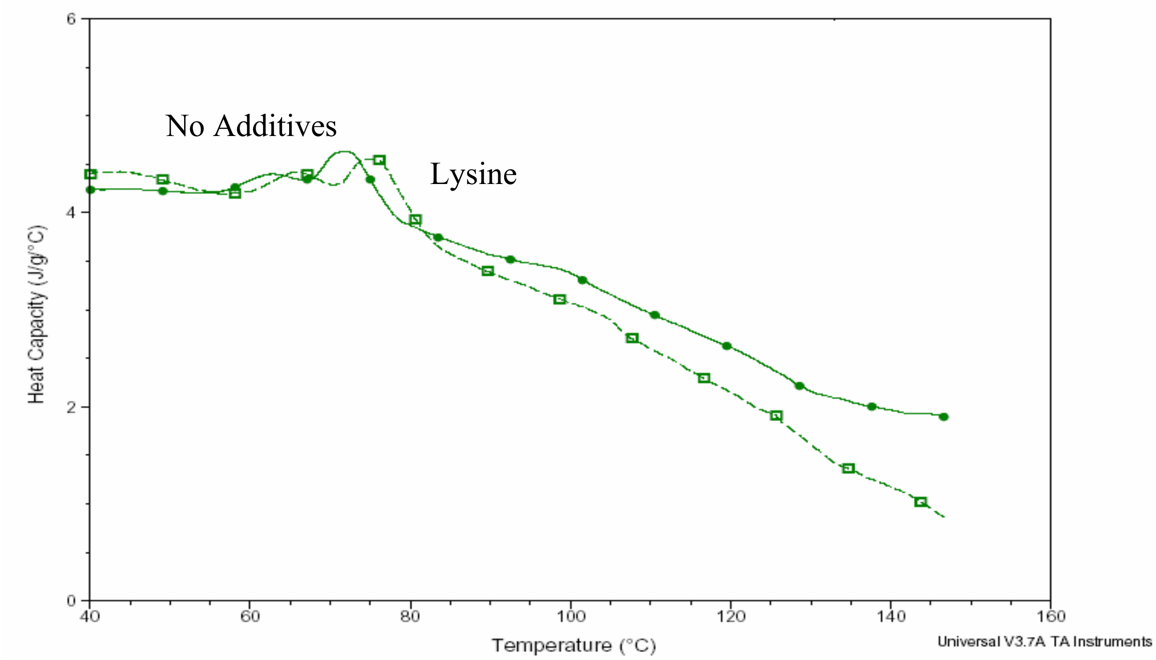

Figure 5.7 DSC Analysis of Sigma Rice Starch Treated with Ozone for 30 minutes

5.3.3 Effects of Pure Oxygen and Ozone on Thermal Properties of White Starch Isolate (WSI)

Unlike Sigma rice starch, both pure oxygen and ozone treatments for 15 minutes of WSI reduced gelatinization temperatures. However, second transition temperatures of PO15 and PO30 were not significantly different from those of untreated starches. Ozone treatment, on the other hand, decreased second transition temperatures as well. In comparison to pure oxygen, ozone reduced first and second transition temperatures to greater extents (Table 5.2 and Figure 5.8). Different results might be because of different structures of the two starches and/or because of different constituents of white starch isolate, especially the lipids and protein residues even after the isolation process (Table 3.2).With the presence of lysine (6%), all of treated starch samples (PO15, PO30, OZ15, and OZ30) decreased gelatinization temperatures and second transition temperatures compared to untreated starches with lysine. Moreover, PO30 decreased gelatinization enthalpy. Ozone treatment reduced temperatures of first and second endotherms greater than pure oxygen did. In addition, lysine added OZ15 and OZ30 enhanced gelatinization enthalpy, but decreased second enthalpy compared to lysine added non-treated starches (Figure 5.9).

5.3.4 Effects of Lysine on Thermal Properties of White Starch Isolate

The addition of lysine (6%) increased first and second transition temperatures and 1st enthalpy of untreated starches compared to those with no additives (Figure 5.10). However, second transition enthalpy was not significantly different from that of untreated starch with no additives, statistically. In comparison to PO15 and PO30 with no additives, the presence of lysine (6%) also enhanced temperatures of gelatinization and amylose-lipid complex endotherms, but did not change their enthalpies (Figure 5.11 and Figure 5.12). Moreover, the addition of

Table 5.2 Effects of Pure Oxygen and Ozone on Thermal Properties of White Starch Isolate [1, 2, 3]

		First Transition (Gelatinization Endotherm)				Second Transition (Amylose-lipid Complex)			
Treatment	**Minutes**	**To**	**Tp**	**Tc**	**ΔH**	**To**	**Tp**	**Tc**	**ΔH**
		NO ADDITIVES							
None	0	64.7c	70.5bc	82.4ab	6.7cd	97.1cd	103.4cd	109.7bcd	0.46ab
Pure oxygen	15	61.9de	66.8ef	76.1de	7.22bcd	95.3de	101.8de	107.8d	0.43ab
	30	63.9cd	69.3cd	77.8cd	5.25d	98.6bc	103.3cd	108.4cd	0.33b
Ozone	15	60.7e	65.2f	73.3e	8.8abc	92.8e	98.8e	103.5e	0.5ab
	30	63.9cd	69.3cd	77.8cd	5.25d	98.6bc	103.3cd	108.4cd	0.33b
		LYSINE							
None	0	67.4a	73.0a	85.2a	7.0b	101.3ab	107.7a	114a	0.45ab
Pure oxygen	15	65.1b	69.8b	79.1bc	7.36b	99.0c	105.3bc	111.4bc	0.59a
	30	67.1a	72.0a	80.1b	5.26c	102.5a	106.8ab	111.8ab	0.34bc
Ozone	15	63.8bc	68.2c	77.1c	9.5a	99.8bc	104.4c	108.6d	0.34bc
	30	63.7c	68.0c	77.2c	9.3a	100bc	104.6c	109cd	0.29c

[1]To=onset temperature; Tp= peak temperature; Tc= conclusion temperature; ΔH (Enthalpy)
[2]Units: Temperature (°C), Enthalpy (J/g, dry matter)
[3]Different letters within column indicate means are significantly different at the level of $p \leq 0.05$

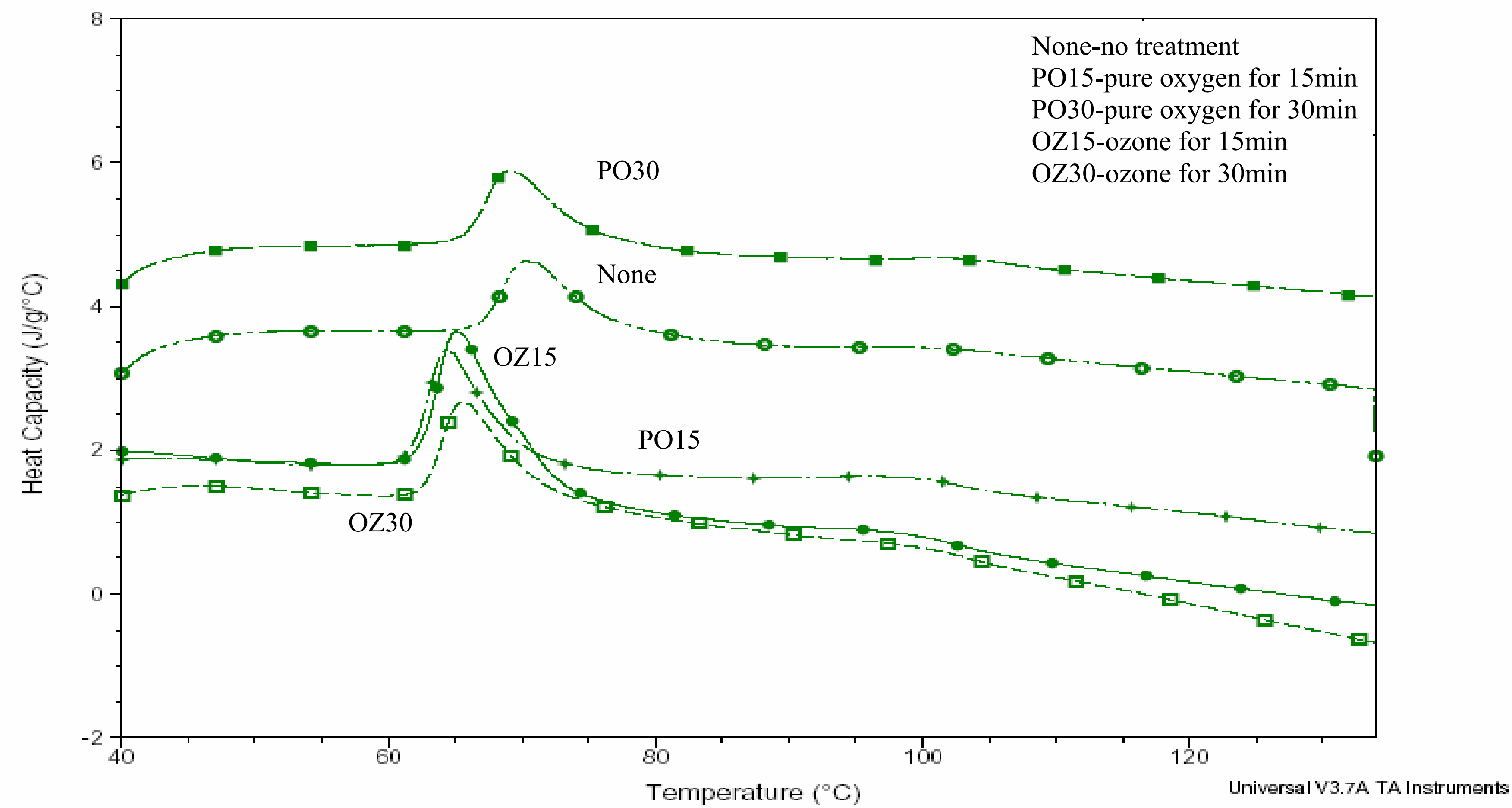

Figure 5.8 DSC Analysis of White Starch Isolate with no Additives

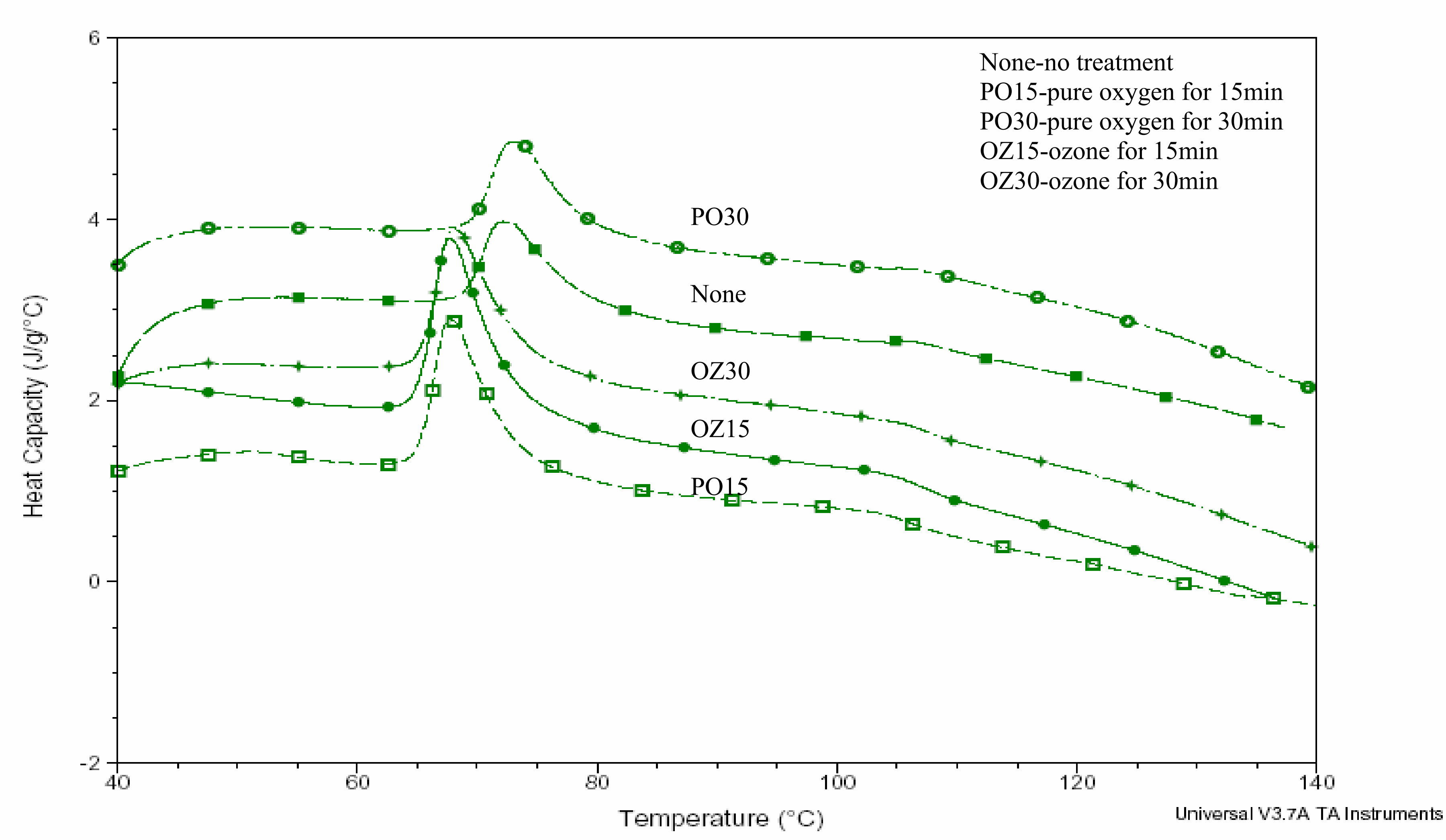

Figure 5.9 DSC Analysis of White Starch Isolate with Lysine

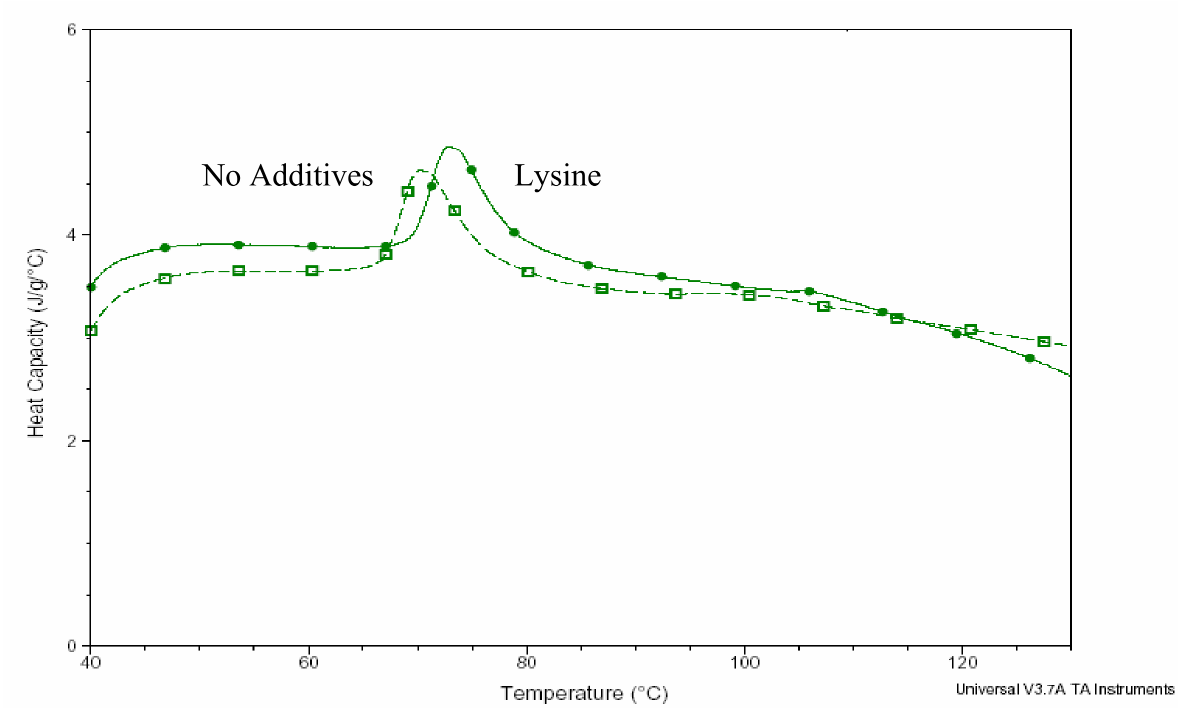

Figure 5.10 DSC Analysis of White Starch Isolate with no Treatment

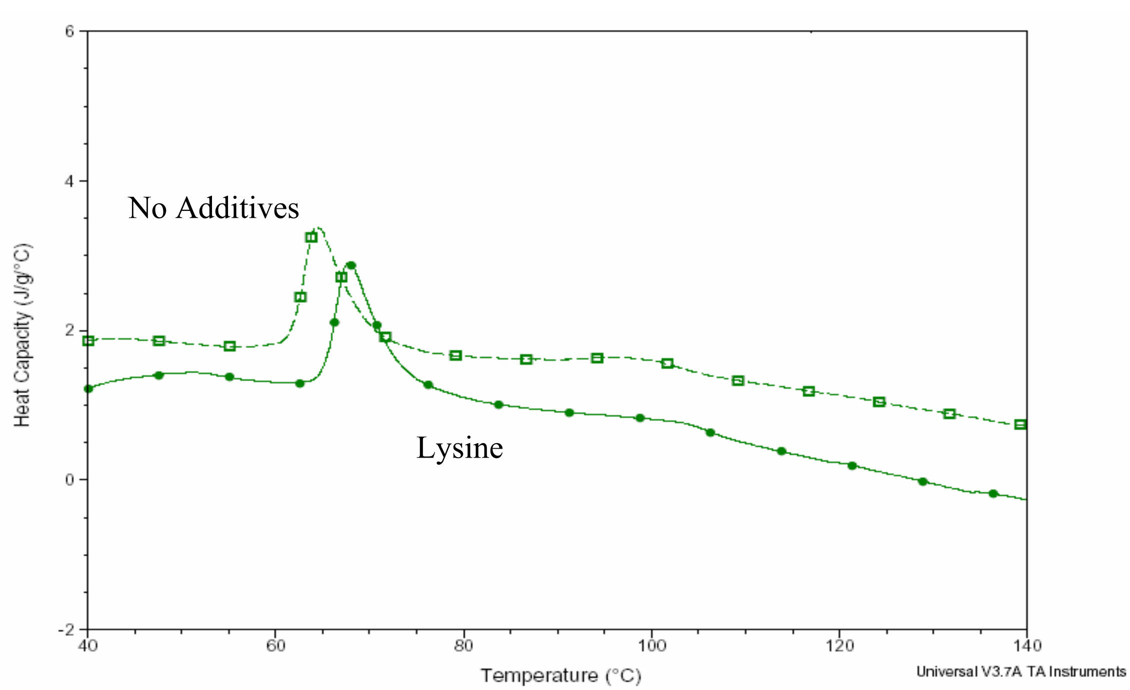

Figure 5.11 DSC Analysis of White Starch Isolate Treated with Pure Oxygen for 15 minutes

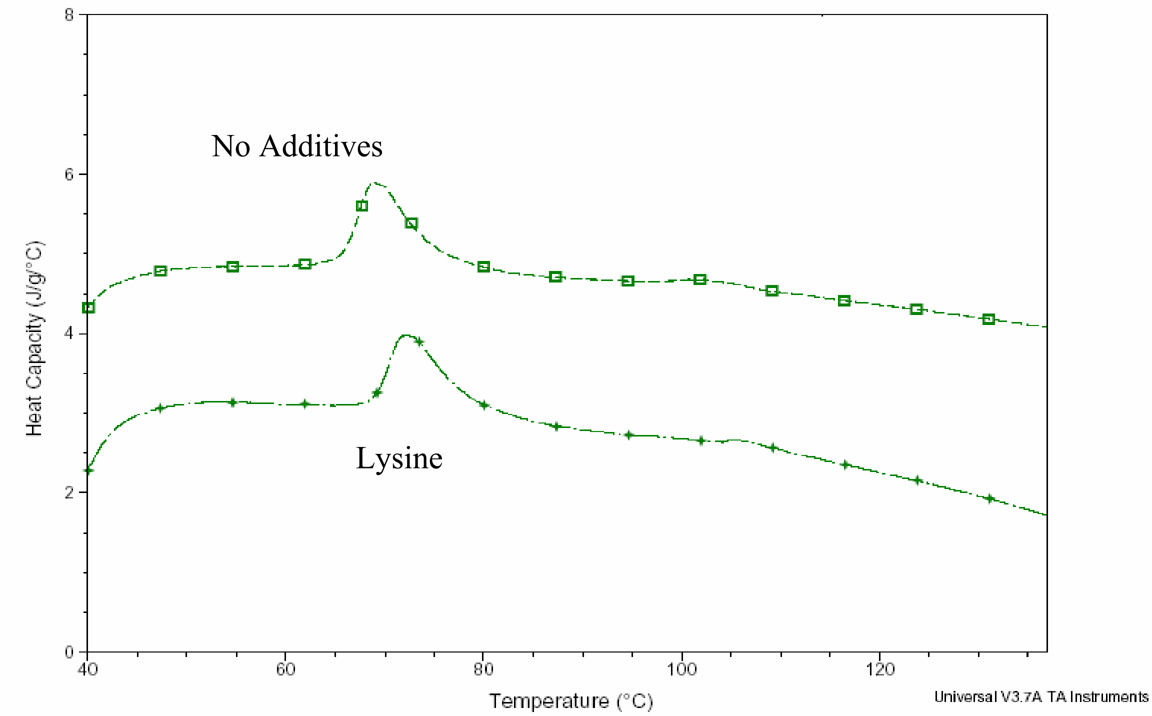

Figure 5.12 DSC Analysis of White Starch Isolate Treated with Pure Oxygen for 30 minutes

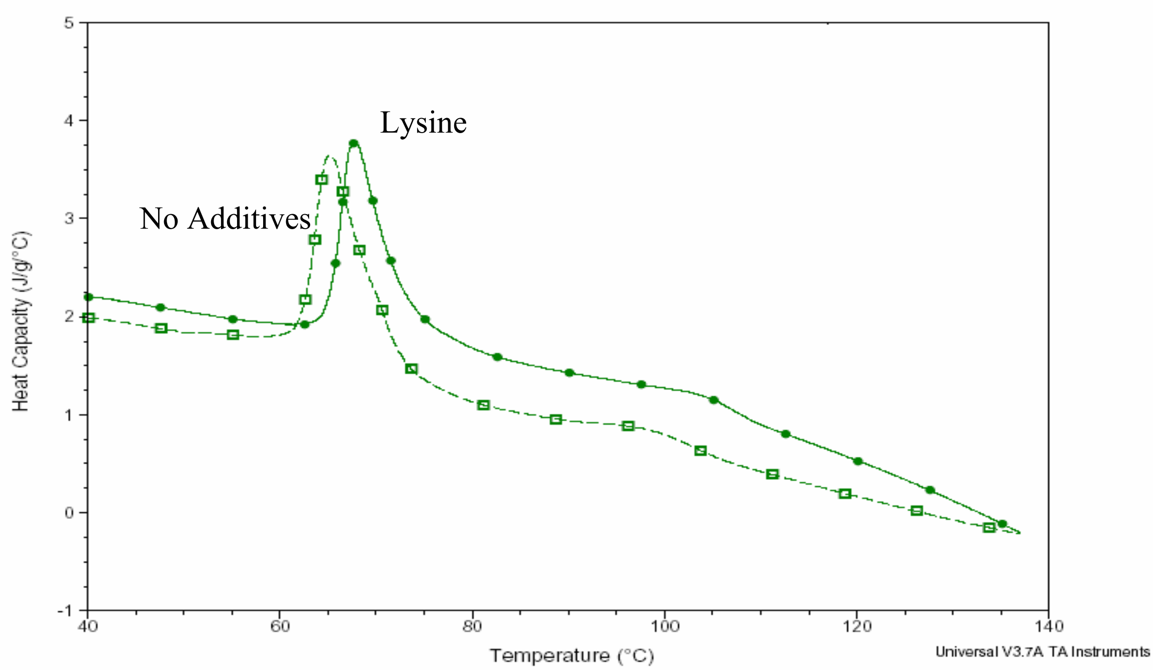

Figure 5.13 DSC Analysis of White Starch Isolate Treated with Ozone for 15 minutes

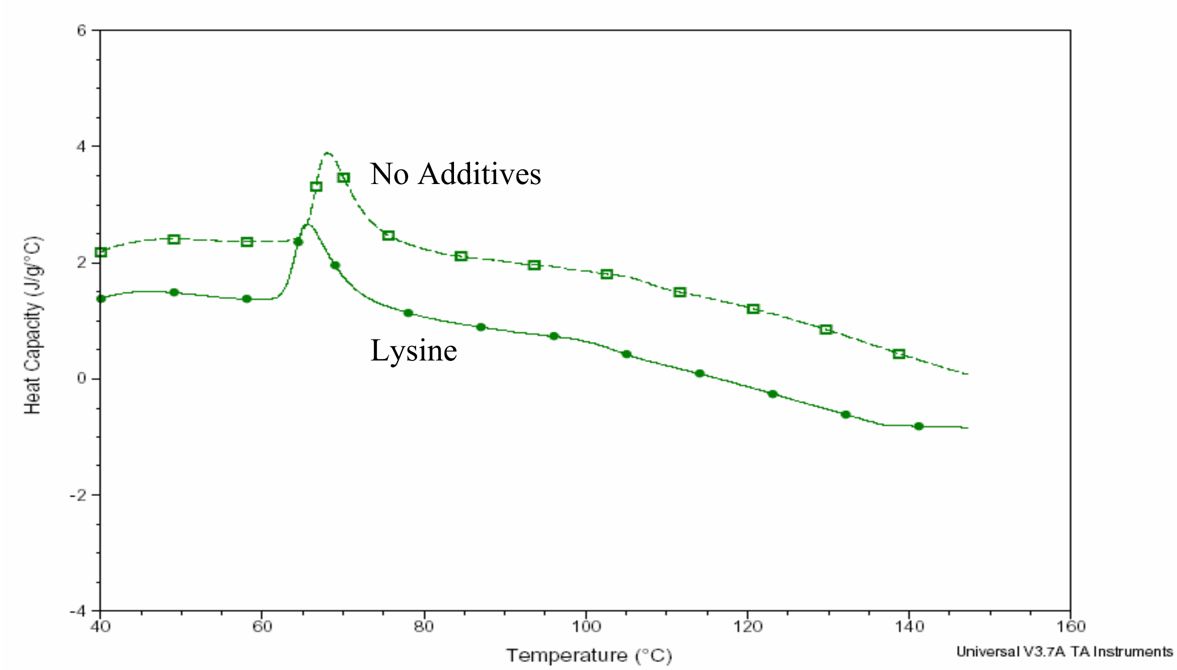

Figure 5.14 DSC Analysis of White Starch Isolate Treated with Ozone for 30 minutes

lysine (6%) in OZ15 showed higher gelatinization temperatures and second transition temperatures (Figure 5.13). OZ30 with lysine presented a higher gelatinization enthalpy than OZ30 with no additives, whereas lysine (6%) on OZ15 did not affect it significantly (Table 5.2 and Figure 5.14).

Among the treatments, ozonation with lysine showed the highest gelatinization enthalpy but the lowest second transition enthalpy. The results were similar to those of ozonated Sigma rice starch.

5.4 CONCLUSION

For Sigma rice starch, ozonation reduced 1^{st} onset and conclusion temperatures and 2^{nd} transition temperatures including enthalpy, but increased 1^{st} peak temperature and enthalpy. Pure oxygen treatment also increased 1^{st} endotherm including enthalpy indicating heat stable starch that more energy is required to cook. This result agreed with our data from pasting characteristics, where pure oxygen treated starches increased cooking stability. Moreover, the presence of lysine (6%) increased gelatinization endotherm and the combination with ozonation decreased second enthalpy even more than any of them itself.

For white starch isolate, ozonation decreased 1^{st} transition temperatures. It could be concluded that oxidative degradation might have occurred during ozonation resulting in starch granule damage; therefore, starch swells more and faster. The lower second enthalpy with addition of lysine on ozonated starch might be due to the fact that starch-protein interaction became competitive with amylose-lipid complexes on damaged starch granules.

CHAPTER 6

EFFECTS OF OZONATION AND ADDITION OF LYSINE ON CRYSTALLIZATION OF RICE STARCHES USING X-RAY DIFFRACTION (XRD)

6.1. INTRODUCTION

Starch granule consists of both crystalline and amorphous regions. Amylose fraction is attributed to amorphous regions, which is destroyed during cooking since amylose leached out from starch granules in hot water. On the other hand, amylopectins are responsible for crystalline structure, which shows a well developed XRD pattern from waxy-maize starch (Steeneken, 1984). Under electron microscopy, starch granule consists of pronounced concentric rings, which are amorphous composition and semi-crystalline, where the semi-crystalline rings are stacks of alternating crystalline and amorphous lamellae (Yamaguchi et al., 1979). The crystalline structure consists of a radial arrangement of amylopectin clusters, and each cluster contains short chain segments of formed double helices (the crystalline lamellae) and highly branched amorphous lamella (Germani et al., 1983). Moreover, clusters of short chain molecules that are interlinked and regularly spaced were confirmed by X-ray diffraction (Zobel, 1988a). It is known that waxy (0.5-1% amylose) starches present higher crystallinity and swell more than regular starches (Vasanthan et al., 1999). On the other hand, amylose takes an important role in retrogradation upon cooking. Crystallization is caused by the strong tendency of hydrogen bonds formation between hydroxyl groups on starch molecules (Germani et al., 1983). Retrogradation of starch is generally related to linear amylose depending on the formation of interchain hydrogen bonds, while recrystallization of amylopectin is slowed by its branched structure.

The crystal structure is characteristic of the plant source (Katz, 1937), and the starch crystal forms can be distinguished as A, B, C or V by their XRD patterns (Zobel, 1988b). Generally, cereal starches such as wheat, maize, and rice starches are usually of the A pattern, while starches from tubers such as potatoes tend to exhibit B-pattern crystallinity (Zobel, 1988b). In addition, C-pattern starch, found in some legumes, root and seed starches, is a mixture of the A and B patterns and amylose-lipid complexes give V-type pattern (Czuchajowska et al., 1991). Each type of X-ray diffraction pattern has distinguishing features. A-type XRD pattern shows three strong peaks at 5.8, 5.2, and 3.8 Angstroms (Å), whereas B-type XRD shows a peak at 15.8-16.0 Å, a broad medium intensity line at about 5.9 Å, a strong line at 5.2 Å and a medium intensity doublet at 4.0 and 3.7 Å. C-type XRD pattern is similar to A-type XRD pattern with the addition of the medium to strong peak at about 16.0Å. Finally, V-type XRD pattern is typically shown by the amylose complex formation and the peaks appear at 12, 6.8, and 4.4 Å (Zobel, 1988b).

Starch granule XRD patterns of the B or C type are known to be more resistant to acidic or enzymatic hydrolysis; therefore, to digestion by pancreatic amylase (Fuwa et al., 1980; Jane, 1997). In addition, some studies have shown that the degree of crystallinity affects other important starch physicochemical properties such as reactivity towards reagent during chemical modification (Zobel, 1988b). Retrograded starch characteristically forms the B-type XRD pattern (Cairns et al., 1990), although amylose fragments in RS fractions showed less crystallinity, indicating poor crystalline structures compared to native starch (Sievert et al., 1991; Berry et al., 1988). Furthermore, X-ray diffraction (XRD) showed different patterns among the crystallites formed at different temperatures. According to Eerlingen et al. (1993a), resistant starch (RS) formed at 100°C presented A- pattern, which was different from that formed by incubation at 0 or 68°C (B pattern). Moreover, RS with two storage steps (0° and 68°C or 0° and 100°C) to favor nucleation and the propagation produced B type diffraction as well (Eerlingen et al., 1993a). It was postulated that RS might be formed by aggregation of amylose helices in a crystalline B type structure (Eerlingen et al., 1993b). Shamai et al. (2003) agreed with different XRD patterns resulting from different temperature treatments, in which retrogradation at 40°C lead to the formation of B-type XRD pattern, while incubation at 95°C produced a mixture of A and V-type XRD pattern.

It was reported that amylose –lipid complexes are highly crystalline giving V-type polymorph (Czuchajowska et al., 1991). Complexes reduced solubility of amylose and resulted in a conformational hindrance to enzymatic digestion from the V helix form (Holm et al., 1983). XRD patterns of normal rice immediately after cooking showed a V-type pattern that may be attributed to helical complexes of amylose with lipids in starch. Moreover, V-type pattern intensity changed to B-type implying that the starch-lipid complexes were metastable and changed to more stable structure partly characterized by B-type X-ray pattern via an amorphous state (Hibi et al., 1990). According to Hizukuri (1985), starch crystallinity varies with botanical sources owing to different amylose/amylopectin ratio and with chain length of amylopectin, in which A-type starch shows shorter amylopectin chain lengths than that of B-type starch. For example, potato starch is more reactive than cereal starches in modification since B-type crystal structure is less stable than the A-type crystals found in cereal starches (Zobel, 1992).

Modification of starch has been shown to change the XRD pattern. Steeneken (1984) reported that heat-moisture treatment of potato starch primarily affects the amylopectin fraction; therefore, the X-ray pattern was changed and shifted from type B via C to A. Sair (1967) also found the change of XRD pattern with heat-moisture treatment of potato starch, which gave A + C X-ray diffraction instead of the B pattern typical of potato starch. It was explained that increased intermolecular association from rotation of starch molecules resulted in the physical changes and that the rearrangement of starch molecules was shown by the change in the XRD pattern. In addition, it was found that cross-linked starch produced more crystalline bread due to extensive crystallization, which was corresponding in bread crumb firmness. Moreover, it was revealed that the proportion of B-type X-ray pattern was greater in gels of cross-linked starch than unmodified starch (Zobel and Senti, 1959).

It was suggested that the protein quality of the flour was correlated to the rate of firming and that the effect of protein on bread firming might be explained by interactions among swollen starch granules, partial solubilization of starch molecules, and protein (Maleki et al., 1980). Furthermore, several studies proposed that protein and starch interaction might influence the quality and the staling of the final products and the availability of starch to digesting enzymes (Dreese et al., 1988; Holm et al., 1985; Bjorck et al., 1986). In fact, it was showed that surface proteins in starch might hinder the access of amylolytic enzymes or might interact with them, causing a modification of their surface distribution (Greewell et al., 1985).

There have been studies on X-ray diffraction of oxidized starch molecules to see if oxidation influenced crystallinity and conflicting results have been presented. Han and Ahn (2002) found a decrease in relative crystallinity and a change in X-ray diffraction pattern with NaOCl with higher than 0.5% active Cl/g. Thus, oxidation occurred not only in amorphous regions but in crystalline part of starch. Moreover, they also implied that there might have been a change in molecular structure. On the other hand, it was reported that there was no X-ray diffraction change when oxidation was conducted on potato, corn, and rice starches with sodium hypochlorite. The explained reason was that oxidation took place mainly in the amorphous region (Kuakpetoon and Wang, 2001).

The objectives of this study were 1) to study the XRD pattern of ozonated rice starches; 2) to determine the influence of lysine on ozonated starch granule XRD patterns

6.2 MATERIALS AND METHODS

6.2.1. Materials

Sigma rice starch was purchased from Sigma Chemical Co. (S7260) while white rice flour was obtained from Riviana Foods Inc. (Abbeville, LA). Positive charged amino acid (lysine), potato amylose (A0512), amylopectin (A8515), and protease (P5147) were purchased from Sigma Chemical Co. (St. Louis, MO).

6.2.2. Sample Preparation and Pure Oxygen and Ozone Treatments

For details of sample preparation, pure oxygen and ozone treatments, refer to Chapter 3.

6.2.3. X-Ray Diffraction

The treated starch samples that were freeze-dried and ground into powder were hydrated at 75% relative humidity (RH) in a sealed vessel using saturated NaCl before the X-Ray Diffraction test. Sodium chloride was added to distilled water until there was no dissolution point. The starch samples with the solution in a vessel were left overnight at room temperature. About 1g of hydrated sample was pressed into a 10X25 mm pellet with a hydraulic press and X-ray diffraction pattern was obtained using Siemens D5000. X-ray diffractograms was obtained under conditions of 40 KV, 30mA, with the scanning angle 2θ set from 2° to 36° at a scanning rate of 0.6°/min. Relative crystallinity (RC) of the starches was determined by the method of Hermans and Weidinger (1948), as described by Nara et al (1978) as following:

$$a_c = x\ A_c$$

Where a_c is the area of the crystalline fraction,

x is crystallinty,

A_c is the diffraction area for a 100% crystalline substance. The area of the crystalline fraction in raw Sigma rice starch XRD pattern was used as the value of A_c (Dragsdorf and Varriano-

Marston, 1980). All the peaks were according to d-spacings and the data was recorded and compared.

6.3 RESULTS AND DISCUSSION

The raw Sigma rice starch presented several peaks at 3.9, 5.2, and 5.8 Å, which indicates a typical A-type pattern of XRD (Figure 6.1). In general, A- pattern XRD is recognized as cereal starch crystal form (Zobel, 1988a, b; Zobel and Senti, 1959). Gelatinized commercial rice starch showed one peak at 4.4 Å, which might have resulted from amylose-lipids complex (Figure 6.1). The crystallinity of amylopectin is destroyed during gelatinization; however, lipids present in starch form a helical inclusion complex with amylose molecules, which presents V-pattern XRD (Zobel, 1988b). The relative crystallinty (RC) of gelatinized rice starch was 46.7% compared to raw starch.

6.3.1 Effect of Pure Oxygen and Ozone Treatment on XRD Diffraction Pattern of Gelatinized Rice Starches

In comparison to untreated Sigma rice starch, pure oxygen for 30minutes (PO30) increased the intensity of the peak at 4.4 Angstroms, and a new peak at 5.2 Å appeared (Figure 6.2). Moreover, RC was enhanced by 18.2%. The intensity pattern of ozone treatment for 30minutes (OZ30) had similar trend to PO30, in which two different peaks at 4.4 and 5.2 Å were shown. In addition, there was a small peak at 4.0 Angstroms, indicating B+V type pattern XRD. The RC was also increased by 8%, but lower than that of PO30 compared to gelatinized starch with no treatment.

The XRD of white starch isolate is shown in Figure 6.3. The intensity of untreated WSI was presented at 4.4 Å, and the RC was 54.6%. Pure oxygen reduced the 4.4 Å peak compared to untreated white starch isolate. On the other hand, PO30 induced a peak of 5.2 Å, and the RC was

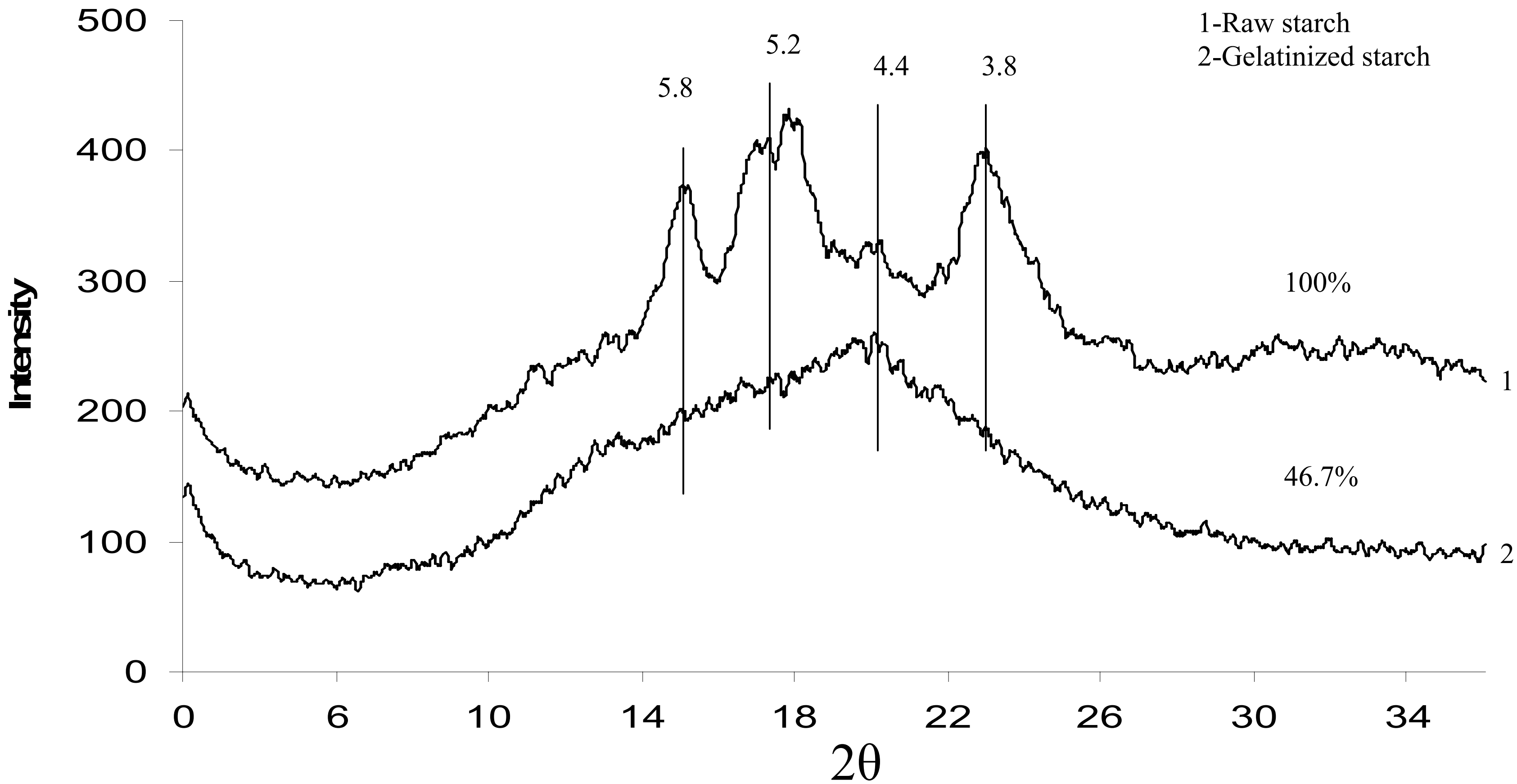

Figure 6.1 X-ray Pattern of Raw and Gelatinized Sigma Rice Starch

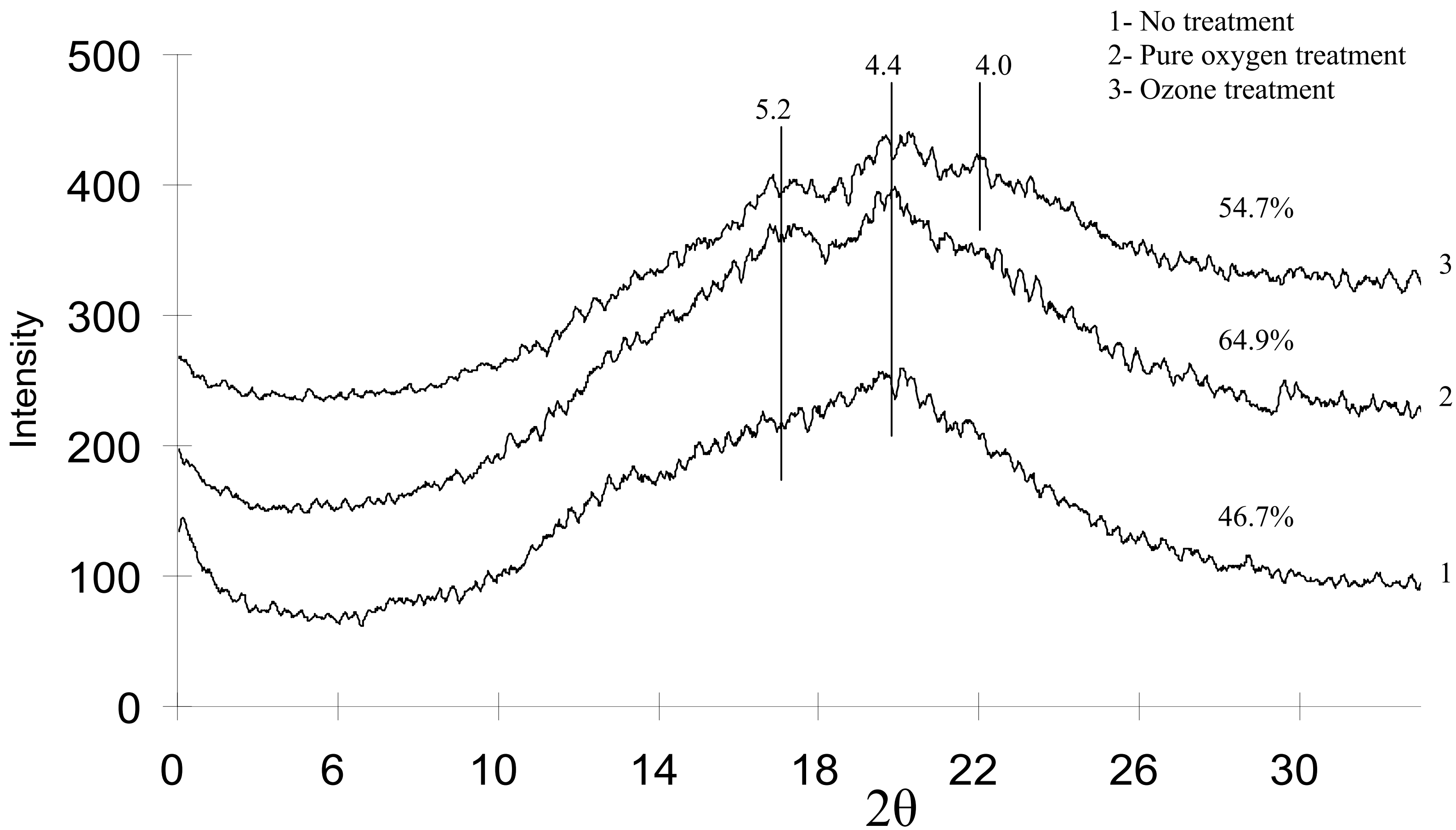

Figure 6.2 X-ray Pattern of Gelatinized Sigma Rice Starch Treated with Pure Oxygen or Ozone for 30 minutes

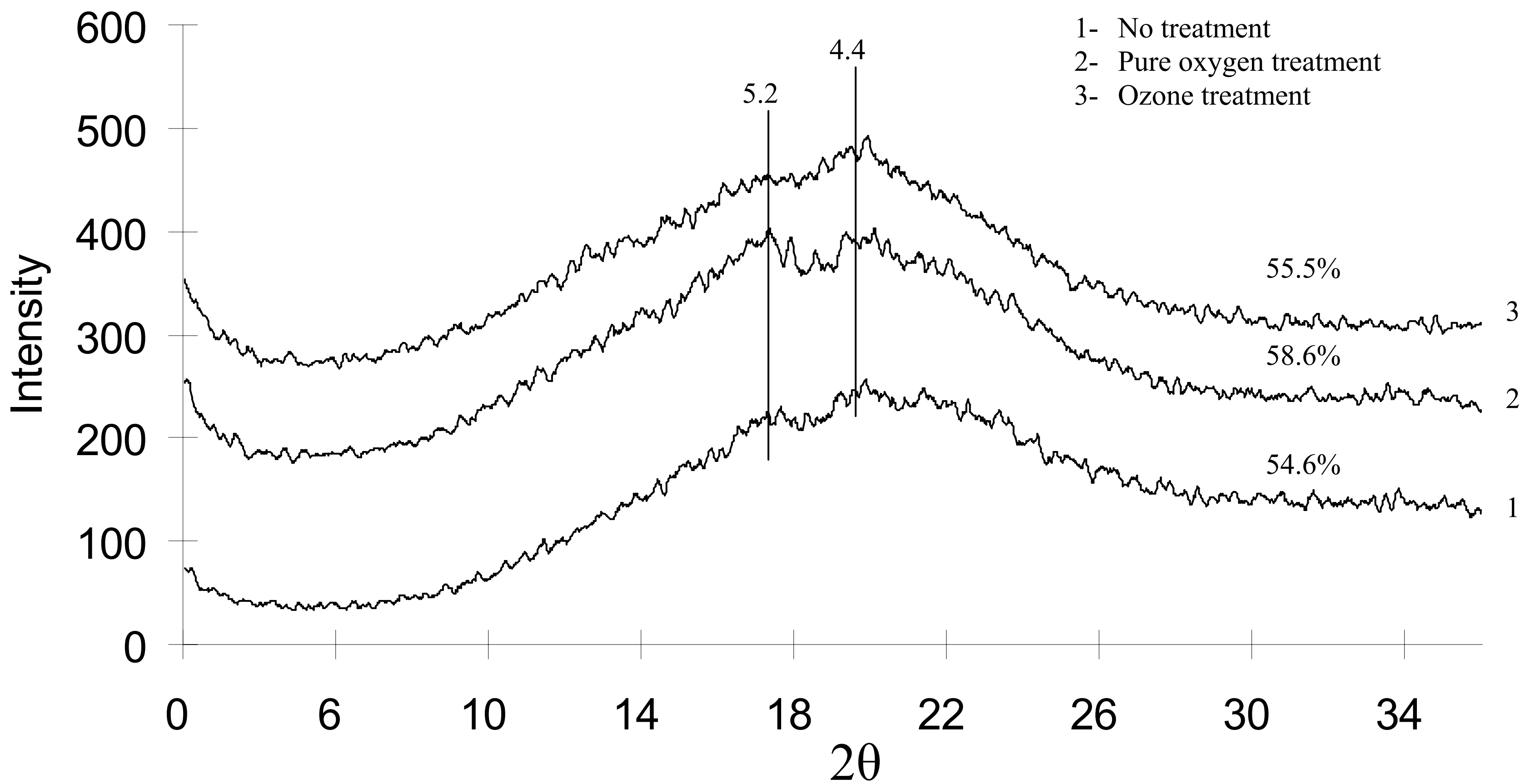

Figure 6.3 X-ray Pattern of Gelatinized White Starch Isolate (WSI) Treated with Pure Oxygen or Ozone for 30 minutes

increased by 4%. The crystallinity of OZ30 was similar to that of untreated white starch isolate with a peak at 4.4 Å. The RC of OZ30 on WSI was increased by only 1%, but lower than that of PO30.

6.3.2 Effect of Lysine on XRD Diffraction Pattern of Gelatinized Rice Starches

In comparison to untreated with no additives, the presence of lysine (6%) induced peaks at 4.0 and 5.2 Å (Figure 6.4). In addition, the RC was enhanced by 11%. Compared to OZ30 with no additives, the intensity of crystallinity at 4.0 and 5.2 Å were enhanced when lysine was added (Figure 6.4). Besides that, there were new weak peaks at 3.8 and 5.8 Å, which presented A + B pattern XRD. Moreover, lysine (6%) increased the relative crystallinity (RC) by 8.6%. However, the peak at 4.4 Å did not change with the presence of lysine. Furthermore, when ozonated Sigma rice starch with lysine was compared to untreated starch with lysine, apparently, OZ30 induced peaks at 3.8 and 5.8 Å, and the RC of ozone with lysine added starch was higher than that of untreated sample by 5.6 %.

The presence of lysine (6%) on ozonated WSI resulted in a decrease at the peak of 4.4 Å, but induced a strong peak at 5.2 Å. In addition, the RC was increased by 4.2 % compared to OZ30 with no lysine (Figure 6.5). In comparison to untreated WSI with no additives, the addition of lysine (6%) induced two peaks at 4.0 and 5.2 Å, which was also presented for Sigma rice starch. The effect of ozone with lysine on WSI showed a stronger peak at 5.2 Å, and enhanced the RC by 3.3 %.

6.4 CONCLUSION

Gelatinization by RVA (Rapid Visco-Analyzer) destroyed typical A-type XRD pattern, and induced V-type XRD pattern, which exhibited amylose-lipid complexes. Ozone treatment on gelatinized Sigma rice starch changed the XRD from V to B+V with the new peaks at 4.0 and 5.2

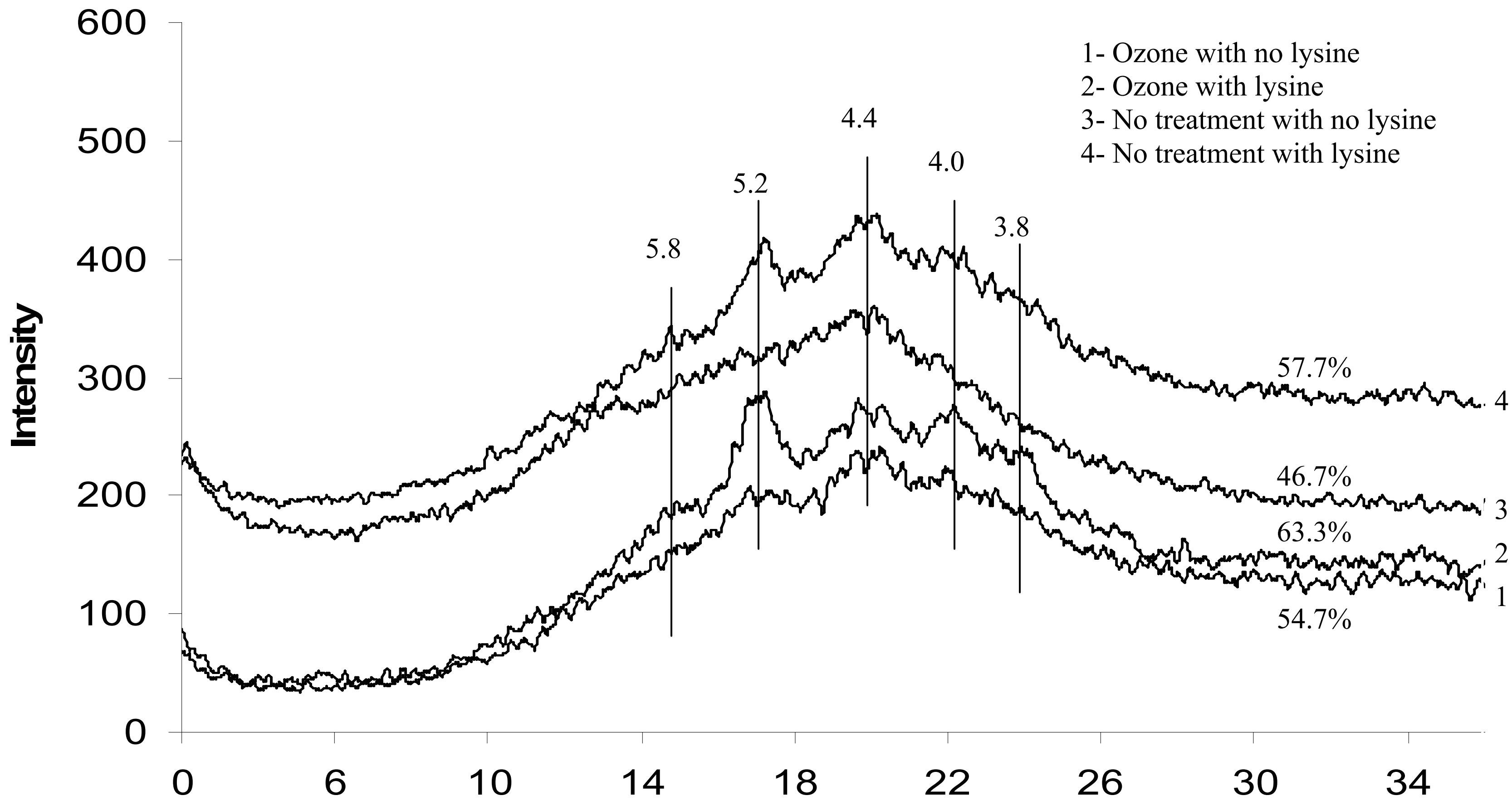

Figure 6.4 X-ray Pattern of Gelatinized Sigma Rice Starch with or without Lysine

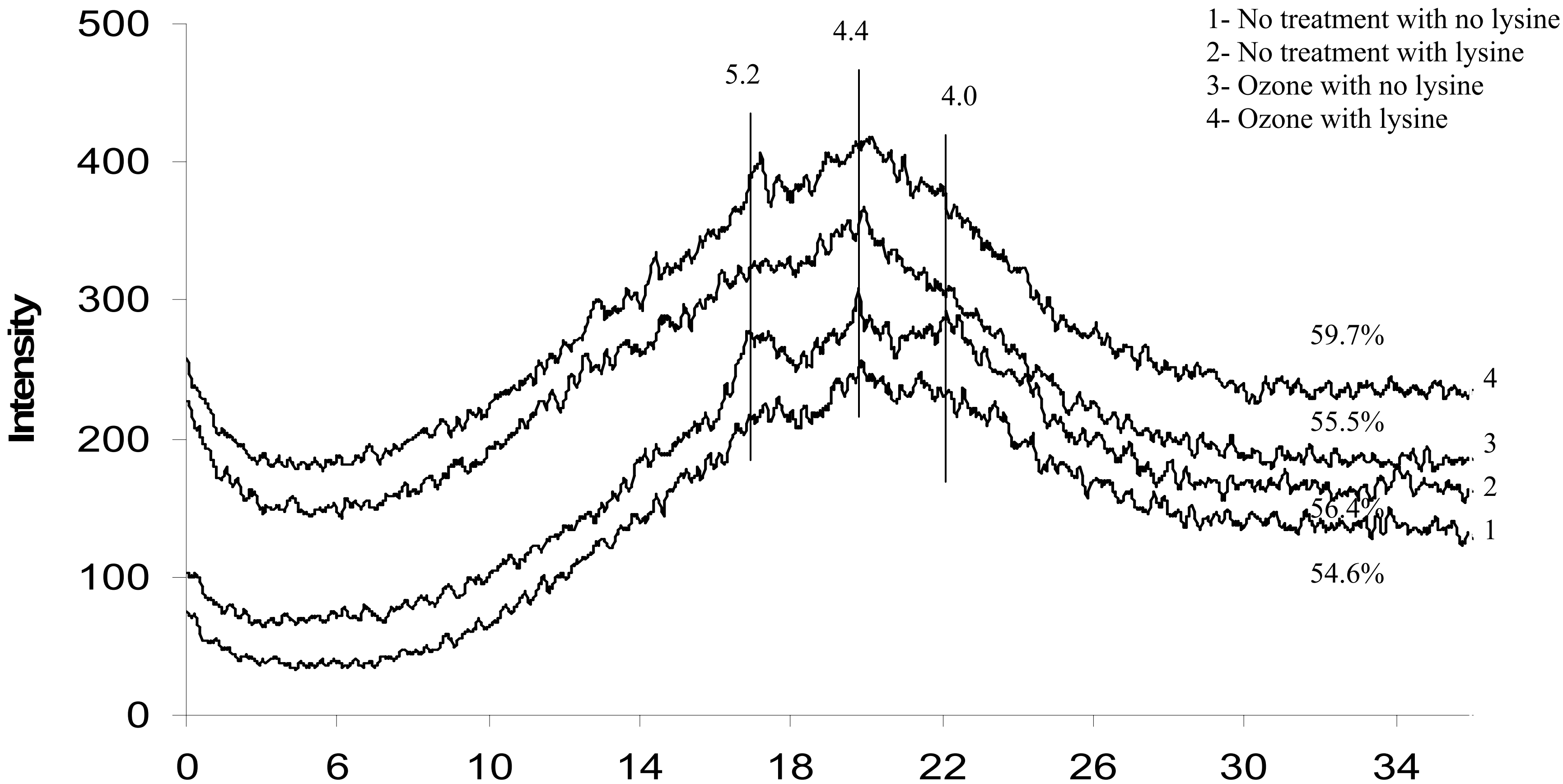

Figure 6.5 X-ray Pattern of Gelatinized White Starch Isolate (WSI) with or without Lysine

Å. Furthermore, the combination of ozone and lysine created the A+B XRD pattern with the peaks at 3.8, 4.0, 4.4, 5.2, and 5.8 Å. Moreover, the RC of ozonated starch with lysine that had been gelatinized was increased by 16.9% compared to gelatinized starch with no treatment. These results might have related to crystalline resistant starch which usually shows B type XRD pattern. It was revealed that retrograded amylose fragments in resistant starch are composed of amorphous regions connected with small or imperfect crystallites (Cairns et al., 1990; Berry et al., 1988). In addition, it was postulated that resistant starch might be reassociation of amylose helices in B type crystalline structure (Eerlingen et al., 1993b).Thereby, the hypothesis also could be applied to this study since there was an increase in resistant starch content with ozone treatment and the addition of lysine (Table 4.1and Table 4.4). On the other hand, ozone treatment and the addition of lysine did not increase the RC on treated WSI as much as they did on Sigma rice starch. This fact also corresponds to the result from resistant starch content on WSI, in which the RS of ozonated WSI was not different from the untreated WSI (Table 4.5 and Table 4.8).

CHAPTER 7

EFFECT OF OZONATION ON SCANNING ELECTRON MICROSCOPY (SEM) OF RICE STARCHES

7.1. INTRODUCTION

Modified starches have exhibited physical changes, and some studies have revealed that the birefringence of starch was influenced by starch modification seen by SEM. Shamekh et al. (1998) revealed that most of the untreated barley starch lost their birefringence and that few intact granules were observed at 60°C when seen by polarized light microscopy. However, many intact granules were present in the lipid-hydrolyzed treated sample and it was explained that less swollen granules resulted from less gelatinization in the presence of phospholipase.

Sievert and Pomeranz (1989) found that retrograded resistant starch produced with 4 autoclave cycles of heating/cooling treatment had more compact formation of granules resulting from stabilization than one cycled starch. Moreover, they also reported that vacuumed dried RS residue formed an open, fluffy structure with much higher melting enthalpy by DSC compared to that of oven-dried RS. This was due to a better hydration capacity of vacuum dried RS and some modification of the crystalline structure during prolonged drying. Han et al. (2003) found that corn starch with six heating/cooling cycles showed increased expansion and no spherical-polygonal starch granules due to the gelatinization. Mangala et al. (1999b) studied SEM of resistant starch from differently processed rice and ragi and found that popped rice starch granules were blown up and fragmented into a thin film due to an increased expansion of the endosperm. They also found that malted ragi showed occasional pits or pinholes on the granular surface since enzyme action took place deep in the granules through these pits. For autoclaved rice and ragi, the granular size increased with temperature because of excessive water imbibed.

Finally, irregular granule folding was seen and swollen granule disintegration was observed as the temperature increased to 90°C and above.

There have been some studies with SEM to see if oxidation influenced the starch granule physically. Forssell et al. (1995) found that phase separation of amylose and amylopectin took place and domains of amylose and amylopectin were seen in barley starch gel at a lower hypochlorite oxidation. It was also revealed that the amylose-rice domains were variable in size and the largest visible were discontinuous. However, fewer granules of gelatinized barley starch remained at a higher degree of oxidation. Wing (1994) reported that 2 hours oxidation at ambient temperature with sodium hypochlorite showed some granular disruption, while commercial oxidized starch showed intact granular structure by SEM. In addition, oxidized starches prepared by instantaneous high temperature procedures such as jet cooking and drum drying resulted in complete gelatinization yielding water soluble products. Boruch (1985) found that hypochlorite oxidation was more effectively visible on potato starch molecules with big grain size compared to potato starch with smaller grain size and that external change and distinct granule damage were seen on the surface of big grains by SEM.

On the other hand, Kuakpetoon and Wang (2001) reported that the appearance of sodium hypochlorite oxidized starch was not different from that of non-oxidized starch. Moreover, it was revealed that the surfaces of regular corn and potato starch granules were not influenced by hypochlorite oxidation up to about 6% active chlorine, but with some apparent change at the 8% level (Rutenberg and Solarek, 1984). They also found that oxidation caused an increase in diameter of wheat starch granules about 16%, while it did not change that of waxy corn starch granules. Han and Ahn (2002) reported that oxidized corn starch with sodium hypochlorite did not change the shape of starch granules seen by photomicrographs since oxidation occurred on amorphous regions of starch. However, they found some breakages on the surfaces of oxidized potato starch as the concentration of oxidation increased. It was explained that oxidation occurred not only on the surface but the inside of the starch granules. They also found some dents and pits on oxidized starch granules and several small pieces that were smashed off of the granules observed by SEM.

The objectives of this study was 1) to determine the physical effect of ozonation on rice starch granules and 2) to study the physical effect of addition of lysine on ozonated rice starches

7.2. MATERIAL AND METHODS

7.2.1. Materials

Sigma rice starch was purchased from Sigma Chemical Co. (S7260) while white rice flour was obtained from Riviana Foods Inc. (Abbeville, LA). Positive charged amino acid (lysine), potato amylose (A0512), amylopectin (A8515), and protease (P5147) were purchased from Sigma Chemical Co. (St. Louis, MO).

7.2.2. Sample Preparation and Pure Oxygen and Ozone Treatments

For details of sample preparation, pure oxygen and ozone treatments, refer to Chapter 3.

7.2.3 Scanning Electron Microscopy (SEM) Analysis

Pure oxygen or ozone treated rice starches were freeze dried and ground for further analysis. Gelatinized ozonated starches by RVA (Rapid Visco-Analyzer) with or without lysine were kept at 4˚C, and they were freeze dried and ground before analysis. The granules of treated rice starches were sprinkled to aluminum specimen mounts with adhesive tabs. Samples were coated with gold: palladium 60:40 in an Edwards S-150 sputter coater and imaged with a Cambridge S-260 SEM at accelerating voltages of 10KV and below.

7.3. RESULTS AND DISCUSSION

7.3.1 Effects of Pure Oxygen and Ozone on SEM of Rice Starches

Uncooked rice starches with different treatments are shown in Figure 7.1 through Figure 7.6. Untreated Sigma rice starch shows typical rice starch granules which consist of mostly polygonal shape, some flat and some spherical shape granules (Figure 7.1). In addition, some pinholes were seen on several rice starch granules. On the other hand, pure oxygen treated Sigma rice starch presented some distorted granules and several granules were smashed on the exterior of the granules (Figure 7.2). Moreover, ozonated Sigma rice starch exhibited some very deep pits on the granules, and there were many small pieces of granules that were probably broken off of large particles (Figure 7.3). Several investigators reported that oxidized starch showed physical damage and disruption both inside and outside of starch granules as oxidizer concentration increased (Han and Ahn, 2002; Boruch, 1985; Rutenberg and Solarek, 1984).

Non-treated WSI showed similar shapes to that of untreated Sigma rice starch that most of the granules were polygonal shapes with some pinholes on them (Figure 7.4). However, pure oxygen treated WSI presented some dents and bumpy surfaces, and some of the granules showed fissures on the edge of the granules (Figure 7.5). In addition, ozone treatment resulted in many small pieces of particles, which was seen in ozonated Sigma rice starch as well (Figure 7.6). Ozonated WSI also exhibited some granules out of configuration.

7.3.2 Effects of Lysine on SEM of Rice Starches

Gelatinized rice starches lost their polygonal granular shape compared to uncooked starch (Figure 7.7), and were fragmented into thin and flat particles. In the presence of lysine (6%), the size of thin particles of ozonated Sigma rice starch seemed to be bigger than that of treated starch with no lysine (Figure 7.8). Mangala et al. (1999b) reported an increase in granule size since

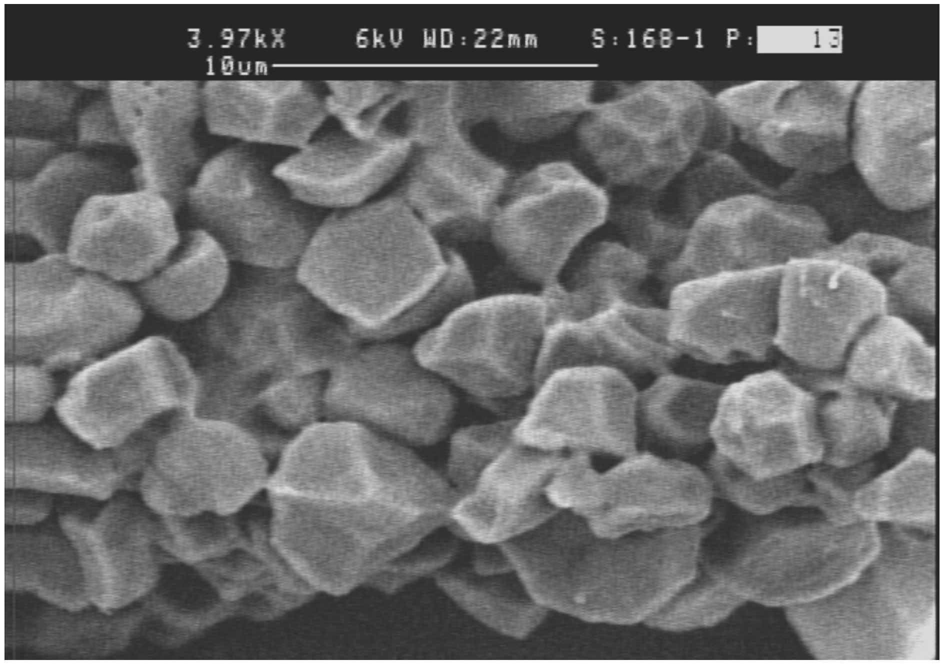

Figure 7.1 SEM of Untreated Sigma Rice Starch

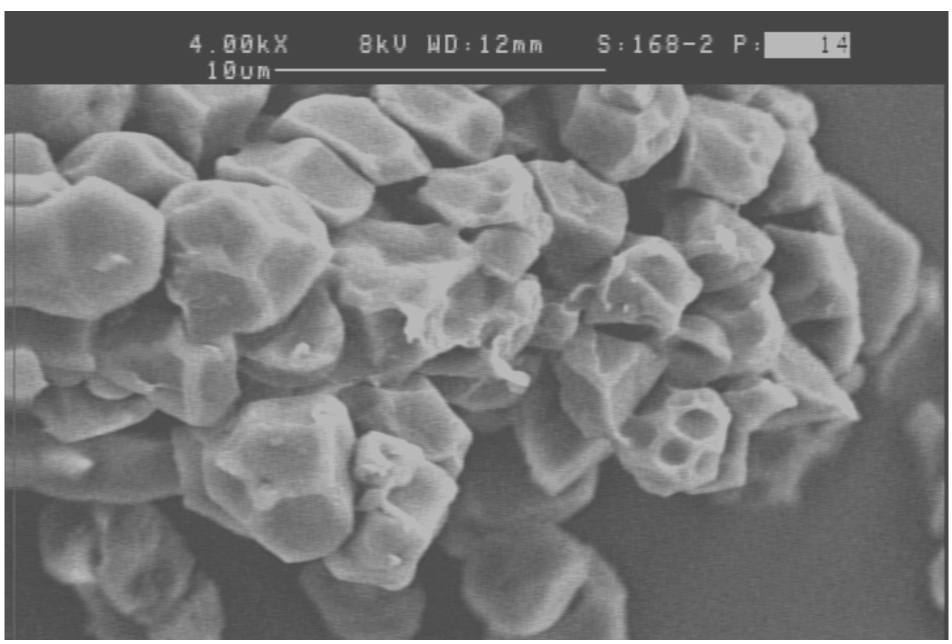

Figure 7.2 SEM of Sigma Rice Starch Treated with Pure Oxygen for 30 minutes

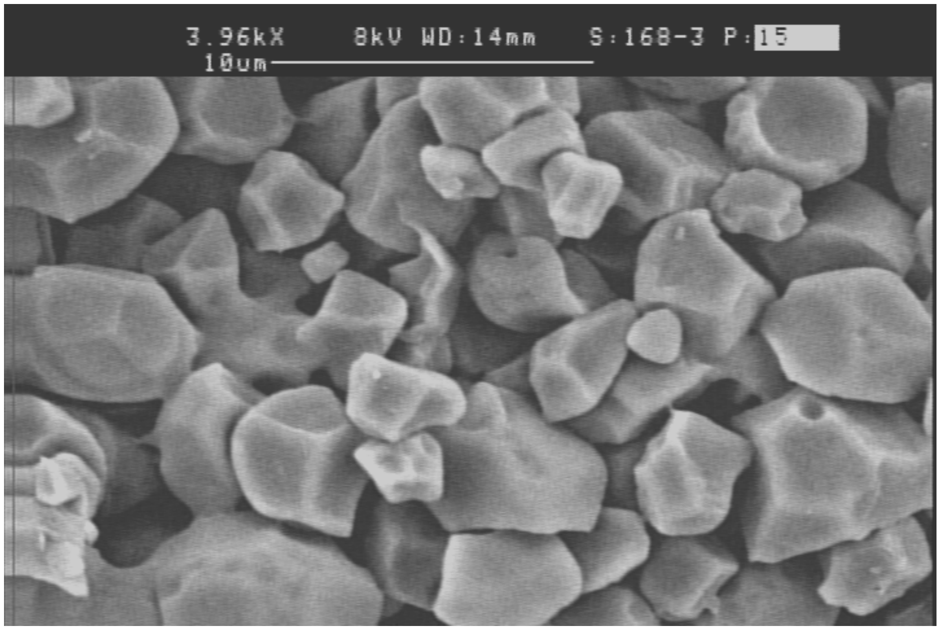

Figure 7.3 SEM of Sigma Rice Starch Treated with Ozone for 30 minutes

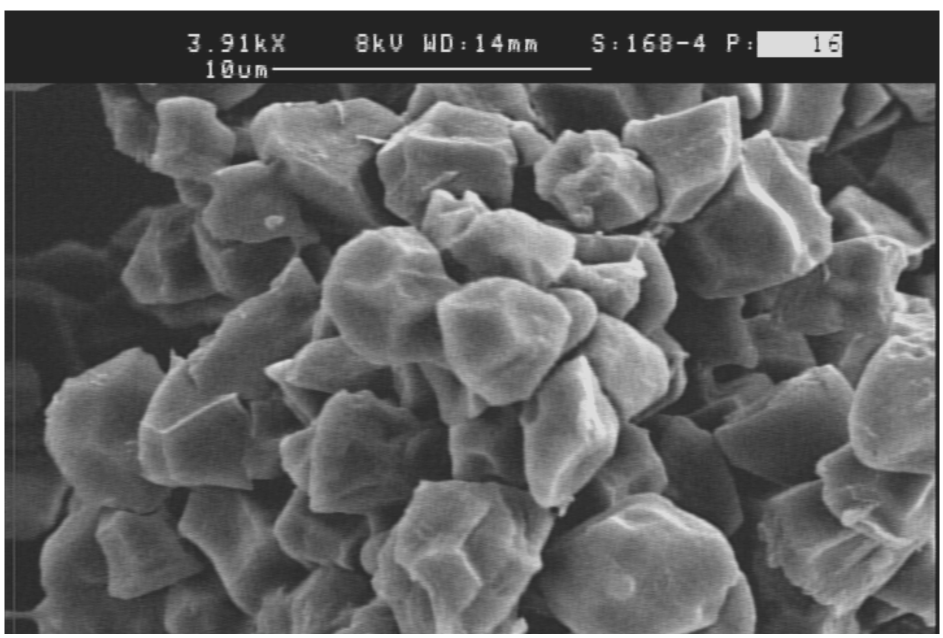

Figure 7.4 SEM of Untreated White Starch Isolate (WSI)

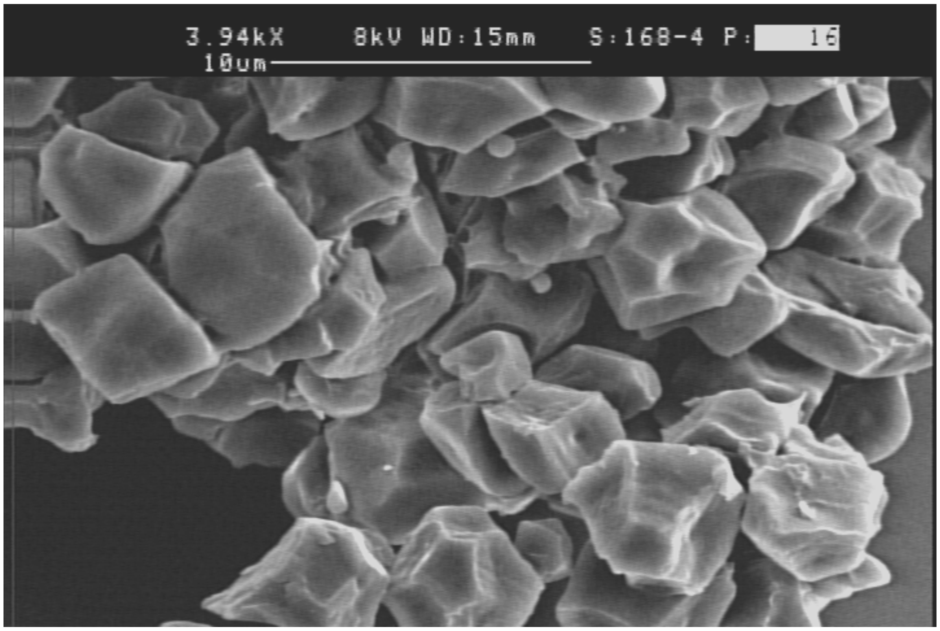

Figure 7.5 SEM of White Starch Isolate Treated with Pure Oxygen for 30 minutes

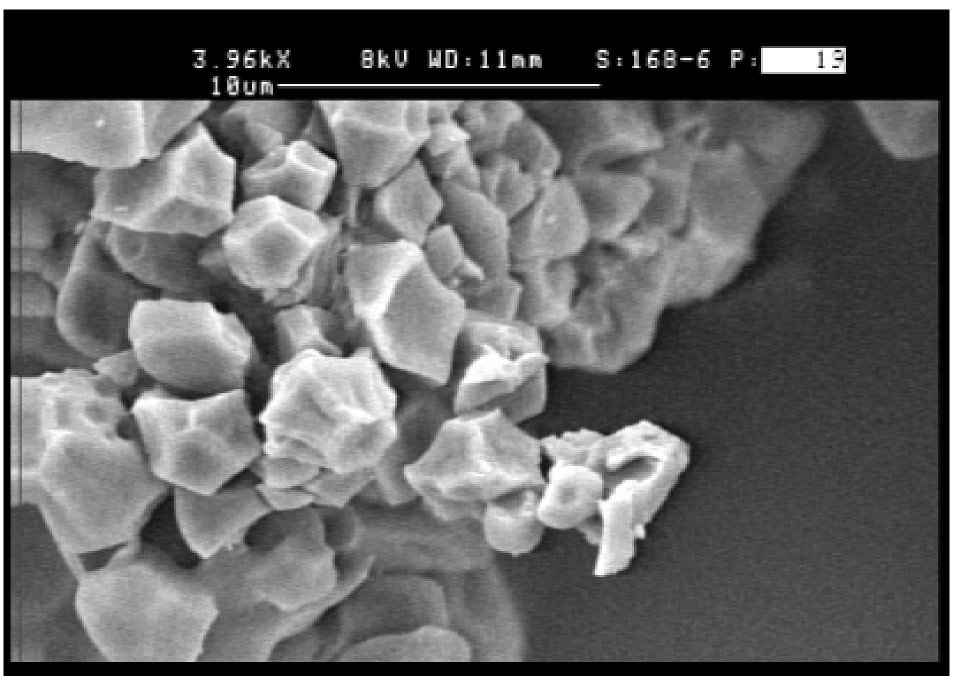

Figure 7.6 SEM of White Starch Isolate Treated with Ozone for 30 minutes

autoclaved rice imbibed excessive water during heating. Moreover, Escarpa et al. (1997) found that protein bound to starch in a same way that hydrogen bonds are formed between amylose chains during starch retrogradation. Thus, starch and lysine might have combined and aggregated each other during gelatinization and retrogradation. Ozonated WSI with lysine showed a similar pattern with bigger particles than with no lysine. It was reported that ionic interaction occurred between free radicals and the formation of oxygen and hydroxyl radicals occurred when a promoter was added during oxidation (Paterson et al., 1996).

7.4. CONCLUSION

The results indicated that ozone resulted in some physical damage on rice starch granules, which might be related to higher amylose content in ozonated rice starch (Table 3.2). Several investigators reported physical disruption and breakage on oxidized starches (Han and Ahn, 2002; Wing, 1994; Boruch, 1985). Ozonation also resulted in breakage and many small pieces of granules. It might be related to hydrolytic degradation, a side reaction during oxidation (Boruch, 1985; Hebeish et al., 1989), in which glycosic scission took place on starch molecules.

This study also showed that the addition of lysine on ozonated rice starches increased the size of granules after gelatinization. It might be related to starch-amino acid interaction, in which they bound to each other during gelatinization and retrogradation.

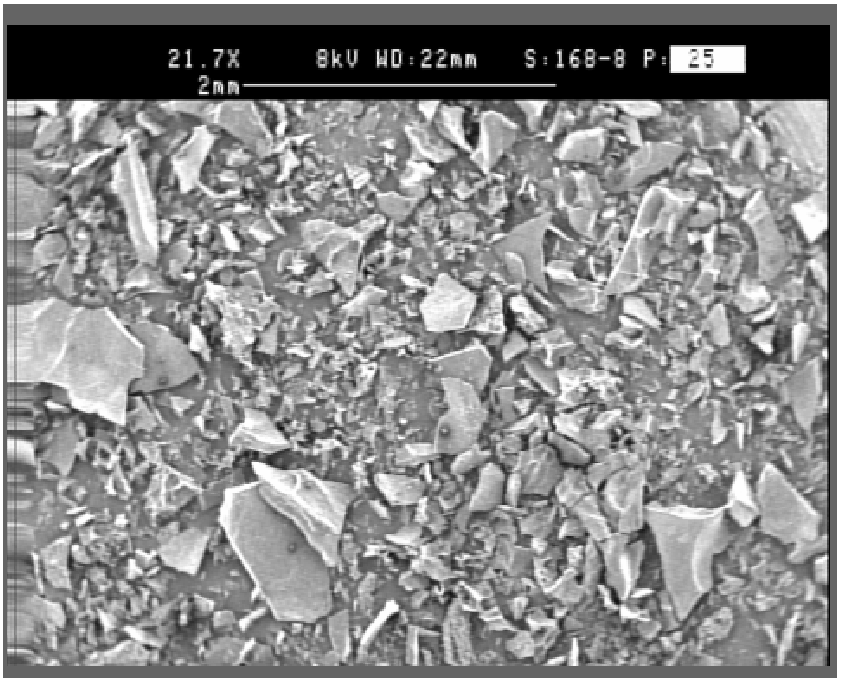

Figure 7.7 SEM of 30 minutes Ozonated Sigma Rice Starch without Lysine (Gelatinized)

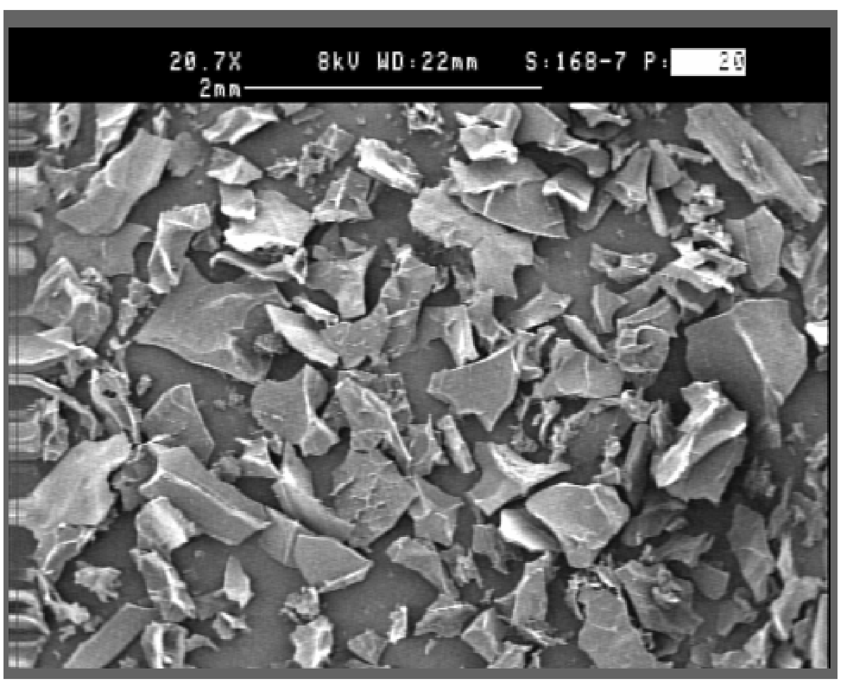

Figure 7.8 SEM of 30 minutes Ozonated Sigma Rice Starch with Lysine (Gelatinized)

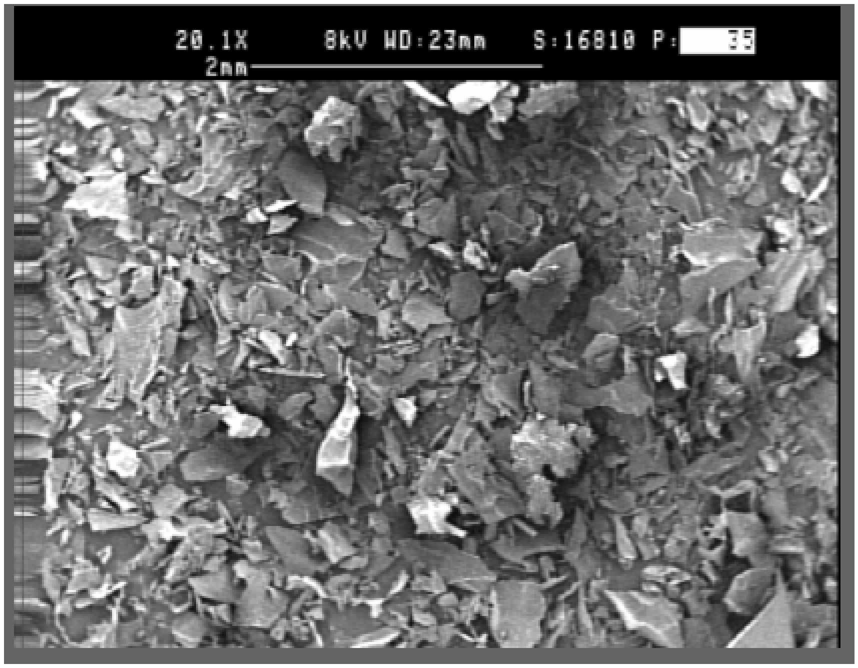

Figure 7.9 SEM of 30 minutes Ozonated White Starch Isolate without Lysine (Gelatinized)

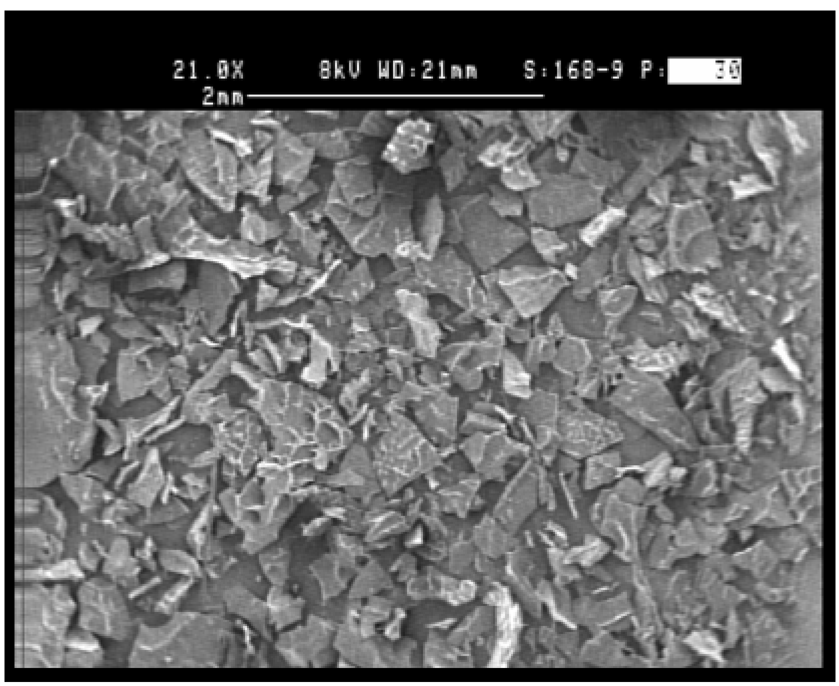

Figure 7.10 SEM of 30 minutes Ozonated White Starch Isolate with Lysine (Gelatinized)

CHAPTER 8. GENERAL CONCLUSIONS AND RECOMMENDATIONS

Chemically modified starches have been produced to overcome natural properties and alternated characteristics for paste viscosity, gelatinization temperature and enthalpy, retrogradation tendency, and digestion to amylolytic enzymes by using chemical agents.

Results from this study indicated that ozonated Sigma rice starch enhanced amylose content; thereby, increased resistant starch yield resulting in B type XRD pattern. Moreover, ozonation caused physical damage on granules seen by SEM and increased gelatinization temperature. Pure oxygen treated starches enhanced the cooking stability but decreased retrogradation tendency, while ozonated rice starches increased swelling extent and pasting time with weak cooled paste. Furthermore, ozonation decreased onset gelatinization temperature indicating that ozonated rice starch was easily swollen, which might be related to hydrolysis resulting from oxidation.

Addition of amino acids changed properties of ozonated rice starches as well. The presence of lysine in white starch isolate decreased the swelling extent and pasting time significantly, resulting in better cooking stability and lower pasting viscosities. In addition, enthalpy of amylase-lipid complexes decreased in the presence of lysine probably because of competitive reaction between lipid and added lysine. The addition of lysine on ozonated rice starch also enhanced crystallinity and the granule diameter seen by SEM due to starch-amino acid aggregation. Furthermore, the addition of leucine and aspartic acid (6%) increased resistant starch yield of ozonated starches. Thereby, processing commercial rice starch or starch isolate with the combination of ozonation and amino acids could be used as new starch ingredients with various functionalities without using typical chemical modifications. Lysine added ozonated rice starch with comparably higher resistant starch content and low paste viscosities could be substituted for commercial oxidized starch with nutritious benefits.

The modification of corn starch to produce health functional food ingredients has resulted in a 10-fold increased return on sales of corn starch. The same could be done through development of resistant rice starch. Rice has a lower risk for allergic potential than products such as wheat or soybeans and it is also high in lysine, an essential amino acid, which is not contained in high levels in other grains (Prepared Foods, 1993). The results of this research provide a new way of increased monetary returns for broken rice kernels, which makes up 15% of milled rice in the U.S., by their utilization to produce value-added food ingredients. Rice producers can reap similar benefits that corn producers due through value-added work on utilization of rice starch as a food ingredient, just like corn starch. The improved cooking stability, based on the results of this study, may also lower the lack acceptability by consumers due to negative cooking characteristics. As a result, it could benefit the Louisiana rice farming and processing industries by providing a new utilization for rice that could result in an increase its national competitiveness and demand in the food ingredient and product market. This in turn increase the economic value of rice and increase the amount of production and processing done by the existing industry and result in new facilities being opened.

NMR (Nuclear Magnetic Resonance) spectroscopy is a useful technique to identify modified chains and to locate the positions of substituents. Therefore, it could be utilized to trace any changes that ozonation might have caused in the internal structures.

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APPENDIX 1
COMMERCIAL STARCH RVA ANALYSIS RAW DATA

Treat	Type	Minutes	Additives	Rep	PV	MV	BKD	FV	SBK	TSB	PTime	PT
1	None	0	NA	1	1719	1460	259	2524	805	1064	6.51	70.3
1	None	0	NA	2	1632	1383	249	2387	755	1004	6.51	69.25
1	None	0	NA	3	1634	1383	251	2477	843	1094	6.45	71.2
1	None	0	NA	4	1679	1391	288	2502	823	1111	6.54	70.9
1	None	0	NA	5	1645	1370	275	2468	823	1098	6.48	70.4
1	None	0	NA	6	1682	1419	263	2497	815	1078	6.48	70.5
2	None	0	ASP	1	1932	1290	642	2087	155	797	6.61	70.45
2	None	0	ASP	2	1935	1269	666	2066	131	797	6.61	70.55
2	None	0	ASP	3	1794	1189	605	1973	179	784	6.61	71.55
2	None	0	ASP	4	1849	1238	611	2027	178	789	6.67	71.49
2	None	0	ASP	5	1853	1228	625	2009	156	781	6.58	70.9
2	None	0	ASP	6	1876	1237	639	2042	166	805	6.61	71.07
3	None	0	LEU	1	1654	1351	303	2517	863	1166	6.32	68.85
3	None	0	LEU	2	1661	1379	282	2501	840	1122	6.48	69.2
3	None	0	LEU	3	1718	1412	306	2555	837	1143	6.42	69.6
3	None	0	LEU	4	1682	1386	296	2549	867	1163	6.47	70.08
3	None	0	LEU	5	1666	1369	297	2475	809	1106	6.48	71.15
3	None	0	LEU	6	1667	1386	281	2534	867	1148	6.54	71.29
4	None	0	LYS	1	1631	1359	272	2015	384	656	5.86	70
4	None	0	LYS	2	1572	1314	258	1954	382	640	5.83	70.35
4	None	0	LYS	3	1546	1284	262	1890	344	606	5.99	71.3
4	None	0	LYS	4	1574	1321	253	1941	367	620	5.81	71.9
4	None	0	LYS	5	1575	1293	282	1942	367	649	5.8	71.6
4	None	0	LYS	6	1586	1308	278	1978	392	670	5.94	71.4
5	PO15	15	NA	1	1660	1467	193	2357	697	890	6.35	75.5
5	PO15	15	NA	2	1577	1425	152	2227	650	802	6.41	73.85
5	PO15	15	NA	3	1547	1414	133	2053	506	639	6.58	75.85
5	PO15	15	NA	4	1620	1438	182	2049	429	611	6.61	75.4
5	PO15	15	NA	5	1608	1451	157	2199	591	748	6.45	76.75

5	PO15	15	NA	6	1629	1468	161	2215	586	747	6.45	75.95
6	PO15	15	ASP	1	1741	1349	392	1943	202	594	6.28	77.6
6	PO15	15	ASP	2	1764	1380	384	1964	200	584	6.28	77.95
6	PO15	15	ASP	3	1687	1337	350	1923	236	586	6.29	76.65
6	PO15	15	ASP	4	1663	1340	323	1942	279	602	6.27	76.4
6	PO15	15	ASP	5	1631	1281	350	1846	215	565	6.25	75.85
6	PO15	15	ASP	6	1637	1290	347	1850	213	560	6.28	77.35
7	PO15	15	LEU	1	1790	1636	154	2282	492	646	6.61	77.05
7	PO15	15	LEU	2	1707	1561	146	2175	468	614	6.51	76.65
7	PO15	15	LEU	3	1625	1484	141	2020	395	536	6.71	76.3
7	PO15	15	LEU	4	1684	1509	175	2091	407	582	6.59	75.3
7	PO15	15	LEU	5	1620	1478	142	2065	445	587	6.61	74.65
7	PO15	15	LEU	6	1617	1479	138	2055	438	576	6.58	76.35
8	PO15	15	LYS	1	1728	1254	474	2453	725	1199	5.27	77.15
8	PO15	15	LYS	2	1723	1239	484	2447	724	1208	5.24	77.4
8	PO15	15	LYS	3	1567	1163	404	2273	706	1110	5.31	77.15
8	PO15	15	LYS	4	1594	1147	447	2281	687	1134	5.19	77.2
8	PO15	15	LYS	5	1640	1207	433	2251	611	1044	5.37	77.15
8	PO15	15	LYS	6	1608	1169	439	2257	649	1088	5.27	77.3
9	PO30	30	NA	1	1559	1402	157	2226	667	824	6.42	75.85
9	PO30	30	NA	2	1597	1419	178	2281	684	862	6.28	75.5
9	PO30	30	NA	3	1599	1432	167	2234	635	802	6.38	75.1
9	PO30	30	NA	4	1650	1479	171	2301	651	822	6.45	75.25
9	PO30	30	NA	5	1598	1434	164	2206	608	772	6.38	75.85
9	PO30	30	NA	6	1624	1450	174	2236	612	786	6.38	76.25
10	PO30	30	ASP	1	1725	1367	358	1951	226	584	6.32	77.5
10	PO30	30	ASP	2	1705	1342	363	1899	194	557	6.28	77.45
10	PO30	30	ASP	3	1694	1339	355	1924	230	585	6.22	76.2
10	PO30	30	ASP	4	1686	1352	334	1967	281	615	6.35	76.26
10	PO30	30	ASP	5	1698	1337	361	1932	234	595	6.28	77.05
10	PO30	30	ASP	6	1694	1338	356	1929	235	591	6.38	77.45
11	PO30	30	LEU	1	1729	1577	152	2212	483	635	6.55	75.85
11	PO30	30	LEU	2	1709	1567	142	2161	452	594	6.64	75.1

11	PO30	30	LEU	3	1675	1528	147	2175	500	647	6.58	78.05
11	PO30	30	LEU	4	1644	1508	136	2113	469	605	6.58	75.95
11	PO30	30	LEU	5	1680	1533	147	2145	465	612	6.55	76.55
11	PO30	30	LEU	6	1694	1547	147	2138	444	591	6.5	75.8
12	PO30	30	LYS	1	1675	1230	445	2346	671	1116	5.34	75.8
12	PO30	30	LYS	2	1689	1220	469	2377	688	1157	5.27	77.85
12	PO30	30	LYS	3	1616	1176	440	2272	656	1096	5.31	77.5
12	PO30	30	LYS	4	1603	1178	425	2225	622	1047	5.37	77.45
12	PO30	30	LYS	5	1683	1220	463	2367	684	1147	5.37	77.15
12	PO30	30	LYS	6	1662	1242	420	2349	687	1107	5.4	77.5
13	OZ15	15	NA	1	2077	1635	442	1967	-110	332	6.97	73.9
13	OZ15	15	NA	2	2024	1627	397	1923	-101	296	6.97	73.95
13	OZ15	15	NA	3	1892	1532	360	2048	156	516	6.9	71.55
13	OZ15	15	NA	4	1918	1547	371	2082	164	535	6.87	71.64
13	OZ15	15	NA	5	1908	1517	391	2059	151	542	6.74	71.55
13	OZ15	15	NA	6	1879	1499	380	2027	148	528	6.84	71.15
14	OZ15	15	ASP	1	2044	1564	480	1936	-108	372	6.77	74.35
14	OZ15	15	ASP	2	2007	1517	490	1916	-91	399	6.74	74.65
14	OZ15	15	ASP	3	1893	1369	524	1919	26	550	6.51	74.55
14	OZ15	15	ASP	4	1967	1438	529	1994	27	556	6.67	74.27
14	OZ15	15	ASP	5	1954	1403	551	1972	18	569	6.55	73.15
14	OZ15	15	ASP	6	1982	1425	557	1993	11	568	6.58	73.7
15	OZ15	15	LEU	1	2048	1624	424	2010	-38	386	6.97	74.65
15	OZ15	15	LEU	2	2021	1612	409	1982	-39	370	6.87	74.71
15	OZ15	15	LEU	3	1810	1435	375	2029	219	594	6.61	73.85
15	OZ15	15	LEU	4	1958	1571	387	2149	191	578	6.67	73.79
15	OZ15	15	LEU	5	1956	1554	402	2140	184	586	6.71	73.55
15	OZ15	15	LEU	6	1899	1496	403	2083	184	587	6.61	74.35
16	OZ15	15	LYS	1	1301	779	522	1195	-106	416	5.05	78.35
16	OZ15	15	LYS	2	1316	766	550	1203	-113	437	5.01	76.65
16	OZ15	15	LYS	3	1343	721	622	1202	-141	481	4.92	76.7
16	OZ15	15	LYS	4	1359	748	611	1218	-141	470	4.97	76.81
16	OZ15	15	LYS	5	1404	740	664	1203	-201	463	4.95	77.4

16	OZ15	15	LYS	6	1401	714	687	1190	-211	476	4.88	77.6
17	OZ30	30	NA	1	1880	1412	468	1727	-153	315	6.74	71.55
17	OZ30	30	NA	2	1875	1401	474	1718	-157	317	6.68	70
17	OZ30	30	NA	3	2126	1602	524	1963	-163	361	6.64	72.3
17	OZ30	30	NA	4	2126	1651	475	1988	-138	337	6.84	72.3
17	OZ30	30	NA	5	2053	1359	694	1634	-419	275	5.24	72.05
17	OZ30	30	NA	6	2143	1388	755	1690	-453	302	5.24	71.2
18	OZ30	30	ASP	1	1853	1301	552	1647	-206	346	5.96	71.95
18	OZ30	30	ASP	2	1849	1387	462	1644	-205	257	6.25	73.55
18	OZ30	30	ASP	3	2068	1587	481	1941	-127	354	6.74	72.55
18	OZ30	30	ASP	4	2055	1497	558	1873	-182	376	5.93	72.4
18	OZ30	30	ASP	5	1942	1385	557	1772	-170	387	5.99	71.15
18	OZ30	30	ASP	6	1926	1386	540	1771	-155	385	6.09	72.65
19	OZ30	30	LEU	1	1860	1384	476	1718	-142	334	6.61	71.55
19	OZ30	30	LEU	2	1822	1381	441	1694	-128	313	6.74	69.35
19	OZ30	30	LEU	3	2083	1560	523	1932	-151	372	6.58	72.55
19	OZ30	30	LEU	4	1998	1506	492	1858	-140	352	6.61	72.3
19	OZ30	30	LEU	5	2019	1535	484	1936	-83	401	6.64	71.55
19	OZ30	30	LEU	6	1954	1518	436	1876	-78	358	6.81	73.6
20	OZ30	30	LYS	1	951	373	578	657	-294	284	4.69	79.15
20	OZ30	30	LYS	2	1002	409	593	699	-303	290	4.72	77.4
20	OZ30	30	LYS	3	1216	491	725	836	-380	345	4.72	77.7
20	OZ30	30	LYS	4	1190	478	712	815	-375	337	4.69	78.3
20	OZ30	30	LYS	5	1178	454	724	785	-393	331	4.69	77.5
20	OZ30	30	LYS	6	1158	460	698	786	-372	326	4.69	78.75

APPENDIX 2
WHITE STARCH ISOLATE RVA ANALYSIS RAW DATA

Treat	Type	Time	Additives	Rep	PV	MV	BKD	FV	SBK	TSB	PTime	PT
1	None	0	NA	1	1497	1170	327	1560	63	390	6.12	70.15
1	None	0	NA	2	1474	1133	341	1528	54	395	6.09	70.85
1	None	0	NA	3	1449	1142	307	1538	89	396	6.14	70.54
1	None	0	NA	4	1524	1157	367	1572	48	415	6.21	70.29
1	None	0	NA	5	1533	1131	402	1579	46	448	5.99	70.85
1	None	0	NA	6	1544	1129	415	1592	48	463	5.93	70.8
2	None	0	ASP	1	1612	1109	503	1496	-116	387	6.28	70.85
2	None	0	ASP	2	1628	1133	495	1521	-107	388	6.25	70.85
2	None	0	ASP	3	1676	1084	592	1583	-93	499	6.27	70.81
2	None	0	ASP	4	1723	1149	574	1634	-89	485	6.31	70.48
2	None	0	ASP	5	1652	1031	621	1452	-200	421	6.09	70.9
2	None	0	ASP	6	1641	1020	621	1437	-204	417	6.12	71.15
3	None	0	LEU	1	1413	1030	383	1499	86	469	5.96	70.05
3	None	0	LEU	2	1464	1060	404	1536	72	476	6.02	70.6
3	None	0	LEU	3	1584	1194	390	1649	65	455	6.08	70.8
3	None	0	LEU	4	1628	1179	449	1681	53	502	6.14	70.47
3	None	0	LEU	5	1594	1178	416	1660	66	482	6.09	70.8
3	None	0	LEU	6	1630	1192	438	1690	60	498	5.96	70.9
4	None	0	LYS	1	1473	992	481	1339	-134	347	5.8	72.65
4	None	0	LYS	2	1522	1031	491	1350	-172	319	5.82	72.55
4	None	0	LYS	3	1657	1129	528	1476	-181	347	5.79	72.59
4	None	0	LYS	4	1642	1157	485	1482	-160	325	5.84	72.37
4	None	0	LYS	5	1630	1118	512	1498	-132	380	5.76	72.35
4	None	0	LYS	6	1597	1086	511	1477	-120	391	5.7	72.4
5	PO15	15	NA	1	1776	1329	447	1767	-9	438	6.09	69.35
5	PO15	15	NA	2	1751	1324	427	1736	-15	412	6.09	69.95
5	PO15	15	NA	3	2377	1640	737	2534	157	894	6.19	70
5	PO15	15	NA	4	2415	1628	787	2537	122	909	6.12	70.35
5	PO15	15	NA	5	2121	1436	685	2335	214	899	6.25	70.8
5	PO15	15	NA	6	2073	1458	615	2364	291	906	6.28	70.4

6	PO15	15	ASP	1	1897	1269	628	1667	-230	398	6.15	70.4
6	PO15	15	ASP	2	1906	1265	641	1694	-212	429	6.15	71.25
6	PO15	15	ASP	3	2244	1204	1040	1820	-424	616	6.12	70.35
6	PO15	15	ASP	4	2317	1259	1058	1880	-437	621	6.12	71.3
6	PO15	15	ASP	5	2100	1083	1017	1679	-421	596	6.15	71.25
6	PO15	15	ASP	6	2104	1071	1033	1657	-447	586	6.09	71.55
7	PO15	15	LEU	1	1796	1314	482	1809	13	495	6.09	70.05
7	PO15	15	LEU	2	1809	1329	480	1812	3	483	6.06	70.05
7	PO15	15	LEU	3	2327	1561	766	2458	131	897	6.15	70.85
7	PO15	15	LEU	4	2380	1572	808	2479	99	907	6.15	70.9
7	PO15	15	LEU	5	2187	1460	727	2350	163	890	6.22	70.9
7	PO15	15	LEU	6	2180	1462	718	2342	162	880	6.22	70.85
8	PO15	15	LYS	1	1857	1278	579	1654	-203	376	5.83	71.55
8	PO15	15	LYS	2	1844	1268	576	1644	-200	376	5.8	72.1
8	PO15	15	LYS	3	2558	1924	634	2676	118	752	6.06	72.4
8	PO15	15	LYS	4	2524	1946	578	2655	131	709	6.22	72.4
8	PO15	15	LYS	5	2311	1803	508	2472	161	669	6.15	72
8	PO15	15	LYS	6	2374	1821	553	2514	140	693	6.06	72.05
9	PO30	30	NA	1	1764	1311	453	1749	-15	438	6.06	70
9	PO30	30	NA	2	1762	1323	439	1755	-7	432	6.12	70.4
9	PO30	30	NA	3	1562	1206	356	1595	33	389	6.22	70.3
9	PO30	30	NA	4	1599	1199	400	1630	31	431	6.06	70
9	PO30	30	NA	5	1698	1274	424	1698	0	424	6.09	70.75
9	PO30	30	NA	6	1668	1189	479	1666	-2	477	6.02	68.85
10	PO30	30	ASP	1	1911	1265	646	1675	-236	410	6.19	70.6
10	PO30	30	ASP	2	1927	1263	664	1684	-243	421	6.06	69.95
10	PO30	30	ASP	3	1694	1096	598	1513	-181	417	6.09	70.85
10	PO30	30	ASP	4	1681	1085	596	1502	-179	417	6.19	70.25
10	PO30	30	ASP	5	1771	1150	621	1569	-202	419	6.19	70.95
10	PO30	30	ASP	6	1708	1064	644	1487	-221	423	6.15	69.95
11	PO30	30	LEU	1	1799	1323	476	1812	13	489	6.02	69.6
11	PO30	30	LEU	2	1814	1329	485	1820	6	491	6.02	70.5
11	PO30	30	LEU	3	1597	1210	387	1651	54	441	6.06	70.2

11	PO30	30	LEU	4	1592	1207	385	1630	38	423	6.09	70.05
11	PO30	30	LEU	5	1663	1193	470	1693	30	500	6.02	70.05
11	PO30	30	LEU	6	1594	1203	391	1646	52	443	6.12	69.7
12	PO30	30	LYS	1	1838	1274	564	1656	-182	382	5.7	72.05
12	PO30	30	LYS	2	1842	1259	583	1648	-194	389	5.83	71.9
12	PO30	30	LYS	3	1615	1158	457	1509	-106	351	5.83	72.4
12	PO30	30	LYS	4	1634	1141	493	1518	-116	377	5.76	72
12	PO30	30	LYS	5	1713	1184	529	1568	-145	384	5.8	72.05
12	PO30	30	LYS	6	1632	1161	471	1507	-125	346	5.83	72.4
13	OZ15	15	NA	1	2039	1555	484	2171	132	616	6.48	70.05
13	OZ15	15	NA	2	2027	1529	498	2153	126	624	6.45	69.65
13	OZ15	15	NA	3	1995	1521	474	2108	113	587	6.45	70.65
13	OZ15	15	NA	4	1984	1515	469	2134	150	619	6.42	70.4
13	OZ15	15	NA	5	1965	1399	566	2096	131	697	6.45	70.9
13	OZ15	15	NA	6	1940	1415	525	2077	137	662	6.45	70.9
14	OZ15	15	ASP	1	1977	1267	710	1795	-182	528	6.28	70.05
14	OZ15	15	ASP	2	1942	1273	669	1792	-150	519	6.32	70.35
14	OZ15	15	ASP	3	1873	1218	655	1705	-168	487	6.35	70.8
14	OZ15	15	ASP	4	1955	1269	686	1777	-178	508	6.32	70.35
14	OZ15	15	ASP	5	1934	1192	742	1769	-165	577	6.22	70.9
14	OZ15	15	ASP	6	1892	1181	711	1760	-132	579	6.28	70.8
15	OZ15	15	LEU	1	1955	1437	518	2077	122	640	6.35	70
15	OZ15	15	LEU	2	2022	1480	542	2133	111	653	6.42	70.85
15	OZ15	15	LEU	3	1895	1403	492	2028	133	625	6.32	70.25
15	OZ15	15	LEU	4	2004	1486	518	2122	118	636	6.32	70.35
15	OZ15	15	LEU	5	1887	1342	545	2024	137	682	6.45	70.9
15	OZ15	15	LEU	6	1907	1354	553	2043	136	689	6.38	70.9
16	OZ15	15	LYS	1	1354	960	394	1286	-68	326	5.63	72.4
16	OZ15	15	LYS	2	1391	963	428	1310	-81	347	5.67	72.35
16	OZ15	15	LYS	3	1398	966	432	1294	-104	328	5.7	72.4
16	OZ15	15	LYS	4	1387	958	429	1283	-104	325	5.63	71.6
16	OZ15	15	LYS	5	1266	886	380	1227	-39	341	5.67	71.55
16	OZ15	15	LYS	6	1268	913	355	1245	-23	332	5.67	72.4

17	OZ30	30	NA	1	1549	1189	360	1537	-12	348	6.38	69.5
17	OZ30	30	NA	2	1543	1175	368	1532	-11	357	6.41	69.6
17	OZ30	30	NA	3	1692	1279	413	1695	3	416	6.42	69.55
17	OZ30	30	NA	4	1698	1269	429	1690	-8	421	6.38	69.6
17	OZ30	30	NA	5	1681	1152	529	1685	4	533	6.22	68.85
17	OZ30	30	NA	6	1625	1188	437	1682	57	494	6.45	70
18	OZ30	30	ASP	1	1523	1019	504	1341	-182	322	6.28	70.05
18	OZ30	30	ASP	2	1538	1012	526	1352	-186	340	6.28	70
18	OZ30	30	ASP	3	1655	1061	594	1465	-190	404	6.25	69.95
18	OZ30	30	ASP	4	1668	1072	596	1466	-202	394	6.29	70.05
18	OZ30	30	ASP	5	1604	960	644	1428	-176	468	6.02	69.2
18	OZ30	30	ASP	6	1608	1027	581	1460	-148	433	6.22	70
19	OZ30	30	LEU	1	1565	1141	424	1548	-17	407	6.35	69.6
19	OZ30	30	LEU	2	1506	1108	398	1483	-23	375	6.38	70
19	OZ30	30	LEU	3	1660	1211	449	1673	13	462	6.32	70.1
19	OZ30	30	LEU	4	1689	1220	469	1690	1	470	6.25	70
19	OZ30	30	LEU	5	1646	1107	539	1659	13	552	6.22	69.25
19	OZ30	30	LEU	6	1694	1127	567	1683	-11	556	6.27	70.2
20	OZ30	30	LYS	1	851	516	335	725	-126	209	5.37	72.4
20	OZ30	30	LYS	2	852	506	346	719	-133	213	5.27	72.4
20	OZ30	30	LYS	3	984	606	378	842	-142	236	5.34	72.4
20	OZ30	30	LYS	4	992	603	389	842	-150	239	5.4	72.5
20	OZ30	30	LYS	5	996	643	353	906	-90	263	5.4	72
20	OZ30	30	LYS	6	963	621	342	870	-93	249	5.47	72.1

APPENDIX 3
COMMERCIAL STARCH RESISTANT STARCH RAW DATA

Treat	Type	Minutes	Additives												
1	None	0	NA	6.46	5.44	5.28	5.34	4.64	4.98	5.76	8.18	5.04	5.38	5.46	4.88
2	None	0	ASP	5.02	5.24	4.9	5.14	5.46	7.1	5.42	4.88	5.06	5.7	5.8	5.84
3	None	0	LEU	5.8	5.24	4.76	4.66	3.9	4.36	5.38	5.68	5.84	3.82	5.2	4.72
4	None	0	LYS	5.1	5.74	4.62	5.56	4.64	5.4	6.34	6.38	4.94	5.32	5.78	5.54
5	PO15	15	NA	7.96	8.72	8.2	7	7.14	7.6	9.26	9.4	6.78	7.32	7.12	6.7
6	PO15	15	ASP	8.4	7.56	9.9	8.1	11.92	9.06	9.72	9.22	7.74	7.76	8.22	8.3
7	PO15	15	LEU	7.78	8.52	7.38	5.48	6.76	6.56	7.22	6.82	7.24	6.86	7.16	6.44
8	PO15	15	LYS	6.76	7.08	8.88	8.14	6.34	9.26	8.3	6.32	7.1	6.66	7.9	6.7
9	PO30	30	NA	7.94	8.82	6.88	6.76	8.8	9.4	7.5	7.44	7.94	8.7	8.24	9.34
10	PO30	30	ASP	6.66	7.84	9.54	9.92	7	8.04	9.92	9.32	7.1	7.52	8.5	9.42
11	PO30	30	LEU	7.3	6.38	7.5	6.82	6.8	6.68	6.56	9.44	8.68	8.16	7.54	8.72
12	PO30	30	LYS	6.7	9.42	7.42	8.78	7.72	7.46	9.42	7.68	7.44	7.16	7.08	6.34
13	OZ15	15	NA	8.32	8.24	7.48	8.22	7.6	9.66	8.82	8.82	8.34	7.26	7.7	9.58
14	OZ15	15	ASP	8.8	7.4	6.78	7.66	10.2	9.64	7.86	9.72	7.4	8.28	10.9	8.52
15	OZ15	15	LEU	7	7.76	7.56	8.06	9.4	7.5	10.34	8.24	7.24	8.02	7.2	7.7
16	OZ15	15	LYS	6.8	7.32	7.82	6.8	7.82	8.88	7.66	8.44	6.94	7.92	6.42	9.08
17	OZ30	30	NA	8.72	9.44	8.56	10.3	7.92	7.82	8.8	7.94	7.7	8.7	7.58	7.56
18	OZ30	30	ASP	8.92	8.36	7.66	7.92	8.24	8.28	8.2	8.98	7.72	7.76	8.4	7.88
19	OZ30	30	LEU	8.16	8	7.8	8.28	9.2	10.9	9.98	9.68	7.58	7.3	11.4	11.32
20	OZ30	30	LYS	9.78	9.2	8.68	7.68	9.16	11.38	7.26	8.22	7.04	7.78	7.42	8.68

APPENDIX 4
WHITE STARCH ISOLATE RESISTANT STARCH RAW DATA

1	None	0	NA	7.9	8.42	9.4	9.6	8.76	8.24	8.58	8	9.5	9.2	8.6	10.46
2	None	0	ASP	9.54	9.34	9.56	9.08	9.88	10.54	8.62	9.2	9.84	9.26	8.06	8.04
3	None	0	LEU	9.3	8.9	6.04	5.5	7.8	7.88	7.86	6.96	6.7	0	8.42	8.34
4	None	0	LYS	9.64	11.94	6.22	5.86	8.7	7.9	7.9	9	9.16	8.5	9.48	11.84
5	PO15	15	NA	9.66	7.96	8.6	7.7	7.94	8	9.16	8.74	9.36	8.36	7.02	7.62
6	PO15	15	ASP	8.24	7.48	7.36	7.48	6.08	8.54	9.06	9.28	7.46	7.34	6.34	6.26
7	PO15	15	LEU	8.16	8.92	7.66	6.6	9.62	6.3	9	9.66	8.12	8.28	7.08	7.62
8	PO15	15	LYS	7.88	8.16	7.82	8.5	7.38	6.06	10.84	9.18	9.34	7.44	6.34	6.1
9	PO30	30	NA	8.2	9.4	8.64	11.56	10.4	9.5	8.9	9.36	9.74	10.6	8	8.34
10	PO30	30	ASP	9.2	10.84	8.72	10.92	8.8	7.92	9.1	8.28	7.78	8.4	7.54	7.72
11	PO30	30	LEU	9.36	9.42	8.04	7.94	7.52	8.28	10.18	9.04	8.08	7.64	8	8.86
12	PO30	30	LYS	7.48	9.68	9.9	9.24	8.08	7.32	8.24	7.56	7.62	7.06	6.84	7.74
13	OZ15	15	NA	6.68	7.24	6.06	7.18	7.04	6.52	6.78	6.98	6.98	6.96	10	6.46
14	OZ15	15	ASP	7.56	6.66	6.2	6.4	7.92	8.08	6.14	5.86	6.92	7.06	6.36	7.4
15	OZ15	15	LEU	9	9.48	7.36	9.02	7.84	7.56	7.32	6.36	6.9	7.32	5.86	7.08
16	OZ15	15	LYS	9	7.4	6.04	6	7.6	9.2	6.3	10.64	6.9	8	6.32	5.54
17	OZ30	30	NA	6.1	6.8	6.28	6.88	7.16	6	6.94	6.32	6.48	6.9	5.4	6
18	OZ30	30	ASP	7.66	7.68	8.82	9	5.6	5.28	8.64	7.94	10.88	8.04	8.26	6.94
19	OZ30	30	LEU	6.2	10.8	6.66	5.74	5.38	5.68	6.86	6.46	6.06	6.04	7.54	6.2
20	OZ30	30	LYS	6.84	6.08	6.16	6.94	4.88	6.46	6.7	8.24	5.78	5.84	5.5	6.34

APPENDIX 5
COMMERCIAL STARCH DSC RAW DATA

					First transition Gelatinization endotherm				Second transition Amylose-lipid complex			
#	Treat	Min	Additives	Rep	**To**	**Tp**	**Tc**	**ΔH**	**To**	**Tp**	**Tc**	**ΔH**
1	none	0	NA	1	61.61	69.03	86.88	6.593	96.7	103.45	110.55	0.9889
1	none	0	NA	2	63.07	69.67	82.08	4.338	97.37	103.06	110.15	0.6756
1	none	0	NA	3	60.62	69.38	85.22	6.268	94.67	100.87	108.83	0.9826
1	none	0	NA	4	57.46	68.79	82.91	6.089	95.95	101.42	107.81	0.8234
1	none	0	NA	5	60.76	70.33	84.01	5.919	95.47	103.31	110.29	0.9702
1	none	0	NA	6	57.6	68.6	82.91	6.022	96.14	103.14	110.57	1.035
2	none	0	LYS	1	62.93	71.26	87.2	6.617	103.83	107.97	111.97	0.2577
2	none	0	LYS	2	62.99	71.11	86.01	6.306	102.38	107.05	112.3	0.5011
2	none	0	LYS	3	63.1	71.31	86.54	6.383	102.55	107.87	113.12	0.4662
2	none	0	LYS	4	60.62	70.31	84.89	6.726	103.69	107.36	113.45	0.5422
2	none	0	LYS	5	62.44	71.05	85.88	6.146	100.56	107.28	115.27	0.9115
2	none	0	LYS	6	59.08	70.67	84.12	6.194	105.35	107.35	114.17	0.7402
3	PO15	15	NA	1	70.03	77.24	90.25	7.022	98.34	104.8	112.79	0.8676
3	PO15	15	NA	2	67.39	80.08	89.35	6.711	101.07	108.04	113.45	0.5631
3	PO15	15	NA	3	70.36	77.36	89.35	6.241	98.26	103.53	109.82	0.4779
3	PO15	15	NA	4	70.69	77.37	89.02	6.101	97.6	105.26	112.3	0.7716
3	PO15	15	NA	5	69.7	78.13	89.18	6.69	96.78	104.01	111.97	0.7741
3	PO15	15	NA	6	69.37	77.07	88.03	6.447	95.46	105.02	110.64	0.9049
4	PO15	15	LYS	1	72.51	79.47	92.15	6.997	100.41	107.36	114.11	0.7146
4	PO15	15	LYS	2	63.43	78.08	90.83	7.722	99.09	105.77	112.3	0.5106
4	PO15	15	LYS	3	64.25	78.77	89.84	8.809	100.24	106.48	112.79	0.5521
4	PO15	15	LYS	4	65.82	78.78	88.74	6.931	97.6	105.94	111.47	0.4524
4	PO15	15	LYS	5	65.41	77.79	91.16	8.334	97.44	104.82	109.82	0.5606
4	PO15	15	LYS	6	62.01	74.31	84.53	8.748	97.17	102.59	107.28	0.3166
5	PO30	30	NA	1	69.53	77.06	88.69	6.642	97.6	104.91	111.8	0.8284
5	PO30	30	NA	2	63.51	76.92	87.97	7.16	94.91	103.41	111.86	0.9669
5	PO30	30	NA	3	65.71	77.19	88	7.347	98.46	105.25	111.39	0.7647
5	PO30	30	NA	4	71.02	77.77	89.84	6.037	97.44	103.35	111.8	0.6457

5	PO30	30	NA	5	69.86	71.43	90.5	6.816	95.46	103.08	110.81	0.7899
5	PO30	30	NA	6	70.39	77.43	91.17	6.445	98.87	105.16	111.39	0.6123
6	PO30	30	LYS	1	63.9	79.04	89.71	8.296	98.18	105.92	111.86	0.9748
6	PO30	30	LYS	2	63.39	74.25	83.38	7.964	95.56	102.28	108.89	0.8416
6	PO30	30	LYS	3	65.71	78.41	89.65	8.135	100.38	107.49	113.73	0.7781
6	PO30	30	LYS	4	64.42	78.44	91	8.518	97.27	105.99	111.64	0.7481
6	PO30	30	LYS	5	64.58	78.37	91.33	8.928	100.08	106.68	111.8	0.4155
6	PO30	30	LYS	6	62.69	78.87	91.3	9.492	100.11	105.36	111.53	0.5046
7	OZ15	15	NA	1	56.03	72.56	80.19	9.708	93.35	98.4	103.18	0.6826
7	OZ15	15	NA	2	55.46	72.56	80.22	8.08	92.24	98.99	105.2	0.6778
7	OZ15	15	NA	3	55.93	72.6	80.69	8.861	93.42	99.92	106.51	1.079
7	OZ15	15	NA	4	56.88	75.09	84.22	5.222	99.31	106.84	111.8	0.9923
7	OZ15	15	NA	5	56.03	72.55	80.78	9.092	95.32	99.8	104.95	0.4035
7	OZ15	15	NA	6	55.93	72.5	78.33	6.964	91.77	98.05	103.32	0.6873
8	OZ15	15	LYS	1	58.78	75.46	84.71	10.12	99.84	103.71	108.68	0.1918
8	OZ15	15	LYS	2	58.29	75.19	82.1	8.681	98.13	103.06	106.62	0.2795
8	OZ15	15	LYS	3	56.96	74.98	81.77	8.906	95.79	102.58	107.74	0.6884
8	OZ15	15	LYS	4	58.53	74.92	82.57	8.562	97.19	102.57	107.56	0.38
8	OZ15	15	LYS	5	58.53	75.12	81.39	7.902	96.48	103.35	106.85	0.4325
8	OZ15	15	LYS	6	56.18	75.07	82.34	7.763	98.84	103.55	106.38	0.289
9	OZ30	30	NA	1	55.93	72.35	78.56	6.916	93.65	99.35	104.97	0.6284
9	OZ30	30	NA	2	56.88	72.25	78.56	6.184	93.89	99.04	103.32	0.3526
9	OZ30	30	NA	3	56.88	73.22	81.16	7.867	96.95	101.75	105.68	0.3914
9	OZ30	30	NA	4	54.99	72.73	80.22	8.736	95.3	99.94	103.55	0.3371
9	OZ30	30	NA	5	53.81	72.92	86.34	8.903	95.79	101.04	108.27	0.6326
9	OZ30	30	NA	6	53.81	72.51	80.45	8.406	92.95	99.55	105.2	0.6232
10	OZ30	30	LYS	1	58.39	75	81.37	8.655	98.07	102.83	106.12	0.2625
10	OZ30	30	LYS	2	58.06	75.31	82.34	9.475	100.02	103.91	107.33	0.198
10	OZ30	30	LYS	3	58.98	75.61	82.94	10.18	98.27	103.39	106.91	0.1999
10	OZ30	30	LYS	4	60.18	75.95	82.1	6.975	99.55	104.62	107.56	0.306
10	OZ30	30	LYS	5	58.53	75.44	83.04	9.219	97.9	103.87	107.56	0.3042
10	OZ30	30	LYS	6	57.35	75.79	83.75	9.322	97.42	103.95	108.5	0.3425

APPENDIX 6
WHITE STARCH ISOLATE DSC RAW DATA

					First transition Gelatinization endotherm				Second transition Amylose-lipid complex			
#	Treat	Min	Additives	Rep	**To**	**Tp**	**Tc**	**ΔH**	**To**	**Tp**	**Tc**	**ΔH**
11	none	0	NA	1	65.16	70.66	82.77	6.836	97.63	103.48	110.43	0.4325
11	none	0	NA	2	63.79	69.95	79.33	5.567	96.09	99.87	105.75	0.4779
11	none	0	NA	3	66.13	71.65	84.15	7.043	98.87	105.29	111.25	0.3462
11	none	0	NA	4	64.89	70.51	83.46	7.214	97.22	105.29	112.08	0.5362
11	none	0	NA	5	63.65	70.22	80.43	6.21	94.47	101.87	107.26	0.4483
11	none	0	NA	6	64.75	70.06	84.42	7.46	98.18	104.79	111.53	0.4972
12	none	0	LYS	1	67.5	73.24	85.94	6.477	99.42	106.38	113.59	0.4932
12	none	0	LYS	2	68.21	73.14	85.88	7.078	102.89	108.04	114.28	0.3283
12	none	0	LYS	3	67.39	73.28	87.04	7.918	101.56	108.11	113.29	0.48
12	none	0	LYS	4	66.78	72.98	82.41	6.051	101.4	106.42	112.13	0.3604
12	none	0	LYS	5	68.05	73.29	87.04	7.586	101.89	109.46	117.08	0.6715
12	none	0	LYS	6	66.68	72.32	82.64	6.853	100.93	107.46	114.01	0.395
13	PO15	15	NA	1	65.28	69.17	76.71	5.208	97.05	103.12	108.12	0.3061
13	PO15	15	NA	2	63.92	69.12	78.29	5.488	98.92	106.89	109.98	0.259
13	PO15	15	NA	3	60.4	64.31	73.5	7.868	93.49	99.7	106.13	0.6571
13	PO15	15	NA	4	63.39	68.99	78.66	6.329	99.2	102.8	108.25	0.2574
13	PO15	15	NA	5	58.56	64.42	74.65	10.69	93.72	99.46	106.82	0.5235
13	PO15	15	NA	6	59.94	64.59	74.65	7.727	89.13	98.87	107.51	0.5781
14	PO15	15	LYS	1	67.09	71.8	79.74	5.324	98.71	107.2	113.15	0.641
14	PO15	15	LYS	2	66.28	71.8	81.85	6.743	101.31	108.18	114.93	0.5427
14	PO15	15	LYS	3	63.53	67.79	77.47	8.341	97.58	103.4	109.11	0.5269
14	PO15	15	LYS	4	63.53	67.39	77.47	8.431	98.39	102.57	108.04	0.6209
14	PO15	15	LYS	5	65.72	69.62	79.3	7.912	98.5	104.61	110.6	0.569
14	PO15	15	LYS	6	64.24	70.19	78.49	7.371	99.62	105.85	112.29	0.629
15	PO30	30	NA	1	64.48	68.94	77.85	5.559	101.09	105.43	111.23	0.2442
15	PO30	30	NA	2	62.96	68.98	79.47	6.403	99.15	102.13	110.02	0.3578
15	PO30	30	NA	3	62.44	68.65	77.85	4.589	97.58	101.45	106.23	0.1812
15	PO30	30	NA	4	63.39	69.12	77.44	5.319	97.58	103.48	107.31	0.3628

15	PO30	30	NA	5	65.55	69.9	77.58	4.836	98.66	103.66	108.79	0.3694
15	PO30	30	NA	6	64.89	70.35	76.86	4.776	97.49	103.42	106.99	0.45
16	PO30	30	LYS	1	67.7	72.17	80.23	5.298	103.96	106.49	111.25	0.2439
16	PO30	30	LYS	2	67.09	71.56	80.24	5.596	101.04	106.64	113.18	0.4037
16	PO30	30	LYS	3	66.77	71.96	80.01	5.086	102.98	107.11	112.44	0.5225
16	PO30	30	LYS	4	66.36	71.76	80.15	5.192	101.63	106.5	111.36	0.3681
16	PO30	30	LYS	5	67.64	71.93	79.75	5.242	102.45	106.83	111.12	0.2504
16	PO30	30	LYS	6	67.23	72.29	80.13	5.129	102.86	107.23	111.34	0.2593
17	OZ15	15	NA	1	61.12	65.73	73.85	9.508	92	98.27	102.14	0.592
17	OZ15	15	NA	2	60.5	65	73.37	8.078	93.48	99.24	103.65	0.4188
17	OZ15	15	NA	3	60.62	65.03	72.34	9.795	91.64	98.22	104.05	0.6712
17	OZ15	15	NA	4	61.12	65.45	73.85	7.384	92.95	99.95	104.97	0.5851
17	OZ15	15	NA	5	60.62	65.07	72.57	9.988	93.71	98.93	104.97	0.5322
17	OZ15	15	NA	6	60.37	64.91	74.04	8.188	93.21	98.19	101.39	0.2178
18	OZ15	15	LYS	1	64.28	68.92	77.37	9.461	100.57	105.36	108.92	0.392
18	OZ15	15	LYS	2	63.15	67.63	74.87	8.563	99.92	104.5	108.42	0.3206
18	OZ15	15	LYS	3	64.07	68.5	79.01	10.4	99.92	104.79	108.88	0.3505
18	OZ15	15	LYS	4	63.51	67.72	75.77	9.311	100.47	104.73	108.51	0.2613
18	OZ15	15	LYS	5	63.99	68.48	79.55	10.37	98.41	102.61	108.07	0.3851
18	OZ15	15	LYS	6	63.84	67.74	75.79	8.931	99.92	104.49	108.65	0.3395
19	OZ30	30	NA	1	61.83	65.47	72.67	6.249	95.3	100.14	104.73	0.3243
19	OZ30	30	NA	2	60.41	64.82	73.14	7.414	94.12	99.36	104.5	0.4264
19	OZ30	30	NA	3	60.65	65.35	73.61	6.729	93.65	99.45	102.85	0.3503
19	OZ30	30	NA	4	60.65	65.43	72.67	6.587	92	97.15	101.43	0.1642
19	OZ30	30	NA	5	60.45	65.4	75.54	9.93	95.34	99.75	104.06	0.3405
19	OZ30	30	NA	6	60.18	64.88	73.85	7.312	90.59	97.4	103.32	0.7305
20	OZ30	30	LYS	1	63.75	68.29	76.48	10.48	99.82	104.41	109.72	0.3421
20	OZ30	30	LYS	2	63.62	67.64	75.72	8.594	99.1	104.6	108.72	0.3505
20	OZ30	30	LYS	3	63.75	68.36	79.31	10.31	101.71	105.26	109.01	0.2018
20	OZ30	30	LYS	4	63.71	67.98	77.15	8.092	99.78	105.47	109.21	0.2913
20	OZ30	30	LYS	5	63.52	68.22	78.6	11.11	101.23	104.95	109.25	0.2368
20	OZ30	30	LYS	6	63.71	67.77	75.74	7.482	98.37	103.13	108.03	0.3244

VITA

The author was born in Pusan, South Korea, in 1975. She finished her bachelor's degree in agronomy at Dong-A University. She started her graduate study in food science at Louisiana State University in 1999 and got a Master of Science in food science in fall, 2001. Ms An is a candidate for the degree of Doctor of Philosophy in food science, which she will receive in August 2005.